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Invited Speaker
Abstracts
PROGRESS WITH NATIONAL PLANNING AND COUNTRY EXPERIENCES WITH THE ROLL OUT OF GAPP

S. Aboubaker, Geneva, Switzerland

The Global Action Plan for Pneumonia Prevention and Control (GAPP) was developed by WHO and UNICEF in response to a call to action during ISPPD-5. GAPP aims to call attention to need for rapidly scale up the use of interventions of proven benefit in reducing morbidity and mortality from pneumonia, by a broad coalition of global and national policy-makers, donor agencies and civil society. Following the last ISPPD in Tel Aviv, World Health Assembly endorsed the GAPP in May 2010 in a resolution (WHA63.24) urging Member States to implement the strategies outlined in GAPP.

Many countries have used UNICEF/WHO joint statements for managing children with pneumonia and diarrhoea to increase access to care through trained and supervised community health workers. By 2010, 30 countries had adopted the community case management of pneumonia policy.

To accelerate the implementation of GAPP, WHO with national ministries of health, UNICEF and other partners conducted four regional workshops in Africa and South-East Asia during 2011. In these workshops, representatives from child health, immunization, nutrition, malaria programmes and national health planning cells from 22 countries in Africa and 11 countries in South-East Asia got together to jointly address policy and programmatic gaps and initiate action to scale up the effective pneumonia interventions. Countries with plans to introduce pneumococcal conjugate vaccines nationally were targeted to use as a catalyst to implement GAPP. Country follow-up activities demonstrate improvements in coordination across programmes and increasing implementation of GAPP and diarrhoea control strategies and identifying barriers to overcome them.
KENYA ACTION PLAN FOR THE PREVENTION AND CONTROL OF PNEUMONIA


Background: At the current under five mortality of 74 deaths per 1000 live births, Kenya remains far from achieving its MDG 4 target of 32 deaths per 1000 live births. Pneumonia remains one of the highest causes of these deaths at 20%.

Method: Child survival interventions including the GAPP strategy have been adopted. The Kenya Action for Prevention and Control of Pneumonia (KAPP) working group was established in November 2010. Kenya hosted an inter-country GAPP meeting where 8 countries developed plans of actions for scaling up implementation of the outlined interventions.

Results: Exclusive breastfeeding (including Code of Marketing Breastmilk Substitutes), complementary feeding and hand washing activities have been intensified.

Pneumococcal vaccine was launched on 14th February 2011. Rotavirus vaccine will be introduced in 2013. Virtual elimination of Mother to Child Transmission of HIV activities are being scaled up. IMCI is delivered at health facilities with identification and referral of severe cases at community level. A Community Strategy has been adopted which includes Community Case Management. Intensive policy dialogue with major policy makers and stakeholders is on-going to avail antibiotics at the community. Capacity building for research on maternal newborn and child health is in progress.

Conclusion: Kenya is in advanced stages of the implementation of the Action Plan for Prevention and Control of Pneumonia despite existing challenges. Facilitating factors include good policy environment, high level commitment, renewed interest in control of diarrhea, pneumonia and malnutrition by the Government and partners and public awareness of the right to health.
IMPACT OF A PNEUMONIA INTERVENTION IN LATIN AMERICA: LESSONS FROM THE COMPAS TRIAL

X. Saez-Llorens, Panama, Panama

Grossly, 1.5 million episodes of pneumococcal pneumonia occur annually in LA in children < 5 years. Although several interventions (breast feeding, infant nutrition, air cleaning, sanitary access, medical management) reduce disease morbidity and mortality, the single most effective short-term strategy is vaccination. Up to now, we only had evidence of the efficacy of one commercially available PCV on pneumonia. Since PCV7 was tailored to the serotypes most prevalent in US, there were doubts about its potential usefulness in a developing region with different epidemiological variables. The COMPAS trial was designed, utilizing the gold standard double-blind, randomized, controlled study model, to explore the efficacy of a vaccine (VE) that included 3 additional serotypes (PHID-CV) in Argentina, Colombia and Panama. After overcoming multiple research challenges, we demonstrated a 26% VE (pp) against WHO-defined community-acquired pneumonia (C-CAP) and, more importantly from a public health perspective, a 7% reduction on suspected clinical pneumonia. Building on the lessons from a previous PCV trial conducted in South Africa, an innovative primary outcome (likely bacterial CAP or B-CAP) was introduced to improve the sensitivity of the pneumonia definition. B-CAP is a mixture of C-CAP and other abnormal radiological findings plus C-reactive protein >40 mg/L. Although the VE for B-CAP was lower (23%) than for C-CAP, more cases were averted. Incorporating a PCV in our infant immunization calendar will have a tremendous impact on the burden of pneumonia and other pneumococcal diseases in LA. The associated herd effect of these vaccines will likely improve the efficacy figures.
RECENT IMPROVEMENTS IN THE DIAGNOSIS OF PNEUMOCOCCAL PNEUMONIA

W.C. Albrich, Aarau, Switzerland

Historical data from the pre-antibiotic era and more recent latent class analyses or vaccine-probe studies indicate that the burden of pneumococcal pneumonia is largely underestimated by current diagnostic methods. The usefulness and added value of serology, urinary antigen testing and molecular methods on sterile and respiratory samples are discussed. Real-time polymerase chain reaction (rtPCR) provides quantification of bacterial DNA loads and correlates with quantitative culture results. Critical colonization densities (8x10^3–10^4 lytA or Spn9802 copies/ml from nasopharyngeal samples) seem to distinguish pneumonia from asymptomatic carriage. Applying an 8x10^3 copies/ml lytA cut-off measured by rtPCR from nasopharyngeal swabs for quantitative colonization density might prove an important diagnostic improvement as it uses an easily obtainable specimen and increased the etiologic fraction attributable to pneumococcus by 34% in HIV-infected adults. A different (battlefield) hypothesis assumes that the bacterial-to-leukocyte ratio is higher in pneumonia than asymptomatic colonization. Optimized cut-offs showed high sensitivity and negative predictive values for pneumococcal etiology. High levels of the biomarkers procalcitonin and C-reactive protein improve the specificity of radiographic findings for pneumococcal pneumonia. Multi-detection assays including multiplex rtPCR, mass spectrometry and microarrays hold promise for the future.
DETECTION OF MULTIPLE SEROTYPES IN THE NASOPHARYNX

C. Satzke¹, K.P. Klugman²,3, E.K. Mulholland¹,4,5, ¹Parkville, VIC, Australia, ²Atlanta, GA, USA, ³Johannesburg, South Africa, ⁴Darwin, NT, Australia, ⁵London, UK

Background and aims: Multiple serotype carriage (MSC) is common and important for understanding the link between pneumococcal immunisation, carriage and disease. Traditional serotyping methods typically identify the major serotyping present in a sample, overlooking minor serotypes that may be important in pneumococcal epidemiology, particularly as conjugate vaccines are introduced into countries with a high burden of pneumococcal carriage and disease.

Methods: In recent years, several methods for detection of MSC have been developed. This talk will highlight several of these including molecular approaches, such as standard PCR (including characterisation of products by standard methods, mass spectrometry, IRFLP or sequencing), quantitative PCR, microarray; and antibody-based methods, such as latex agglutination or high-throughput bead-based assays.

Results: Methods for detection of MSC will be described, contrasted, and compared. The advantages and disadvantages of particular methods will be discussed. Challenges including technical issues, how to determine the optimal method, and how to best employ methods for MSC detection in the future, will be outlined.

Conclusions: Despite many advances in technology and increased recognition of the importance of MSC, challenges remain. Accurate detection of MSC will enhance our abilities to study pneumococcal carriage and epidemiology and monitor the impact of vaccination on carriage.
A MULTI-SITE ANALYSIS OF SEROTYPE-SPECIFIC CHANGES IN INVASIVE PNEUMOCOCCAL DISEASE AFTER CONJUGATE VACCINE INTRODUCTION AMONG CHILDREN < 5 YEARS OLD

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Background: To evaluate the issue of serotype replacement, at WHO’s request we undertook a systematic review and analysis of changes in incidence of serotype-specific invasive pneumococcal disease (IPD) after 7-valent pneumococcal conjugate vaccine (PCV7).

Methods: We identified datasets from literature review and recommendations of pneumococcal experts. Original data were collected in standardized tables. Summary measures of changes in serotype-specific IPD rates among hospitalized children < 5 years old were calculated as rate ratios (RR) by dividing rates of IPD at 3-4 and ≥5 years after PCV7 introduction by the average pre-PCV7 IPD rate. Datasets included in the final analysis were restricted to those with ≥2 years pre- and ≥3 years post-PCV7 introduction, ≥70% PCV7 coverage, and no major changes in surveillance systems.

Results: Among 31 datasets received, 17 met inclusion criteria, from North America (n=6), Europe (n=9) and Australia (n=2). A summary of changes in IPD rates is shown below.

<table>
<thead>
<tr>
<th>Serotypes</th>
<th>3-4 years after PCV7 introduction (N=17)</th>
<th>≥5 years after PCV7 introduction (N=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV7</td>
<td>Decreases all sites</td>
<td>Decreases all sites</td>
</tr>
<tr>
<td></td>
<td>Median RR (range) 0.087 (0.030-0.37)</td>
<td>Median RR (range) 0.058 (0.015-0.17)</td>
</tr>
<tr>
<td>Non-PCV7</td>
<td>Increases all sites</td>
<td>Increases all sites</td>
</tr>
<tr>
<td></td>
<td>Median RR (range) 1.7 (1.1-3.4)</td>
<td>Median RR (range) 2.8 (1.9-4.6)</td>
</tr>
<tr>
<td>All</td>
<td>Decreases all sites</td>
<td>Decreases all sites</td>
</tr>
<tr>
<td></td>
<td>Median RR (range) 0.50 (0.31-0.82)</td>
<td>Median RR (range) 0.83 (0.30-0.95)</td>
</tr>
</tbody>
</table>

The greatest increase in non-PCV7 serotypes was among those in 13-valent PCV (5+ years post-introduction, non-PCV7 serotypes in PCV13 RR=3.74, other nonPCV7 serotypes RR=1.87).

Conclusions: Despite consistent increases in non-PCV7 serotypes suggestive of serotype replacement, PCV7 led to a net benefit by reducing overall IPD among children in multiple countries.
A MULTI-SITE ANALYSIS OF SEROTYPE-SPECIFIC CHANGES IN INVASIVE PNEUMOCOCCAL DISEASE AFTER CONJUGATE VACCINE INTRODUCTION AMONG PERSONS ≥5 YEARS OLD

M. Moore¹, D. Feikin², The Serotype Replacement Study Team, ¹Atlanta, GA, ²Baltimore, MD, USA

Background: To evaluate serotype replacement, we performed a systematic review of changes in incidence of serotype-specific invasive pneumococcal disease (IPD) among persons ≥5 years old after 7-valent pneumococcal conjugate vaccine (PCV7) introduction.

Methods: We identified datasets from literature review and expert recommendations. Investigators provided serotype-specific IPD case counts and census data in standardized tables. Changes in serotype-specific IPD rates were estimated using rate ratios (RR) at 3-4 and ≥5 years after PCV7 introduction compared to mean pre-PCV7 rates. Final datasets included those with ≥2 years pre- and ≥3 years post-PCV7 introduction, ≥70% PCV7 coverage, and no major changes in surveillance systems.

Results: Among 31 datasets received, 15 met inclusion criteria (North America, n=5; Europe, n=8; Australia, n=2). The mean annual number of cases pre-PCV7 ranged from < 20 (3 sites) to >1000 (7 sites). PCV7-type IPD declined in most sites while non-PCV7-type IPD increased in all (table). Six sites reported outbreaks of serotypes 1, 5, 8 and 12F after PCV7 introduction.

<table>
<thead>
<tr>
<th>Serotypes</th>
<th>3-4 years post-PCV7 (N=15)</th>
<th>&gt;4 years post-PCV7 (N=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Summary trend: n sites</td>
<td>Median RR (range)</td>
</tr>
<tr>
<td>PCV7</td>
<td>Decreases: 14 Increase: 1</td>
<td>0.40 (0.16-1.1)</td>
</tr>
<tr>
<td>Non-PCV7</td>
<td>Increases: All</td>
<td>1.4 (1.0-2.9)</td>
</tr>
<tr>
<td>All</td>
<td>Decreases: 8 Increases: 7</td>
<td>0.93 (0.66-2.2)</td>
</tr>
</tbody>
</table>

[Trends in IPD among Persons >4 Years Old]

Conclusions: Serotype replacement is occurring among persons not targeted for PCV7; some increases are also likely related to serotype-specific outbreaks and surveillance methodology. Multivariable modeling will be done to account for outbreaks, pre-PCV7 trends, and small sample sizes.
MOLECULAR EPIDEMIOLOGY OF PNEUMOCOCCAL DISEASES IN LATIN AMERICA (LA): THE LAST TWENTY YEARS

A. Corso, PAHO-SIREVA II Group, Ciudad Buenos Aires, Argentina

For the last twenty years, SIREVAII Program (PAHO/WHO) has provided relevant information on invasive pneumococcal serotypes, antibiotic susceptibility patterns, and genetic relationship among S.pneumoniae isolates from children.

Data collected from 19 LA countries during 2006-2010 indicate that thirteen serotypes accounted for 86% of the isolates: 14,6B,1,5,18C,19F,23F,6A,19A,7F,9V,3 and 4, with serotype 14 being the leading isolate in most countries. Last data (LA-2010) showed that for meningitis 44.7% were penicillin resistant (MIC > 0.12 mg/ml) and for non-meningitis 10.8% (MIC > 2 mg/ml) for non-meningitis isolates. Resistance was high for trimethoprim/sulfamethoxazole (SXT) (60%) and erythromycin (32%) but low for chloramphenicol (1.4%). Decreased susceptibility to penicillin (DSP: penicillin MIC > 0.12 mg/ml) was associated to serotypes 14, 23F, 19A and 6B.

Spain 9V-3 sequence type (ST) 156, was associated with DSP and SXT resistance and represents the main clone spread in LA. Spain 6B-1-ST81 was associated with DSP and cefotaxime/SXT/chloramphenicol resistance in Mexico. Colombia 19-ST289, penicillin/erythromycin susceptible but tetracycline/chloramphenicol/SXT resistant was detected in 15 countries. Spain 6B-2-ST90, Colombia 6B-26-ST338 and Taiwan 23F-15-ST242 were identified in different countries. Erythromycin resistance in Argentina was associated with the spread of England 14-9-ST9, Poland 14-3-ST304, Spain 14-3-ST156, Spain 6B-2-ST90, and Spain 23F-1-ST81 clones. Serotype 19A represents 8.7% of the isolates in LA in 2010 and it is associated with CC320 in Mexico, ST1118 in Brazil, and ST1131 (SLV-ST172) in Argentina.

Forty percent of penicillin susceptible serotypes 14,6B,5,1,23F,7F and 3 from Argentina, Brazil, Colombia, Mexico and Uruguay belonged to ST289, ST191, ST242 and ST304.

Continuing surveillance of dominant clones is helpful to evaluate the influence of antimicrobial selective pressure and the impact of PCVs in LA.
PCV7 IN THE U.S.: LESSONS FROM THE FIRST-USE COUNTRY

C. Whitney, Atlanta, GA, USA

In 2000, the U.S. became the first country to introduce 7-valent pneumococcal conjugate vaccine (PCV7). Four PCV7 doses were recommended for infants; catch-up doses were recommended for all children under 2 years. PCV7 was quickly seen to be as effective in routine use as had been demonstrated in an earlier clinical trial, as a rapid drop in vaccine-serotype invasive disease was seen in the first year of introduction. Surveillance data from the second year demonstrated more pronounced effects among young children and a drop in vaccine-serotype disease in older persons, the first signs of herd effects that later became the strongest contributor to the vaccines overall benefits. Vaccine shortages occurred, resulting in missed doses; the shortage allowed a demonstration that both 3- and 4-dose schedules were effective. Subsequent studies showed reductions in antibiotic use, vaccine-serotype carriage, otitis media, meningitis, and pneumonia hospitalizations. As vaccine serotypes disappeared, some non-vaccine serotype strains partially filled the void; an antibiotic-resistant serotype 19A clone became common. Data from indigenous groups showed conflicting results; in Alaska, non-vaccine serotypes eroded vaccine benefit but significant replacement was not seen among Navajo and Apache. New serotypes were discovered, as some 6A strains that continued to cause disease in spite of demonstrated cross protection from the 6B antigen were shown to be a 6A varianta serotype coined 6C. In 2010, the U.S. joined dozens of countries introducing the next generation conjugate vaccines. The coming years hold promise for improved prevention and new exploration of vaccine effects around the world.
SEVEN LESSONS FROM AN ABBREVIATED SCHEDULE COUNTRY

E. Miller, P. Kaye, London, UK

**Background and methods:** The UK introduced PCV7 in 2006 as a 2/4/13 month schedule with a single dose toddler catchup based on immunogenicity data from this schedule, supported by a dynamic model, an economic analysis and practical considerations. Enhanced national surveillance of IPD was established in 1996 and supported by carriage studies conducted pre/post-PCV7 introduction. Surveillance of non-invasive disease endpoints relied on computerised databases.

**Results:** The 2+1 schedule was highly effective in reducing IPD in children and generated herd protection in older agegroups. Serotype replacement was more marked than reported in the US but overall impact on IPD was still substantial. No reduction in non-bacteraemic pneumonia was seen in older agegroups or vaccinated children in high risk groups. Vaccination of individuals >= 65 years with 23-valent vaccine did not mitigate serotype replacement effects. The antibody level predicting protection was shown to vary between serotypes. Carriage studies conducted in parallel with IPD surveillance suggested that the lower case/carrier ratios in some of the non-vaccine serotypes would reduce the potential for replacement disease with higher valency vaccines. Parameterisation of the HPA dynamic model with UK post-PCV surveillance data produced markedly different results than when parameters were estimated from US data. An individual-based model incorporating immunity would allow a more realistic representation of the competition between serotypes in carriage.

**Conclusions:** Proper understanding of the complexities of the epidemiological impact of conjugate vaccines requires a multidisciplinary approach incorporating a variety of different data sources and careful interpretation of trends over time.
SEVEN LESSONS FROM A COUNTRY WITHOUT A CONJUGATE BOOSTER DOSE

P. McIntyre¹, V. Krause², R. Menzies¹, C. Chiu¹, H. Cook²
¹Sydney, NSW, ²Darwin, NT, Australia

Background: 7 valent pneumococcal conjugate vaccine (7vPCV) was funded for Indigenous children in Australia in 2001, including a booster dose of 23 valent polysaccharide vaccine (23vPPV) in high incidence regions. In 2005, 7vPCV at 2, 4, and 6 months with no booster became universal, with catch-up to 2 years of age and 23vPPV was funded for all persons > 65 years.

Lessons:

2. By 2006, high 7vPCV coverage and reduction in IPD due to 7 valent types comparable to settings with a dose in second year of life.
3. Small numbers of vaccine failures, predominantly 19F.
4. Significant overall IPD reductions consistent with herd effects from 7vPCV in all age groups >5 years, especially > 65 years, but not among Indigenous adults in high incidence regions.
6. Indigenous children eligible for 23vPPV booster had higher 19A incidence pre 7vPCV which did not increase post PCV7.
7. Significant reductions in operations for ventilation tube insertion (children) and ICD coded pneumonia (all ages) in non-Indigenous but for Indigenous only ICD coded pneumonia in children.
Vaccination is one of the most successful interventions in public health and it is a main contributor to human health, equity and safety. It has been one of the pillars in the Pan-American Health Organization's (PAHO) technical cooperation.

SIREVA II represents the first international \textit{S. pneumoniae} prospective laboratory-based surveillance program in the world with more than 37,000 isolates characterized since its creation in 1994.

In 19 countries in LA and one Center in the Caribbean, SIREVA II has become an internationally recognized laboratory based surveillance network.

The pneumococcal conjugate vaccine was implemented in several national immunization programs across the Latin America and Caribbean region (LAC) since 2007. By 2010, a total of fifteen countries and two territories used conjugate pneumococcal vaccines in their national vaccination program.

For the Invasive Pneumococcal Disease, laboratory based-evidence distribution of serotypes in children < 5 years in LAC showed a potential coverage of 58%, 73% and 86% for the 7-valent, 10-valent and 13-valent conjugate vaccines, respectively.

This presentation will focus on the surveillance data that has been gathered through SIREVA II, describing the historical fluctuations in serotypes as well as the changes observed after vaccine introduction, comparing the various sub regions and countries.

In order to contribute for vaccine decision making and monitoring, SIREVA II, in close coordination with Immunization Programs, is extending its effort in improving a cost/effective sentinel surveillance, developing new and more efficient molecular technologies for diagnosis and strain characterization in children, with an extension to adults and most at risk populations.
OUT OF AFRICA: LESSONS LEARNT AND CHALLENGES IN EVALUATING THE EFFECTIVENESS OF PNEUMOCOCCAL CONJUGATE VACCINE (PCV)

S.A. Madhi, Johannesburg, South Africa

Contributing to the burden of pneumococcal disease in Africa are underlying conditions such as HIV and malaria. Consequently, interventions targeted at these underlying risk factors, which coincide with the introduction of PCV, may compromise the ability to attribute reductions in the burden of pneumococcal disease solely to vaccination.

The best study design to evaluate the impact of PCV in Africa is yet to be determined. Initial ecological studies have reported an anticipated decline in vaccine-serotype IPD following introduction of PCV. However, the magnitude of replacement disease due to non-vaccine serotypes has been greater than observed in developed countries. In addition, changes in management and/or prevention of perinatal-HIV-transmission and malaria which coincided or immediately preceded the introduction of PCV, may have contributed to reduction in pneumococcal disease independent of vaccination. The impact of childhood PCV immunization on pneumococcal transmission has also been explored in Africa, with indications that there may be an interruption of transmission of vaccine-serotypes even in settings which previously reported high prevalence of colonization among adults. However, PCV may be less effective in reducing the risk of vaccine-serotype acquisition in some high-risk groups, such as HIV-infected children.

Although data on the effectiveness of PCV against pneumonia in African settings are still sparse, initial studies have raised questions as to the best study design with which this can be evaluated in Africa. A case-control study on pneumonia in South Africa has also highlighted the discordant effect which choices of controls may have on vaccine-effectiveness estimates.
PCVS EFFECTIVENESS IN LATAM, LESSONS AND EXPERIENCE

A. Gentile, Ciudad Autónoma de Buenos Aires, Argentina

Adopted by world leaders in the year 2000 and set to be achieved by 2015, the Millennium Development Goals (MDGs) provide concrete, numerical benchmarks for tackling extreme poverty in its many dimensions.

In 1990-2003, which covers about half the time allowed for achieving the targets, the health of the American Region's population improved significantly, particularly in children. However, the regional infant mortality averages mask wide disparities between countries so vaccines to combat preventable diseases are the goal to be achieved.

One of the most important disease is pneumonia, one pneumococcal disease form, causes almost 1 in 5 deaths of children under five worldwide — more than 1.6 million children each year; but Streptococcus pneumoniae (SP) causes others clinical conditions, such as invasive disease, meningitis, bacteremia, sepsis and acute otitis media (AOM).

A strong surveillance system should be developed using different endpoints: a- invasive disease rates, b- the bacterial resistance and antibiotics use, c-population-based studies on community-acquired pneumonia and laboratory surveillance systems. d- Carriage and herd immunity e- AOM: serotypes distribution, antibiotic use.

Ecological studies, such as temporal series analysis represents a good methodology however its imply a strong surveillance system (using similar indicators and same methodology) with baseline data (three years data pre vaccine introduction) and a health system so stable that we could measure the impact.

Pneumococcal disease is preventable disease; bacterial resistance to common antibiotics continues to expand worldwide so the need to promote effective pneumococcal vaccines has never been greater. LATAM is introducing PCVs very fast so it is very important to measure this impact.

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THE PNEUCARRIAGE PROJECT - OPTIMIZING CARRIAGE DETECTION METHODS TO MONITOR THE IMPACT OF PNEUMOCOCCAL VACCINE INTRODUCTION

K. Mulholland¹, C. Satzke², ¹London, UK, ²Melbourne, VIC, Australia

Pneumococcal conjugate vaccines (PCVs) are currently being rolled out at a rapid rate into developing countries, particularly Africa. In virtually all settings where PCVs have been used, serotype replacement has occurred to some extent. In most settings there is some reduction in effectiveness associated with replacement, but the overall effect is positive. No data exist for developing countries, and on first principles it could be expected that replacement effects would be more profound in these settings.

There are three means of monitoring the effectiveness of PCVs and the population biology changes that may indicate replacement - monitoring invasive pneumococcal disease (IPD), radiological pneumonia, and carriage.

Few developing countries have any system for monitoring invasive pneumococcal disease rates. Following vaccine introduction there will usually be improvements in surveillance and microbiology, so it is to be expected that the number of IPD cases will remain the same or even increase.

Monitoring changes in pneumococcal carriage remains the only option for most developing countries. To provide useful information to guide pneumococcal vaccine use, it is important to use the best available methods. The PneuCarriage project is a global project funded by the Bill & Melinda Gates Foundation which will identify the best method to detect multiple serotype carriage. This will be important for monitoring population biology changes associated with vaccine introduction.
COMPARING PNEUMOCOCCAL CLONE EVOLUTION THROUGH WHOLE GENOME ANALYSIS

S.D. Bentley¹, N.J. Croucher², ¹Hinxton, UK, ²Cambridge, MA, USA

Whole genome sequences representing populations of bacteria can be analysed to give a detailed view of evolutionary changes from gross rearrangements through to single nucleotide changes. Coupled with clinical and epidemiological data, such analyses can advance our understanding of critical pathogen characteristics such as person-to-person transmission, geographic spread, acquisition of resistance to antimicrobial agents and evasion of vaccines.

We first applied this high throughput sequencing approach to Streptococcus pneumoniae clone PMEN1 (also known as Spain23F-1), renowned as the first recognized multidrug resistant clone to spread to most parts of the globe, and detailed the impact of recombination on the acquisition of further drug resistance and the evasion of conjugate vaccines through capsule switching. Application of this approach to other major clones has revealed differences in characteristics such as geographic structure, rates of recombination and mutation, acquisition of drug resistance markers and recombination hotspots. These features will be summarized and discussed.
A HIGH-RESOLUTION VIEW OF GENOME-WIDE PNEUMOCOCCAL TRANSFORMATION

N.J. Croucher¹, S.R. Harris², L. Barquist², J. Parkhill², S.D. Bentley², ¹Boston, MA, USA, ²Cambridge, UK

Transformation is an important mechanism of microbial evolution that allows bacteria to rapidly adapt in response to clinical interventions; examples include facilitating vaccine evasion and the development of penicillin resistance in the major respiratory pathogen Streptococcus pneumoniae. To characterise the process in detail, the genomes of 124 S. pneumoniae isolates produced through in vitro transformation were sequenced and transformation events detected. Those recombinations importing the selected marker were independent of unselected events elsewhere in the genome, the positions of which were not significantly affected by local sequence similarity between donor and recipient or mismatch repair processes. However, both types of recombinations were sometimes mosaic, with multiple segments originating from the same strand of donor DNA. The lengths of the unselected events were exponentially distributed with a mean of 2.3 kb, implying that recombinations are stochastically resolved with a fixed per base probability of 4.4x10⁻⁴ bp⁻¹. The distribution of recombination sizes, and the observed under representation of large insertions within them, suggests transformation may act to reduce the size of bacterial genomes rather than acting as an efficient mechanism for the uptake of accessory genomic loci.
HOST-MICROBIAL INTERACTIONS: THE KEY TO PNEUMOCOCCAL DISEASE?

D. Bogaert, Utrecht, The Netherlands

Respiratory diseases are caused by viruses and bacteria like Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis and Staphylococcus aureus. Asymptomatic colonization of the upper respiratory tract with both viral and bacterial pathogens occurs frequently, though it is also prerequisite for consecutive infections. At any given time, the nasopharyngeal niche is colonized with a vast range of (commensal) bacteria, and as such constantly subject to synergistic and competitive interspecies and microbiome-host interactions. We hypothesize that disturbances in this equilibrium due to for instance new acquisitions of bacteria and viruses, antimicrobial therapy, vaccination and environmental factors may play a major role in susceptibility to consecutive infections. A better understanding of the dynamics of the nasopharyngeal microbiota and its interplay with host and environment may give us more insight into pathogenesis of respiratory diseases.
AGING AND CHRONIC LUNG INFLAMMATION INCREASES SUSCEPTIBILITY TO PNEUMOCOCCAL PNEUMONIA THROUGH INCREASED BACTERIAL LIGAND EXPRESSION IN THE LUNGS

C.J. Orihuela, San Antonio, TX, USA

Background and aims: Advanced age is associated with chronic inflammation due to increased numbers of DNA-damaged cells, which secrete pro-inflammatory cytokines, and increased susceptibility to community-acquired pneumonia. As Streptococcus pneumoniae binds to host proteins that are produced in response to pro-inflammatory cytokines (i.e. Platelet activating factor receptor [PAFr], Polymeric immunoglobulin receptor [plgR], Laminin receptor [LR] and following DNA damage (i.e. Keratin 10 [K10]), we investigated the expression of these bacterial ligands in the lungs of aged animals.

Methods: Lung sections from healthy young, mature, and aged humans and mice were examined for PAFr, LR, plgR, and K10 levels. Young mice were infused with physiologically relevant levels of tumor necrosis factor (TNF) and levels of these proteins measured in the lungs; susceptibility to pneumococcal infection was also assessed. DNA damaged senescent lung cells were examined for the expression of bacterial ligands, permissiveness to pneumococcal infection, and their ability to induce ligand expression on normal lung cells in a paracrine manner.

Results: Aged lungs had significantly increased levels of PAFr, LR, plgR, and K10. Young mice infused with TNF had increased levels of PAFr and plgR and dramatically increased susceptibility to pneumonia. Senescent lung cells had increased LR and K10 and were able to induce expression of PAFr on normal cells. Senescent cells and normal cells exposed to senescent cell supernatant were permissive for pneumococcal infection.

Conclusions: Aging, chronic inflammation, and DNA damage was associated with increased permissiveness for pneumococcal infection due to increased bacterial ligand expression on lung cells.
THE IMMUNOLOGY OF COLONISATION
S.B. Gordon, Liverpool, UK

Pneumococcal colonisation is essential to transmission and precedes disease. Early life exposures and vaccination reduce colonisation, transmission and disease by inducing a combination of humoral and cellular responses in the mucosal and systemic compartment. Repeated pneumococcal exposure during adult life boosts these responses. This presentation will review epidemiological and immunological data from infant studies and present new data from experimental pneumococcal inoculation of adult volunteers. The immunological importance of repeated pneumococcal exposure in adult life will be discussed.
COMPARISON OF THE IMMUNOLOGY OF POLYSACCHARIDE AND CONJUGATE VACCINES IN HUMANS

D. Goldblatt, London, UK

Pneumococcal capsular polysaccharides, the key components of all licensed pneumococcal vaccines are considered T-independent antigens when administered to the immune system in a purified form. The polysaccharide vaccine (PPV) available today was licensed in 1983 and contains a combination of 23 different capsular polysaccharides. Much of our understanding of polysaccharide immunology comes from observations of vaccinated individuals. The recognised limitation of T dependent antigens and in particular their relatively poor immunogenicity in early life led to attempts to modify the immune handling of bacterial polysaccharides. The chemical conjugation of polysaccharides to carrier proteins has been successful and protein-polysaccharide-conjugate-vaccines (PCV's) are highly immunogenic in early life and induce memory. These immunological characteristics of PCV's have been translated into unequivocal benefits in vaccinated populations; reduced nasopharyngeal carriage and reduced pneumococcal disease. The immunological basis of reduced carriage remains unclear. Is reduced carriage mediated by local immune mechanisms or serum-derived antibody acting on mucosal surfaces and are such effects seen with PPV? When PPV and PCV are studied head to head in adult populations, differences in responses between the two are not as dramatic as anticipated. It is unclear whether this is due to the fact that most adults are antigen experienced, having encountered the pneumococcus via carriage or whether the formulations are not directly comparable. Finally, information about the immune modulatory properties of capsular polysaccharides is emerging as evidence for reduced responses to vaccine following infection or while carrying the homologous serotype are described. The mechanism remains unclear.
NOVEL PNEUMOCOCCAL VACCINES AND THEIR REGULATORY PATHWAYS

G.R. Siber\textsuperscript{1,2}, 1Baltimore, MD, \textsuperscript{2}Cambridge, MA, USA

Serologic correlates of protection for polysaccharide antibodies have facilitated the development of new pneumococcal conjugate vaccines. Pneumococcal protein vaccines are simpler and less expensive to manufacture and could substitute for PCV or be added to PCV to enhance coverage. However, clinical efficacy will need to be demonstrated in head-to-head trials with PCV, which would be extremely large.

It would be desirable to de-risk development by showing proof-of-concept for efficacy before Phase 3 trials. For surface proteins which are antibody targets, induction of opsonizing antibodies is critical. For other antibody targets such as pneumolysin or adherence factors other functional assays are needed. Pneumococcal proteins which mediate colonization resistance via Th17 cells have recently been discovered.

Showing reduction in colonization in a phase 2 clinical study in humans is the most convincing and relevant way to de-risk development of protein vaccines. In order to match the efficacy of the conjugates which protect unimmunized people via herd immunity, protein vaccines will have to show an impact on colonization. Decreased colonization is likely to reduce otitis media, pneumonia and invasive disease. A powerful approach is to combine proteins which reduce both colonization and invasive disease thus providing protection at multiple phases of pathogenesis.

Since colonization is not in itself a medically significant disease it may not be sufficient to serve as the primary outcome for regulatory approval. However, an impact on colonization may serve as a basis for a provisional approval followed by large scale phase 4 studies showing efficacy against non-PCV types.
IMMUNOLOGY AND PROTECTIVE MECHANISMS OF PNEUMOCOCCAL PROTEIN AND WHOLE CELL VACCINES

R. Malley, Boston, MA, USA

The advent and success of conjugate pneumococcal vaccines brought about the hope of reducing, or even potentially eliminating, Streptococcus pneumoniae as a major scourge of humanity. Since then, much has been learned about the ability of anticapsular antibodies to reduce the burden of pneumococcal diseases both directly and indirectly via the establishment of herd protection. However, pneumococcus has demonstrated its capacity to adapt to changes in the environment, overcome immunological pressures, and thus potentially reduce the overall impact of an effective vaccine strategy. Over the past several decades, scientific investigations focused on the discovery of novel approaches to provide broad systemic and mucosal immunity to this pathogen. These efforts remain an urgent priority to this day, both for developing and developed countries. Several approaches are under intense investigation, including variations of the polysaccharide-protein conjugate strategy, protein-based strategies, and whole cell pneumococcal vaccines. This talk will review these various strategies as well as their immunological basis.
PROGRESS TOWARDS DEVELOPMENT OF A PROTEIN-BASED PNEUMOCOCCAL VACCINE

D. Borys, W. Hausdorff, F. Fierens, F. Godfroid, V. Verlant, Wavre, Belgium

Background/aims: The burden of pneumococcal disease and childhood pneumonia remains high worldwide, as does the risk of serotype replacement. Novel pneumococcal vaccines are needed to prevent disease regardless of S. pneumoniae serotype.

Methods: Highly conserved proteins, expressed by all serotypes, preferentially involved in pathogen growth or virulence and having potential to elicit a protective immune response would be ideal antigen candidates. Our research has identified detoxified pneumolysin (dPly) and pneumococcal histidine triad (PhtD) as promising candidates. They were selected from other candidates including choline binding protein A (CbpA), pneumococcal surface protein A (PspA), pneumococcal surface adhesion A (PsaA). Preclinical data showed that PhtD elicited protection against several S. pneumoniae serotypes in various mouse models and that a combination of dPly and PhtD protected monkeys from pneumococcal pneumonia. Clinical studies are ongoing to assess candidate protein antigens, with or without pneumococcal polysaccharide conjugates, in adults and children. Additionally, impact on nasopharyngeal carriage is being evaluated in a study in Gambia (partnership with PATH, MRC, LSHTM). Available safety and immunogenicity data in adults and toddlers to date will be presented (abstracts: 250, 372 and 374).

Results: All investigational vaccine formulations containing pneumococcal dPly and PhtD, with or without pneumococcal polysaccharide conjugates, were well tolerated by adults and toddlers. Proteins were immunogenic and did not seem to alter the immune responses elicited by conjugates when given as a combined formulation in toddlers.

Conclusions: These results for dPly and PhtD are promising and support potential inclusion in a protein-based pneumococcal vaccine to be developed.
Poster Shift 1
THE EFFECTIVENESS OF BENZYL PENICILLIN FOR TREATMENT OF SEVERE PNEUMONIA AMONG KENYAN CHILDREN

A. Agweyu¹, D. Gathara¹, N. Muinga¹, J. Oliwa¹, T. Edwards², M. English¹,²,³, Severe Pneumonia Trial Study Group, ¹Nairobi, Kenya, ²London, ³Oxford, UK

Background: For over 20 years, case management has been a key strategy in the management of childhood pneumonia in low income countries. However, the introduction of the Haemophilus influenzae type B (HiB) and pneumococcal conjugate vaccines targeting the major causes of bacterial pneumonia, the effect of HIV on the pattern of childhood pneumonia and concerns of increasing antibiotic resistance all raise questions regarding the current effectiveness of the recommended treatments. We studied the effectiveness of benzyl penicillin for treatment of severe childhood pneumonia as a prelude to a randomized controlled trial comparing benzyl penicillin with amoxicillin for treatment of severe pneumonia.

Methods: We recruited children aged 2 - 59 months from seven primary referral hospitals across Kenya using a prospective observational design. Eligible children were managed in hospital and followed up for the primary outcome of treatment failure at 48 hours.

Results: A total of 220/820 (26.8%) screened fulfilled the eligibility criteria. A majority of those excluded had signs of very severe pneumonia. HIV testing was offered to children and performed on 217/220 (98.6%) children. Of those who received the HIV rapid test, 11 (5.1%) tested positive. The risk of treatment failure at 48 hours was estimated at 10.0% (95% CI 6.3 - 14.9%). Three deaths were reported giving a mortality risk of 1.36% (95% CI 0.28 - 3.93).

Conclusion: These results suggest that benzyl penicillin remains effective for the treatment of severe pneumonia. Further work is needed to explore the aetiology of childhood pneumonia in the population studied.
Poster No 2

ACUTE PYOGENIC MENINGITIS: COMPARISON OF VARIOUS ANTIBIOTIC REGIMENS IN THE TREATMENT IN A SOUTH ASIAN POPULATION

B. Ahmed, A. Valliani, B. Zulfiqar, Karachi, Pakistan

Object: Aim of this study is to compare various antibiotics in setting of acute pyogenic meningitis.

Design: A quasi-experimental study.

Patients and methods: Study was conducted in department of medicine Military Hospital Kohat from Dec 2006 to March 2009. A total of sixty patients with provisional diagnosis of acute pyogenic meningitis between 13 - 50 years age belonging to heterogenous group of population mainly recruits from various military training centers were included in the study. Ten patients were excluded from study because of negative cerebrospinal fluid findings. These patients were started on group A antibiotics (Ceftriaxone, Ampicillin plus Chloramphenicol), group B (Ampicillin plus Chloramphenicol) and group C (Ceftrixone) at random.

Result: The analysis of results of treatment with three different treatment regimens was done by noting complete recovery, complications and death. Twenty patients who were given group A regimen showed 90% complete recovery, one patient developed fulminant menigococcemia and another patient developed deafness with gradual recovery (10% complications). Amongst fifteen patients in group B regimen 87% patients showed complete recovery, one patient developed seizure, another patient developed hydrocephalus (13% complications). Fifteen patients who were started group C regimen 93.33% patients fully recovered and one patient developed fulminant meningococcemia (7% complications). The results of three treatment regimens were compared by using chi square test and were almost comparable (p-value > 0.05).

Conclusion: In the empirical treatment of acute pyogenic meningitis, ceftriaxone alone cheap, safe and is as effective as combination of ceftriaxone plus ampicillin plus chloromphenicol or combination ampicillin plus chloromphenicol.
WHOLE GENOME SEQUENCING SUGGESTS COTRIMOXAZOLE IS BECOMING INCREASINGLY INEFFECTIVE AT PREVENTING INVASIVE PNEUMOCOCCAL DISEASE IN THE MALAWIAN POPULATION

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Background: Cotrimoxazole (trimethoprim-sulphamethoxazole) is recommended by the WHO for prophylaxis in HIV/AIDS to limit opportunistic infections including S. pneumoniae. Sulfadoxine/Pyrimethamine was widely used in Malawi to treat malaria between 1993 and 2007. Since the introduction of cotrimoxazole in 2002 >94% of Malawian pneumococci have been resistant. We explored the molecular basis of this resistance under this dual antibiotic pressure.

Methods: 140 Malawian pneumococci were subjected to Illumina sequencing. In silico techniques were used to identify resistance mechanisms.

Results and discussion: Phylogenetic analysis suggested clonal dissemination was responsible for the spread of cotrimoxazole resistance. All sulphamethoxazole resistant isolates displayed an insertion of 3-6bp in folP, resulting in the insertion of 1-2 amino acids in the sulphonamide-binding site of DHPS.

The molecular basis of trimethoprim resistance was more complex. The majority of isolates (56%) contained I100L mutation (critical in causing trimethoprim resistance) with an additional mutation at residue 92 (increases but alone is not sufficient to cause resistance), while 10% contained I100L alone. 34% of Malawian isolates that were resistant to trimethoprim(>32µg/ml), showed a mutation at residue 92 but did not contain I100L. This suggests Malawian pneumococci are employing a novel trimethoprim resistance mechanism. The folA genes showed an annual increase in sequence divergence from 2004, arguably due to the strong selective pressure exerted by extensive cotrimoxazole use.

These data suggest that Cotrimoxazole is likely to become increasingly ineffective at preventing IPD in the population and that the clinical efficacy of cotrimoxazole should be re-evaluated.
REGIONAL VARIATION ON ANTIPNEUMOCOCCAL AND ANTIOXIDANT ACTIVITY OF LAUREL AND MYRTLE ESSENTIAL OILS

E. Eriotou, D. Koulougliotis, I. Kokosi, A. Amitsi, C. Roussa, E. Makariadou, Y. Samaras, Kefalonia, Zakynthos, Greece

Background and aims: Laurus nobilis and Myrtus communis are characterized by a large biotype variability among plants. The aim of this work was to determine and compare the chemical composition as well as the antipneumococcal and antioxidant activities of essential oils derived from both plant species in 3 different islands of the Ionian Sea in Greece.

Methods: Essential oils (EOs) were obtained by hydrodistillation of native plants collected from the islands Kefalonia, Zakynthos and Leukada during the last 10 days of August. Their chemical composition was determined by gas chromatography (GC). The bacterial strains used were: Streptococcus pneumoniae ATCC 6303, 6305, 27336, 49136, 49150 and 49619. The antipneumococcal activity was determined by using the disc assay and MIC. The antioxidant activity was determined by monitoring the decrease of the DPPH radical via EPR spectroscopy.

Results: Laurel EO from the island of Zakynthos showed significantly higher antipneumococcal and antioxidant activities relative to the laurel EOs obtained in the other two islands. Myrtle EO showed similar such activities in all three islands and on average lower than laurel EO. A comparison of the chemical compositions of each plant EO between the three islands depicts certain differences. An effort is made to associate this variability with the observed antipneumococcal and antioxidant activities.

Conclusions: Data indicate that both laurel and myrtle essential oils can be used as antimicrobials for the control of pneumococcus, as well as antioxidants. Furthermore, it is apparent that plant biotype is crucial for the antimicrobial and antioxidant activity.
ANTIPNEUMOCOCCAL ACTIVITY OF THYME ESSENTIAL OIL’S COMPONENTS

E. Eriotou1, D. Koulougliotis2, A. Amitsi1, I. Kokosi1, A. Mourelatou1, I. Riga1, Y. Samaras1, 1Kefalonia, 2Zakynthos, Greece

Background and aims: Plants of importance in modern agriculture and trade are not restricted to traditional food, forage and fiber crops, but increasingly include species with secondary metabolites in their essential oils (EOs) having therapeutic qualities. Among the EOs examined, thyme EO shows significant antipeumonococcal activity. In this work, 14 major chemical components of thyme EO were individually tested for antimicrobial activity against pneumonococcus.

Methods: The major components of thyme EO tested for antipeumonococcal activity were: carvacrol, caryophyllene, g-terpinene, p-cymene, eugenol, (-) limonene, terpinyl acetate, eucalyptol, linalyl acetate, myrtenol, a-pinene, (+) limonene, a-terpineol, (-) carveol. Purity of substances was determined by gas chromatography (GC). Bacterial strains used were: Streptococcus pneumoniae ATCC 6303, 6305, 27336, 49136, 49150 and 49619. The tests used were the disc assay and the MIC.

Disc Assay: Bacteria cells mixed with soft agar were placed onto sheep blood agar plates. Sterile filter discs were placed onto the surface and 5 µl of essential oils were pipetted onto the discs.

MIC: Each strain at a concentration of $10^5$ cfu/ml in sheep blood broth was aliquoted into ELISA plate wells, where essential oil of different concentrations was added.

Results: Carvacrol, eugenol and myrtenol showed the largest antimicrobial activity, whereas p-cymene and a-pinene exhibited none. The remaining substances showed variable and overall significantly lower antimicrobial activity.

Conclusions: Data indicate that the antipeumonococcal activity of thyme EO can be attributed to specific oil components. Further research is required to determine possible synergistic and/or antagonistic activity among the chemical constituents.
Poster No 6

PNEUMONIA ONLINE: USING SOCIAL MEDIA TO BUILD ALLIES

M. Feldman, J. Younkin, L. Privor-Dumm, M. Knoll, O. Levine, Baltimore, MD, USA

Background: Pneumonia kills more children than AIDS, TB and Malaria combined, yet each disease attracts significantly more attention and funding than pneumonia. The highest social engagement has come from simple messages with clear actions. Our goals were to build awareness regarding prevention and treatment of childhood pneumonia using social and digital media.

Methods: We updated the World Pneumonia Day website, Facebook page and Twitter account by adding video and interactive content to increase communication of key pneumonia messages. We measured traffic and sharing across geographies and organizations before and after additions.

Results: In 30 days prior to WPD, >3000 people (>60-fold increase) “liked” the Prevent Pneumonia Facebook page, and more than 25% of these users commented on and shared our content, above the threshold considered to be an “actively engaged” base, with 120907 people seeing content associated with the page. Real-time conversations increased >8-fold on Twitter and attracted followers from >12 countries (29% in India, 19% in US, 7% in Nigeria) and from influential organizations. Key pneumonia messages reached >550,000 Twitter users via these efforts. Conclusions: These data suggest social media is effective at increasing attention to child pneumonia. To increase traffic and expand reach, future engagement needs to build on these successes and to target activists already interested in similar issues.
PERFORMANCE OF BINAXNOW FOR DETECTING STREPTOCOCCUS PNEUMONIAE IN PLERUAL FLUID FROM CHILDREN IN THE DOMINICAN REPUBLIC


Background: Streptococcus pneumoniae is a leading cause of pediatric pneumonia with effusion. Diagnosis is challenging since culture is insensitive and molecular methods are not widely available. We evaluated performance of the immunochromatographic test BinaxNOW for pneumococcal detection in pleural fluid (PF) from children.

Methods: From 7/2009 to 6/2011, we collected PF from children < 15 years old admitted to Robert Reid Cabral Children's Hospital in Santo Domingo, Dominican Republic with pneumonia and pleural effusion. PF was tested by culture, PCR and BinaxNOW (as both point-of-care test and repeated in CDC-Atlanta laboratory). We calculated sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of BinaxNOW and culture compared with PCR.

Results: Among 120 enrolled cases, 111 had PF tested by lytA real-time-PCR, culture, and point-of-care BinaxNOW; 61 (55.0%) were positive by PCR. Sensitivity, specificity, PPV, and NPV of point-of-care BinaxNOW relative to PCR were 100% (95%CI 94.1-100), 86.0% (95% CI 73.3-94.2), 89.7% (95% CI 80.0-95.8), 100% (95%CI 91.8-100.0); those of laboratory-performed BinaxNOW (n=100) were 98.2% (95%CI 90.3-100), 97.8% (95%CI 88.2-99.9), 98.2% (95%CI 90.3-99.9), 97.8% (95%CI 88.2-99.9), respectively. Sensitivity, specificity, PPV, NPV of culture relative to PCR were 29.5% (95%CI 18.5-42.6), 100% (95%CI 93.0-100), 100%, (95%CI 81.5-100), 54.3%, (95%CI 43.7-64.6), respectively. Fifty percent (56/112) of cases received antibiotics prior to sample collection.

Conclusions: BinaxNOW is a practical, valid diagnostic tool for clinical care and surveillance in settings with limited laboratory capacity and where prior antibiotic use is common. Further studies are recommended in developing countries to assess cost-effectiveness and validate feasibility.
Poster No 8

STREPTOCOCCUS PNEUMONIAE INVASIVE INFECTIONS IN CHILDREN. SENSITIVITY AND CLINICAL COURSE

M. Medina, G. Ferrucci, Ramos Mejia, Argentina

Introduction: Streptococcus pneumoniae is the etiologic agent of invasive infections with significant morbidity and mortality in pediatric patients.

Objectives:
1. To assess the antibiotic sensitivity of isolates from blood cultures in hospitalized patients with invasive disease by S. pneumoniae.
2. Discuss the clinical course of patients.

Material and methods: Prospective study lasting 20 months (January 2010 to September 2011). Entered the study, male and female pediatric patients aged 1 month to 14 years who had invasive infections with positive blood culture for S. pneumoniae. Sensitivity was studied by diffusion on Mueller-Hinton agar supplemented with sheep blood according to standard CLSI% and penicillin MICs was performed by E-test.

Clinically monitor progress and response to treatment and need for antibiotic schema change.

Results: Seventeen patients with invasive infections caused by S. pneumoniae, 11/2010; 6/2011. In 2010, penicillin MICs were performed at 9 of the isolates with MICs < 0.2, half of whom also had accompanying resistance to TMS. In 2011 6 isolates, 3 MIC of 0.006 ug/ml, one had MIC of 1 ug/ml, another was not viable strain and was identified by specific latex, the last the diagnosis was made by urinary antigen. One was associated TMS resistant.

Seventeen patients, average age 46 months. Prevenar 7 in 9; 11 had pneumonia with effusion, without effusion 2, 3 occult bacteremia, mastoiditis 1.

Comment: Our population had pneumonia complicated by stroke even with isolates susceptible to penicillin.
EPIDEMIOLOGICAL PROFILE OF COMMUNITY-ACQUIRED PNEUMONIA IN CHILDREN ADMITTED TO A BRAZILIAN CENTER IN THE PRE PNEUMOCOCCAL CONJUGATE VACCINE ERA


Background and aims: A 10-valent pneumococcal conjugate vaccine (VP10v) was universally introduced in Brazil in 2010, but studies of its impact on community-acquired pneumonia (CAP) admissions in children are scarce. This study describes CAP-related hospitalization rates and the characteristics of children < 5 years old hospitalized with CAP prior to the conjugate vaccine introduction.

Methods: A cross-sectional retrospective study was conducted. Children (> 1 month and < 5 years old) admitted with CAP to a pediatric referral center in northeast Brazil between January 2008 and December 2009 were assessed for age, gender, month of hospitalization, length of stay, outcome and origin.

Results: 2051 children were enrolled, with similar number of CAP admissions in 2008 (1002) and 2009 (1049). Most children came from Greater Recife (70%), had < 2 years old (74%), were male (54%) and stayed in hospital for < 8 days (70%). The monthly distribution of CAP hospitalization can be seen in Figure 1. There were 13 (1.3%) CAP-related deaths in 2008 and 58 (5.5%) in 2009.

Conclusion: CAP was a frequent cause of hospitalization in children < 2 years. The differences in mortality between 2008 and 2009 might be related to the 2009 circulation H1N1 Influenza virus. Knowing pre VP10v immunization baseline characteristics of CAP admitted children in Brazil will inform the assessment of immunization effectiveness.

Figure 1 – Seasonal distribution in the frequency of hospitalization for CAP in children under 5 years. IMIP – 2008 and 2009.
Poster No 10

PNEUMOCOCCAL PNEUMONIA IN HIV/AIDS CUBANS PATIENTS

T. García, D. Verdasquera, J. Pérez, I. Martínez, D. Salazar, M. Pérez, Havana, Cuba

Background: Human immunodeficiency virus (HIV)-infected persons have a high incidence of pneumonia and pneumococcal disease. To identify *Streptococcus pneumoniae* by non-invasive microbiological techniques as a cause of pneumonia in HIV/AIDS and associated risk factors, we performed a prospective study of HIV-infected patients in Institute of Tropical Medicine "Pedro Kouri", Cuba.

Methods: We studied 85 HIV infected patients diagnosed with bacterial pneumonia based on clinical criteria, radiological and laboratory between November 2007 and April 2008. Prior to antimicrobial treatment, each patient was collected from blood and sputum samples for microbiological culture. The identification and antimicrobial susceptibility was performed by miniApi semiautomated system (bioMérieux, France). The association between various risk factors was measured by calculating the hazard ratio (significant values above 1.5, 95% confidence interval).

Results: *S. pneumoniae* was identified in 40.5% of patients. High percentage of organisms resistant to penicillin (83%) was reported; also 17% of pneumococci with intermediate susceptibility were identified in this study. On the other hands, 56.6% of pneumococcal isolates of multidrug was described. Prevailed individuals under 50 years of age (91.7%), smoking (65.9%), with a lymphocyte count below 200 cells/mm$^3$ TCD4 (64.7%) and that used highly active antiretroviral therapy (54.7%). No statistically significant association was found between these risk factors and pneumococcal pneumonia.

Conclusions: In HIV / AIDS cubans patients there is still a high percentage of pneumococcal pneumonia and sputum culture is a useful technique for diagnosis. Antimicrobial resistance of *S. pneumoniae* should be seen in the treatment of these patients.
Poster No 11

A LANDSCAPE ANALYSIS OF RECENT AND ONGOING CHILDHOOD PNEUMONIA ETIOLOGY STUDIES

Z. Gilani¹, Y.D. Kwong¹, O.S. Levine¹, M. Deloria-Knoll¹, J.A.G. Scott²,³, K.L. O’Brien¹, D.R. Feikin¹,⁴, ¹Baltimore, MD, USA, ²Kilifi, Kenya, ³Oxford, UK, ⁴Atlanta, GA, USA

Background: While Streptococcus pneumoniae and Haemophilus influenzae type b cause a majority of the 1.6 million annual childhood pneumonia deaths, widespread use of conjugate vaccines is changing the overall pneumonia burden and etiology profile. We describe the current landscape of recent pneumonia etiology studies in children ≤5 years in the developed and developing world.

Methods: We conducted a literature review and a survey of researchers in the field of childhood pneumonia to identify studies with pneumonia etiology data in children ≤5 years since the year 2000.

Results: We identified 153 studies (88 published and 65 ongoing or recently completed) being conducted in 63 countries. A substantial fraction of studies were from Africa (19.0%) and South Asia (24.8%), regions with the greatest burden of childhood pneumonia deaths. The most commonly used case definitions were the WHO IMCI definitions (pneumonia 23.8%, severe pneumonia 32.5% and very severe pneumonia 5.3%); most (51.0%) case definitions included a radiologic component. Most studies included inpatients (94.9%). Physicians determined study inclusion in 77.0% of studies and nonphysicians in the remainder. Blood culture was the most common lab method employed (72.3%), while 55.4 % used PCR of nasopharyngeal swabs or aspirates. Thirteen studies (8.5%) employed case-control methodology.

Conclusions: Recent pneumonia etiology studies in children vary by case definitions used, levels of clinician involvement, facility types, specimen collection and laboratory techniques. There is a need for standardizing methods, analyses and reporting for pneumonia etiology studies to reduce confounding and thereby optimize the understanding of true etiologic variability across settings.
SUSTAINED DECLINE IN PNEUMONIA HOSPITALIZATIONS FOLLOWING INTRODUCTION OF PCV7 IN 2000, US CHILDREN < 2 YEARS OF AGE

M. Griffin¹, C.G. Grijalva², M.R. Moore², Y. Zhu¹, C.G. Whitney², ¹Nashville, TN, ²Atlanta, GA, USA

Background and aims: We evaluated whether the decline in pneumonia hospitalizations for US children age < 2 years following PCV7 introduction in 2000 were sustained through 2009.

Methods: We used Nationwide Inpatient Sample and Census data to estimate annual all-cause and pneumococcal pneumonia hospitalization rates for persons ≥65 years old in pre-PCV7 (1997-1999) and recent post-PCV7 (2007-2009) years. Pneumonia hospitalizations had a pneumonia code as primary diagnosis or a sepsis or meningitis code as primary and pneumonia as secondary. Pneumococcal pneumonia also had a pneumococcal disease code.

Results: Between pre- and recent post-PCV7 years, all-cause pneumonia declined 43% (95% CI 35% to 50%) in children age < 2 years, a difference of 551 (95% CI 430 to 672) hospitalizations per 100,000 children annually. Pneumococcal pneumonia declined 61% (95% CI 47% to 72%), accounting for a difference of 17 (95% CI 12 to 21) hospitalizations per 100,000. For children 2-4 years, respective declines were 13% (95% CI 3% to 22%) and 29% (95% CI 12% to 42%) for all-cause and pneumococcal pneumonia hospitalizations.

Conclusions: Dramatic declines in pneumonia in young were sustained 9 years following PCV7 introduction in the US.
US Pneumococcal Pneumonia Hospitalization Rates
Age <2 Years

<table>
<thead>
<tr>
<th>Year</th>
<th>Rate per 100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>29.3</td>
</tr>
<tr>
<td>1998</td>
<td>24.5</td>
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<tr>
<td>2001</td>
<td>11.2</td>
</tr>
<tr>
<td>2002</td>
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</tr>
<tr>
<td>2003</td>
<td>8.85</td>
</tr>
<tr>
<td>2004</td>
<td>9.62</td>
</tr>
<tr>
<td>2005</td>
<td>12.4</td>
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<td>2007</td>
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<td>12.3</td>
</tr>
<tr>
<td>2009</td>
<td>10.3</td>
</tr>
</tbody>
</table>

[US Pneumococcal Pneumonia Hospitalization Rates A]
Background and aims: Whether replacement disease erodes the indirect benefits of PCV7 use over time is unknown. We assessed pneumonia hospitalization rates for older adults up to 9 years after PCV7 introduction.

Methods: We used Nationwide Inpatient Sample and Census data to estimate annual pneumococcal and all-cause pneumonia hospitalization rates for persons ≥65 years old in pre-PCV7 (1997-1999) and recent post-PCV7 (2007-2009) years. Pneumonia hospitalizations had a pneumonia code as primary diagnosis or a sepsis or meningitis code as primary and pneumonia as secondary. Pneumococcal pneumonia also had a pneumococcal disease code.

Results: Recent pneumococcal pneumonia rates were 42-54% lower than pre-PCV7 rates and all-cause pneumonia rates were 7-23% lower. There was an absolute average annual decline of 255 (95% CI 114 to 396) hospitalizations per 100,000 persons age ≥65 years.

Conclusions: Parallel declines in pneumococcal and all-cause pneumonia hospitalizations among older adults long after PCV7 introduction suggest a persistent indirect benefit of PCV7 infant immunization.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>1997-1999</th>
<th>2001-2006</th>
<th>2007-2009</th>
<th>%Decline</th>
<th>Rate Difference (95% CI)</th>
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<tbody>
<tr>
<td>65-74</td>
<td>72</td>
<td>44</td>
<td>42</td>
<td>42%</td>
<td>30 (25, 35)</td>
</tr>
<tr>
<td>75-84</td>
<td>127</td>
<td>71</td>
<td>63</td>
<td>51%</td>
<td>65 (56, 74)</td>
</tr>
<tr>
<td>≥85</td>
<td>212</td>
<td>115</td>
<td>98</td>
<td>54%</td>
<td>113 (97, 129)</td>
</tr>
</tbody>
</table>

[Pneumococcal Pneumonia Rates per 100,000]

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>1997-1999</th>
<th>2001-2006</th>
<th>2007-2009</th>
<th>%Decline</th>
<th>Rate Difference (95% CI)</th>
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</thead>
<tbody>
<tr>
<td>65-74</td>
<td>1290</td>
<td>1264</td>
<td>1203</td>
<td>7%</td>
<td>87 (7, 167)</td>
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<td>2753</td>
<td>2610</td>
<td>2392</td>
<td>13%</td>
<td>361 (193, 530)</td>
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<tr>
<td>≥85</td>
<td>5692</td>
<td>5202</td>
<td>4389</td>
<td>23%</td>
<td>1303 (950, 1656)</td>
</tr>
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</table>

[All-cause Pneumonia Rates per 100,000]
Poster No 14

A STUDY OF PNEUMONIA ETIOLOGY AMONG HOSPITALIZED CHILDREN IN KILIFI, KENYA - A PILOT STUDY FOR PERCH

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Background: Pneumonia is the leading cause of childhood death in the developing world. Better etiology data are required to reduce this burden.

Methods: We conducted a case-control study of pneumonia etiology among children aged 1-59 months in rural Kenya. Cases were hospitalized with WHO-defined severe pneumonia (SP) or very severe pneumonia (VSP); controls were outpatients without pneumonia. We collected blood for culture, induced sputum for culture and multiplex PCR, and oropharyngeal swabs for multiplex PCR from cases, and serum for serology and nasopharyngeal swabs for multiplex PCR from cases and controls.

Results: 810 (85%)/984 eligible cases were enrolled; 232 (29%) had VSP. Blood cultures were positive in 52 (7%)/749 cases; most commonly for Streptococcus pneumoniae (n=30). A predominant potential pathogen was identified in sputum culture in 70 (17%)/417 cases; most commonly S. pneumoniae and M. catarrhalis (n=16). A virus was detected by PCR of nasopharyngeal swabs in 486 (60%)/805 cases and 172 (47%)/369 controls. Only RSV showed a significant association between detection in the nasopharynx and pneumonia hospitalization (odds ratio 12.5, 95%CI: 3.1, 51.5). Among 257 cases in whom all specimens (excluding serology) were collected, bacteria were identified in 24 (9%), viruses were identified in 24 (9%), viruses in 137 (53%), mixed viral/bacterial infection in 39 (15%), and no pathogen in 57 (22%); bacterial causes outnumbered viral causes when the case-control analysis results were considered.

Conclusions: A potential etiology was detected in >75% of children hospitalized with SP or VSP. Except for RSV, the case-control analysis did not detect an association between viral infection of the nasopharynx and hospitalized pneumonia.
PENICILLIN DISK DIFFUSION FOR PREDICT SUSCEPTIBILITY OF STREPTOCOCCUS PNEUMONIAE TO $\beta$-LACTAMS IN MENINGITIDIS ISOLATES: AN IMPORTANT TOOL FOR RESOURCE-LIMITED SETTINGS

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Background and aims: Streptococcus pneumoniae is a predominant pathogen causing meningitis. In recent years, the resistance of $S$.pneumoniae to penicillin and third-generation cephalosporins has increased and this has restricted the use of ceftriaxone for empirical treatment of bacterial meningitidis. We evaluated the use of penicillin disk diffusion for predict the susceptibility of $S$.pneumoniae to $\beta$-lactam in meningitidis isolates.

Methods: In 571 isolates of $S$.pneumoniae from healthy carriers under two years, we determined the correlations between inhibition zone of disk diffusion and minimum inhibitory concentration (MIC) of penicillin. We determined the MIC of penicillin and ceftriaxone.

Result: Of the 571 isolates, 314 (54.9%) showed resistance to penicillin (MIC $\geq$ 0.12 $\mu$g/ml o PE-meningitidis). 91 isolates (15.9%) showed intermediate resistance to ceftriaxone (MIC = 1 $\mu$g/ml), and 33 (5.7%) showed complete resistance (MIC $\geq$ 2 $\mu$g/ml). By correlating the inhibition zone and MIC PE-meningitidis a high level of concordance was observed ($k$=0.8239) in contrast to the PE- non meningitidis where there was a weak level of concordance ($k$=0.0269).

Conclusion: The rate of penicillin resistance was high for $S$.pneumoniae (PE-meningitidis), meanwhile the resistance to ceftriaxone was low but non negligible. High correlation was observed between disk diffusion and MIC PE meningitidis. Based on our results, penicillin disk diffusion could be used as a predictor of resistance of $S$.pneumoniae to ceftriaxone in meningitidis isolates. Furthermore, this method is quick and cost-effective to be performed in routine clinical laboratories, as a consequence physicians could modify treatment in case of resistance to ceftriaxone.
THE ROLE OF ECHINACEA PURPUREA AGAINST UPPER RESPIRATORY INFECTIONS

G. Eslami, S. Taheri, F. Kaghazchi, N.B. Monafred, Tehran, Iran

Infectious disease such as Upper Respiratory Infections (like Otitis, Pharyngit, …) have great role in our daily life & antibiotics cause some problem such as medical resistance & side effects. Therefore using Herbal product may help greatly to cure infections.

Echinacea purpurea which is being used in traditional medicine due to healing properties in USA & Europe. Now this herb was used in some products in Iran.

Echinacea purpurea was collected from researching farm of haljerd (karaj) in summer of 2004. Air dried aerial, root and flower parts of plants were ground and subjected to percolation. diagnostic tests were applied on samples chosen from hospitalized patients to compare antibacterial effects of extract with synthetic antibiotics. To do this, disk diffusion and agar dilution methods were used and an antibiogram was prepared.

Antimicrobial activity was determined by measuring the growth inhibitory zone. The results, show that extract of Echinacea purpurea has mild antibacterial effects.
Poster No 17

**NASOPHARYNGEAL CARRIAGE SURVEILLANCE - A CORRELATE OF HEALTH OUTCOMES IN MONITORING HOUSING AND HYGIENE PROGRAMS IN REMOTE AUSTRALIA**

**A.J. Leach, H. Smith-Vaughan, K. Hare, L. McDonald, R. Bailie, P. Morris, J. Carapetis, Darwin, NT, Australia**

**Background:** The early age of acquisition, high prevalence, co-colonisation, serotype diversity, density and persistence of pneumococcal and non-typeable *H. influenzae* (NTHi) carriage in Indigenous populations are associated with high rates of respiratory infection.

Overcrowding and inadequate washing facilities in the home may be important causes of disease.

A 10 year, billion dollar initiative in new and renovated housing, designed to reduce crowding and improve poor living conditions and the health of Indigenous people, is underway. Nasopharyngeal (NP) carriage surveillance has the potential to capture a critical shift in health status of Indigenous children in response to such programs.

**Methods:** Ongoing surveillance of otitis media and nasal carriage of important respiratory bacteria in children 0 to 6 years of age living in remote communities. Data linkage with housing programs is planned.

**Results:** Post PCV, between 2003 and 2009, carriage of pneumococci and NTHi were each consistently ~80% in all age groups. Fewer than 10% children had bilateral normal middle ears and ~20% had perforations. The overall hierarchy of top ten serotypes is now 16F>>19A>19F>11A>23B>6C>10A>6A>23F>33F; all except 6C&33F had isolates with resistance to penicillin, azithromycin, or both.

**Conclusions:** No reduction in nasopharyngeal carriage or ear disease has been achieved in remote Indigenous communities over many years. Ongoing surveillance and data linkage may assist in determining whether current housing or other programs are sufficient at the individual, household and community level to reduce crowding, cross infection and thus pneumococcal and NTHi disease in these high-risk children.
ISPPD-8
Poster Shift 1: Monday, March 12, 2012 – Tuesday, March 13, 2012

Poster No 18

SUSTAINED DECREASES IN PNEUMONIA HOSPITALISATIONS IN INDIGENOUS AUSTRALIAN CHILDREN
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Background and aims: In 2001, 7-valent pneumococcal conjugate vaccine (7vPCV) at 2, 4 and 6 months of age was introduced for all Indigenous children in Australia. Following universal funding in 2005, 7vPCV coverage in Indigenous children significantly increased. We examined rates of hospitalisations coded as pneumonia (P), acute lower respiratory infection (ALRI), and bronchiolitis (B) before and after implementation of this immunisation program in Australia.

Methods: Hospitalisations coded as Indigenous among children < 2 years of age for P, ALRI, and B were identified in a national electronic database for the jurisdictions where a 23vPPV booster is also given to Indigenous children in the second year of life (Northern Territory (NT), North Queensland (Qld), Western Australia (WA) and South Australia (SA)) for 4 time periods: Jul98-Jun00 (baseline); Jul02-Jun04 (targeted PCV); Jul05-Jun07 (early universal PCV); and Jul08-Jun10 (later universal). Hospitalisation rates were determined for each diagnostic category and jurisdiction.

Results: Rate ratios for each diagnostic category by jurisdiction, diagnostic category and time period are shown in Figure.

Hospitalisation rate ratios compared to baseline by coding group and region

[Hospitalisation rate ratios]

Conclusion: A significant decrease in Indigenous hospitalisations coded for pneumonia was consistent across jurisdictions and sustained after 5 years of universal funding of 7vPCV in Australia. Decreases in ALRI were seen in 3 of 4 jurisdictions but no changes in B. This is suggestive of a sustained vaccine effect.
DIAGNOSTIC VALUE FOR RAPID URINARY ANTIGEN TEST (BINAX NOW) S. PNEUMONIAE IN URTI & LRTI IN CHILDREN TEHRAN, IRAN

S. Noorbakhsh, M. Farhadi, A. Tabatabaei, Tehran, Iran

Background: S. Pneumoniae is one of the most common bacterial pathogens causing meningitis; CAP; rhinosinusitis in Iranian children and adolescents.

S. pneumoniae is the second most common cause of bacterial meningitis in our children. Massive immunization of children with H. influenzae and pneumococcal vaccines is not used in our country.

Objective: We searched the prevalence of S. pneumoniae by rapid urinary test in children with upper and lower respiratory tract infection and compared with healthy children.

Materials and methods: A case-control study done in 3 tertiary care centers in Rasul hospital, Tehran, Iran (2006-2007). The urinary antigen for S. Pneumoniae tested for 54 CAP; 56 acute rhinosinusitis and 50 healthy children.

Results: Antigenuria detected in 31.5% of CAP; 31.5% of rhinosinusitis cases; and 6% (3/50) of controls with significantly differences in CAP and rhinosinusitis cases (P =0.01, 0.01). None cases with non-pneumococcal CAP had antigenuria. In compare with blood culture the specificity for Pneumococcal antigenuria test was 94% but sensitivity is undetectable (high negative culture in cases).

Conclusion: The nasopharyngeal carrier state for pneumococci in our country is very lower than other developed countries (3-7%).

Nasopharyngeal carrier states for S. pneumonia in healthy control are very low (6%). We recommend rapid urinary antigen test add to conventional cultural methods for early diagnosis of pneumococcal respiratory infection.
THE EFFECTS OF ENVIRONMENTAL TOBACCO SMOKE ON PNEUMONIA RISK IN CHILDREN UNDER 7 YEARS IN NORTHERN NIGERIA

E.O. Odiase, Children/Adolescents As Smoke Free Examples-CASE, Ibadan, Nigeria

Background: The numerous adverse effects of Environmental Tobacco Smoke (ETS) on the non-smoking public have being evidenced through decades of research. This does not only affect adults but children. ETS effects on children have shown to be grave as it worsens asthma conditions, increases pneumonia cases and causes Sudden Infant Death Syndrome (SIDS). This study considers pneumonia risk on children under age 7 in Northern Nigeria exposed to ETS.

Methods: Most residents in Kano State of Northern Nigeria took part in a population-based large-scale cross-sectional survey in Kano state from 2007-2010. Demographic information coupled with socioeconomic status, smoking status and house environment was collected. Pneumonia cases reported among children below 7 in each household in the previous 18 months were recorded based on parent's/guardian’s report.

Results: Out of 528, 800 people resident in 102,334 homes, 52,888 (10%) were children aged 7 years and below. While the prevalence of ETS exposure on children was 81%, the prevalence of reported pneumonia cases was 3.5%. Multiple logistic regression analysis showed that exposure to ETS was independently associated with reports of pneumonia cases (adjusted odds ratio 1.55, 95% CI 1.25 to 1.92). The prevalence of tobacco smoking was higher among men than women (63.5% vs 44.1%). It is estimated that 32.7% of childhood pneumonia in the northern region of Nigeria is attributable to ETS.

Conclusions: Attention should be given to reduction to children’s exposure to ETS not only in Nigeria but in all affected areas mostly all parts of the world.
A PREDICTION MODEL FOR OUTCOME IN CHILDREN WITH PNEUMONIA IN RURAL WEST AFRICA

Background and aim: Pneumonia causes about 1.6 million deaths every year. Symptom recognition and identification of children needing urgent referral is a key step in reducing mortality. We developed a model predicting mortality in children admitted with pneumonia.

Methods: We conducted a prospective observational study in children aged 2 to 59 months at health facilities in the Basse Demographic Surveillance Area from 2009 to 2011. Presenting symptoms and signs were recorded for patients meeting screening criteria. Haemoglobin, malaria testing, blood cultures and chest radiographs were done where required. Data was analysed with multivariate analysis and logistic regression.

Results: 3198 records were included in the analysis. The final multivariate model produced the following (table 1).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Odds ratio</th>
<th>95% Confidence Interval</th>
<th>95% Confidence Interval (bootstrap)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unable to drink or sit</td>
<td>7.93</td>
<td>5.25, 11.99</td>
<td>5.08, 12.39</td>
</tr>
<tr>
<td>Clinically severe malnutrition</td>
<td>5.49</td>
<td>3.57, 8.44</td>
<td>3.58, 8.43</td>
</tr>
<tr>
<td>Oxygen saturation</td>
<td>0.95</td>
<td>0.93, 0.97</td>
<td>0.92, 0.98</td>
</tr>
<tr>
<td>Grunting</td>
<td>2.21</td>
<td>1.44, 3.39</td>
<td>1.43, 3.40</td>
</tr>
</tbody>
</table>

18% of observed variation was explained by the model. If age in months was added to the model the odds of death were 0.99 (95% CI; 0.97, 1.01) for each increasing month of age. Internal validation by bootstrap sampling produced insignificant changes in coefficient standard errors.

Conclusion: The model provided modest prediction of mortality. Internal validation suggests that our model is robust. However external validation of the model is needed and updating with additional predictive variables would be useful.

Acknowledgements: Basse Health Centre, Basse DSS residents
THE GLOBAL COALITION AGAINST CHILD PNEUMONIA: INTEGRATED SOLUTIONS, COORDINATED ACTIONS TO ACHIEVE POLITICAL SUPPORT & FINANCIAL SUSTAINABILITY

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Pneumonia is a leading killer of children globally. Despite available tools to prevent and treat this disease, increased political and financial commitment is needed to significantly reduce child deaths. In 2009, WHO and UNICEF led the charge to gain consensus from the technical community and issue the Global Action Plan for the Prevention and Control of Pneumonia (GAPP). The Global Coalition Against Child Pneumonia was formed to build public and political support to address this problem.

In the summer of 2009, the coalition met to plan the first World Pneumonia Day (WPD, observed annually on November 12th) and support the GAPP, which called for advocacy and action. The coalition agreed on strategies, objectives, and tactics to raise awareness, call for funding of programs and action by key constituencies through use of traditional and digital media, small grants, briefings and events. The coalition also used GAPP as a basis for messaging to political leaders, decision makers, funders and international bodies.

By 2010, 125 organizations (+25% vs. 2009) had joined the coalition and events were held in 42 countries (+16% vs. 2009). WPD was an opportunity to mobilize policy makers in developing and donor countries. New partnerships and innovative activities including PneumoniaFighters, Pneumonia's Last Syrah and Blue Jeans Day helped expand the advocate base. An approach based on GAPP led to coordinated efforts and a strong voice to bring global focus to the problem of child pneumonia and improve the likelihood of financial sustainability by generating political support.
ANALYSIS OF DISEASE BURDEN AND MORTALITY SPECIFICALLY RELATED TO PNEUMONIA IN VENEZUELA

A. Risquez, L. Echezuria, J.S.M. Castro, Caracas, Venezuela

The purpose of this study is an analysis of pneumonia-specific mortality in Venezuela to meet the burden of disease, the risk of death according to mortality rates, years of potential life lost (YPLL) as an indicator of preventable deaths.


Results: The risk of dying by age group is very different, with the extremes of life risk 10 to 500 times greater than the average for the general population.

Children under 1 year and adults over 85 years, remain at risk adults older than 60 years. Impact on YPLL is concentrated in younger age groups to 4 years.

Conclusions: Mortality from pneumonia is a public health problem for all age groups: children, adults, and seniors. The years of potential life lost shows the relevance of infant deaths from this disease, raising its relative burden to almost 50% of the total, is preventable with vaccination in children and adults. Reducing deaths in this age group substantially improved life expectancy, with a potential impact on infant mortality.
AN 가치의 예: Overuse and misuse of antibiotics in the treatment of community acquired pneumonia (CAP) in Vietnam has contributed to alarming antibiotic resistance rates. The aims were to describe the antibiotics prescribed for inpatient CAP, and estimate the cost of prescribed antibiotics with those recommended in the clinical practice guideline (CPG).

Methods: A random sample of 300 hospitalised children aged 2m to < 5 years with an ICD10 code consistent with pneumonia in 2010 were included. Data on demographic and clinical features, and antibiotics prescribed were extracted from medical records. Pneumonia severity (WHO criteria) was allocated by a senior doctor, who also determined adherence to the CPG, and recommended which antibiotics should have been used. Costs of antibiotics were sourced. A blinded radiologist re-read all radiographs (WHO criteria).

Results: 54.3% were admitted from home. Moderate, severe, and very severe pneumonia accounted for 41%, 55%, and 2% of all cases. Pre-treatment with antibiotics occurred in 49%. Very few children (8%) had primary end point consolidation. The median LOS was 7 days. Ceftazadime was the most commonly prescribed antibiotic (43.3%), followed by cefotaxime (30%). Amoxycillin and oxacillin were rarely prescribed. Complicated combinations of antibiotics were common. The median cost of antibiotics prescribed was more than 2 times higher than the cost that was estimated if appropriate antibiotics were prescribed.

Conclusions: Adherence to the CPG was reasonable. However, inappropriate prescribing was most common among cases with moderate pneumonia. The costs of antibiotics prescribed were unnecessarily high.
ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS FROM NATIVE PLANTS OF GREECE ON PNEUMOCOCCUS

Y. Samaras¹, D. Koulougliotis², A. Amitis¹, I. Kokosi¹, C. Roussa¹, E. Eriotou¹, ¹Kefalonia, ²Zakynthos, Greece

Background and aims: There is a trend in western society to consume fewer synthetic products and prefer products with small environmental impact. In this work, the antimicrobial activity of 12 plant essential oils on 6 strains of the Streptococcus pneumoniae was determined.

Methods: Essential oils (EOs) screened for antimicrobial activity were those of the following plants collected in Greece: Pistacia lentiscus, Crithmum maritimum, Rosmarinus officinalis, Pelargonium capitatum, Lavandula angustifolia, Foeniculum vulgare, Smyrnium rotundifolium, Vitex agnus-cactus, Thymus vulgaris, Ocimum basilicum, Origanum majorana and Mentha spicata. All EOs were obtained by hydrodistillation of local plants and their chemical composition was determined by gas chromatography (GC). The bacterial strains used were: Streptococcus pneumoniae ATCC 6303, 6305, 27336, 49136, 49150 and 49619.

Disc assay: Bacteria cells mixed with soft agar were placed onto sheep blood agar plates. Sterile filter discs were placed onto the surface and 5 µl of essential oils were pipetted onto the discs.

MIC: Each strain at a concentration of 10⁵ cfu/ml in sheep blood broth was aliquoted into ELISA plate wells, where essential oil of different concentrations was added.

Results: The essential oils of T. vulgaris, M. spicata, Oc. basilicum and P. capitatum were the most active against all strains tested with both assays. The remaining essential oils showed variable and overall significantly lower antimicrobial activity.

Conclusions: The data indicate that specific essential oils can be used as antimicrobials for the control of pneumococcus. Further research is required to determine their mode of action at the cellular/molecular level.
Pneumonia is one of the major killers in the developing countries and affects millions of people every year. Despite significant advancement in therapeutics, pneumonia is still a big challenge for the health care providers which demand for a global campaign against pneumonia. However, there are many hurdles/problems which should be overcome. Translational medicine provides approaches which can accelerate the therapeutics development and can provide support in fight against pneumonia. Translational medicine approaches includes: 1) There should be close communication and interaction between clinician and microbiologist to share each other's knowledge and observations and to put mutual efforts for the development of effective therapeutics. 2) Databases and libraries for strains for the individual geographical area should be established to target effective treatment against specific strain. 3) New bioinformatics and biostatistics tools and methods should be developed to extract the useful information from the large amount of collected data. 4) Community involvement and education are also important factors for achieving the targets against pneumonia. 5) Active cooperation between academics, industry and government should be established. 6) New funding sources should be explored. We suggest the translational medicine approaches to achieve the global initiatives for control of pneumonia.
SYNTHESIS AND MICROBIOLOGICAL ACTIVITY OF NEWLY SYNTHESIZED HALO- AND ALKYL- FLAVANONES

I. Stojanovic, B. Ivkovic, M. Sokovic, Belgrade, Serbia

Aims: Structure-properties relationship study of antibacterial activity of synthesized halo- and alkyl - flavanones

Methods: The compounds were synthesized in Claisen-Schmidt’s reaction between 2-hydroxacetophenone and ortho-substituted benzaldehyde (substituents: metoxy, methyl, fluoro and chloro) in alkal solution and they were cyclizated by the influence of alkal at high temperature (Picture 1). In order to investigate the antimicrobial activity of the synthesized compounds, the modified microdilution technique was used. All determinations were performed in duplicate and two positive growth controls were included.

![Picture 1.]

Results: The MICs of flavanones are summarized in Table 1. The MICs were defined as the lowest concentrations of tested compounds which completely inhibited bacterial growth.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MIC (µmol/ml) fluoro-flavanone</th>
<th>MIC (µmol/ml) chloro-flavanone</th>
<th>MIC (µmol/ml) methyl-flavanone</th>
<th>MIC (µmol/ml) metoxy-flavanone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus (ATCC 6538)</td>
<td>0.4</td>
<td>0.4</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Bacillus cereus (humane isolate)</td>
<td>0.4</td>
<td>0.4</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Micrococcus flavus (ATCC 10240)</td>
<td>0.4</td>
<td>0.4</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Listeria monocytogenes (NCTC 7973)</td>
<td>0.4</td>
<td>0.4</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (ATCC 27853)</td>
<td>0.8</td>
<td>0.8</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Enterobacter cloacae (humane isolate)</td>
<td>0.8</td>
<td>0.8</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Salmonella typhimurium (ATCC 13311)</td>
<td>0.8</td>
<td>0.8</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Escherichia coli (ATCC 35210)</td>
<td>0.8</td>
<td>0.8</td>
<td>0.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

[Table 1. MIC (µmol/ml) of compounds]

Conclusion: All of these compounds showed significant antibacterial activity. Compounds with alkyl and metoxy groups showed better activity towards Gram positive bacteria.
Poster No 28

SEASONALITY OF CLINICAL AND PNEUMOCOCCAL PNEUMONIA IN THE AFRICAN MENINGITIS BELT, TOGO 2010-2011


Background and aims: Streptococcus pneumoniae is known to substantially contribute to hyperendemic meningitis incidence during the dry season in the African meningitis belt. We explored whether a similar seasonality exists for pneumococcal pneumonia.

Methods: From May 2010 to April 2011, we enrolled all patients admitted for clinical pneumonia at the five hospitals in Dapaong (northern Togo). We obtained blood for culture and evaluated chest radiographs. Endpoint pneumonia was defined as lobar infiltrate or pleural effusion as agreed upon by two readers or by one reader and the arbiter.

Results: Among 643 enrolled patients, the median age was 22 years (range 0-90) and 39.3 % (253/643) had endpoint pneumonia. The annual incidences of clinical and endpoint pneumonia were 200 and 85 per 100,000 population and were highest among persons 30+ and < 5 years of age. Monthly incidence rates peaked in January and February for endpoint and clinical pneumonia at 19 and 46 per 100,000 population, respectively, with a nadir during July. The same seasonality was found for bacteremic pneumococcal pneumonia, which accounted for 6% of cases (N=40) with serotype 1 predominating (7/13 isolates; serotyping ongoing).

Conclusions: Clinical, lobar, and bacteremic pneumococcal pneumonia occur with high incidences in the African meningitis belt with a seasonality that parallels that of meningitis. The moniker “meningitis belt” may incompletely describe bacterial disease epidemiology in the region.
Poster No 29

HOSPITALIZATIONS WITH PNEUMONIA: NATIONWIDE POPULATION-BASED TRENDS IN 30-DAY MORTALITY IN DENMARK 1996-2010

R. Thomsen, M. Søgaard, J.B. Kornum, R.B. Nielsen, H.C. Schønheyder, M. Nørgaard, Aalborg, Denmark

Background and aims: Pneumonia-associated hospitalizations have increased substantially in the Western world. Population-based data on the development in prognosis of hospitalized pneumonia in the PCV-era are scarce. We examined this issue in a large nationwide cohort study.

Methods: We used Denmark’s unique nationwide medical databases to identify 522,299 adults with a hospitalization with pneumonia between 1995 and 2010. We compared trends in 30-day mortality rates over calendar time, using Cox’s regression analyses to adjust for changes in age, gender, and comorbidity.

Results: The overall 30-day mortality in patients with a first-time pneumonia hospitalization remained fairly stable at 15.0% in patients diagnosed in 1996-2000, 15.2% in 2001-2005, and 15.6% in 2006-2010. Advanced age and comorbidity were strong predictors of death. After controlling for increases in patient age and comorbidity over calendar time, the adjusted 30-day mortality rate ratio was 0.98 (95% CI 0.96-1.01) for patients diagnosed in 2001-2005, and 1.00 (95% CI 0.98-1.02) for patients diagnosed in 2006-2010, as compared to 1996-2000. Stratified analyses showed a marked mortality reduction over time among young adults aged 15-39 years, but a small mortality increase over time for adults aged 40-64 years or ≥80 years and for those with high levels of comorbidity.

Conclusions: It is of concern that the adjusted mortality of hospitalized pneumonia has remained practically unchanged over the last 15 years in Denmark. More data on the effectiveness of interventions to prevent pneumonia and improve prognosis among the very elderly and those with co-existing chronic diseases are needed.
Background and aims: Kathmandu University Hospital, located about mid way between Kathmandu and northern Nepal on way to China border (Tibet). The attending children were from the population of about 1.9 million people of six districts. The records showed bacterial pneumonia and meningitis is an important cause of morbidity and mortality. Because of these reasons surveillance of bacterial pneumonia was initiated in Jan 2010 to Sept 2011 to know the status of invasive Streptococcus pneumoniae strains among the children attending Emergency and OPD services of the University Hospital.

Methods: A total of 1620 children who fulfilled the enrollment criteria for suspect of bacterial pneumonia, sepsis or meningitis were enrolled for etiologic studies of severe illness. 317 Blood and 126 CSF cultured to isolate the etiologic agent by WHO procedure manual. Antibiotic susceptibility test for Streptococcus pneumoniae, Haemophilus influenzae and other pathogens was done following NCCLS methodology. Total leucocyte count was also carried out from CSF.

Results: *Streptococcus pneumoniae* yield 5.6% from CSF, 1.2% from blood. Anti-microbial resistance Cotrimoxazole 81%, Penicillin 6.2%. Pneumo ADIP supported SAPNA Nepal reported the fifteen serotypes 1, 2, 5, 6B, 7F, 12A, 16, NT, 19F, 19B, 23F, 14, 32, 39. The most common serotypes 1, 5, 2.

Conclusions: *Streptococcus pneumoniae* is the most common etiologic agent of children bacterial pneumonia, septicemia and meningitis, emerging trend on rise. Four of five recommended drugs showed rising resistant trend. Early immunization with PCV7 plus (PCV7 : 4, 6B, 9V, 14, 18C, 19F & 23F) could prevent pneumococcal infection.
A RANDOMIZED CONTROLLED TRIAL OF ZINC AS ADJUVANT THERAPY IN CHILDREN WITH SEVERE OR NON-SEVERE PNEUMONIA IN BHAKTAPUR, NEPAL

P. Valentiner-Branth1, P.S. Shrestha2, R.K. Chandyo2, M. Mathisen3, S. Basnet2, N. Bhandari4, R. Adhikari2, H. Sommerfelt3, T. Strand1, 1Copenhagen, Denmark, 2Kathmandu, Nepal, 3Bergen, Norway, 4New Delhi, India

Background: Pneumonia is a leading cause of illness and death in young children.

Objective: To measure the effect of zinc supplementation in children with pneumonia in a population where zinc deficiency is common.

Design: In a double-blind placebo-controlled clinical trial, children aged 2-35 months with severe (n=149) or non-severe pneumonia (n=2,479) were randomized to receive zinc (10 mg for children 2-11 months and 20 mg for children from 12 months of age) or placebo daily for 14 days adjuvant to antibiotics. The primary outcomes were treatment failure, defined as a need for change in antibiotics or hospitalization, and time to recovery from pneumonia.

Results: One out of 5 children did not respond adequately to antibiotic treatment; the odds ratio (OR) for treatment failure being 0.95 (95% CI 0.78, 1.2) for non-severe pneumonia and 0.97 (0.42, 2.2) for severe pneumonia. There was no difference in time to recovery between zinc and placebo groups for non-severe (median 2 days; hazard ratio [HR] 1.0, 95% CI 0.96, 1.1) or severe pneumonia (median 4 days; HR 1.1, 95% CI 0.79, 1.5). Regurgitation/vomiting within 15 minutes after supplementation was observed more frequently among children in the zinc group during the supplementation period (37% versus 13%; OR 0.25, 95% CI 0.20, 0.30).

Conclusions: Adjuvant treatment with zinc did neither reduce the risk of treatment failure nor accelerate the recovery in episodes of non-severe or severe pneumonia. Interventions to reduce the burden of pneumonia are needed and may include vaccination strategies against established pathogens as pneumococci.
RISK FACTORS FOR PNEUMONIA AMONG HIV-UNINFECTED YOUNG CHILDREN IN SOWETO, SOUTH AFRICA

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Background: Pneumonia is a leading cause of child morbidity and death in South Africa and globally. Data on risk factors can guide prevention efforts. Within a study on pneumococcal conjugate vaccine effectiveness, we investigated risk factors for probable bacterial pneumonia (PBP).

Methods: PBP cases were HIV-uninfected children aged < 2 years with lower respiratory tract infection and consolidation on chest radiograph or non-consolidated infiltrate with C-reactive protein ≥40 mg/L hospitalized at Chris Hani Baragwanath Hospital (CHBH) in Soweto. HIV-uninfected age-matched community controls were identified using CHBH birth records ±1 week of case birth date. Data collected by interview were analyzed using multivariable conditional logistic regression.

Results: Among 450 cases and 1,242 controls, risk factors for PBP included: male sex (adjusted odds ratio [aOR] 1.32, 95% CI 1.04-1.68), preterm/low birth-weight (aOR 1.64, 95% CI 1.22-2.20), previous hospitalization (aOR 2.52, 95% CI 1.69-3.75), house of non-brick material (OR 1.37, 95% CI 1.04-1.80), and primary care-giver smoking (aOR 3.10, 95% CI 1.38-7.00). Exclusive breastfeeding at age < 4 months was included in the model, but was not significant (aOR 0.79; 95%CI 0.59-1.05). We found effect modification between maternal HIV and crowding (>2 people sleeping in room with child); an HIV-infected mother was a risk factor only when crowding was absent (aOR 1.75, 95% CI 1.21-2.53).

Conclusions: Our findings are consistent with known pneumonia risk factors including male sex, preterm/low birth-weight, low socioeconomic status, and passive smoke exposure. They also contribute to growing evidence of poor health outcomes in HIV-exposed uninfected infants, perhaps through increased exposure to respiratory pathogens.
ACUTE RESPIRATORY TRACT INFECTIONS AMONG WARAO AMERINDIANS IN VENEZUELA IN RELATION TO IMMUNIZATION COVERAGE AND NUTRITIONAL STATUS

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Background: While high prevalence rates of acute respiratory tract infections (ARTI) have been described in Australian and Canadian indigenous populations, few studies on ARTI in South American indigenous populations have been published. We have shown previously that Warao Amerindian children from the Delta Amacuro in Venezuela are at increased risk of being colonized with S. pneumoniae. We now present a cross-sectional survey describing the prevalence of both upper respiratory tract infections (URTI) and acute lower respiratory tract infections (ALRTI) in the Delta Amacuro prior to the introduction of pneumococcal vaccination.

Methods: From December 1, 2009 to May 31, 2010, 487 Warao children aged 0-59 months were included in a cross-sectional survey. Data were obtained through parent questionnaires, vaccination cards and physical examinations.

Results: Forty-seven percent (47%) of children presented with an ARTI. Of these, 60% were URTI and 40% were ALRTI. Overall immunization coverage was low and coverage of Haemophilus influenzae type b conjugate vaccine was only 29%. The prevalence of malnutrition was high (52%). ARTI and ALRTI prevalence diminished with increasing age (OR for ALRTI children aged 25-59 months vs. children younger than 12 months 0.49, 95% CI 0.26 - 0.93). Furthermore, significant differences in ARTI prevalence were seen between villages. No significant associations between immunization status or malnutrition and ARTI prevalence were identified.

Conclusion: A high prevalence of ARTI and chronic malnutrition in combination with a low immunization status highlight the need for an integrated approach including pneumococcal vaccination to improve the health status of indigenous Venezuelan children.
EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF INVASIVE PNEUMOCOCCAL PNEUMONIA IN BRAZILIAN (SÃO PAULO) CHILDREN


Objectives: Clinically and epidemiologically characterize children hospitalized with a diagnosis of invasive pneumococcal pneumonia before the introduction of PCV-10. The 10-valent conjugate vaccine was introduced into the National Immunization Program in April 2010.

Methods: From January 2003 to October 2011, a retrospective study of hospitalized children with a diagnosis of pneumococcal pneumonia. Criteria for inclusion were: age ≥ 29 days and < 15 years, radiological and clinical diagnosis of pneumonia, and isolation of *S. pneumoniae* in blood cultures and/or pleural effusion.

Results: The study included 150 children. 68% of the isolates occurred in the mouths of June to October. Eighty four were male (56%), median age was 23 months (52% ≤ 24 months). Regarding the place of isolation of pneumococcus, the blood was 109, the fluid pleural was 26 and in both pleural and blood was 15. The most common serotypes isolated were 14 (40.2%), 5 (13.6%), 1 (12.9%), 6B (6.1%), 19A (4.5%), 4 (3%), 6A (3%) and 3 (3.3%). Median age for each of the most prevalent serotypes was 21 months (serotype 14), 42 months (serotype 5), 69 months (serotype 1) and 18 months (serotype 6B). The proportion of identified serotypes contained in 10-valent and 13-valent conjugate vaccines was 85.6% and 96.2% respectively. Pneumococcal strains were sensitive to penicillin (MIC ≤ 2 µg/mL) in 93.9% and intermediate resistance (MIC = 4 µg/mL) in 6.1%. Fifty eight percent of the cases developed complications. The median of length of hospital was 7 days.

Conclusions: The invasive pneumococcal pneumonia occurs predominantly ≤ 24 months and conjugate vaccine (10-V and 13-V) providing appropriate coverage. Furthermore, susceptibility testing results show that penicillin is still the treatment choice for invasive pneumonia in our setting.
Poster No 35

SEROTYPE-SPECIFIC DISEASE CAPACITY FOR PNEUMOCOCCAL PNEUMONIA IN HIV-INFECTED SOUTH AFRICAN ADULTS

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Background/aims: The serotype-specific disease-potential for non-bacteremic pneumococcal pneumonia in adults is unknown. The aim of this study was to assess if critical colonization density differentiating pneumonia from asymptomatic colonization in adults is serotype-specific.

Methods: Etiology was considered pneumococcal in South African HIV-infected adults if any of sputum culture or Gram stain, urine antigen, blood culture, whole blood lytA real-time (rt) PCR revealed pneumococcus or if nasopharyngeal (NP) quantitative lytA rtPCR was >8000cfu/ml. Quantitative serotype-specific rtPCR was performed on NP swabs for the serotypes of the 13-valent pneumococcal conjugate vaccine in pneumonia patients and asymptomatic HIV-infected controls.

Results: There was no asymptomatic pneumococcal colonization with serotypes 1, 5, 7F, 18C. For most remaining PCV13-serotypes, serotype-specific NPS colonization densities of >1000-5000 cfu/ml had sensitivities of >90% and specificities of 50-75% for pneumococcal pneumonia versus asymptomatic colonization. Several serotypes (4, 5, 14, 18C, 19A, 23F) were never or rarely found in pneumonia patients whose etiology was considered non-pneumococcal. Colonization densities in pneumococcal CAP were lowest for serotypes 5 and 18C, which were similar or even lower compared to the density in asymptomatic carriage of other serotypes (6A, 19A, 19F).

Conclusions: Mean NP colonization densities varied between serotypes, but were invariably higher in pneumonia than in asymptomatic carriage, particularly for “invasive” serotypes. Colonization density in pneumococcal pneumonia was lowest for serotypes which have higher invasive potential. We propose NP identification of some serotypes (1, 5, 7F, 18C) and identification of critical NP colonization densities for other serotypes as novel means to diagnose pneumococcal pneumonia.
POPULATION-BASED CROSS-SECTIONAL SURVEY OF PNEUMOCOCCAL CARRIAGE IN CHILDREN USING MOLECULAR TECHNIQUES: IMPORTANCE FOR VACCINATION IMPACT ASSESSMENT

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Background and aims: Pneumococcal nasopharyngeal colonization (NPC) assessment is an approach for monitoring conjugate vaccine impact. We assessed NPC in a cross-sectional population-based survey conducted 6-9 months after 10-valent pneumococcal conjugate vaccine (PCV-10) introduction in Brazil.

Methods: During December/2010-March/2011, 1291 children aged <2 years living in Goiania (~1,200,000 inhab), Brazil, were randomly sampled. Nasopharyngeal swabs were collected in STGG storage medium (STGG-NPC specimens) and immunization status obtained during home visits. NPC was assessed by lytA-targeted real-time-PCR. Bacterial loads were identified by PCR cycle-threshold (PCR-Ct). Specimens with high PCR-Ct were subjected to broth-enriched culture. Capsular-types for lytA or culture-positive specimens were determined by conventional-multiplex-PCR of DNA extracted from stored STGG-NPC or broth-enriched STGG-NPC specimens. Factors independently associated with co-colonization were identified using logistic regression.

Results: NPC prevalence was 55.9%(n=722). Serotype was ascertained for 548(75.9%) lytA-positive children and vaccine-serotypes represented 40.3% of them. Prevalence was higher in non-vaccinated children (67%), when compared to incompletely (57%) or fully-vaccinated (52%) children. Most frequent serotypes/serogroups were 6B, 14, 19F, 23F, 15B/15C, 6A/6C, 18, 19A and 11A/11D. Vaccine-types were associated with higher pneumococcal DNA load (p< 0.01). Co-colonization (2-4 serotypes) was detected in 87(15.9%;87/548) children with vaccine-types identified among 62(71.3%) co-colonized children. Non-vaccine-types were not preferentially associated with detected co-colonization after adjusting for confounders (OR=4.895;95%CI:2.9-8.0).

Conclusions: Use of molecular tests in NPC survey studies is expedient and highly sensitive, especially for detecting co-colonization. As PCV-10 was introduced in mid-2010 in Brazil, our results can be used as baseline for future assessments of vaccination impact.

VALIDATION OF MULTIPLEX ASSAYS FOR QUANTITATION OF ANTIBODY RESPONSES TO PNEUMOCOCCAL VACCINES

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Background & Aim: Multiplex technologies for the simultaneous quantitation of anti-pneumococcal serotype-specific IgG are increasingly used in studies of vaccine efficacy and in the investigation of immune competence. To date there has been limited validation of these assays against the 'gold standard' third generation WHO ELISA. We compared the measurement of serotype-specific anti-pneumococcal IgG by xMAP® Pneumo 14 multiplex, Meso Scale Discovery® technology and ELISA.

Methods: Two hundred serum samples from children and adults taken pre- and post-PCV7 and post-23vPPV immunization were analyzed by each method. Results for serotypes 1, 4, 6B, 9V, 14, 18C, 19F and 23F were included in this analysis.

Results: Both multiplex technologies correlated poorly with the ELISA. The IgG GMC for the majority of serotypes was significantly higher when samples were assayed by multiplex as compared to ELISA. In post-PCV7 immunization samples, high titres of serotype-specific IgG to non-PCV7 serotypes were detected by multiplex but not ELISA.

Using the APIIEG (2009) and AAAAI clinical guidelines for an adequate response to 23vPPV immunization, 28% infants were identified as having an inadequate response by ELISA but an adequate response by multiplex: possible antibody deficiency would have been missed in these infants. There was agreement between the methods for 21 of 26 (81%) adult paired sera.

Conclusion: Method-specific thresholds for vaccine efficacy must be established if alternative multiplex platforms are to be used. Similarly, method-specific criteria for an adequate response to pneumococcal polysaccharide vaccine are required for clinical application of multiplex assays.
QPCR-BASED DETECTION OF STREPTOCOCCUS PNEUMONIAE IN CULTURED TRANSORAL NASOPHARYNGEAL SAMPLES SIGNIFICANTLY INCREASES COLONIZATION RATES IN ADULTS


Background: The main reservoir for Streptococcus pneumoniae in humans is the upper respiratory tract. Here, we applied conventional and molecular methods to identify the presence of pneumococci in nasopharyngeal samples from adults.

Methods: Transoral and transnasal nasopharyngeal samples from 326 parents of 24-month old children were assessed for pneumococcal presence. A person was classified as colonized when live pneumococci were recovered from either of these cultures. The culture-enriched samples were further tested with quantitative-PCR targeting lytA and piaA genes specific for S. pneumoniae. They were considered positive if a signal for both genes was detected.

Results: Of the 326 parents, 66 (20%) appeared positive for pneumococcus with conventional culturing. The molecular approach was tested on a subset of 160 paired parental samples. With the conventional culture method 46 of these parents were identified as colonized. Although significantly more transnasal (n=43/160) compared to transoral (n=10/160) samples proved culture-positive for pneumococci (p=< 0.001), the opposite trend was observed when the quantitative-PCR approach was applied (43/160 vs. 63/160, p=0.024). Eventually, a quarter of pneumococcus-negative transoral cultures from non-carriers (58 of 231 processed in this study) became S. pneumoniae-positive by quantitative-PCR. Extrapolation of these results towards the overall study population suggests adult colonization rates of over 38%, approximately double the number detected by the conventional culture.

Conclusions: Our data suggest relatively high rates of pneumococcal colonization in parents of young children. The use of a culture-enriched quantitative-PCR approach in analysis of transoral nasopharyngeal samples seemed superior over a transnasal approach in this adult population.
PNEUMOCOCCAL ETIOLOGY AND SEROTYPE DISTRIBUTION IN HOSPITALISED CHILDREN WITH COMMUNITY-ACQUIRED PNEUMONIA IN BELGIUM. EVALUATION BY CULTURE, RT-PCR AND SEROTYPE-SPECIFIC-IGG&IGA-SEROLOGY

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Background and aims: Knowledge on pediatric pneumococcal CAP burden and causative serotypes (ST’s) is limited. We evaluated pneumococcal etiology and ST-distribution in children with CAP, using standard (SDT) and recently developed detection techniques (RDDT).

Methods: Patients: children (0-15y) hospitalised with X-ray confirmed CAP. SDT +/- RDDT were performed on 211 selected cases.

[Diagram patient selection for evaluation by RDDT]
SDT: blood culture +/- culture/RT-PCR on pleural fluid. Quellung reaction for ST-determination.

RDDT: LytA-gene RT-PCR with ST-determination for PCV13-ST’s (Prevnar-13®, Pfizer), serotype-specific-IgG&IgA-serology against ST’s 1, 5, 6B, 7, 9N+, 14, 19A, 19F, 23F. Seroconversion definition: >3x rise in Ab-concentrations for IgG and/or IgA, with an Ab-concentration ≥600 pg in the convalescent sample. Whenever seroconversion for ≥2 ST’s, the ST with the highest Ab-concentration was retained.

The causative ST was determined using the ranking: quellung reaction > RT-PCR > serotype-specific-IgG-serology > serotype-specific-IgA-serology.

Results: Pneumococcal etiology was found in 156/211 (73.9%) cases; 66 (100%) proven P-CAP, 43/57 (75.4%) suspected P-CAP, 47/88 (53.4%) random group, and established by SDT, RT-PCR and serotype-specific-IgG&IgA-serology in 66/211 (31.3%), 54/154 (35.1%) and 104/171 (60.8%), respectively. Adjustment of these findings to the entire study population resulted in an overall pneumococcal etiology rate of 61.7%. ST 1, 5, and 7 were predominant, accounting for 68.5% of cases.

Conclusions: Addition of RDDT significantly increases the sensitivity for pneumococcal etiology and provides knowledge on ST-distribution in non-bacteremic childhood CAP.
NEED FOR CONTROLS TO INTERPRET DIAGNOSTIC RESULTS FROM NASOPHARYNGEAL AND OROPHARYNGEAL SPECIMENS FOR DETERMINING PNEUMONIA ETIOLOGY IN PERCH

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Background and aims: Sensitive PCR diagnostics often detect pathogens in up to 60% of nasopharyngeal/oropharyngeal specimens from children hospitalized with severe or very severe pneumonia. Knowing how often these tests detect the same pathogens in the upper respiratory tract of healthy controls is essential when assigning causation based on these specimens. We describe methods to select controls for the Pneumonia Etiology Research for Child Health (PERCH) project.

Methods: We considered issues including whether to enroll hospital or community controls, whether to exclude controls with upper respiratory tract infection (URTI) or non-severe pneumonia, and matching criteria. We summarize the advantages and disadvantages of options considered, the rationale for the methods selected for PERCH, and remaining implications and limitations.

Results: For PERCH, we will randomly select community controls since hospital controls may be biased with respect to circulating pneumonia-causing pathogens, enroll HIV-infected controls at high HIV-prevalence sites to enable HIV-stratified analyses, and frequency-match on age and date since pathogen circulation can vary by age and seasonality. We will not match on residence so that controls can also be used for determining pneumonia risk factors. URTI and non-severe pneumonia are not control exclusion criteria since not all URTIs or non-severe pneumonias are on the causal pathway to severe pneumonia, and because some pathogens cause URTIs but rarely severe pneumonia.

Conclusions: By enrolling controls, randomly sampled from the community and along the continuum of respiratory illness, PERCH aims to minimize bias and improve interpretation of results from nasopharyngeal/oropharyngeal specimens regarding causation of pneumonia.
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QUANTITATIVE DETECTION AND MOLECULAR SEROTYPING OF STREPTOCOCCUS PNEUMONIAE IN LUNG ASPIRATES FROM GAMBIAN CHILDREN UNDER 5 YEARS WITH SEVERE PNEUMONIA


Background and aims: An intrinsic difficulty with pneumonia aetiology studies is the low yield from blood culture. Lung aspiration with culture provides a higher diagnostic yield and is more specific for causative pathogen, is safe and provides reliable diagnostic information. Laboratory detection and serotyping of these pathogens is desirable to monitor vaccine impact and coverage.

Methods: Lung aspiration was carried out on a total of 31 children under 5 years with radiological focal, lobar or segmental consolidation between December 2009 and November 2010. Bacterial culture and quantitative PCR for bacterial detection were performed. Pneumococcal DNA detection was done targeting the lytA gene. Bacterial loads were calculated using genomic DNA extracted directly from the lung aspirate. Serotyping of Streptococcus pneumoniae was by molecular methods.

Results: Pneumococcal DNA was detected in 24 (77.42%) samples using qPCR, while culture detected only 5 (16.13%) out of the 31 samples. The most common pneumococcal serotypes detected were serotypes 4 and 5 which accounted for 25% of invasive pneumococcus. Additional bacterial species were detected by qPCR including Staphylococcus aureus in 14 samples (45.16%), and Haemophilus influenzae type B in 8 samples (21.81%). Co-infections were detected in 13 patients (41.94%) and co-infection of S. pneumoniae with S. aureus was highest (53%).

Conclusions: Molecular analysis of lung aspirates increased the detection of pathogens causing invasive pneumococcal disease (IPD). S. pneumoniae was the predominant cause of IPD, serotypes 4 and 5 were the most common pneumococcal serotypes. Co-infections of S. pneumoniae with Staphylococcus aureus and Haemophilus influenzae occurred.
DESIGN OF A MULTIPLEX PCR FOR *STREPTOCOCCUS PNEUMONIAE* SEROTYPING

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**Background and aims:** Easy and complete (all serotypes) serotyping techniques are needed for the control and the knowledge of the epidemiology of pneumococcal infections. The aim of this work was to develop a multiplex-PCR that enabled a fast, specific and objective determination of all *S. pneumoniae* serotypes directly from clinical samples or their isolates from both patients and healthy carriers.

**Methods:** Different fluorophore-labelled primers common to several serotypes or serotype specific were designed on the basis of the pneumococcal capsular gene sequences and included in a multiplex PCR. Amplicon sizes were determined by capillary electrophoresis of fragments using a DNA sequencer (GeneMapper v 4.1, Applied Biosystems). The size of the specifically amplified products or the combination of amplicons allowed the identification of each serotype. *S. pneumoniae* autolysine and pneumolysine genes were also detected as control for species identification and to discard inhibitions from direct samples.

**Results:** The new multiplex-PCR was tested against the 92 reference strains of *S. pneumoniae* currently described being all of them correctly identified. The amplicon sizes were the expected and no crossing reactions were observed.

**Conclusions:** The multiplex-PCR showed to be a fast, easy and effective method for *S. pneumoniae* serotyping. Further studies are being conducted on clinical isolates, clinical samples and nasopharyngeal carriers to evaluate the role of this technique as an alternative to the more laborious serotyping techniques currently performed.
EPIDEMIOLOGY IN GIPUZKOA (SPAIN) OF STREPTOCOCCUS PNEUMONIAE SEROTYPES 6C AND 6D (1980-2010): IMPORTANCE OF PNEUMOARRAY AND PCR IN THEIR IDENTIFICATION

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Aim: Assess the epidemiology of serotypes 6C and 6D in Gipuzkoa Northern Spain and the effectiveness of PneumoArray® in its identification.

Methods: Pneumococcal serogroup 6 isolates were tested by the Quellung, PneumoArray and PCR of wciN region. PneumoArray® can detect 83 of the 92 serotypes described up to now.

Results: Overall 607 serogroup 6 isolates were detected: 179, 337 and 91 serotype 6A, 6B and 6C respectively (29.5%, 55.5%, 15%) and none serotype 6D. PCR and PneumoArray serotyping results were identical. The time required for performing was: 15 min./isolate for the PCR (batch of 14 strains) and 2.5 min./isolate for the PneumoArray (batch of 12 strains). Most Serotype 6C isolates was recovered after PCV7 introduction. By MLST and PFGE Serotype 6C isolates showed a great heterogeneity.

Conclusions: Serotype 6C was present since 1988 but most of them were detected after 2002. PneumoArray proved to be fast and effective for S. pneumoniae serotyping.
THE CONFIRMATION OF PSAA BY PCR IN THE DIFFERENT SEROTYPES OF STREPTOCOCCUS PNEUMONIAE ISOLATED FROM NASOPHARYNX OF HEALTHY CHILDREN

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Recent attention has focused on the role of pneumococcal proteins, including the pneumococcal surface adhesion A, (PsaA], as a virulence factor in the pathogenesis of infections with Streptococcus Pneumoniae. Immunization with these proteins may provide long lasting protection against virulent pneumococci.

Objective: To detect the psaA gene in different serotypes of S. pneumonia found in the upper respiratory tract of healthy children and to evaluate the potential usefulness of the psaA PCR assay as a possible diagnostic method for Pneumococcal disease.

Methods: In this study, nasopharyngeal swabs were taken from healthy children under 10 years old recruited from randomly selected daycare centers and primary schools in Tehran. These swabs were tested for the presence of Pnuemococci by both culture and the psaA PCR assay. To detect the gene we used a PCR-amplified internal fragment of the psaA gene.

Results: Samples were collected from 485 children. Streptococcus pneumoniae were isolated from 228, (47%), samples; fifteen different serotypes were identified. PCR detected the psaA gene in 164 specimens, (70%).

Conclusion: Our results confirm that psaA is present and detectable in heterologous serotypes of Streptococcus pneumoniae. These results indicate that PsaA can be used for detection of invasive pneumococcal serotypes in carriers and may be for vaccination development in different areas.
CONTRIBUTION OF PCR AND PNEUMOCOCCAL ANTIGEN TESTING OF BLOOD CULTURES AND SERA FOR THE DIAGNOSIS OF PNEUMOCOCCAL BACTEREMIA IN CHILDREN

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Background and aims: The diagnosis of Streptococcus pneumoniae infection relies heavily on blood cultures. To enhance the detection of pneumococcal bacteremia we used the Binax NOW® immunochromatographic test (ICT) on negative blood cultures and PCR on blood culture broth and sera samples of the patients.

Methods: Study population: 49 children under five years old with an Acute Lower Respiratory Tract Infection and a negative blood culture. Blood cultures were tested for the presence pneumococcal antigen by the ICT. Positive broths and sera samples of the same patients were further tested for the presence of the pneumococcal DNA by a PCR targeting the polysaccharide C and autolysin. Results were compared with an ICT on urine samples.

Results: ICT was positive in 21 (42%) of the 49 negative blood cultures. PCR on sera confirmed 2 (9.5%) of the ICT positive blood cultures. PCR on the blood cultures was negative for all 21 ICT positive samples. The ICT urine antigen test was positive in 24 of the 49 patients including 15 patients with an ICT positive blood culture.

Conclusions: PCR is significantly less sensitive than the ICT for the detection of S. pneumonia in blood culture media or serum. We estimate that at least 15 out of 49 patients (30.6%) (ICT positive of blood culture and urine) suffered from pneumococcal invasive disease. We discuss the infectious agent for the other 34 patients basing us on sensitivity and specificity of the urine antigen test and results of nasopharyngeal carriage in these children.
APPLICATION OF PCR IN THE DIAGNOSIS OF OTITIS MEDIA WITH EFFUSION. EFFECT OF STREPTOCOCCUS ORALIS ON STREPTOCOCCUS PNEUMONIAE VIRULENCE

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Otitis media with effusions (OME) can lead to significant hearing loss in childhood. The aim of the present study was to: a) investigate the presence of Streptococcus pneumoniae (Sp), Moraxella catarrhalis (Mc) and Haemophilus influenzae (Hi) in the clinical materials from OME; b) determine the effect of commensal bacteria isolated from healthy children (Streptococcus) on the biofilm formed by Sp isolated from OME; c) the effect of commensal bacteria on the necrosis induced by Sp on peripheral blood polymorphonuclear (PMN). Effusion obtained from 38 patients aspirated from the mid-ear were analyzed bacteriologically and PCR assay. Studies of inhibition of biofilm formation were performed by the technique of crystal violet and the PMN necrosis was measured with IP by flow cytometry. The detection of pathogens was significantly higher by PCR than by bacteriological cultures: Sp (p=0.004), Hi (p=0.002), Mc (p< 0.002). Commensal bacteria (p=0.34) or their supernatants (p=0.73) are biofilm inhibitory capacity of Sp. The isolated bacteria are more capable of inducing necrosis of PMN that their supernatants (p < 0.001). Commensal bacteria and their supernatants potentiate the induction of necrosis induced by Sp. PCR technique is more specific and sensitive in detection of bacteria in middle-ear effusion of OME. We found that commensal bacteria and their supernatants studied not inhibit the Sp biofilm formation in Vitro. The bacterial pathogen could promote the action of SP stimulating the development of OME. To obtain inhibition of patogens like Sp it requires further studies with other bacteria in commensal flora of healthy children.
PRELIMINARY ASSESSMENT OF THE CONTRIBUTION OF STREPTOCOCCUS PNEUMONIAE TO THE BURDEN OF COMMUNITY ACQUIRED PNEUMONIA HOSPITALIZATIONS IN MIDDLE TENNESSEE

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Background/aims: Hospitalization for community-acquired pneumonia (CAP) is common and often thought to be due to Streptococcus pneumoniae. More than 10 years after introduction of pneumococcal conjugate vaccines, we evaluated the contribution of S. pneumoniae to CAP.

Methods: We prospectively identified patients hospitalized with CAP from 9 counties in middle Tennessee. Tests for S. pneumoniae included blood culture, whole blood polymerase chain-reaction (PCR for lyt-A), urinary pneumococcal antigen detection (adults only) and pleural fluid culture/PCR (when available).

Results: From January-December 2010, 172 children and 269 adults with CAP were enrolled. From available results, 2% (6/346) of blood cultures were positive for S. pneumoniae (1% (1/131) and 2% (5/215) for children and adults, respectively). Blood PCR was positive in 1% (1/121) and 1% (2/245) of children and adults, respectively. Urine antigen testing was positive in 9% (21/232) of adults with available samples. Four (27%) of 15 pleural fluids (14 available for cultures/PCR, respectively) tested positive. Among patients with ≥1 S. pneumoniae test available, the proportion positive was 3%/4 (159) for children and adults, respectively. Sixty two percent (216/346) of blood cultures were collected before in-hospital antibiotic administration (53%/69 for children and adults, respectively).

Conclusions: Using an expanded array of tests, preliminary results from this ongoing study suggest the current contribution of pneumococcus to CAP hospitalizations is smaller than previously reported. Limited sensitivity of available tests in presence of antibiotic use could partially explain these findings.
NOVEL INSIGHTS INTO MULTIPLE SEROTYPE CARRIAGE, SEROTYPE DIVERSITY AND NON-TYPEABLES THROUGH WIDESPREAD UTILIZATION OF A STREPTOCoccus PNEUMONIAE MOLECULAR SEROTYPING TOOL

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Serotyping pneumococcal isolates provides surveillance of pre- and post-vaccination populations, enabling the epidemiology of disease association, vaccine introduction and serotype replacement to be investigated. In studies worldwide, the molecular serotyping microarray technology has demonstrated an enhanced ability to detect multiple serotype carriage and determine the relative abundance of serotypes present. Furthermore, the added capacity to assess genetic relatedness of isolates, monitor antibiotic resistance markers and detect co-colonizing pathogens enables a comprehensive single analysis of samples.

To date, around 3,000 samples have been analyzed from numerous investigations conducted in distinct geographic locations. Broadly these studies have investigated circulating pneumococcal carriage, assessed the impact of vaccine or other factors upon carriage, and further characterized atypical isolates of interest. The molecular serotyping tool has revealed the level and nature of multiple serotype carriage in these studies, as well as indicating potential serotype variants and the origin of non-typeable organisms. Whilst these unique insights have benefited each individual study, further power comes from the meta-analysis of multiple datasets to reveal more widespread findings. Examples of this include the geographic distribution of potential serotype variants within vaccine types, linking non-typeable organisms with typeable lineages and identifying the extended gene pool available for genetic exchange between related Streptococcus species.

An underlying complexity of nasopharyngeal carriage, masked or missed by other methods, has been revealed by the microarray approach. The improved understanding of pneumococcal carriage, transmission and disease gained using this technology is important for modeling and monitoring the effects of ongoing international vaccine roll-out programmes.
NEW METHOD FOR TESTING OF ANTIBIOTIC RESISTANCE IN BIOFILM-POSITIVE PNEUMOCOCCI

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*Streptococcus pneumoniae* often colonizes the nasopharynx of healthy individuals. Respiratory infections are responsible for the death of 4 million people each year, and *S. pneumoniae* is the predominant species in these infections. *S. pneumoniae* is also responsible for diseases such as chronic otitis media, pneumonia, bacteremia, and meningitis. Biofilm formation either on the surface of the mucosa or on the surface of medical devices represents a serious problem that even is magnified with the increasing use of prosthetic devices. The biofilm-forming bacteria are difficult to eradicate with antibiotics and often cause chronic infections, such as otitis media or other infections of the upper respiratory tract in pneumococci. Biofilm-forming bacteria have different susceptibility profiles, than those obtained with Minimum Inhibitory Concentration (MIC) assessment. The Minimum Biofilm Eradication Concentration (MBEC) was measured on grown biofilm. The aim of this study was to assess applicability of the novel method for testing of antibiotic resistance in biofilm-positive pneumococci. We determined the resistance profiles in biofilm-positive and biofilm-negative *Streptococcus pneumoniae* isolates by means of new approach in detection of viable cells after antibiotic treatment. The MBEC was assessed in colorimetric medium indicating metabolic activity of surviving cells and the changes of the medium were detected spectrophotometrically. The biofilm-positive strains showed higher resistance to all tested antibiotics, when examined in biofilm form. The method of resistance detection by means of metabolically active cells after antibiotic treatment is cheap and applicable for wide use.

The research was supported by project INGO LA 10037.
Poster No 50

CLINICAL EVALUATION OF A RAPID IMMUNOCHROMATOGRAPHIC TEST (ODK-0901) FOR DETECTING PNEUMOCOCCAL ANTIGEN IN MIDDLE EAR FLUIDS AND NASOPHARYNGEAL SECRETIONS

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Background: Since the incidence of penicillin-resistant Streptococcus pneumoniae has been increasing at an astonishing rate throughout the world, the need for accurate and rapid identification of pneumococci has become increasingly important to determine the appropriate antimicrobial treatment. We have developed a novel immunochromatographic test (ODK-0901) that detects pneumococcal antigen in middle ear fluids (MEFs) and nasopharyngeal secretions (NPSs).

Method: A total 532 samples, 264 MEFs and 268 nasopharyngeal swabs were obtained from patients with AOM and used in this study. We identified H. influenzae in both specimens by immunochromatographic antigen detection, real-time PCR and conventional bacterial culture. Informed consent was obtained by patients themselves, or their guardians in case of infant patients.

Results: The sensitivity and specificity of the ODK-0901 test were 81.4% and 80.5%, respectively, for MEFs from patients with acute otitis media (AOM). In addition, the sensitivity and specificity were 75.2% and 88.8%, respectively, for NPSs from patients with acute rhinosinusitis.

Conclusion: The ODK-0901 test provides a rapid and highly sensitive evaluation of the presence of S. pneumoniae. The rapid immunochromatographic test is attractive for identifying S. pneumoniae in MEFs and nasopharyngeal swabs in the era of antimicrobial resistance and thus will be a promising method of identifying pneumococci in MEFs and NPSs.
DISCRIMINATION BETWEEN A-HEMOLYTIC STREPTOCOCCI BASED ON SORTING OF THEIR DBP MASS SPECTRA

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Background: Accurate species-level identification of alpha-hemolytic (viridans) streptococci is very important for understanding of their pathogenicity and virulence. However, an extremely high level of the similarity between viridans streptococci, especially of Streptococcus pneumoniae/mitis/oralis group (S.pneumoniae, S.mitis, S.oralis and S.pseudopneumoniae), often results in misidentification of these organisms, so there is an urgent need of novel approaches to species identification.

Methods: 90 randomly selected clinical isolates of alpha-hemolytic streptococci from upper respiratory tract were collected in Moscow (Russia) in 2009 and characterized using a set of routine phenotypic methods (alpha-hemolysis, colony morphology, Gram stain, bile and optochin susceptibility tests, latex agglutination test and serotyping) and modern proteomic and genetic approaches: the direct bacterial profiling (DBP) by means of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) technique and multilocus sequence analysis (MLSA) (http://viridans.emlsa.net/).

Results: By means of the combination of all methods applied, we discriminated our isolates on S.pneumoniae (n=40), S.oralis (n=4) and S.mitis (n=46). Diagnostic models based on the DBP mass spectra were created using the ClinProTools 2.1 software (Bruker Daltonics, Germany) allowing to classify the pools of mass spectra of different species on the set of their minor mass peaks.

Conclusion: In spite of the fact that an accurate identification of α-hemolytic streptococci to species by MALDI-TOF MS profiling usually does not yield a good result, the possibility to “sort” them using mathematical optimizing and classifying algorithms could be one more step to the creation of powerful tool of viridans streptococci discrimination.
ESTIMATING THE BURDEN OF NON-BACTEREMIC PNEUMOCOCCAL PNEUMONIA AMONG ADULTS: RESULTS FROM A META-ANALYSIS


Background and aims: Pneumococcal pneumonia causes significant morbidity and mortality among adults, especially the elderly and immunocompromised. Given limitations of diagnostic tests for nonbacteremic pneumococcal pneumonia, most studies report the incidence of bacteremic pneumococcal pneumonia or invasive pneumococcal disease (IPD), and thus, grossly underestimate the burden of pneumococcal pneumonia. The availability of a urine antigen assay and systematic study methods provide a new opportunity to understand the burden of non-bacteremic pneumococcal pneumonia.

Methods: We performed a comprehensive literature review of studies providing information on the relative yield of the Binax streptococcal urine antigen test (UAT) with blood and/or sputum culture in the diagnosis of pneumococcal pneumonia. We estimated the ratio of nonbacteremic to bacteremic pneumococcal pneumonia, the proportion of community-acquired pneumonia (CAP) attributable to pneumococcus, and the additional contribution of the Binax streptococcal UAT beyond conventional diagnostic techniques, using bootstrapping and random effects meta-analytic methods.

Results: We included 35 studies in the analysis, predominantly from developed countries. The estimated ratio of nonbacteremic to bacteremic pneumococcal pneumonia was 3.75 (95% percentile CI 2.51-5.59). The estimated proportion of CAP attributable to pneumococcus was 27.5% (95% CI: 24.1%-31.3%). The Binax streptococcal UAT diagnosed an additional 11.3% (95% CI: 9.5-13.4%) of CAP patients with pneumococcal disease beyond those identified by blood and sputum culture alone.

Conclusions: Bacteremic pneumococcal pneumonia burden significantly underestimates the true burden of disease in adults with pneumococcal pneumonia. For every case of bacteremic pneumococcal pneumonia, there are at least 3.75 additional cases of nonbacteremic pneumococcal pneumonia.
THE CHOICE OF MUELLER-HINTON AGAR SIGNIFICANTLY AFFECTS THE PENICILLIN MIC OF PNEUMOCOCCI

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Susceptibility to penicillin is determined by measuring the MIC, often by using the E-test on Mueller Hinton (MH) agar with horse blood. Using this method and MH agar from Oxoid, we noticed that strains falling into the intermediately resistant category could have lower penicillin MIC than expected, despite following the recommendations from the manufacturer. The only difference we found was the type of MH-agar.

Penicillin non-susceptible clinical isolates and reference strains were tested for penicillin MIC using the E-test on Mueller Hinton agar from two manufacturers, Becton Dickinson (BBL Mueller Hinton II Agar) and Oxoid (Mueller-Hinton Agar). Both agars were supplemented with horse blood.

The penicillin MIC was consistently higher with the MH agar from Becton Dickinson, especially for strains with penicillin MIC between 0.5-1.0 mg/l, where the penicillin MIC for these strains was often 2-4x higher than on Oxoid MH-agar. Strains with penicillin MIC of 0.5-1.0 mg/l on the Oxoid agar, and classified as amenable to oral treatment (according to the CLSI criteria) would often have MIC of 1.5-3 mg/l on the Becton Dickinson agar, and should therefore be classified as resistant. The results using the Becton Dickinson agar were more in agreement the results from other reference laboratories.

The choice of MH agar significantly influenced the penicillin MIC and may therefore be clinically important and affect the prevalence rates of penicillin resistance in surveillance studies.
MULTIPLEX PCR FOR DETECTION OF MULTIPLE CARRIAGE OF PNEUMOCOCCI IN NASOPHARYNGEAL SAMPLES FROM CHILDREN ATTENDING DAY CARE CENTERS

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Carriage of multiple pneumococcal serotypes favours genetic exchange between strains. Conventional culture and serotyping methods are insensitive for detecting multiple carriage. The aim was to analyze rate of carriage and multiple carriage in healthy children in Iceland using multiplex PCR (mPCR) method.

Nasopharyngeal swabs from 516 pre-school children sampled in 2009 were subjected to direct mPCR parallel to conventional culture and serotyping. Primers for serotypes included in the 13-valent conjugated vaccine and other common serotypes (11A, 15B/C, 22, 23A) were used and cpsA as a control. Serogroup 6 was separated into 6A, 6B, 6C and 6D.

The carriage rate was 78% using mPCR compared to 72% using culture. The mPCR detected serotypes in 86% of the swabs that yielded pneumococci by culture and detected pneumococci in 20% of nasopharyngeal samples that were negative by culture. The samples that were negative by mPCR and positive by culture, either had serotypes not included in the mPCR panel or uncapsulated strains. The mPCR detected more than one serotype in 21% of the samples compared to 8% by culture. Up to 4 serotypes were detected in a single sample by the mPCR. The most prevalent serotypes were 23F, 19A, 6B, 6A, 19F, 14 and 15B/C.

The prevalence of multiple carriage is considerably higher than we have previously detected and the possibility of genetic exchange is accordingly high. This also has implications for the evaluation of vaccine efficacy.
STREPTOCOCCUS PNEUMONIAE DIAGNOSIS FOR NESTED-PCR IN HOSPITALIZED CHILDREN WITH COMMUNITY-ACQUIRED PNEUMONIA

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Background: Streptococcus pneumoniae (Sp) is the main etiological agent of pneumonia, but it is undoubt-edly underdiagnosed. The culture has less positivity because of low pneumococcus concentration and previous antibiotic-based therapy, while PCR can detect a small proportion of pathogens.

Objectives: To assess a Nested PCR for Sp detection in whole blood (WB), plasma (P), buffy coat (BC) and serum (S). To examine clinical-epidemiological aspects.

Methods: From 2008-2010, 240 hospitalized patients < 14 years old with suspected bacterial pneumonia were surveyed. A Nested-PCR detecting fragments of the pneumolysin gene was used. 89 confirmed patients were evaluated.

Results: From 240 samples, 25.8% were positive: 38.7% in BC, 51.6% in WB, 40.3% P, 29% S. 24 showed more than 1 positive result; 14 in two, 8 in three, and 2 in four samples. Pre-sampling antibiotic: 81.4% (median 3.7 days). Age distribution: < 1 year: 19; 1-5: 30; 6-14: 9. Male 52.6%. Respiration rate: 49/min; reduced vestibular noise: 94%; retraction: 53.6%; rales: 87%; subcrepitant: 67.3%, and crepitant: 29.1%; cough: 77.2%; wheeze: 32.1%; median oxygen saturation: 93.3%. Radiology findings: 47.5% air trapping, 47.5% interstitial infiltrate, 10.2% pleural effusion, 44.2% multilobular and 55.8% unilobular alveolar infiltrate. Leukocytosis: 84.2%. 62.7% requires oxygen. Deceased: 0. In ICU: 2. 75% applied an ampicillin-based treatment regimen.

Conclusions: Higher positivity was found in WB, P and BC. Using WB alone, 46.8% of the cases would have remained unconfirmed. All four blood fractions are vital to reach a definite diagnosis of all cases.
MOLECULAR DETECTION AND SEROTYPING OF PNEUMOCOCCAL PNEUMONIA FROM HOSPITALISED PATIENTS WITH SEVERE ACUTE RESPIRATORY ILLNESS, SOUTH AFRICA, 2009-2010

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Background and aims: In South Africa PCV7 was introduced in April 2009 with a novel schedule of 6, 14 weeks, and 9 months. We used molecular methods to assess the early impact of PCV7 on serotype distribution in hospitalised pneumococcal pneumonia cases.

Methods: 5130 patients were enrolled from May 2009 through December 2010 as part of a prospective hospital-based pneumonia surveillance programme. Detection of lytA in blood specimens and serotyping was performed by real-time PCR. Vaccination status was obtained from the vaccination card on admission.

Results: Overall 7% (371/5130) of patients were lytA positive. Of 1696 children < 2 years, lytA positivity decreased from 6% (43/723) in 2009 to 4% (36/973) in 2010 (p=0.03). 368 lytA-positive specimens were available for serotyping and of these 21% (79/368) were PCV7 serotypes, accounting for 25% (19/77), 11% (2/18) and 21% (58/273) in patients < 2, 2-4, and ≥5 years of age, respectively. Of 842 children eligible to receive PCV7 with available lytA results and vaccination status, 48% (407), 25% (210) and 27% (225) received 0, 1 or 2-3 doses at least 14 days before hospitalisation for pneumonia, respectively. In this group, 41 lytA-positive cases were identified and PCV7 serotypes accounted for 33% (7/21), 18% (2/11) and 11% (1/9) in children who received 0, 1, or 2-3 doses, respectively (p=0.45).

Conclusions: PCR assays are an important tool for detection and serotyping of pneumococcal pneumonia. Preliminary results are consistent with vaccine effectiveness against vaccine types, although more data are needed to test statistical significance.
Poster No 57

EVALUATION OF THE S. PNEUMONIAE IMMUNO-CHROMATOGRAPHIC TEST AND REAL-TIME LYTA PCR FOR DIAGNOSING INVASIVE PNEUMOCOCCAL DISEASE IN MALI


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Background: Prior antibiotic use, limited blood volume, and laboratory processing delays reduce sensitivity of blood cultures (BCX) for diagnosing invasive pneumococcal disease (IPD). We evaluated antigen- and molecular-based tools for improved detection of pneumococci from blood.

Methods: Hospitalized children < 16 and outpatients < 3 years with temperature ≥39°C or suspected invasive bacterial disease (SIBD) in Bamako, Mali had automated BCX, immuno-chromatographic testing (ICT) on cultured BCX broth and real-time lytA PCR on cultured BCX broth and whole blood. We tested serum for antimicrobial activity based on growth inhibition of a pan-susceptible bacterial strain. Specimens positive by lytA PCR were serotyped using sequential multiplex PCR.

Results: Over 12 months, we selected 381 patients (91% < 5 years; 67% hospitalized) based on their BCX results: 75 BCX-positive for Spn, 100 BCX-positive for other organisms, and 206 BCX-negative of which 10 were alarm-positive by automated BCX machine.

ICT and PCR of cultured broth were 100% sensitive and specific in BCX-positive subjects but detected no additional pneumococcal cases among BCX-negatives, including alarm-positives. PCR on whole blood was 81% sensitive and 100% specific in BCX-positive cases and identified 3 pneumococcal cases among BCX-negatives, all with prior antibiotic use, of which 1 was alarm-positive. All 27 lytA-positive broth and 21/26 whole blood specimens were successfully serotyped by conventional or real-time PCR.

Conclusions: ICT and lytA PCR on cultured BCX broth were highly sensitive and specific among BCX-positive cases. PCR on whole blood had slightly lower sensitivity but yielded additional probable Spn cases among BCX-negatives.
NO LOSS OF PNEUMOCOCCUS IN STGG OVER 8 HOURS BY QUANTITATIVE PCR OR CULTURE BUT YIELD MAY DEPEND ON SEROTYPE

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Background: WHO guidelines for nasopharyngeal swab sampling for pneumococcus include immediate inoculation into skim-milk-tryptone-glucose-glycerol (STGG) transport medium and transport in a cool box with ice packs for up to 8 hours before laboratory reception for culture or PCR.

Aims: To test for loss of viability of pneumococcus in STGG transport media over 8 hours.
To test for loss of detectable pneumococci in STGG transport media by PCR over 8 hours.

Methods: Thirty clinical pneumococcal isolates, 3 of each vaccine-serotype, and a quality control strain, were spiked into STGG at 5 concentrations, and held in a cool-box in a tropical climate for 8 hours. Aliquots were taken from each vial at 0, 2, 4, 6 and 8 hours for culture, and for quantitative real-time lytA PCR (qPCR). Analysis: linear regression of log-transformed mean counts at 2 hour intervals in colony-forming-units (CFU) and by qPCR; Wilcoxon rank-sum test of concentration by CFU and qPCR.

Results: qPCR measured a greater pneumococcal quantity than colony-counting for every serotype (p < 0.0001) although the yield from the spiked-in concentration varied considerably between serotypes.

Conclusions: Culture and colony counting for pneumococcal detection from swabs in STGG yields lower concentrations than by qPCR, presumably due to the ability to detect nonviable organisms by PCR. Over 8 hours, there was no detectable loss in ability to detect pneumococcus.
Making Standards for Quantitative Real-Time Pneumococcal PCR

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Background: Many labs perform quantitative real-time lytA PCR (qPCR) but methods for making standards vary. We tested the assumption that measuring a standard suspension of \textit{Streptococcus pneumoniae} by counting colony-forming-units (CFU)/mL or DNA measurement and calculation of genome-copies/mL should be the same.

Methods: \textit{S. pneumoniae} ATCC 49619 was grown to log phase in brain heart infusion broth, or harvested from a young culture plate and suspended to 3.0 McFarland. \textit{Escherichia coli} ATCC 25923 was used as a comparator, because it does not undergo autolysis. The suspensions were serially diluted for CFU/mL, underwent DNA extraction (Qiagen, USA) and measurement (NanoDrop, ThermoScientific, USA) to calculate genome-copies/mL, 12 times per organism.

Results: Spearman's correlation between concentration in CFU/mL and in genome-copies/mL, for \textit{S. pneumoniae} was 0.64, for \textit{E. coli} was 0.20. The mean ratio of CFU/genome-copies for \textit{S. pneumoniae} was less than for \textit{E. coli} regardless of the suspension method [from solid media; means (sd) 0.63(0.66), 2.10(1.54), \(p = 0.058\), broth; means 0.86(1.13), 2.52(1.29), \(p = 0.039\)]. The coefficient of variation for \textit{S. pneumoniae} in CFU was 119% and in genomecopies was 48%; for \textit{E. coli} it was 67% in CFU and 52% in genome-copies.

Conclusions: The NanoDrop method was less variable than the CFU method but poor correlation and within-method variability means that neither method results in absolute quantification that would be expected to be comparable between laboratories. Readers of the medical literature should be aware that the method used for assigning values to qPCR standards will affect the results obtained.
Poster No 60

PNEUMOCOCCAL ANTIBODIES IN DRIED BLOOD SPOT SAMPLES AS A METHOD FOR LARGE-SCALE SERO-SURVEILLANCE

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\textbf{Background:} Introduction of conjugate pneumococcal vaccines in childhood vaccination programmes has led to increased interest in epidemiological surveillance of serotype distribution to determine vaccine impact. However, these studies are mainly based on determination of nasopharyngeal pneumococcal carriage using swaps, which only reflects current carriage, besides being subject to sampling errors. Determination of serotype specific antibodies reflects current and past exposure, but logistic problems associated with tapping, treatment, and shipping of venous blood samples hamper use of such samples in field studies. Dried blood spot samples (DBSS, finger stick whole blood on filter paper) are easy to collect and store, as the spot samples can be transported and stored at room temperature. Yet, this method has not been used in pneumococcal studies. We compared this minimally invasive method of pneumococcal antibody detection with antibody detection using conventional venous blood samples.

\textbf{Method:} Paired serum and DBSS samples where collected from 40 healthy Danish adults and children below 6 years of age chosen at random. Antibodies to the 13 serotypes included in the PCV-13 were determined in the two set of samples using the Luminex principle and pairwise correlations between the two were calculated.

\textbf{Results:} Preliminary data show very high degree of correlation (correlation coefficients >0.95). Further testing is on going.

\textbf{Conclusion:} The DBSS method appears an easy and reliable method to determine antibody response to pneumococci and other pathogens, which is particularly helpful in large-scale epidemiological studies in remote settings with limited laboratory resources. This may facilitate vaccine trials in developing countries.
**ISPPD-8**  
**Poster Shift 1: Monday, March 12, 2012 – Tuesday, March 13, 2012**

**Poster No 61**

**EFFECT OF ANTIMICROBIAL USE ON PNEUMOCOCCAL (PNC) DIAGNOSTIC TESTS IN ELDERLY PATIENTS WITH COMMUNITY ACQUIRED PNEUMONIA (CAP)**

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**Background and aims:** Antimicrobial treatment decreases yields of conventional culture methods. We studied results of different pneumococcal diagnostic tests in CAP patients with and without exposure to antimicrobials.

**Methods:** We studied 323 cases aged ≥65 with radiologically confirmed CAP. The effects of use of antimicrobials at the acute visit (before sputum sampling) and 2 weeks before it on pneumococcal CAP case definition (Table) and individual assays (culture and real-time PCR of sputum and nasopharyngeal swab, urine antigen test and serology) were analysed.

**Results:** Records of antimicrobial use 2 weeks before and at the visit were available for 98% and 94% of the cases, respectively. Results are presented in the table.

<table>
<thead>
<tr>
<th></th>
<th>No antimicrobial exposure at the visit or within 2 weeks before it</th>
<th>Antimicrobial exposure within 2 weeks before the visit</th>
<th>p, chi-square</th>
<th>Antimicrobial exposure at the visit only</th>
<th>p, chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture pnc +</td>
<td>4/185 (2%)</td>
<td>0/60 (0%)</td>
<td>0.25</td>
<td>5/54 (9%)</td>
<td>0.02</td>
</tr>
<tr>
<td>High quality sputum culture, encapsulated pnc +</td>
<td>26/104 (25%)</td>
<td>3/29 (10%)</td>
<td>0.09</td>
<td>6/33 (18%)</td>
<td>0.42</td>
</tr>
<tr>
<td>CbpA or PsaA antibodies, 2-fold increase</td>
<td>23/155 (15%)</td>
<td>6/48 (13%)</td>
<td>0.69</td>
<td>13/45 (29%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Pnc urine antigen test +</td>
<td>13/164 (8%)</td>
<td>4/48 (8%)</td>
<td>0.93</td>
<td>10/52 (19%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Low quality sputum culture, encapsulated pnc +</td>
<td>4/33 (12%)</td>
<td>0/10 (0%)</td>
<td>0.25</td>
<td>0/9 (0%)</td>
<td>0.27</td>
</tr>
<tr>
<td>Nasopharyngeal swab (NPS) culture, encapsulated pnc +</td>
<td>28/178 (16%)</td>
<td>2/57 (4%)</td>
<td>0.02</td>
<td>2/53 (4%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Sputum autolysin (lytA)-PCR +</td>
<td>34/138 (25%)</td>
<td>3/39 (8%)</td>
<td>0.02</td>
<td>13/41 (32%)</td>
<td>0.37</td>
</tr>
<tr>
<td>NPS lytA-PCR +</td>
<td>19/178 (11%)</td>
<td>2/57 (4%)</td>
<td>0.10</td>
<td>4/53 (8%)</td>
<td>0.51</td>
</tr>
<tr>
<td>Pnc CAP (1 OR 2 OR any two of 3, 4, and 5)</td>
<td>36/186 (19%)</td>
<td>4/60 (7%)</td>
<td>0.02</td>
<td>16/57 (28%)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

**Conclusions:** Antimicrobial exposure before the visit reduced culture and PCR-based detection of pneumococcus. The higher prevalence of blood culture, serology and urine antigen among those with exposure at the visit suggests a higher Pnc CAP prevalence in these patients.
DEVELOPMENT OF A REAL-TIME MULTIPLEX PCR ASSAY FOR THE TYPING OF 40 STREPTOCOCCUS PNEUMONIAE SEROTYPES DIRECTLY FROM SPECIMENS

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Background and aims: The serotype identification of Streptococcus pneumoniae is primordial for the surveillance for pneumococcal circulation and assessment of vaccine impact. We report herein the development of a multiplex real-time PCR assay for the identification of the 40 main circulating S. pneumoniae serotypes worldwide.

Methods: The sensitivity and the specificity of the real-time multiplex assay were validated on S. pneumoniae isolates serotyped with the Neufeld Quellung method. This semi-quantitative assay sensitivity was estimated between 1 and 100 cfu/ml depending on the serotype tested.

Results: For the 40 serotypes tested, the assay showed no cross reactivity, except for the 6C and 12F serotypes which were detected by 6A/B and 10A primers/probe sets, respectively. In this study, the assay was evaluated for its capacity to serotype S. pneumoniae directly from clinical samples. Both nasal as well as pleural effusion and blood specimens from pneumonia patients hospitalized in Brazil, France and South Africa were tested. A different S. pneumoniae serotype distribution was observed in these different cohorts. The serotypes 6A/B, 14, 19F were predominant in Brazil and South Africa whereas in France the most prevalent serotypes were 7F, 1 and 3. Serotype 1 was also found in a high percentage in South Africa.

Conclusions: We showed that the invasive serotypes found in the blood or pleural effusions samples were also found as colonizing isolates in the nasal samples. This assay is specific, sensitive and can be a quantitative tool to monitor S. pneumoniae circulation and evaluate vaccine effectiveness.
STANDARDIZATION OF MULTIPLEX RT-PCR MELTING TEMPERATURE ANALYSIS OF PRODUCTS FOR PNEUMOCOCCAL SEROTYPING

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Pneumococcal diseases are among the most frequent causes of vaccine preventable death. Serotyping is necessary to evaluate vaccine coverage and pneumococcal epidemiology, as a need for establishment of health policies. We developed a multiplex RT-PCR analysis of PCR products by Sybr Green, using the sets of primers described by PAI et al 2006. Four of the most relevant serotypes were included in the reaction, 19F, 14, 9V and 6 A/B/C/D (one isolate for each serotype was tested eight times). Melting temperature of amplicons was evaluated in Applied 7500 Real-Time PCR System, and samples were previously serotyped by conventional PCR. We set a reaction including the following mean melting temperatures: 19F (77.23 +/- 0.52 °C), 14 (74.98 +/- 0.18 °C), 9V (78.13 +/- 0.16 °C) and 6A/B/C/D (79.01 +/- 0.33 °C). Agarose gel electrophoresis confirmed the amplification of target fragments. The reaction was reproducible and able to distinguish each serotype with a mean melting temperature difference of 1.0°C. ANOVA analysis showed statically difference between all groups (p< 0.05). The assay was able to rapidly and cost-effectively identify the serotypes tested. This is the first step in the establishment of a comprehensive approach to serotype pneumococci by RT-PCR. Such procedure provides conditions to monitor emerging serotypes and to evaluate the pneumococcal epidemiology after the implementation of vaccine programs.

Financial support: CNPq, FAPERGS.
MULTIPLEX PCR ASSAY FOR DETECTION OF PNEUMOCOCCAL SEROTYPES IN NASOPHARYNGEAL SAMPLES OF HEALTHY CHILDREN; TEHRAN, 2009-2010

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Background and aims: Streptococcus Pneumoniae, a major pathogen causing invasive disease, colonizes the nasopharynx constituting a potential source of infection in both children and adults.

Aim: To identify the rate of pneumococcal nasopharyngeal colonization in healthy infants < 2 years of age and to define the prevalent serotypes.

Methods: This cross-sectional study was performed for 3 months in June- August 2009 on healthy children, aged 6, 12 and 18 months visiting Health centers for routine vaccinations.

Trained personnel collected nasopharyngeal samples through flexible nasal swabs and sent the specimens for isolation of pneumococci; after DNA extraction, microorganisms were serotyped by Multiplex PCR assay.

Results: One thousand three hundred-one infants were enrolled; Streptococcus pneumoniae was isolated from 34%. A total of 762 isolates of S. pneumoniae, belonging to 30 different serotypes were recovered from 440 positive nasopharyngeal specimens. Serotypes 19, 6, 14, 19F, 17, 21, 20, 12F, 11 and 3 were most common, isolated in frequencies of 8.2, 7.6, 7.2, 7.2, 6.4, 5.3, 5.1, 4.8, 4.5 and 4.3% respectively. There was no significant difference in the serotypes isolated from the three age groups.

Colonization with more than one strain was seen in 228 samples, (52% of carriers).

Eleven serotypes, constituting about 38.5% of isolates, are included in the 13-valent pneumococcal conjugate vaccine, (PCV 13).

Conclusion: Limited coverage of the current PCV13 emphasizes the need to manufacture vaccines with an optimal formulation that would provide effective protection against serotypes prevalent in the community.
ADDED VALUE OF DIRECT PCR METHODS IN THE DIAGNOSIS AND SEROTYPING OF PNEUMOCOCCAL MENINGITIS IN SALVADOR CITY, BRAZIL

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Background and aims: Accurate diagnosis of pneumococcal meningitis remains a challenge due to the inadequate sensitivity of conventional diagnostic tests. The aim of this study was to evaluate PCR-assays for diagnosis and serotyping of pneumococcal meningitis cases.

Methods: A prospective clinical observational study of consecutively enrolled patients with meningitis was performed at Couto Maia Hospital, the State infectious diseases referral center. Along with routine testing, CSF samples were tested by a pneumococcal-specific real-time PCR (lytA) and Multiplex PCR for serotype deduction. A Micrococcus bioassay was used to test for the presence of antimicrobial agents in CSF.

Results: Pneumococcal infection was diagnosed in 60 of the 639 (9.4%) specimens. For 46/60 (76.6%) cases, culture and real-time PCR were both positive. Twenty-three percent (14/60) were pneumococcal culture-negative and lytA-positive. Twelve of 14 lytA-positive specimens were serotypeable using multiplex PCR. Pneumococcal lytA-positive, culture-negative results were more frequently found among patients older than 5 years old (12/14=86%). From this group 21% (3/14) of the specimens were positive for antimicrobial activity. Among the 60 meningitis cases, 56 (93%) had capsular serotype determined. The most commons serogroups were: 6A/B/C (18%), 14 (18%), 18 (14%) and 23F (12%). Overall 53% of the cases were caused by vaccine serotypes (PCV10). In patients < 5 years old the estimated PCV-10 coverage was 80%.

Conclusion: PCR-based diagnostics increased the yield of pneumococcal etiology and pneumococcal serotype information from 75% of cases to 95%. This added information contributes considerably to efforts in monitoring disease burden, vaccine impact, and serotype replacement.
PNEUCARRIAGE PROJECT: COMPARISON OF NEW AND TRADITIONAL METHODS OF DETECTING MULTIPLE SEROTYPE CARRIAGE

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Background and aims: Multiple serotype carriage (MSC) is common and important for understanding the link between pneumococcal immunisation, carriage and disease. However, there is no standard laboratory method to detect MSC. A variety of new methods show promise, but lack data describing and comparing their performance. The PneuCarriage project addresses this issue in a coordinated, global fashion.

Methods: We have developed a set of reference samples containing 81 spiked samples (containing 0-4+ isolates in varying numbers and proportions in STGG transport medium) and 250 nasopharyngeal (NP) samples from sites including South Africa, Kenya, The Gambia, Fiji, Papua New Guinea and Bangladesh. Groups developing new serotyping methods will test the spiked samples, and the top 3-5 methods will progress to field sample testing.

Results: Over 20 different methods have been applied to the spiked samples. The sensitivity and positive predictive values (PPV) were determined. Results indicated that a culture step prior to testing, increased sensitivity of detecting serotypes that were present in low proportion. Some methods were unsuitable for detecting multiple serotype carriage (sensitivity < 70% and/or PPV < 90%). Others performed well but will not be pursued due to technical limitations of the assay. Four top-performing methods have been sent field samples for further testing. From their results, we will be able to recommend the best currently available method to detect multiple serotype carriage.

Conclusions: The PneuCarriage project will identify the best method to detect MSC according to set criteria including scientific performance and applicability to resource-poor settings.
Poster No 67

LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) FOR DETECTION OF HAEMOPHILUS INFLUENZAE B AND STREPTOCOCCUS PNEUMONIAE

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Background and aims: Loop-mediated isothermal amplification (LAMP) method is now elucidated as a powerful tool for amplification of target genes especially for diagnostic purposes. We set out to establish LAMP methods for detection of \textit{Haemophilus influenzae} b (Hib) and \textit{Streptococcus pneumoniae} (Sp).

Methods: For development of LAMP primers, we targeted selected target genes including the capsulation locus region II for Hib and lytA for Sp. LAMP reactions were run under standardized, controlled conditions for detection of both Hib and Sp.

Results: In the case of each gene target, the LAMP method was able to detect 10 copies of purified reference DNA reflecting a detection limit over 1,000-fold higher than that of conventional PCR of the same target genes. Moreover, the detection was target specific; no other control bacterial species were detected.

Conclusions: In the case of Sp LAMP, our method can discriminate \textit{S. pneumoniae} from very closely related species. The LAMP method can substantially raise the detection rate of each bacterial species in clinical CSF specimens. Further work is now ongoing to develop LAMP for point-of-care testing of bacterial pathogens important in developed and developing countries.
DETECTING AND QUANTIFYING PNEUMOCOCCAL CWPS POLYSACCHARIDES IN HUMAN SERUM

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Background and aims: The objective was to develop a protocol for detection and quantification of pneumococcal Cell Wall Polysaccharide (CWPS) in serum from bacteraemic patients. Being able to quantify the CWPS opens a possibility of correlating the severity of disease to the concentration of CWPS in serum prospectively, and to follow recovery and/or effect of treatment.

Methods: A protocol for a sandwich ELISA was setup to detect CWPS. Monoclonal anti-CWPS (mouse IgM) and polyclonal anti-CWPS (Rabbit IgG) antibodies were used in the ELISA. A standard serum test panel based on CWPS polysaccharides from SSI Diagnostica was created, in which different known concentrations of CWPS were added to human serum free from natural infectious CWPS. Using dilution series of CFU in serum, also the impact of whole Streptococcus pneumonia bacteria on the setup was tested. Furthermore, other streptococcal species were tested in the ELISA setup to reveal possible crossreactions. Sera from patients with diagnosed bacteraemia were tested and compared to sera from healthy patients.

Results: The method, following a necessary serum sample dilution of 1:2 in EDTA, was able to detect CWPS in human bacteraemic serum with a limit of 0.3ng/mL. Of seven human bacteraemic serum samples, four showed a conc. between 0.48ng/mL - 0.78ng/mL, and three showed a concentration below 0.31ng/mL. Only minor crossreactions were seen with other Streptococcus species.

Conclusion: The data showed that it was possible to detect CWPS in human serum. The method is to be further tested on a larger sample collection.
LYTA PCR AND SPN9802 PCR APPLIED TO SPUTUM FOR RAPID DETECTION OF PNEUMOCOCCAL PNEUMONIA

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Background/aims: We aimed to evaluate if PCR for the two Streptococcus pneumoniae specific genes lytA and Spn9802 applied to sputum could be used for rapid detection of pneumococcal aetiology in community-acquired pneumonia (CAP).

Methods: In a prospective study of adult patients hospitalised for radiologically confirmed CAP, sputum samples of high quality were available in 78 cases. These samples were subjected to DNA extraction with an automatic extraction system and were run with two different quantitative real-time PCR assays, one for lytA and one for Spn9802. The time for analysis from original sample to result was < 2½ h for either method.

Results: Thirty-two patients had pneumococcal aetiology according to blood culture, sputum culture, and/or urinary antigen test. The table shows the quantitative PCR results in different CAP patient categories. For lytA PCR, a cut-off of $10^5$ DNA copies/mL gave a sensitivity of 94% and a specificity of 96%. For Spn9802 PCR, no cut-off provided both high sensitivity and high specificity. However, the area under the receiver operating characteristic curve was similar for lytA PCR (0.957) and Spn9802 PCR (0.942).

<table>
<thead>
<tr>
<th>PCR test</th>
<th>Cut-off limit DNA copies/mL</th>
<th>Pneumococcal pneumonia (n=32)</th>
<th>Non-pneumococcal pneumonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>lytA</td>
<td>10⁵</td>
<td>31 (97)</td>
<td>Pneumococcal carriage (n=4) positive 1 (25) positive 1 (25) positive</td>
</tr>
<tr>
<td></td>
<td>10⁴</td>
<td>31 (97)</td>
<td>3 (75) 1 (4.3) positive 2 (12) positive</td>
</tr>
<tr>
<td></td>
<td>10³</td>
<td>31 (97)</td>
<td>2 (50) 1 (4.3) positive 1 (5.9) positive</td>
</tr>
<tr>
<td></td>
<td>10²</td>
<td>39 (94)</td>
<td>2 (50) 0 0</td>
</tr>
<tr>
<td></td>
<td>10¹</td>
<td>23 (72)</td>
<td>2 (50) 0 0</td>
</tr>
<tr>
<td>Spn9802</td>
<td>10⁵</td>
<td>31 (97)</td>
<td>4 (100) 1 (4.3) positive 3 (18) positive</td>
</tr>
<tr>
<td></td>
<td>10⁴</td>
<td>31 (97)</td>
<td>4 (100) 1 (4.3) positive 3 (18) positive</td>
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<td></td>
<td>10³</td>
<td>31 (97)</td>
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</tr>
<tr>
<td></td>
<td>10²</td>
<td>28 (89)</td>
<td>2 (50) 0 0</td>
</tr>
<tr>
<td></td>
<td>10¹</td>
<td>19 (59)</td>
<td>2 (50) 0 0</td>
</tr>
</tbody>
</table>

Conclusion: Both lytA PCR and Spn9802 PCR performed well for rapid detection of pneumococcal pneumonia. However, lytA PCR appeared to be more useful than Spn9802 PCR, as the cut-off of $10^5$ DNA copies/mL made lytA PCR highly sensitive and highly specific.
HIV INFECTION AND INFLUENZA CO-INFECTION INCREASE THE RISK OF ELEVATED BLOOD PNEUMOCOCCAL LOADS AND ASSOCIATED MORTALITY IN HOSPITALISED PNEUMONIA PATIENTS

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Background and aims: There is a lack of sensitive assays for making an etiologic-specific diagnosis of pneumococcal pneumonia. We determined the prevalence of pneumococcal DNA in blood and factors associated with high bacterial load and death in patients with hospitalised pneumonia.

Methods: 5130 patients were enrolled from May 2009 through December 2010 as part of a hospital-based pneumonia surveillance programme in six South African hospitals. Streptococcus pneumoniae in whole blood was quantified by lytA real-time PCR (RT-PCR). Nasopharyngeal swabs/aspirates were tested for influenza by reverse-transcription RT-PCR. HIV status was determined by ELISA/PCR.

Results: Overall 7% (372/5130) tested lytA positive. Pneumococcal prevalence was 5% (77/1683), 6% (25/392), 1% (2/167), 10% (188/1936) and 8% (80/952) in the < 2, 2-5, 6-18, 19-44 and ≥45 years age groups respectively. On multivariable analysis the lytA-positive patients with higher blood pneumococcal loads had a higher prevalence of HIV [adjusted odds ratio (AOR): 2.5, 95% confidence interval (CI): 1.6-3.8], influenza co-infection [AOR: 1.4, CI: 1.2-1.7] and were more likely to be treated with supplemental oxygen [AOR: 1.6, CI: 1.1-2.4]. Amongst lytA-positive patients increased risk of death was associated with pneumococcal loads of ≥10,000 DNA copies/ml [AOR: 3.9, CI: 1.9-8.1], controlling for oxygen treatment and late presentation to the hospital.

Conclusions: HIV and influenza infections are significant risk factors for elevated pneumococcal loads in blood, which was also associated with an increased risk of death. High pneumococcal loads at time of diagnosis may have a role in future as a prognostic marker for pneumococcal pneumonia.
CARRIAGE OF HIGH PNEUMOCOCCAL LOAD IS ASSOCIATED WITH AN INCREASED RISK OF DEVELOPING INVASIVE PNEUMOCOCCAL DISEASE

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Background and aims: In South Africa PCV7 routine immunisation at 6, 14 and 36 weeks was introduced in April 2009. We investigated factors associated with pneumococcal carriage and high density of nasopharyngeal colonisation (NPC) in hospitalised pneumonia patients.

Methods: 4433 patients were enrolled in 2010 as part of hospital-based pneumonia surveillance. Nasopharyngeal swabs/aspirates were tested by real-time PCR (RT-PCR) for ten respiratory viruses. Quantitative pneumococcal lyA RT-PCR was performed on all blood specimens (77%, 3415/4433) and systematically selected (every 4th) respiratory specimens (24%, 1065/4433).

Results: The prevalence of pneumococcal carriage was 55% (584/1065); 67% (320/481), 79% (19/24) and 44% (245/560) in patients < 5, 5-12 and >12 years, respectively. To date molecular serotyping was performed on 49% (288/584) of lyA-positive respiratory specimens. 39% (111/288) of patients carried ≥2 serotypes. On multivariable analysis, controlling for hospital and age, HIV [adjusted odds ratio (AOR):1.5, 95% confidence interval (CI): 1.0-2.2], influenza [AOR: 1.9, CI: 1.0-3.7], adenovirus [AOR: 1.7, CI: 1.1-2.8] and rhinovirus [AOR: 1.7, CI: 1.2-2.4] co-infections were associated with an increased risk of carriage while NPC was less prevalent in patients with current underlying tuberculosis [AOR: 0.4, CI: 0.3-0.6]. Controlling for age, high density of NPC was associated with increased blood lyA positivity [AOR: 2.2, CI: 1.3-3.9]. Preliminary data indicate that in HIV-negative children, ≥2 PCV7 doses provided protection against vaccine-type carriage [AOR: 0.7, CI: 0.1-3.9], although not significantly.

Conclusions: HIV and respiratory virus co-infections are associated with increased risk of pneumococcal carriage. Invasive pneumococcal disease was associated with elevated NPC density.
DEVELOPMENT OF AN AUTOMATED AND MULTIPLEXED SEROTYPING ASSAY FOR STREPTOCOCCUS PNEUMONIAE

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Background: Streptococcus pneumoniae expresses more than 90 capsule types and serotyping pneumococcal isolates is important for developing and deploying pneumococcal vaccines. Yet, serotyping pneumococcal isolates has remained a significant technical challenge.

Method: We have devised 3 reactions for the Luminex® assay platform. The three reactions require only six well defined reagents and share one pneumococcal lysate. One reaction is an inhibition type immunoassay that uses a mixture of 26 monoclonal antibodies and 26 Luminex® beads coated with 26 different pneumococcal capsule types. The 26 serotypes include all the capsule types in the 23-valent polysaccharide vaccine and serotypes 6A, 6C and 11E. The other two reactions are designed to identify all other remaining serotypes by detecting DNA that is serotype specific. To obtain serotype specific DNA, two different multiplex PCR reactions are performed with the lysate. The PCR products were identified with Luminex® beads coated with serotype-specific DNA probes.

Results: The assay system has been validated with a panel of pneumococci expressing all known pneumococcal serotypes and clinical isolates. The assay was found to be easily transferable to another laboratory located at a distant site.

Conclusion: Pneumococcal serotyping has been largely automated with the use of multiplex assays.

The work was supported by funding from NIH (N01-AI-30021).
Poster No 73

CLINICAL DATA AND SEROTYPES AMONG CHILDREN HOSPITALIZED IN TWO SENTINEL HOSPITALS IN BRAZIL IN THE PRE-IMUNIZATION ERA

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Aims: We have evaluated clinical characteristics of pneumococcal pneumonia (PP) in pre-immunization era in Brazil.

Methods: PP was defined as Sp isolated from Blood or pleural Fluid and X Ray findings of pneumonia among infants and children 0 to 156 months of age hospitalized in 2 sentinels hospitals (S. Paulo and Uberlandia) from 1999 to 2010.

Results: We included 225 patients. 58 (26%) have history of previous underlying disease like Asthma or wheezing child, history of prematurity, Hemoglobinopathy, Neurologic disease, Neoplasia, HIV infected and others. Sp was isolated from: Blood culture in 125 patients, Pleural Fluid in 95 and in both in 7. Age distribution was: less than 24 ms - 67%, 2 to 5 years - 20% and over 5 years 13%. The diagnosis were Bacteremic pneumonia in 109 patients, and Pneumonia with pleural effusion in 116. The most important serotypes were: In patients with bacteremic pneumonia (N= 109): serotype 14 in 65 patients (60%), serotype 6B in 16 patients (16%) and serotype 9V in 6 (5.5%) In patients with pleural effusion : (N= 116) serotype 14 was present in 51 patients (44%), serotype 1 in 15 (13%) and serotype 6B in 7 (6%). A total of 39 patients were admitted in Intensive Care. From the patients admitted in Intensive care 14 have history of underlying disease. Patients with underlying disease had most frequent intensive care admissions.

Conclusion: Pneumococcal pneumonia is a severe disease with high frequency of Intensive care admissions.
Poster No 74

HOSPITAL-BASED SURVEILLANCE OF SEROTYPES ASSOCIATED WITH PNEUMOCOCCAL INVASIVE DISEASE (PID) AMONG PATIENTS OVER 50 YEARS

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Background and aims: Pneumococcal invasive disease (PID) is associated to significant morbidity and mortality in Brazil. The aim of this study was to describe the distribution of serotypes associated with pneumococcal invasive disease among adults over 40 years old of age in a general hospital of Sao Paulo.

Methods: We performed a 6-year (2005 - 2010) hospital-based surveillance in patients admitted due to PID, with isolation of S. pneumoniae.

Results: During the period 2005-2011 there were 63 patients admitted due to PID. Pneumonia was the most frequent diagnosis. The serotypes most frequent were: ST14 present in 8 patients, ST 23F in 7, ST3 in 5, ST6B in 4 and ST 20 in 5 patients. 70 % of the serotypes are included in the 13V vaccine. In 2005 we admitted 6 patients over 40 years old (15% of all the PID diagnosed). In 2010 there were 17 patients over 40 years old (38 % of all the PID diagnosed).

Conclusions: Based on our data, the 13V-pneumococcal conjugate vaccine would potentially prevent 70% of the PID cases among adults >40 years. The diagnosis of PID in this age increased.
PREVALENCE OF INVASIVE PNEUMOCOCCAL SEROTYPES FROM OLD ADULTS DURING 11 YEARS BEFORE THE INTRODUCTION OF THE PHID-CV IN BRAZIL

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Background and aims: \textit{S.pneumoniae} (Spn) is an important cause of morbidity and mortality among old adults. In Brazil, the 23-valent polysaccharide vaccine (PV-23) was introduced in 2000 and is available for the elderly (≥60y), adults and children (≥2y) with chronic conditions. The PV-23 coverage is estimated in 9%. We report the prevalence of serotypes isolated from old adult patients during an 11-year laboratorial surveillance, before the PHID-CV introduction in Brazil.

Methods: Spn isolates are sent to IAL for serotyping and other testing. Serotyping is performed by Quellung reaction.

Results: From 2000 to 2010, IAL studied 1,372 invasive pneumococcal isolates from patients aging ≥50y (48% from 50-59y; 52% from ≥60y).

The 23 prevalent serotypes from ≥50y patients were 3(11%), 14(8%), 23F(7%), 19F(6.2%), 12F(6%), 4(5.3%), 6B(4.7%), 9V(4%), 7F(3.7%), 18C(3.6%), 10A(3.6%), 6A(3.4), 22F(2.7%), 19A(2.6%), 5(2%), 8(2%), 9N(1.8%), 11A(1.7%), 1(1.5%), 16F(1.2%), 6C(1.2%), 23B(1.1%), 15A(1.1%); and 32 other types(13.8%) and NT(1%) isolates. Other less frequent PV-23 serotypes were 17F(1%), 15B(0.9%), and 20(0.6%); Serotype 2 and 33F isolates were not found. The PV-23 (plus 6A) showed an estimated impact of 83.3%, 81.7% and 84.2% for ≥50y, 50-59y, and ≥60y, respectively. Differences in the serotypes distribution between 50-59y and ≥60y age groups were related to 6C,15A,15B,15C,23B and 28A.

Conclusions: In 2010, the PHID-CV was introduced in the national immunization program for children ≤2y. Because the Spn conjugate vaccines induce indirect herd effect, it is essential to monitor Spn serotypes in all age groups.

Acknowledgment: SIREVAl-PAHO for supplying the pneumococcal antisera; CNPQ for grant support to MCCBrandileone(no.302175/2010-5).
Poster No 76

EPIDEMIOLOGY OF STREPTOCOCCUS PNEUMONIAE (SPN) AND HAEMOPHILUS INFLUENZAE (HI) NASOPHARYNGEAL CARRIAGE IN UNVACCINATED HEALTHY CHILDREN IN BRAZIL

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Background and aims: Investigation of Spn carriage is of worth to evaluate the epidemiology of Spn and to assess the pneumococcal conjugate vaccine impact after its introduction. The 10-valent pneumococcal conjugate vaccine (PHiD-CV) was introduced in the National Immunization Program in 2010. We studied the prevalence of Spn and Hi nasopharyngeal (NP) carriage and potential risk factors in children.

Methods: During March and August/2010, nasopharyngeal swabs were collected from 1,540 healthy unvaccinated children, aged 1<2y (n=965) and 4<5y (n=575), in 16 immunization rooms located in the 5 regions of the municipality of São Paulo, Brazil. Demographic data and potential risk factors were obtained during the NP sample collection. Logistic regression was applied to assess potential risk variables associated to Spn/Hi carriers.

Results: Spn, Hi and Spn+Hi carriage rates were 19.2%, 16.7% and 32.1%, respectively; for the age-group 1<2y, the respective rates were 21%, 15.9%, and 28.6% while for 4<5y the corresponding prevalence was 16%, 18.2% and 38%. Non-typeable Hi accounted for 96.7% of Hi isolates. Day-care attendance (OR=2.47; 95%CI:1.83-3.20), age/4<5y (OR=1.55-1.16-2.03) and mothers' schooling (OR=1.52:1.09-2.10) remained independently associated with pneumococcal carriage in multivariable models. Children with simultaneous colonization with Hi, mostly non-typeable Hi, were significantly associated with Spn carriage (adjusted OR=2.66:2.13-3.32).

Conclusions: Crowded environment and mothers' educational level contribute significantly for pneumococcal nasopharyngeal colonization in children. This investigation provides a baseline for assessment of the epidemiology of Spn carriage and the impact of PHiD-CV on Spn and non-typeable Hi colonization.

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NON VACCINE-RELATED CHANGES OF SEROTYPES AMONG INVASIVE PNEUMOCOCCAL ISOLATES IN URUGUAY

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Background and aim: Temporal trends of serotypes causing invasive pneumococcal disease (IPD) have been reported. In the post conjugate vaccines era, it may be difficult to assess which changes are due to these trends or to vaccination selective pressure. In this study, the variation in incidence for serotypes 1 and 5 invasive pneumococcal disease (IPD) was estimated over time in Uruguay.

Methods: A retrospective study based on national laboratory surveillance was undertaken, from 1994 to 2009, the last year before PCV13 introduction. IPD cases were analyzed in 3 age groups: 0-4 years, 5-14 years and > 15 years old.

Results: The incidence of serotype 5 IPD changed along the study period, with to peaks in 1999-2000 and 2004-2005, observed in all age groups. The incidence per 100 000 children 0 to 4 years varied between 11.5 (2005) to 0 (2007). The same situation was observed for serotype 1 IPD, although the 2 peaks were in 2000 and 2005-2006. The incidences for the same age group were between 3 (1998 and 2002) to 11.7 per 100 000 (2005). Overall, a tendency of decreasing incidence was documented for both serotypes.

Conclusion: Serotypes 5 and 1 have a periodicity with significant changes in incidence. The short-term effect of pneumococcal conjugate vaccines including serotypes 1 and 5 may be affected by the incidence of IPD caused by these serotypes in the moment of vaccine introduction.
SEROTYPE DISTRIBUTION, ANTIBIOTIC RESISTANCE AND COVERAGE OF PNEUMOCOCCAL CONJUGATE VACCINES (PCV) BEFORE THEIR INTRODUCTION IN THE NATIONAL SCHEDULE: ARGENTINA 2000-2011


Background: *Streptococcus pneumoniae* (Spn) is a prevalent cause of invasive diseases in children, justifying continuous surveillance Programs.

Aim: to determine serotype distribution, antibiotic resistance and coverage of PCV of Spn causing invasive disease in < 6y.o. before the incorporation of the PCV in the National Schedule in January 2012.

Methodology: Spn isolated from sterile fluids (55 hospitals, 17 provinces and Buenos Aires) from January 2000 to August 2011 were serotyped by Quellung. MIC was performed by agar dilution (CLSI).

Results: 2402 Spn (63.1%< 2y.o.) were evaluated.

Diagnosis: pneumonia(53.7%), meningitis(23.1%), sepsis(9.2%), other(13.9%). Fifty serotypes(%) were identified: 14(26.3), 1(12.7), 5(12.3), 6B(4.9), 19A(4.7), 7F(4.6), 18C(3.9), 6A(3.5), 9V(3.1), 23F(2.9), 19F(2.6), others(15.9). Changes in serotypes were observed: decrease in serotypes 14(35.3%-22.6%) and 6B(7.5%-4.2%) and increase in 1(7.5%-14.7%), 19A(2.6%-5.1%), 6A(2%-4.8%), 3(1.5%-3.4%) and 7F(3.5%-6.1%). Penicillin no susceptibility (PNS: MIC≥0.12mg/L) was 33.7% (25.3%MIC=0.12-1mg/L; 8%MIC=2mg/L; 0.4%MIC=4mg/L), remaining stable. Main serotypes associated with PNS were: 14(72.1%),6B(68.5%),19A(67.9%),6A(48.2%),9V(34.2%),23F(23.5%),19F(25%) and 9N(15.4%). No-susceptibility was: cefotaxime 13.6% (meningeal isolates), 1.1%(non-meningeal isolates); amoxicillin 0.6%; meropenem 8.4%; tetracycline 11%; chloramphenicol 0.6%; ofloxacin, rifampicin and vancomycin 0%. Erythromycin resistance was 18.5% increasing from 6.3%(2000) to 28.2%(2011) and trimethoprim-sulfamethoxazole decreased from 54%(2000-03) to 41%(2008-11). Serotype coverage of PCV-10/PCV-13 during 2008-2011 for < 2y.o. was: 68.2%/82.4%(all diagnosis); 72.1%/87%(pneumonia); 70.2%/79.5%(meningitis).

Conclusions: PNS keeps stable in the last years but erythromycin resistance increased. Serotype 14 decreased through the time but remain been the most prevalent. The increased in the last decade of the prevalence of serotypes related to PCV10-13 (1,19A,6A,3 and 7F) deserves the introduction of a PCV with a highest coverage.
COMPARISON BETWEEN INVASIVE DISEASE (ID) AND NASOPHARYNGEAL CARRIAGE (NC) OF S. PNEUMONIAE (SPN) AMONG NON VACCINATED ARGENTINEAN CHILDREN DURING 2007/08


Background and aim: Spn usually colonizes the nasopharynx and carriage is related to the development of invasive disease. The aim was to compare serotype distribution and antimicrobial susceptibility of Spn isolated from children < 3 y.o. with ID and NC during the same period of time.

Methods: During 2007/08, 362 Spn isolates causing ID (37 hospitals, 18 cities, National Surveillance Program SIREVA) and 415 Spn from NC from children attending 7 daycare centers (7 cities) from Argentina were studied. Spn were serotyped by Quellung, MIC was performed by agar dilution (CLSI), and macrolide-resistant phenotypes (MLSb and M) by disk diffusion.

Results: Serotype distribution was: 14(26%), 5(15%), 1(8%), 7F(7%), 19A(6%), 6A(5%), 12F(4%), 18C(4%), 6B(3%), 19F(2%), others(20%) for ID and: 6A(12%), 15B(10%), 19F(9%), 14(8%), 6B(7%), 23F(7%), 9V(6%), 19A(4%), 15C(4%), 11A(4%), others(29%) for NC. Significant differences (p: < 0.05) were observed in serotypes 14, 6A, 6B and 19F prevalent in both groups. Serotypes 5, 1, 7F, 12F and 18C were prevalent in ID but not in NC(p: < 0.001), whereas serotypes 15B, 23F, 9V, 15C and 11A were prevalent in NC(p: < 0.001). No-susceptibility was: penicillin MIC≥0.12mg/L: 27.2%ID/40.7%NC (MIC=0.12-1mg/L:19.6%/ID/36.1%/NC, MIC≥2mg/L: 7.6%/ID/4.6%/NC); cefotaxime MIC≥1mg/L: 7.8%/ID/6.3%/NC (MIC=2mg/L 1.4%/ID/1%/NC, MIC≥4mg/L: 0%/ID/0%/NC); amoxicillin 0%/ID/1.9%/NC, meropenem 9.5%/ID/7%/NC, trimethoprim-sulfamethoxazole 45.4%/ID/48.2%/NC, 0% ofloxacin and vancomycin. Eritromycin resistance was: 21.6%/ID/19.8%/NC with dominance of M phenotype 79.2%/ID/63.4%/NC. Serotype 19A isolates with PEN MIC≥0.12mg/L were 60%/ID/81%/NC.

Conclusions: Different serotypes were associated with ID and NC. Penicillin no-susceptibility was higher in NC than in ID. The new pneumococcal conjugate vaccines will improve the coverage of serotypes and antibiotic resistance of Spn from ID and NC.
S. pneumoniae (Sp) is a major cause of community-acquired bacterial infections, and serotype 14 is among the capsular types most frequently associated with invasive disease and with antimicrobial resistance. Understanding the strain distribution associated with Sp disease may assist in generating prevention and treatment strategies. In this work, we evaluated antimicrobial susceptibility and genetic diversity of 221 serotype 14 Sp isolates recovered between 1990 and 2011 from diverse clinical sources and Brazilian locations. A total of 129 (58.4%) isolates were penicillin non-susceptible (Pen-NS; MIC ≥0.12µg/ml). Twenty-four (10.8%) isolates were erythromycin-resistant and 51 (23.1%) were tetracycline-resistant, primarily associated with *erm*B and *tet*M, respectively. PFGE and MLST (46 different sequence types determined) revealed that 3 clonal complexes (CCs), associated with international clones Spain9V-3, England14-9 and Tennessee14-18, accounted for the vast majority of the isolates. Sixty-four selected isolates were also analyzed by Multiple-Locus Variable number tandem repeat Analysis (MLVA), generating 58 profiles and 4 major groups. Sp strains presenting identical STs and PFGE profiles could be distinguished by MLVA, indicating its potential as a complementary genotyping tool for Sp strains. The predominant CC observed (encompassing 90 isolates) was associated with ST156, MLVA type 7, PFGE profile Pen-H, and has been detected among isolates from a variety of clinical sources in different Brazilian cities since 1995. Invasive disease caused by these 3 major CCs of serotype 14 was essentially eliminated within the United States vaccinated population by implementation of PCV7. We feel it is reasonable to project that vaccination in Brazil could effect similar results.
AGE-RELATED SEROEPIDEMIOLOGY OF ANTI-PNEUMOCOCCAL PROTEIN ANTIBODIES (APPAB) IN CHILDREN OF TWO DISTINCT ETHNIC POPULATIONS IN SOUTHERN ISRAEL

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Background: Immunity against pneumococcal protein antigens (PPAg) is important but not sufficiently characterized. We aimed at determining the seroepidemiology of APPAb against 16 vaccine-candidate PPAgs, with 2 objectives: 1) To determine the dynamics of APPAb with age; and 2) to compare APPAb dynamics between Bedouin children (BC; a population with high pneumococcal carriage) and Jewish children (JC; a population with lower carriage).

Methods: Sera of JC (n=254) and BC (n=378) < 5 years old (equally distributed among age-groups [0-3, 4-7, 11-14, 17-20, 24-32, ≥33 months]), prospectively collected in the pre-PCV7 era. The sera were tested by a multiplex-based immunoassay (Tal et al, submitted to 8th ISPPD).

Results: 1) For all 16 proteins, maternally-derived APPAb decreased from age 0-3 to 4-7m; 2) from age 4-7m to ≥33m all APPAb increased ≥2-fold; 3) in all age groups, APPAbs among BC were ≥2-fold higher than JC (including maternally-derived APPAb); and 4) from age 4-7m to ≥33m, the fold increase among JC was significantly higher than among BC for all proteins. About 2/3 of proteins have high APPAb titers at younger ages that increase with age and 1/3 have very low APPAb titers that also increase with age in the two populations, which correlates with the age at which anti-pneumococcal immunity is gained.

Conclusions: We have confirmed an age-related response for all 16 vaccine-candidate PPAgs. Furthermore, the titers were several-fold higher in BC (with a much higher pneumococcal exposure) than JC. We are currently investigating the APPAb responses in systemic and mucosal pneumococcal infections.
POPULATION SNAPSHOT OF STREPTOCOCCUS PNEUMONIAE ISOLATED FROM SOUTH AFRICAN CHILDREN LESS THAN 5 YEARS, PRIOR TO PCV7 INTRODUCTION

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Background/Aims: Routine PCV7 immunization began in April 2009. Limited genotypic baseline data exist for vaccine serotype pneumococci prior to PCV7 introduction. We genetically characterised strains expressing PCV13+6C serotypes from children < 5 years.

Methods: 579 isolates, collected in 2007 as part of national, laboratory-based surveillance for invasive pneumococcal disease, were characterised by MLST. ST relationships were determined by eBURST.

Results: 4744 IPD cases were reported. Age was known in 4531 (96%) and children (< 5y) represented 32% (1468/4531). PCV7 and -13 serotypes accounted for 59% (637/1085) and 85% (924/1085), respectively. STs were available for 364/579 (63%) strains. Twenty-seven clonal groups representing 111 (including 67 new) STs, and 51 singletons (including 41 new STs) were compiled. Serotype 1 was ST217 (35/43, 81%) or SLV ST612 (8/43, 19%). Serotype 5 (n=8) was ST289 or SLVs ST5659 or ST6370. Serotypes 6A (36/42) and 6B (34/46) comprised 7 and 10 clonal groups, respectively. ST185, ST1094 and ST2285 were shared between 6A and 6B. Serotype 6C comprised 1 clonal group (3/5) and 2 singletons. Serotype 14 was ST63 (or one of 3 SLVs) (11/58, 19%), or ST 230 (or one of 8 SLVs) (32/58, 55%). ST2062 (or one of 13 SLVs) accounted for 92% (54/59) of serotype 19A.

Conclusions: Several strains were unique as indicated by the significant proportion of new STs. Global clones Sweden1 and Columbia5 were prevalent. South Africa3b was rare. Sweden15 (ST63) and Denmark14 (ST230) were prevalent among serotype 14 strains. The serotype 19A clone appears to be unique to South Africa.
SEROTYPES AND ANTIMICROBIAL RESISTANCE IN COLOMBIAN ISOLATES OF STREPTOCOCCUS PNEUMONIAE 2008-2011

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Background: Antimicrobial resistance in Streptococcus pneumoniae has increased worldwide. The introduction of the seven-valent pneumococcal conjugate vaccine (PCV7) has been associated with a significant decline in the incidence of invasive pneumococcal infections and in rates of antibiotic resistance in many countries.

Objective: To analyze antimicrobial resistance and serotypes distribution from laboratory surveillance of S. pneumoniae invasive Colombian isolates recovered from 2008 to June 2011.

Materials and methods: Database of invasive isolates of S. pneumoniae, sent to the Microbiology Group through the national surveillance laboratory network. The isolates had epidemiological data, serotyping and antimicrobial susceptibility patterns.

Results: The data of 1179 isolates from different regions of the country were analyzed. The isolates were recovered from blood cultures (71.2%), cerebrospinal fluid (19.4%) and others (9.4%), with diagnosis of meningitis (20.1%) and non-meningitis (79.9%). The resistance to penicillin and ceftriaxone was 30.3% and 10.9% for patients diagnosed with meningitis and 11.4% and 10.4% for non-meningitis, respectively. Overall, 38.8% of the isolates presented resistance to trimethoprim-sulfamethoxazole, 16.3% to tetracycline, 8.2% to erythromycin and 3% to chloramphenicol. Serotypes 14, 6B, 23F, 19F and 19A were the most prevalent and represented 88.3%, 96%, 77.9% and 62.1% resistance to penicillin, ceftriaxone, trimethoprimsulfamethoxazole and tetracycline, respectively. Otherwise, the serotypes 19A, 6A, 6B, 19F and 23F displayed 81.4% resistance to erythromycin.

Conclusions: Antimicrobial resistance is mainly associated with PCV7 serotypes. However, these results showed antimicrobial resistance in non PCV7 serotypes as 19A and 6A. This information is necessary to implement strategies for prevention of pneumococcal disease.
INVASIVE PNEUMOCOCCAL DISEASE, MORTALITY AND SEROTYPE IN CHILDREN HOSPITALIZED IN A TERTIARY CENTER IN EL SALVADOR

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**Background:** Streptococcus pneumoniae (SP) contributes with a high proportion of the disease burden secondary to vaccine preventable diseases worldwide. To support local policy decisions on pneumococcal disease prevention, knowledge about local disease burden is important.

**Objective:** To analyze disease burden secondary to invasive pneumococcal disease in children admitted to a tertiary pediatric center.

**Methods:** Retrospectively reviewed demographic, clinical and microbiological information of patients admitted during 2006-2011 in which SP was isolated from a sterile site. SP was identified and serotype using routine microbiologic procedures. Antimicrobial susceptibility to commonly used antibiotics was determined by E-test and minimum inhibitory concentration.

**Results:** During the study period 142 SP isolates were obtained from different sterile sites; 113 (80%) were serotype. The most common diagnoses were meningitis (35%) and bacteremic pneumonia (34%) followed by empyema (19%), and sepsis (12%). The average age of the patients was 34.5 months. A high proportion of the patients died (30.2%), being meningitis and sepsis the diagnosis associated with the highest mortality (50%). Patients with a diagnosis of meningitis showed a highest sequel (85.6%). The most common pneumococcal serotype were 14 (24%), 19 (21%), 18 (16%), 6 (10%), 7 and 9 (6% each). Potential vaccine coverage for 7V, 10V and 13V pneumococcal vaccines would be 79%, 58% and 93% respectively. Susceptibility of recovered pneumococcal strains demonstrated high resistance to penicillin (52.5%).

**Conclusions:** Invasive pneumococcal disease represents an important cause of mobility and mortality in children in El Salvador. Introduction of conjugated pneumococcal vaccine can reduce the disease burden.
MULTILOCUS SEQUENCE TYPING OF STREPTOCOCCUS PNEUMONIAE USING MASS SPECTROMETRY

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Background and aims: Streptococcus pneumoniae frequently colonises the nasopharynx of children and causes diseases such as pneumonia and otitis media. S. pneumoniae can be classified by multilocus sequence typing (MLST), an important tool for the global surveillance of bacterial pathogens performed by comparing the sequences of seven housekeeping genes. We developed and tested a novel mass spectrometry-based method for MLST of S. pneumoniae and used it to characterize isolates carried by children in Papua New Guinea, a region with high rates of pneumococcal disease.

Methods: PCR amplicons were subjected to in vitro transcription and base-specific cleavage, followed by analysis of the resultant fragments by using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Comparison of the cleavage fragment peak patterns to a reference sequence set permitted automated identification of alleles.

Results: Validation experiments using 29 isolates of S. pneumoniae revealed that results of MALDI-TOF MS MLST matched those obtained by traditional sequence-based MLST for 99% of alleles, and that the MALDI-TOF MS method accurately identified single-nucleotide variations. The MADLI-TOF MS method was then used for MLST analysis of 43 S. pneumoniae isolates from PNG children. The majority of the isolates present in this population were not clonal and contained seven new alleles and 30 previously unreported sequence types.

Conclusion: MALDI TOF MS MLST is an accurate and efficient method for genetic typing of S. pneumoniae. Data provide a population snapshot of S. pneumoniae sequence types carried by children in PNG prior to widespread pneumococcal vaccination.
Poster No 86

STREPTOCOCCUS PNEUMONIAE (PNC) DENSITY OF NASOPHARYNGEAL CARRIAGE IN UNIMMUNIZED 6-10 YEAR OLD CHILDREN: COMPARISON BETWEEN 2 ETHNIC POPULATIONS


Background: We compared the prevalence and density of Pnc carriage in general, and that of PCV7+6A serotypes (VT-SP) in particular, in children 6-10 years old from 2 distinct ethnic populations residing side-by-side in southern Israel: Jewish children (JC) (generally resembling a western population), and Bedouin children (BC) (a population with significantly higher crowding, pneumococcal carriage and respiratory diseases). There is almost no contact between the 2 populations. The study was conducted in non-PCV7 immunized children in the immediate post-PCV7 introduction period.

Methods: Nasopharyngeal swabs were obtained from healthy children 6-10 years old. Information on family size and antibiotic use was documented. Density was determined by semi-quantitative cultures using 4-quadrant dilutions on blood agar/gentamicin plates. Density is expressed from 1+ (lowest) to 4+ (highest).

Results: Carriage of pneumococci in general, and PCV7+6A serotypes in particular, was more frequent in BC than in JC (P< 0.001). These differences were also found in density of total Pnc carriage and of PCV7+6A serotypes (P< 0.001).

Figure: Distribution of density rates of S. pneumoniae carriage and PCV7+6A serotypes in the nasopharynx, among unimmunized children 6-10 years old in 2 ethnic populations, one year after the introduction of PCV7 into the NIP

Conclusions: Pnc carriage prevalence and density in general and those of PCV7+6A serotypes in particular are higher in BC than in JC. These findings suggest that indirect protection following PCV7 introduction may differ in the 2 populations, resulting in different rates of disease-burden reduction between the 2 groups.
BURDEN OF CHILDHOOD MORTALITY CAUSED BY STREPTOCOCCUS PNEUMONIAE IN INDIA

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Background and aims: 369,000 Indian children died in year 2005 due to pneumonia; these deaths were not equally distributed among Indian states. To inform national policy decisions prioritizing introduction and scale-up of childhood pneumonia prevention and treatment strategies, we estimated state-level mortality due to Streptococcus pneumoniae, the leading bacterial cause of severe pneumonia, in children age 1-59 months in India.

Methods: We combined pneumococcal disease incidence and case-fatality ratio data identified from a systematic search of the literature to estimate state-level pneumococcal meningitis and non-pneumonia non-meningitis mortality with adjustments for HIV prevalence and access to care. To estimate pneumococcal pneumonia deaths, we applied the average proportion of pneumonia caused by pneumococci from vaccine trials to the number of pneumonia deaths in Indian states estimated from a published nationally representative mortality survey.

Results: In 2005, we estimated pneumococcal disease caused 136,000 deaths (46,000-253,000) comprising 10% of deaths in Indian children aged 1-59 months. The death rate for pneumococci was 106 per 100,000 (36-197), and more than two-thirds of pneumococcal deaths were pneumonia-related. Pneumococcal mortality was highest in the Central and Eastern regions with more than half of all Indian deaths occurring in four states with reported low rates of antibiotic use: Bihar, Madhya Pradesh, Rajasthan, and Uttar Pradesh.

Conclusions: Pneumococcal disease burden in Indian children is substantial and not equally distributed throughout the country. Access to life-saving pneumococcal vaccines and scale-up of antibiotic treatment is critical to improve child survival in India and reduce inequities in disease morbidity.
Poster No 88

SEASONAL VARIATIONS OF PNEUMOCOCCAL MENINGITIS IN 30 YEARS: INFLUENCE OF ATMOSPHERIC CONDITIONS

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Background: Knowledge of seasonal trends in incidence of pneumococcal meningitis may improve the understanding of factors which contribute to onset and development of the diseases.

Methods: Clinic for Infectious and Tropical Diseases in Belgrade is the biggest institution in the country where infections of central nervous system are treated. A database containing results of cerebro spinal fluid (CSF) cultures (January 1981-December 2010) from this Clinic was assembled. The database included monthly counts of inpatient CSF cultures positive for Streptococcus pneumoniae. Results of monthly measuring mean atmospheric parameters for the same period in Belgrade were obtained from national meteorological database.

Results: A total of 1068 inpatient CSF yielding bacterial pathogens, deemed to be causes of meningitis, were reported. Summer season (April-September) was associated with 19.64% fewer pneumococci (137) relative to winter (October-March, 204 isolates). Correlation analysis has been used to compare the total number of pneumococci in CSF and mean atmospheric parameters for each one of 12 months in 30 year period. Positive correlation has been proved with atmospheric pressure (R=0.724, p< 0.01) and negative correlation with temperature (R= -0.751, p< 0.01) and insolation (R= -0.759, p< 0.01), while the correlation with monthly precipitation hasn’t reached the level of statistical significance (R=0.417, p>0.05). Regression model of the occurrence of pneumococci in CSF revealed linear trend of growth, R square=0.319, p< 0.01.

Conclusions: Winter season, higher mean atmospheric pressure and lower monthly outdoor temperature and insolation are associated with substantially increased frequency of pneumococcal meningitis.
ANTIMICROBIAL SUSCEPTIBILITY AND SEROTYPE DISTRIBUTION IN INVASIVE AND COLONISING STREPTOCOCCUS PNEUMONIAE FROM CHILDREN IN ABIDJAN

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Background and aim: Pneumococcal disease is an important cause of childhood morbidity and mortality world-wide. In the developing countries, the effective vaccines against Streptococcus pneumoniae are not in routine use. However, the emergence of antibiotic-resistance has become an increasing problem. The aim of this study was to analyse the resistance patterns and serotypes distribution of invasive Streptococcus pneumoniae disease and nasopharyngeal from children in Abidjan.

Methods: From February 2007 to September 2008, A prospective study was undertaken in children under 5 years in Abidjan. Isolates of Streptococcus pneumoniae were obtained from clinical specimen (cerebrospinal fluid and blood) and nasopharyngeal swabs. After identification by standard bacteriological methods, antimicrobial susceptibility testing and capsular serotyping were performed.

Results: The prevalence of pneumococci with decreased susceptibility to penicillin G (PDSP) was 8.1% and 17.3% respectively from clinical specimen and nasopharyngeal carriage. Antimicrobial resistance to erythromycin (9.8% vs 9.5%), tetracycline (59.8% vs 45.9%) and chloramphénicol (21.2% vs 17.6%) was much more in colonizing isolates than clinical isolates. Among the clinical isolates, the predominant serotypes were 1 (28.1%), 19 (21.9%) and 6 (12.5%). The prevalent serotype pneumococcal carriage were 19 (38.2%), 6 (13.5%) and 14 (10.1%).

Conclusion: these preliminary data should be taken into consideration in the therapeutic choices. Comprehensive serotype and antimicrobial susceptibility can aid in evaluating the impact of immunization program.
COMPREHENSIVE REVIEW CHARACTERIZING THE EPIDEMIOLOGY OF STREPTOCOCCUS PNEUMONIAE IN INDIA

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Background: In 2008 the National Technical Advisory Group on Immunization (NTAGI) of India recommended introduction of pneumococcal conjugate vaccine (PCV), yet PCV remains available only on the private market. To inform decisions for PCV introduction in India, we sought to identify and assess the availability and quality of pneumococcal data in India and neighboring countries.

Methods: Pneumococcal carriage and disease burden data from India, Pakistan, Bangladesh, Sri Lanka, and Nepal were identified from citations in existing reviews and a search of the literature published in January 2005-February 2011. Studies were screened then relevant data were double abstracted and assessed by quality criteria.

Results: We identified N=99 studies with relevant pneumococcal data, n=62 from India. Prevalence of NP colonization with pneumococci was common in infants (>80%) and school children (35-50%). Pneumococci accounted for 5-12% of pneumonia cases across studies; 12-30% of pneumonia cases with a confirmed etiology. Among bacterial meningitis cases, 27-39% were caused by pneumococci. A single study measuring invasive pneumococcal disease (IPD) incidence found a rate of 15 per 1000 child years. Many studies reported high levels of antibiotic resistance to cotrimoxazole in pneumococcal cases. PCV10/13 serotype coverage of IPD in children < 13 years was ~70%. Many Indian studies were older or were limited by reduced case detection due to low specimen collection rates, frequent antibiotic pretreatment, and use of blood culture methods.

Conclusion: The available data suggests pneumococcal disease epidemiology in India is similar to neighboring countries and currently available PCVs could have a significant impact.
HOSPITALIZATIONS ASSOCIATED WITH ALL-CAUSE PNEUMONIA USING NATIONAL HEALTH INSURANCE DATABASE. TAIWAN, 2001-2007

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Background: In 1995, the Taiwan national health insurance system was established. The availability of the population-based administrative hospital discharge database afforded the opportunity to investigate the burden of all-cause pneumonia hospitalizations for Taiwan.

Methods: Pneumonia discharge diagnoses were selected using International Classification of Diseases, 9th revision (ICD-9 CM). Population data were obtained from the Bureau of Statistics, Taiwan, 2001–2007. We estimated the national incidence rates (IR) of hospitalizations and case fatality rates (CFRs) for pneumonia hospitalizations.

Results: The number of hospitalizations associated with pneumonia increased from 186,577 in 2001 to 263,699 in 2005 and 246,767 in 2007. Overall, the national IR of pneumonia hospitalizations increased 29% over this 7-year period (8.3/1,000 to 10.75/1,000). By age group, the IR of pneumonia hospitalizations declined 17.6% in infants aged < 1 year and 23.8% in young adults aged 15-24 years. In contrast, IRs increased in children aged 1-4 years, 5-9 years and elderly aged ≥65 years 38.9%, 53.6%, and 29.3% respectively. CFRs for hospitalized persons with pneumonia ranged from 8.3% in 2001 (n=15,576) to 12.1% in 2007 (n=29,871). Despite the high incidence of pneumonia hospitalizations in 2005, the CFR (9.9%) was lower than that found in 2007.

Conclusions: IRs declined in infant and young adult age groups, while rates among older population increased. Given the large burden of pneumonia in Taiwan, there is a need to consider additional therapeutic and preventive measures for both children and adults.
KNOWLEDGE, ATTITUDES AND BELIEFS ABOUT PNEUMOCOCCAL AND INFLUENZA VACCINATION AMONG ELDERLY AGED 50 AND OLDER, INCHEON, KOREA

S.A. Kim, M.O. Favorov, Seoul, Republic of Korea

Background: Vaccines against pneumonia and influenza (P and I) are effective to prevent hospitalization and death but are underused among people of risk groups in the elderly.

Methods: To evaluate knowledge, attitudes and beliefs about vaccination, cross-sectional interviews were conducted at district senior welfare centers among individuals aged ≥ 50 years, Incheon City, Korea.

Results: A total of 1909 seniors were interviewed; mean age, 72.5±6.1 years, female (70.5%; n=1346) vs male (29.5%; n=563). Nearly all (95%; n=1817) were vaccinated against influenza, while only 8% (n=152) were vaccinated against P and I. Those who were more educated and younger replied with higher knowledge (p<0.001); Vaccines are safe, I know vaccines are recommended to people of my age. Reasons cited by those vaccinated; I heard about it from mass media (71.5%; n=1263), they prevent influenza as well as common cold (64.5%; n=1139), P and I are serious diseases (61.6%; n=1087), but for those who were non-vaccinated; I have no time for vaccination (100%), I am healthy enough (41.7%; n=61). Regarding willingness to get vaccination against pneumonia was 62% (n=1188) and 58% (n=896) would be willing to pay. Decision to get vaccination by themselves was made high in older age group. Gender (female; 1.6 times), age group and underlying disease (1.7 times) were associated with vaccination. Current available information sources were letters from government office, bulletins from the community, and mass media.

Conclusions: Results suggest that public health actions should focus on targeted advocacy activities to increase vaccination among the elderly.
PROSPECTIVE POPULATION-BASED SURVEILLANCE BURDEN OF STREPTOCOCCUS PNEUMONIAE (SP) IN COMMUNITY ACQUIRED PNEUMONIA (CAP) IN OLDER ADULTS, CHRZANÓW POLAND, 2010

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Background and aims: CAP and pneumococcal pneumonia (PP) are important causes of morbidity in older adults. This study addressed the lack of data on non-invasive SP CAP.

Methods: In 2010, the hospital and all clinics in Chrzanów county enrolled all consenting resident patients ≥50 years with clinical symptoms of CAP. Cultures, urine testing (Binax and 13-valent multiplex Urine Antigen Detection-UAD), and a chest x-ray were obtained. Incidence of all-cause CAP and serotype distribution of PP were calculated.

Results: In 2010, there were 2,734 adults enrolled from the population 50+ years of 46,134. Among the 598 with CAP by predefined study criteria, 586 (98%) had radiographically-confirmed CAP and 43.3% were hospitalized. Age distribution of patients with CAP was 29.1% 50-59, 9.0% 60-64, and 61.9% ≥65.

Incidence of CAP was 12.96/1000 persons. Incidence by age: 50-59 = 8.59/1000, 60-64 = 7.41/1000 and ≥65 = 19.90/1000. Highest incidence was in ages ≥80, 27.61/1000. S. pneumoniae was not isolated by blood culture in any patient. Forty-three met study definition for non-invasive PP with an incidence of 0.93/1000. Highest PP incidence was 2.99/1000 (age ≥80). Identified serotypes were 3 (7); 18C (6); 4, 6B, 23F (each 5); 9V (4); 14 (3); 6A, 19A, 19F (each 1), 5 unavailable.

Conclusions: CAP and PP cause substantial burden of vaccine preventable disease in older adults in Chrzanów, Poland. Given the health and social impact of PP, better prevention strategies are needed. Epidemiological data are critical for assessing the potential impact of national immunization programs.
INVASIVE PNEUMOCOCCAL DISEASE (IPD) AMONG CHILDREN FROM ROSARIO CITY (ARGENTINA) BEFORE INTRODUCTION OF PCV13 INTO NATIONAL IMMUNISATION PROGRAMME

S.M. López Papucci¹, A. Badano¹, A.M. Chiossone¹, A. Aletti¹, G. Ensinck¹, G. Agazzini¹, A. Ernst¹, S. Larini¹, M. Regueira², S. Fossatti², Rosario, “Buenos Aires, Argentina

Background and aims: Streptococcus pneumonia (SPN) is the most common cause of invasive bacterial disease, often associated with high morbidity, mortality and progressive antibiotic resistance. We describe the epidemiology of IPD in children from Rosario - Argentina before routine administration of PCV13 in infants beginning in January 2012.

Methods: Prospective observational study of hospitalized children with IPD carried out in Vilela Children’s Hospital from Rosario - Argentina, between January 1st, 1996 and August 31th, 2011. Strains isolated from blood and normally sterile fluids were studied for antibiotic susceptibility and serotyped by Quellung reaction.

Results: 513 children were hospitalized for IPD, representing 0,7% of all outcomes. 57,7% male, 53,6% < 2 years; 17,7% had underlying conditions. Case fatality rate was 4,5% and was not associated with discordant treatment.

Diagnoses were: Pneumonia 61% (1/3 of them with pleural effusion), 0,63% with intermediate resistance to penicillin, and progressive resistance to erythromycin in the last decade from 6,9% to 24,7%; the coverage of PCV13 was 97% in 110 strains analyzed; empyema increased 2,8-fold in the second half of the study period. Meningitis 12%, without high resistance to ceftriaxone, 75% covered by PCV13. Bacteremia/sepsis 16%, 88% covered by PCV13. Peritonitis 8%. Other 3%. Globally, PCV13 coverage was 92,1% and 89,6% in children younger than 6 years and 2 years of age, respectively.

Conclusions: Almost all the strains isolated from pneumonia are represented in the PCV13, resistance to macrolides but not to B-lactams is increasing. We expect a significant reduction in hospitalization for IPD in next years.
PURPURA FULMINANS AS A CLINICAL MANIFESTATION IN STREPTOCOCCUS PNEUMONIAE SEPSIS IN CHILDREN - CASE REPORT

L.M. Luminos, G. Jugulete, A. Draganescu, Bucharest, Romania

Background and aims: Purpura fulminans is an unusual finding in pneumococcal invasive disease. In our clinical experience, over 25 years in PICU, we have met only 6 cases with purpura fulminans associated with pneumococcal disease. Our goal is to remember to the pediatric physicians this event, unfortunately, life threatening.

Methods: We report a case of severe sepsis with Streptococcus pneumoniae with multi organ disfunctions and septic shock in a 2 years old child.

Results/case report: A 2 years old boy, (not vaccinated against pneumococcal infection) from an urban side of our county, was admitted in PICU, after 28 hours from the beginning of the disease for: fever (41 °C degree), coma Glasgow 5, multiple necrosis of fingers, both ears, nose, hands and legs. He presented with disseminated intravascular coagulation, metabolic acidosis, with septic shock and acute renal, pulmonary, cardiac arrest and meningitis. Blood culture and CSF culture were positive for Streptococcus pneumoniae. By PCR we identified serotype 1 A. Quickly he was put on mechanical ventilation, cardiac and renal support and metabolic resuscitation. Unfortunately, despite our efforts to save the life of the child, after 14 days of hospitalisation he died.

Conclusions: The first appearance of a child with purpura fulminans strongly suggest to be a severe meningococcal infection. We wanted to pull the alarm signal for our colleagues, just to have in mind this etiology, knowing the increasing resistance of streptococcus pneumoniae to betalactamic antibiotics. We also insist on antipneumococcal vaccination, the first weapon against the disease.
THE RELATIONSHIP BETWEEN ERYTHROMYCIN RESISTANT GENES AND SEROTYPE IN THE STREPTOCOCUS PNEUMONIAE ISOLATES CAUSING INVASIVE DISEASE IN CHINA

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Background and aims: The rate of erythromycin resistant was high in China but there were rare data about the isolates causing invasive disease. The objective of this paper was to investigate the relationship between erythromycin-resistant genes and serotypes in the streptococcus pneumoniae causing invasive diseases isolates among Chinese children.

Methods: Total 171 strains were isolated from 11 different medical centers through 2006 to 2008. All the strains were characterized by serotyping, antibiotics susceptibility, ermB and mefA genes detecting.

Results: There were 164 (95.9%) strains were resistant to erythromycin. All of them carried ermB gene and showed a high-level resistant (MIC>256µg/ml). Sixty-three(38.42%) strains carried ermB and mefA genes at the meantime, and most of these strains(93.5%) were typed to 19 serogroup (31 strains belong to 19A and 28 strains belong to 19F). The mefA and ermB genes carry rate in the north of China and south was similar. The PCV(pneumococcal conjugated vaccine)7 cover rate in the north of china was 40.38% and south of China was 67.23% (p< 0.05). The PCV10 cover rate in the north of China and south of China is 46.12% and 73.95% respectively(p< 0.05), PCV13 is 86.54% and 89.02% respectively(p>0.05).

Conclusion: The erythromycin resistant rate in China was very high and predominantly mediated by the ermB gene. The most common serogroup carried mefA gene was 19. The PCV7 and PCV10 cover rate in the south of China was higher than north, but there was no significantly different in PCV13.
**Poster No 97**

**ETIOLOGY AND ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIAL PATHOGENS CAUSING ACUTE OTITIS MEDIA (AOM) IN HIV-POSITIVE AND -NEGATIVE CHILDREN IN SOUTH AFRICA**

S. Madhi, K. Dayal, N. Govender, B. Mukherjee, N. van Niekerk, C. Cutland, M. Nunes, P. Adrian, Gauteng, Johannesburg, South Africa, Bangalore, India

**Background and aims:** No published data is available on the etiology of uncomplicated AOM in African children (including HIV+) using tympanocentesis. To evaluate the potential role of available pneumococcal conjugate vaccines (PCV) on AOM in South Africa, this prospective epidemiological study aimed to identify possible differences in bacterial etiology and antimicrobial susceptibility of AOM in children HIV+/HIV− and HIV-exposed and clinically asymptomatic for HIV/AIDS (HEU).

**Methods:** 253 children aged ≥3 months - < 5 years with 260 AOM episodes (onset of symptoms < 3 days) were enrolled; 15.4% (n=40/260) had received PCV. Middle Ear Fluid (MEF) samples were collected by tympanocentesis (94.2% of episodes [n=245/260]) or sampling spontaneous otorrhoea (5.8% of episodes [n=15/260]) for bacterial etiology and susceptibility testing.

**Results:** Overall, in 54.6% of episodes, MEF was positive for at least one bacterial pathogen; most frequent were *H. influenzae* (Hi; 30.8% of episodes) and *S. pneumoniae* (Spn; 20.4% of episodes). Most common Spn serotypes were 19F and 19A (17.9% and 12.8% of episodes, respectively); 98.3% of Hi episodes were non-typeable (NTHi). Co-infection with Hi/Spn was observed in 5.1% of samples. Proportion of Hi+ AOM episodes with (39.1%; 95% CI: 19.7 - 61.5%) or without PCV-history (35.4%; 95% CI: 27.6 - 43.8%) was comparable (p-value: 0.82). 1.4% of Spn+ samples were penicillin-resistant; 12.8% of Hi+ samples were ampicillin-resistant. Data by HIV-status is presented in Table 1.

**Conclusion:** Hi and Spn were the most common etiological agents of AOM in HIV+, HIV− and HIV-exposed children in South Africa. Vaccination strategies targeting both pathogens may significantly reduce AOM disease burden.

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**Table 1**

<table>
<thead>
<tr>
<th>Bacterial agents isolated*</th>
<th>HIV+ (N=103)</th>
<th>HIV− (N=102)*</th>
<th>HEU (N=103)*</th>
<th>Total (N=308)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H. influenzae</strong></td>
<td>48.7%</td>
<td>29.1%</td>
<td>31.7%</td>
<td>30.8%</td>
</tr>
<tr>
<td><strong>S. pneumoniae</strong></td>
<td>13.3%</td>
<td>20.3%</td>
<td>22.2%</td>
<td>20.4%</td>
</tr>
<tr>
<td><strong>S. aureus</strong></td>
<td>13.3%</td>
<td>15.4%</td>
<td>17.5%</td>
<td>15.8%</td>
</tr>
<tr>
<td><strong>A. otitidis</strong></td>
<td>13.3%</td>
<td>3.8%</td>
<td>6.3%</td>
<td>5.0%</td>
</tr>
<tr>
<td><strong>S. pyogenes</strong></td>
<td>6.7%</td>
<td>1.0%</td>
<td>0.0%</td>
<td>1.5%</td>
</tr>
</tbody>
</table>

**Notes:**
- *30 episodes in HIV and 2 episodes in HEU with no etiological agents isolated
- **Total** samples / total number of episodes

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**Figure:** Percentage Colonization Prior, During and After PCV in HIV+ and HIV- Children

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PROSPECTIVE SURVEILLANCE OF *STREPTOCOCCUS PNEUMONIAE* NASOPHARYNGEAL CARRIAGE AMONG INFANTS IN SOUTH INDIA AND ITS ASSOCIATION WITH UPPER RESPIRATORY TRACT INFECTIONS

A. Manoharan, R. Jalagandeeswaran, R. Vedantam, R. Isaac, Vellore, India

**Background and Aim:** *Streptococcus pneumoniae* (Spn) is a leading bacterial cause of upper respiratory tract infections (URI) in infants. The aim of the present study was to analyze the association between URI occurrence and nasopharyngeal (NP) colonization with Spn in Indian infants.

**Methods:** A cohort of 210 babies born over a period of 24 months (2009-2011) in the same district in rural South India were evaluated at birth and at monthly visits with ENT examination and nasopharyngeal swabbing to note the presence of URI and nasopharyngeal colonization with Spn.

**Results:** Overall among 210 neonates Spn colonization rate was 16.5%. The mean and median number of URI episodes in the birth cohort was 3.7 (S.D=2) and 4 respectively. There was a trend towards increasing age based incidence of URI with a maximum point prevalence of 72% at the 9th month. Resistance to co-trimoxazole was high (94.1%), penicillin resistance not observed. The calculated person year risk for having NP colonization in the first year of life was 11.49%. Three major risk factors for URI in the first year of life were NP colonization with Spn (p= 0.001), > 3 children aged < 5 years in the same household (p=0.016) and parental occupation (p=0.034).

**Conclusion:** NP colonization with Spn in the rural birth cohort was 16.5%. The incidence of URI among Indian infants shows a progressive increase from the first to the 9th month of life with NP colonization being the most important risk factor for URI among infants.
Poster No 99

SURVEILLANCE OF INVASIVE PNEUMOCOCCAL DISEASE (IPD) IN THE DEPARTMENT OF PEDIATRICS OF THE HOSPITAL ITALIANO DE BUENOS AIRES

J. Marco del Pont, A. De Cristófano, L. Verdier, C. Faragó, Capital Federal, Argentina

Objective: To follow up and monitoring the IPD in the pediatric patients of a community hospital.

Material and methods: Descriptive and observational study. We included all the patients, admitted at the Hospital Italiano de Buenos Aires with a confirmed diagnosis of IPD, between 1 month and 20 years of age, from 2000 to 2011. The data were processed using EpiInfo version 3.5.3.

Results: We included 117 patients. The median of age was 26 months (11-72). 59% were male. 49.6% were < 2 years. The clinical presentations: lower respiratory tract infection 61.5%, sepsis 16.2%, meningitis 10.2%, peritonitis 5.1% and others 12%. Meningitis was more common in < 2 years (17.2% vs. 1.7%). The 70.9% had bacteremia, this rates were similar across age groups. The 48% had underlying disease. Six patients died (5.1%) (CI 1.9-10.8%) of these 5 have an underlying disease. The deceased had: 2 meningitis, 2 sepsis, respiratory focus and skin and soft tissue 1 each one. There was no difference in mortality in the < or > 2 years. Resistance to penicillin in 2000 decreased 71.5% to 7.1% in 2010-11. Only one isolate was resistant to ceftriaxone (0.9%). The annual rate of infection according to 1000 hospital discharges was 1.95 in 2000 and 2.2 in 2011.

Conclusion: The rate of hospitalization remained stable in the last years, the median age was 26 months. The resistance to penicillin decreased. Penicillin resistance was not a risk factor in evolution. There was no difference in mortality in the < or > 2 years.
MULTILOCUS SEQUENCE TYPING OF S.PNEUMONIAE STRAINS GAINED IN AGED PATIENTS IN FAR EAST OF RUSSIA

A. Martynova, L. Balabanova, A. Sheparyov, Vladivostok, Russia

Despite of the gained results in diagnostic, *Streptococcus pneumoniae* remains the reason of lower respiratory tract morbidity in aged patients of our region. Unfortunately, epidemiology of pneumococcal isolates is not well-documented and molecular sequence typing (MLST) could be valuable instrument to define epidemiologically significant isolates. As there were no any attempts of multilocus sequence typing in our region, we conducted this method in strains of *S.pneumoniae*, isolated in aged patients.

Aim of our project was to perform MLST in a group of aged patients with pneumococcal community-acquired pneumoniae (50 patients) and in carriers of *S.pneumoniae* of the same age (30 patients).

**Materials and methods:** MLST was conducted with housekeeping genes on standard method on recommendations of MC Enright (1998) et al. with previous study of antimicrobial agents resistance and serotyping.

**Results:** Isolates from patients (50 strains) included 24 serotypes (26 of non-typable) and 22 sequence types (ST) according to MLST of which 7 were novel. 8 were of Taiwanese clones (TW-28,19,26) of 19F serotype, 3 were of R6, EU38, SP95. The most prevalent serotype of 6B included 3 newly identified STs. In 30 strains from carriers 17 sequence types, and here are prevailing the strains with similarity to TW50-9V, TW82-14. In strains from patients there were isolated 18% resistant to erythromycin, 16% tetracycline, 4% chloramphenicol. In strains from carriers there were isolated 16,6% resistant to erythromycin, 10% to tetracycline.

**Conclusion:** MLST allows us to define epidemiology of *S.pneumoniae* isolates and to plan preventive measures for aged patients.
SECULAR TRENDS IN SEROTYPES CAUSING INVASIVE PNEUMOCOCCAL DISEASE IN KILIFI, KENYA, 1994-2010

J.C. Moïsi1,2, A. Karani1, V. Kosi1, P. Lewa1, S. Mwarumba1, S. Njenga1, J. Nyiro1, E. Wanjiru1, E. Bauni1, S.C. Morpeth1, J.A.G. Scott1, Kilifi, Kenya, “Paris, France

Background: Serotypes causing invasive pneumococcal disease (IPD) vary by age, syndrome, geographic location and calendar time. We aimed to document long-term trends in IPD-causing serotypes prior to the introduction of pneumococcal conjugate vaccine (PCV) in Kilifi, Kenya.

Methods: From 1994-2010 we conducted continuous surveillance for IPD among children < 15 years of age admitted to Kilifi District Hospital. Blood cultures were performed systematically from 1998 onwards. CSF and pleural fluid were obtained for culture as clinically indicated. Pneumococci were serotyped using the Quellung reaction. The Kilifi Health and Demographic Surveillance Study provided denominator data for incidence calculations.

Results: Among 1029 IPD isolates, serotypes 1 (27%), 14 (12%), 5 and 6B (each 8%), and 6A and 23F (each 7%) were most common overall and in blood (N=963). The rank order of serotypes varied slightly for CSF isolates (N=244) with serotypes 18C and 6B ranking 6th and 7th, respectively. Outbreaks occurred in 2003-4 and 2010 for serotype 1 and in 2003 for serotype 5, coinciding with peak-incidence years. PCV7 serotype coverage was >50% in children 2-23 months of age only whereas PCV10 serotype coverage reached >70% in all age groups.

Conclusion: Long-term IPD surveillance is needed to monitor secular changes in disease-causing serotypes. Pre-vaccine data from Kilifi will allow us to monitor the impact of PCV10 and assess serotype replacement.
THE DETECTION OF STREPTOCOCCUS PNEUMONIA IN NASOPHARYNGEAL CARRIAGE OF CHILDREN

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To define the nasopharyngeal carriage rates, serotype distribution and antimicrobial resistance patterns of Streptococcus pneumonia in healthy children less than 10-year-old in Tehran in order to estimate the prevalence of pathogens that commonly cause infection in Iranian young children.

Methods: This cross-sectional study was performed for three months, (November 2008 to January 2009). Twenty Day care centers and 50 school children under 10 years old were chosen randomly. Nasopharyngeal specimens were collected by a trained investigator.

Results: The carrier rate for Streptococcus pneumoniae was 44.1%. Twenty three isolates, (69.4%), were resistant to one or more class of antimicrobial agents. 38.56% of the isolates belonged to strains covered by the heptavalent pneumococcal vaccine.

Conclusion: This study revealed data about the rate of nasopharyngeal carriage and the serotype distribution of Streptococcus pneumoniae strains in Tehranian children. Our findings have implications on the type and efficacy of pneumococcal conjugate vaccines that should be used for prevention of pneumococcal invasive disease in the Iranian population.
STREPTOCOCCUS PNEUMONIAE AND NON-TYPEABLE HAEMOPHILUS INFLUENZAE: BACTERIAL PATHOGENS CAUSING ACUTE OTITIS MEDIA IN CHILEAN CHILDREN AGED < 5 YEARS

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Background and aims: Acute otitis media (AOM) is a common cause of childhood morbidity and antibiotic use globally. Although bacterial conjugate vaccines have proven to be efficacious against AOM, their impact depends upon etiology and vaccine coverage. Since etiology data from Latin America are limited, this study describes the bacteria and serotypes involved in AOM episodes.

Methods: Children aged 3months-5years with new episodes of AOM (onset of symptoms < 3days) were enrolled (September 2009-2010) following diagnosis and confirmation of suspected cases by ENT specialist upon pediatrician’s referral. Middle-ear-fluid (MEF) samples were collected by tympanocentesis or from spontaneous otorrhea following informed consent from parents/guardians. Bacteria cultured were identified and serotyped.

Results: 164 MEF samples were obtained (tympanocentesis [n=146]; otorrhea [n=18]). Mean-age of children was 27.1months (range: 4-59months); 56.1% were males. Bacteria were cultured from 70.1% (115/164) of samples with at least one pathogen (S.pneumoniae, H.influenzae, M.catarrhalis and S.pyogenes). S.pneumoniae (35.4% [58/164], -serotypes presented in table 1-) and Non-typeable H.influenzae (NTHi) (34.1% [56/164]) were the predominant pathogens among all the isolated samples, 2/164 were positive for both, and 1.8% [3/164] was positive either for M.catarrhalis or S.pyogenes.

Conclusions: S.pneumoniae and NTHi are the leading bacterial causes of AOM episodes in children aged 3months-5years in Santiago, Chile. A broader spectrum vaccine against both pathogens would be the most useful approach to prevent AOM.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Total (n=164)</th>
</tr>
</thead>
<tbody>
<tr>
<td>x2</td>
<td>13</td>
</tr>
<tr>
<td>x3</td>
<td>16</td>
</tr>
<tr>
<td>x5</td>
<td>5</td>
</tr>
<tr>
<td>x6</td>
<td>5</td>
</tr>
<tr>
<td>x10</td>
<td>4</td>
</tr>
<tr>
<td>6B</td>
<td>3</td>
</tr>
<tr>
<td>6A</td>
<td>2</td>
</tr>
<tr>
<td>23F</td>
<td>3</td>
</tr>
<tr>
<td>6C</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>19F</td>
<td>1</td>
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</tr>
<tr>
<td>19F</td>
<td>1</td>
</tr>
<tr>
<td>11A</td>
<td>1</td>
</tr>
</tbody>
</table>

S. pneumoniae was isolated in 158 samples, M. catarrhalis in 44, H. influenzae in 56, S. pyogenes in 33.

[Pneumococcal serotype distribution]
Background: Meningitis is one of the top ten causes of death amongst children under 5 years in Zimbabwe. Paediatric bacterial meningitis (PBM) sentinel surveillance started at Harare Central Childrens Hospital in 2002. In 2008, there was a breakdown of health services at the sentinel site due to frequent industrial actions by health workers and there has been loss of experienced staff to other countries due to poor remuneration.

Methodology: The sentinel site PBM focal team attended training in the Gambia in April 2011. A WHO PBM surveillance meeting was also held in Harare Zimbabwe in July 2010. Since then, there was improved supply of laboratory reagents, production of standard operating procedures and frequent meetings of involved personnel. Training of health personnel was also done including the production of a training manual. In September 2011, 35 culture negative samples were sent to the National Institute of Communicable Diseases (NICD) in South Africa for PCR confirmation.

Results: PBM surveillance has gradually become part of the health worker's routine clinical work. Out of the 35 samples 1 (3 %) had Neisseria meningitidis serogroup W135, 7 (20 %) were PCR positive for Streptococcus pneumonia. Of the S. pneumonia positive, 2 were serotype 14, one serotype 6 and 3 were negative for the 33 serotypes.

Conclusion: Continued support from WHO and the Ministry of Health together with dedicated staff will ensure progress in the surveillance system. The predominant serogroup of S. pneumonia needs to be identified for the purposes of appropriate vaccine implementation.
THE BURDEN OF COMMUNITY-ACQUIRED PNEUMONIA AMONG CHILDREN AGED UNDER 5 YEARS IN SALVADOR, BRAZIL BEFORE USE OF PNEUMOCOCCAL CONJUGATE VACCINE

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Background and aims: Despite the fact that community-acquired pneumonia (CAP) has been a big problem to childhood health, information on CAP incidence has been scarce, mainly in developing countries. Our aim was to estimate the CAP incidence in children aged < 5 years.

Methods: This was a prospective, population-based cohort study conducted in the urban zone of Salvador, Northeast Brazil. Every healthy child aged < 4 years living in the study area was invited to participate in the study. Upon enrollment, information on child's health and life style was obtained. Each child was followed up by home visit or telephone call, weekly during one year. Whenever a child presented cough and respiratory distress, a thorough investigation was performed by a pediatrician, a chest x-ray was taken and read by 2 radiologists blinded to clinical information.

Results: A total of 1,518 children were studied. At recruitment, the median age (months) was 20 months (mean: 21±14); 766 (50.5%) were males. The incidence of CAP was 51/1000 child-year (95%CI: 41-64) (clinical diagnosis), 29/1000 child-year (95%CI: 21-39) (presence of tachypnea according to the WHO) and 20/1000 child-year (95%CI 14-29) (presence of pulmonary infiltrate on chest x-ray agreed by the 2 radiologists).

Conclusions: This study provides information on the CAP burden before the universal introduction of the PCV-10 vaccine when maximal burden of CAP among children under 5 was 6.4% yearly.

Acknowledgments: This study was supported by the Brazilian Council for Science and Technology Development (CNPq) and the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB), Brazil.
**Poster No 106**

**THE DISTRIBUTION OF INVASIVE AND NONINVASIVE PNEUMOCOCCAL INFECTION MONTHLY**

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**Background and aims:** *Streptococcus pneumoniae* is one of the leading etiologic agents of invasive and noninvasive disease. Nonetheless, evidence on pneumococcal infection seasonality is scarce. We aimed to describe the relative frequency of pneumococcal infections per month and compare it.

**Methods:** This investigation included a prospective, laboratory-based surveillance study conducted in Salvador, Brazil. Strains were identified as pneumococcus by the bile solubility and optochin disc tests, 1998-2010. A prospective pneumonia study carried out from 2003 to 2005 was also included. Bacterial infection caused by pneumococcus was investigated by antibody assays to C-polysaccharide and pneumolysin in paired serum. Enzyme immunoassay was performed and a two-fold increase was considered diagnostic. Nonparametric sequence test was used to compare the distribution within each subgroup.

**Results:** A total of 290 cases with invasive disease were detected out of which mean and median age were 17±25ys and 3.7ys, respectively (min 1month, max 97ys) and 171(59%) were males. Pneumonia serologically diagnosed as pneumococcal infection included 39 patients among whom mean and median age were 1.9±1.2ys and 1.8ys, respectively (min 2months, max 4.5ys) and 23(59%) were males. Figure 1 presents the monthly distribution of the cases. The invasive and noninvasive subgroup presented non-random (P=0.02) and random (P=0.2) distribution.

**Fig 1**

**Conclusions:** Invasive pneumococcal infection occurs differently among the months of the year.
LABORATORY-BASED SURVEILLANCE OF STREPTOCOCCUS PNEUMONIAE INVASIVE DISEASE (IPD) IN CHILDREN: SEROTYPE DISTRIBUTION AND ESTIMATION OF VACCINES COVERAGE

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Introduction: Streptococcus pneumoniae is the leading cause of respiratory infections and bacterial meningitis after the neonatal period. PIDs are preventable through vaccination. Available vaccines contain capsular polysaccharides of bacterial cell wall. Currently there are pneumococcal vaccines consisting of 23, 7, 10 and 13 serotypes.

There is variation in the prevalence of pneumococcal serotypes depending on the geographic location, and the time period studied in the same geographical point. There is also variation depending on the clinical form and age.

Objective: To estimate the proportion of pneumococcal serotypes coverage by conjugate vaccines for the prevention of PIDs among children according to age and clinical forms to assess the likely impact of their use in our region.

Methods: Between 1998 and 2005 133 pneumococcal isolates obtained from 164 children < 5 years of age who had PID were serotyped. A total of 103 (77,4%) children had pneumonia, 27 (20,3%) had meningitis and 3 (2,3%) had other forms of disease. The proportion of the 7, 10 and 13-valent conjugate vaccines coverage were determined in young and older children.

Results: The most commonly observed serotypes were: 14 (36.8%); 1 (13.5%); 6A (8.2%); 19A (5.3%); 9V (4.5%) e 23F (4.5%).

Figure 1 shows the distribution of serotypes constituents of conjugated vaccine with 7, 10 and 13 serotype, by age and clinical form.

![Figure1. Distribution of serotype constituents of conjugated vaccine with 7, 10 and 13 serotypes, by age and clinical form.](image)
Streptococcus pneumoniae remains a major cause of childhood morbidity and mortality. Nasopharyngeal colonization serves as the key reservoir for pneumococcal disease. We evaluated pneumococcal carriage among children aged < 6 years in Niterói/RJ, Brazil, during March-June 2010. Nasopharyngeal swabs collected from 242 children (102 from a day-care center and 140 from a hospital) yielded 129 pneumococcal isolates (colonization rate of 49.6%). The carriage rate was higher among children at the day-care center (57.8% vs 42.1%). The most prevalent serotypes determined by Quellung tests and/or multiplex PCR were 6B, 19F, 6A, 14 and 15C which accounted for 56.6% of the isolates. All isolates were susceptible to clindamycin, rifampicin and vancomycin. The highest rate of resistance was observed for trimethoprim/sulfamethoxazole (37.2%). Non-susceptibility to penicillin (Pen-NS) was detected in 26.4% of the isolates (MICs 0.12-4.0 µg/mL), and was strongly associated with serotypes 14 and 23F. Multilocus sequence typing revealed 50 different STs (19 new STs). Four international clones were identified, 3 of them associated with Pen-NS (ST116 serotype 14 variant of Spain9V-3; ST338 Colombia23F-26 and ST177 Portugal19F-21). Three isolates were constitutively erythromycin-resistant (MICs 4-8 µg/mL) and mef(A/E)-positive. Although the estimated coverage of carriage serotypes by the 10-valent vaccine, available in Brazil since 2010, was only 43.4%, the spectrum of targeted serotypes accounts for the majority of the Pen-NS isolates detected in the present study.
PROSPECTIVE, ACTIVE HOSPITAL-BASED EPIDEMIOLOGIC SURVEILLANCE FOR IPD AND PNEUMONIA BURDEN AMONG INFANTS AND CHILDREN IN BANGALORE SOUTH ZONE, INDIA

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Background: Streptococcus pneumoniae (SP) infections are a leading cause of childhood morbidity and mortality. Data from India are scarce. We studied IPD, pneumonia, SP serotypes and antibiotic resistance in children in Bangalore South Zone.

Methods: Prospective, hospital-based surveillance conducted 27/2/2009-26/2/2011. Eligible children resided in surveillance area and were either 28d to <36m+temperature/temperature history ≥39.0°C (within 24h) or clinical suspicion of IPD, or >36m to < 60m+suspected IPD. Blood culture required from all enrolled children, CSF culture in suspected meningitis, CXRs in suspected pneumonia. IPD confirmed by SP identification from sterile site. At-risk population calculated referencing National Polio Surveillance Project data.

Results:
-9,950 subjects/112,483 calculated at-risk population enrolled
-IPD and Pneumonia Incidence:
-40 confirmed IPD cases
-Overall, estimated IPD incidence=17.78/100,000 (28d-60m)
-Highest IPD incidence=49.85/100,000 (6m to < 12m)
-Clinical pneumonia incidence: 5,032.98/100,000 (28d to < 6m); 3,780.67 /100,000 (6m to < 12m)
-CXR-confirmed pneumonia incidence: 1,113.50/100,000 (28d-60m)
-IPD cases, combined serotype distribution, antibiotic resistant cases (Table)
-PCV13 serotype coverage=91.7%

<table>
<thead>
<tr>
<th>IPD cases (N=40): Combined Serotype Distribution and Antibiotic Resistant Cases (N=36)</th>
<th>Antibiotic Resistant Isolates</th>
<th>Antibiotic Resistant Isolates</th>
<th>Antibiotic Resistant Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotype (N_cases)</td>
<td>Serotype (N_cases)</td>
<td>Serotype (N_cases)</td>
<td>Serotype (N_cases)</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>1 (3)</td>
<td>1 TMP-Sulfa</td>
<td>1 Erythromycin</td>
<td>1 Erythromycin/TMP-Sulfa</td>
</tr>
<tr>
<td>3 (1)</td>
<td>1 TMP-Sulfa</td>
<td>19F (1)</td>
<td></td>
</tr>
<tr>
<td>4 (1)</td>
<td>1 TMP-Sulfa</td>
<td>10C (1)</td>
<td>1TMP-Sulfa</td>
</tr>
<tr>
<td>5 (4)</td>
<td>14 (5)</td>
<td>1 Erythromycin</td>
<td>19F (1)</td>
</tr>
</tbody>
</table>
**Conclusions**: Study results provide insight to IPD and pneumonia incidences, SP serotypes and antibiotic resistance distribution. Active surveillance in India should continue.
HEALTH SURVEY OF EPIDEMIOLOGY OF NASOPHARYNGEAL S PNEUMONIAE COLONIZATION IN RURAL SOUTH AFRICAN COMMUNITY WITH A HIGH PREVALENCE OF HIV-INFECTION

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Background and aims: We undertook a baseline survey of Streptococcus pneumoniae nasopharyngeal (NP) colonisation in a rural, African community with a high HIV prevalence in preparation for evaluating the effect of childhood 7-valent pneumococcal conjugate vaccine (PCV-7) immunization on the ecology of NP colonisation.

Methods: NP swabs were undertaken in all consenting members within households from May to September 2009. Standardized microbiology methods for the detection of S. pneumoniae were used and serotyping was done by Quellung method. PCV-7 was introduced into the infant immunisation programme in April 2009 at a 6, 14 and 40 week schedule, without any additional catch-up.

Results: 567 households were sampled of which, 513 had at least one person > 12 years (n=905) and one child ≤ 12 years (n=888). The prevalence of NP S. pneumoniae colonisation is detailed below.

<table>
<thead>
<tr>
<th>Age group</th>
<th>n per age group</th>
<th>Overall pneumococcal colonization (%)</th>
<th>Vaccine serotype (VT) colonization</th>
<th>Non-vaccine serotype (NVT) colonization</th>
<th>Non typeable (NT) colonization</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2 years</td>
<td>357 (19.9)</td>
<td>299 (83.75)</td>
<td>143 (40.05)</td>
<td>149 (41.70)</td>
<td>7 (1.96)</td>
</tr>
<tr>
<td>2- 5 years</td>
<td>241 (13.4)</td>
<td>194 (80.50)</td>
<td>81 (37.85)</td>
<td>110 (45.64)</td>
<td>3 (1.24)</td>
</tr>
<tr>
<td>5- 12 years</td>
<td>269 (15.0)</td>
<td>163 (60.59)</td>
<td>55 (29.44)</td>
<td>106 (39.40)</td>
<td>2 (0.74)</td>
</tr>
<tr>
<td>12- 18 years</td>
<td>145 (8.1)</td>
<td>35 (24.14)</td>
<td>8 (5.51)</td>
<td>25 (17.24)</td>
<td>2 (1.37)</td>
</tr>
<tr>
<td>18- 45 years</td>
<td>562 (31.3)</td>
<td>63 (11.21)</td>
<td>14 (2.49)</td>
<td>42 (7.47)</td>
<td>7 (1.24)</td>
</tr>
<tr>
<td>&gt; 45 years</td>
<td>219 (12.3)</td>
<td>11 (5.02)</td>
<td>4 (1.82)</td>
<td>6 (2.73)</td>
<td>1 (0.05)</td>
</tr>
</tbody>
</table>

Conclusions: This study identified a high prevalence of NP pneumococcal colonization up to 12 years of age. The implications of a high VT colonization in children older than those targeted for PCV-7 immunization on interrupting the transmission of VT, needs to be explored.
INVASIVE PNEUMOCOCCAL DISEASES AMONG HOSPITALIZED ADULT PATIENTS IN LIMA, PERU

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Background and aims: There is no data on the serotype distribution of invasive pneumococcal disease (IPD) strains in adult patients in Peru. The aim of this study was to determine the serotype distribution and antimicrobial resistance of IPD strains in adult patients during the initial period of PCV7 introduction in Lima (2009).

Methods: We conducted a passive surveillance study of IPD in patients (>18 yrs) from 13 public and private hospitals in Lima (2009-2011). All strains were serotyped by a multiplex sequential PCR and by Quellung. We determined the antibiotic resistance rates to penicillin, ceftriaxone and erythromycin by MIC.

Results: 43 IPD strains were isolated: 56% in patients >60yrs. The main diagnoses were pneumonia in 17(40%) and meningitis in 13(30%). We performed the serotyping in 40 strains; the most common were: 19F (15%), 23F (13%), 14 (8%), 6ABCD (8%) and 15BC (8%). We still need to complete the serotype by Quellung in some strains. The distribution of vaccine serotypes was 43% for PCV7, 43% for PCV10, 48% for PCV13 and 55% for PPV23. Resistance to penicillin among meningal strains was 27% and among non-meningal strains 0% (4% intermediate resistance); resistance to erythromycin was 36%. The case fatality rate for patients >60yrs was 33%(8/24).

Conclusions: To our knowledge it is the first study to describe the serotypes among adult patients in Peru. Since new vaccines for the elderly are currently under evaluation, it is important to continue with this type of surveillance in order to measure the impact of future vaccination programs.
COMPARISON OF NASOPHARYNGEAL BACTERIAL ISOLATES FROM MOTHER-INFANT PAIRS DURING THE FIRST 12 WEEKS AFTER BIRTH IN THE GAMBIA

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¹Banjul, The Gambia, ²London, UK

Background and aims: The acquisition rate and sources of carriage of Streptococcus pneumoniae (SP) following birth is not clearly understood. This study aimed at understanding the time of SP acquisition and its relationship to carriage in the mother.

Methods: Thirty-six mother-infant pairs had nasopharyngeal swabs collected at birth and 2-weekly for three months. Standard culture techniques were used to isolate Staphylococcus aureus, SP and for SP serotyping.

Results: At delivery, none of the mother or babies carried SP, but 19 mothers (52.8%) and 4 babies (11.1%) carried S. aureus. Carriage rates of SP increased to 50% at 8-10 weeks of age, while that of mothers ranged from 17 to 22.9%. The maximum carriage rate of S. aureus for babies was 58.9% at 2 weeks while the 52.8% at birth was the highest for the mothers. The 4 leading SP serotypes in the babies were 6A, 19F, non-typeable and 4, while 6A, 20, non-typeable and 11A were the most frequently identified serotypes in mothers. 80.6% of the infants had a discordant SP serotype with their mothers. 50% of the infants carried both SP and S. aureus at one time point.

Conclusions: Bacterial colonisation of the nasopharynx among Gambian infants occurs early and the serotype distribution changes with age. Infants carried serotypes different from their mothers for most of the time. This could imply acquisition of SP through other sources or that immunity and competition between the serotypes and other bacteria may play a role in the differences in carriage observed.
**EPIDEMIOLOGY OF INVASIVE PNEUMOCOCCAL DISEASE IN CHILDREN UNDER 15 YEARS IN PARAGUAY**

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**Introduction:** Invasive pneumococcal disease (IPD) is a common cause of morbidity and mortality in children. The prevalence of serotypes varies geographically and by age.

**Objective:** Knowing the epidemiological and microbiological of IPD in children under 15 years of the Hospital Central IPS.

**Material and methods:** Retrospective study from January 2006 to December 2010 with a review of microbiology records and files of patients with isolation of S. pneumoniae from sterile sites.

**Results:** Of 148 isolates 64 were younger than 15 years, average age 38 months, 55% younger than 2 years, and 61% male. Risk factors identified in 33%. The sites of isolation were 58% blood culture, 23% pleural fluid and 7% in cerebrospinal. Diagnoses of pneumonia were 45, complex 26, and 5 meningitis, with neurological sequelae in 4. It reported a fatal case in a premature infant with sepsis. 40 strains were typed being the prevalent serotypes 14, 1, 5, 6B and 23F. Were penicillin resistant isolates 2/5 and 18/59 meningeal and non-meningeal.

**Conclusions:** The IPD most often affects children under 2 years with male predominance without comorbidities. Pneumonia was the most frequent clinical form, dominating the complex. The neurological disorder was less common but severe sequelae. The prevalents serotypes were 14, 1, 5, 6B and 23F.
Poster No 114

SEROTYPE DISTRIBUTION OF STREPTOCOCCUS PNEUMONIAE FROM NASOPHARYNGEAL SAMPLES BEFORE AND AFTER IMPLEMENTATION THE HEPTAVALENT PNEUMOCOCCAL CONJUGATE VACCINE IN BOGOTA, COLOMBIA

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Background and aims: The introduction of the seven-valent conjugated pneumococcal polysaccharide vaccine (PCV7) could alter the Streptococcus pneumoniae epidemiology. The introduction of PCV7 into the routine infant immunization schedule for children less than two years old started in Bogotá Colombia since 2009. The aim of this study was to determine the rate of nasopharyngeal carriage and serotype distribution of S. pneumoniae before and after implementation vaccination.

Methods: Nasopharyngeal samples from 246 and 146 were collected from children (12 to 18 months of ages) during 2007 and 2011 years before and after massive PCV7 implementation, respectively. S. pneumoniae was identified by multiplex PCR using primers to the genes lytA, 16S rRNA and cpsA. The serotype was determinated by both multiplex PCR and Quellung reaction.

Results: The rate of carriage before and after PCV7 implementation was 55.69% (137/246) and 39.72% (58/146), respectively. The PCV7-serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) decreased from 41.26% to 19%. The frequency of non-PCV7 serotypes varied to both periods. Before-PCV7 the most frequent serotypes were 6A/C (14.7%), 15B (3.5%), 9N (3.5%), 11A (2.8%), 23A (2.8%), 34 (2.1%), 10A (2.1%) and 19A (1.4%) and after-vaccine were 11A (11.7%), 23A (8.3%), 19A (8.3%), 6A (6.7%), 15B (6.7%), 15C (6.7%), 35B (5%), and 13 (3.3%).

Conclusions: The rates of nasopharyngeal carriage presented important differences. The serotype distribution highlights the importance of assessing the effects of pneumococcal vaccines. Understanding the effect of PCV7 on carriage is important to interpreting and predicting the impact of PCV7 on disease.
Poster No 115

COMPARATIVE ANALYSIS OF STREPTOCOCCUS PNEUMONIAE TRANSMISSION IN PORTUGUESE AND FINNISH DAY-CARE CENTERS

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Background: Day care center (DCC) attendees play a central role in maintaining the circulation of Streptococcus pneumoniae (pneumococcus) in the population. The prevalence of pneumococcal carriage is highest in DCC attendees but varies across countries and is found to be consistently lower in Finland than in Portugal. We compared key parameters underlying pneumococcal transmission in DCCs to understand which of these contributed to the observed differences in carriage prevalence.

Methods: Longitudinal data about serotype-specific carriage in DCC attendees in Portugal (N=47 in 3 groups; mean age 2 years) and Finland (N=91 in 7 groups; mean age 4 years) were analysed with a continuous-time event-history model in a Bayesian framework. The degree of between-serotype competition and the monthly rates of clearing carriage, within-group transmission and community acquisition were estimated.

Results: The posterior mean of within-group transmission rate was 1.07 (Portugal) vs. 0.64 (Finland). The smaller rate of clearance in Portugal (0.56 vs. 0.73) is in accordance with the children being younger. The overall community rate of acquisition was larger in Portugal (0.26 vs. 0.12), in agreement with that the groups in Portugal belonged to a larger DCC. Current carriage was found to interfere with colonization, with the same degree of between-serotype competition in both data sets. The model correctly predicted the observed levels of carriage prevalence.

Conclusions: The difference in prevalence of carriage (61% vs. 26%) could be explained by the longer duration of carriage in younger attendees and significantly higher rates of within-group transmission and community acquisition in Portugal.
Poster No 116

CHARACTERISTICS OF OPTOCHIN-RESISTANT STREPTOCOCCUS PNEUMONIAE ISOLATED IN BRAZIL FROM DIFFERENT SETTINGS AND LOCATIONS

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Optochin (Opt) susceptibility is used in diagnostic laboratories for the presumptive identification of Streptococcus pneumoniae (Sp). Opt-resistant (Opt-R) isolates have been reported, however, indicating the potential for misidentification of this important pathogen. Information on the occurrence and diversity of Opt-R variants is still limited. Fourteen Opt-R Sp isolates recovered from carriers or patients in Brazil, including 10 from the Southeast region (Rio de Janeiro and Niterói cities) and 4 from the South region (Porto Alegre city), were characterized by phenotypic (Opt susceptibility, bile solubility, serotyping, antimicrobial susceptibility, determination of Opt MICs) and genetic tests (amplification of the ply, lytA and psaA genes, PFGE profile, sequencing of the atpC gene). Nine isolates consisted of heterogeneous populations of both Opt-R and Opt-susceptible subpopulations, and five were homogeneously Opt-R. They belonged to various serotypes, including vaccine types 6A, 6B, 14 and 23F, and had different susceptibility and genetic profiles. Except for Opt MICs (4 to 64-fold higher among Opt-R variants), Opt-R and Opt-S subpopulations originated from the same culture had identical phenotypic and genetic characteristics. Single substitutions in the atpC sequence were observed only for the Opt-R isolates. Eight of them presented a modification in codon 49, suggesting that this might be a hot spot, while six had substitutions in codons 13, 20, 23, 31 or 45. Opt resistance was detected among Sp isolates from diverse Brazilian locations and clinical settings, was not related to particular serotypes or clonal groups, and was strongly associated with modifications in the atpC gene.
DIFFERENTIAL CLONALITY OF NON-PCV7 SEROTYPES CAUSING OTITIS AND INVASIVE PNEUMOCOCCAL DISEASE AMONG 2 DISTINCT PEDIATRIC ETHNIC POPULATIONS IN SOUTHERN ISRAEL

N. Porat, N. Givon-Lavi, R. Benisty, R. Dagan, Beer Sheva, Israel

**Background and aims:** The relative importance of serotype vs. genotype in determining the potential of pneumococci to cause infection is still debated. The aim of this study was to compare the clonal distribution of non-PCV7 serotypes (non-7VST), isolated from acute otitis media (AOM) and invasive pneumococcal disease (IPD), between 2 distinct ethnic populations, in southern Israel, during the decade (1999-2008) preceding PCV7 implementation.

**Methods:** Pneumococcal AOM and IPD cultures were recovered from Jewish and Bedouin children < 5 years old, which differ in their lifestyle and socioeconomic background. These populations live separately. Molecular typing by pulsed field gel electrophoresis (PFGE) were performed on all strains, and MLST on selected strains from each PFGE cluster.

**Results:** Of 5230 AOM isolates, 2265 (43%) and 2965 (57%) were from Jewish and Bedouin children, respectively (non-7VST, 46%). Of 408 IPD isolates, 133 (33%) and 275 (67%) were from Jewish and Bedouin children, respectively (non-7VST, 54%). 11 serotypes (1,3,5,6A,7F,12F,15B/C,19A,21,33F,35B) constituted 67% of the AOM non-7VST and 66% of the IPD non-7VST. These serotypes constituted 28 AOM clones (≥10 isolates/clone), of which 17 clones were significantly more frequent in the Bedouin children, 10 among Jewish children; only one clone was evenly distributed. The distribution of 6/7 major IPD clones (≥5 isolates/clone) among Bedouin vs. Jewish children resembled that of AOM clones.

**Conclusions:** The significant differences in clonal distribution between the 2 ethnic populations, living in the same area, suggests that lifestyle and microenvironment are more important than geographical location in clonal distribution of disease-causing pneumococcal clones.
Poster No 118

PNEUMOCOCCAL INFECTIONS IN A TERTIARY CARE SETTING IN SRI LANKA; WHERE DO WE STAND?
S. Weerasinghe, M. Dassanayake, K. Karunaratna, T. Thalgaspitiya, R. Premaratna, Ragama, Sri Lanka

Background: Surveillance of invasive pneumococcal diseases in Sri Lanka was commenced as part of the South Asian Pneumococcal Network Alliance (SAPNA) in 2004. This study compares the pneumococcal disease incidence figures of Colombo North Teaching Hospital, Ragama, Sri Lanka (CNTH), for 2009-2010 with data of Lady Ridgeway Children's Hospital in Colombo (LRH) (A study carried out as part of SAPNA; 2004-2007), in order to identify the pneumococcal disease burden and to suggest possible deficiencies in a tertiary care hospital of a low income country.

Method: Data on culture positive pneumococcal disease admitted to CNTH, from December 2009-2010 were retrieved to demonstrate the burden and the disease pattern and compared with previously published data.

Results: During 2009-2010, there were 257 meningitis with 8 deaths, 446 pneumonia with 128 deaths and 344 severe sepsis with 168 deaths. Pneumococcal isolates were found in 10 (0.04%) of meningitis, 3 (0.006%) of pneumonia and 3 (0.009%) of sepsis, compared to LRH figures of 2.2% for meningitis, 1.4% for pneumonia and 4.9% for sepsis.

Discussion: The identification of pneumococcal illness in the CNTH for 2009-2010 is lower compared to figures from LRH, which is also far below the global figures and figures for regional countries. These low incidence figures are questionable due to high rate of pre-admission antibiotics utilization among febrile illness, non-availability of an automated blood culture system due to cost factors and poor awareness of the importance of blood cultures in the management of febrile illness.
DIVERSITY OF PNEUMOCOCCAL SURFACE PROTEIN A (PSPA) AMONG STREPTOCOCCUS PNEUMONIAE CAUSING INVASIVE DISEASE IN CHINESE CHILDREN

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¹Beijing, ²Lanzhou, ³Shenzhen, ⁴Shanghai, ⁵Shenyang, ⁶Nanjing, ⁷Tianjing, ⁸Chongqing, ⁹Wenzhou, China

Background and aims: Pneumococcal surface protein A (PspA) is recognized as a major pneumococcal virulence factor and a possible vaccine candidate for the development of a pneumococcal vaccine that has the potential to offer a broad range of protection. The objective of this paper was to investigate the sequence types (STs) and diversity of surface antigen PspA in 171 invasive Streptococcus pneumoniae isolates from Chinese children.

Methods: A total of 171 pneumococci isolates were isolated from Chinese children with invasive pneumococcal diseases (IPD) in 11 hospitals between 2006 and 2008. The pneumococci samples were characterized by serotyping, PspA classification, and multilocus sequence typing (MLST).

Results: The PspA of these strains could be assigned to two families. The PspA family 2 was the most common (120/171, 70.1%). No PspA family 3 isolates were detected. Family 1 could be subdivided into two clades, with 42 strains in clade 1 and 9 strains in clade 2 and family 2 could be subdivided into clades 3, 4, and 5, which respectively contained 5, 21, and 14 strains. In total, 65 STs were identified, of which ST320 (30/171, 17.5%), ST271 (23/171, 13.5%) and ST876 (18/171, 10.5%) were the most common types.

Conclusions: PspA family 2 and family 1 were dominant among pneumococcal clones isolated from Chinese children with invasive disease. The strains with the same ST always presented in the same PspA family.
MOLECULAR CHARACTERISTICS OF MACROLIDE RESISTANT PNEUMOCOCCAL STRAINS COLONIZING HEALTHY CHILDREN IN MÉRIDA, VENEZUELA

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Macrolides are frequently used to treat pneumococcal respiratory tract infections. As a consequence, pneumococcal macrolide resistance has increased globally over recent years.

Aim: Among 125 pneumococcal isolates colonizing asymptomatic children in Mérida, Venezuela, we investigated the molecular and epidemiological characteristics of macrolide resistance.

Methods: Macrolide phenotypes were investigated using the triple-disc-test described previously (Montanari, 2001 and 2003). Antimicrobial susceptibility was determined following CLSI 2010 recommendations. The occurrence of ermB, mefE, mefA, tetM, cat, aphA-3, int-Tn916, xis-Tn916, tspA-Tn917, tspR-Tn917, and int-Tn5252 genes were studied by PCR. MLST was performed as described by Enright et al., 2001.

Results: Erythromycin and Clindamycin resistance was 43% (54/125) and 34% (42/125), respectively. The observed Macrolide phenotypes were iMcLS (72.2%), M (22.2%) and cMLS (1.8%). Phenotype M strains carried the mefE gene. Phenotype iMcLS and cMLS strains carried the ermB gene. Two phenotype-iMcLS strains carried both the ermB and mefE genes. The ermB-positive strains (N=40) were related with Tn2009 (22/40), Tn3872 (13/40) and Tn6002 (5/40) and the international clones Spain6B-2 (N=13), England1-9 (N=11), Tennessee23F-4 (N=3), Taiwan23F-15 (N=2), Spain23F-1 (N=1), Colombia23F-26 (N=1). Among mefE-positive strains (N=12), we detected Tn2009 (N=5) and 4 PMEN clones: Spain9V-3 (N=1), Spain23F-1 (N=1), Taiwan23F-15 (N=1) and Netherlands15B-1 (N=1). The two ermB+MefE-positive strains carried the Tn2010 and were related with Taiwan19F-14 clone.

Conclusions: We observed a very high prevalence of erythromycin resistance among pneumococcal strains colonizing healthy Latin-Americans children, and this prevalence is most likely due to the dissemination of different PMEN clones carrying a variety of transposons.
PHASE VARIATION AND TYPE 1 PILUS AMONG STREPTOCOCCUS PNEUMONIAE STRAINS COLONIZING HEALTHY CHILDREN IN MÉRIDA, VENEZUELA

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Background and aims: Phase variation and type 1 pilus have been associated with pneumococcal pathogenesis of colonization and infection. We studied the relationship between phase variation, type 1 pilus and the antimicrobial resistance in 125 S. pneumoniae strains isolated from the upper respiratory tract of non-vaccinated healthy children in Mérida, Venezuela.

Methods: Pneumococcal serotypes were determined by Quellung reaction and multiplex PCR. Phase variation was explored using a stereomicroscope with oblique illumination. Phenotypes (transparent, opaque and intermediate) were determined as described previously (Weiser et al., 1994). The frequency of type 1 pilus was determined by PCR using the rrgC gene as described previously (Basset et al., 2008). Susceptibility to 18 distinct antimicrobials was determined following CLSI, 2010 recommendations.

Results: The frequency of heptavalent-vaccine serotypes was 48.8% (61/125) and these serotypes were significantly associated with (multi)drug resistance (p=0.0001). The most common phenotype was transparent (77.6%, 97/125) and this phenotype was significantly associated with heptavalent-vaccine serotypes (p=0.018) and penicillin-resistance (p=0.025). The frequency of the transparent phenotype differed among serotypes, being more frequent among serotypes 19F, 6A, 6B, 23F and 15B. Type 1 pilus was present in 34.4% (43/125) of the strains belonging to serotypes 6B and 15B. This pilus was significantly associated with heptavalent-vaccine serotypes (p=0.0002) and (multi)drug resistance (p=0.001).

Conclusions: We conclude that the presence of type 1 pilus and transparent phenotype might promote the success of heptavalent-vaccine serotypes in colonization and disease, and the persistence and high prevalence of antimicrobial resistance among pneumococcal strains colonizing non-vaccinated children of our community.
CHARACTERIZATION OF STREPTOCOCCUS PNEUMONIAE CAUSING PNEUMONIA IN PANAMA 2001 - 2010

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Background: Pneumonia by Streptococcus pneumoniae is an important cause of morbidity and mortality in children and adults. In Panama there are modest publications about pneumococcal pneumonia.

Objective: Determine the most common serotypes and antimicrobial susceptibility in S. pneumoniae causing pneumonia in Panama from 2001 to 2010.

Methods: A descriptive study of 113 isolates from National Surveillance Network of Microbiology Laboratories from: blood 56 (49.5%), pleural fluid 36 (31.9%) and bronchial aspirates 21 (18.5%). Samples from in children less than 5 years old: 64 (57%), in older than 5 years including over 60 years 49 (43%). Serotyping was performed by Quellung method and antimicrobial susceptibility using minimum inhibitory concentration, according to Clinical and Laboratory Standards Institute (CLSI).

Results: Among the 113 isolates, the most common serotypes for children under 5 years were: 6B (11.5%), 14 (7.1%), 19F (6.2%), 19A (6.2%). In higher or equal to 5 years: 19F (12.5%), 5 (3.5%), 10F (3.5%), 18C (2.7%). High and intermediate resistance was for: 4.4% penicillin, ceftriaxone 43.5%, erythromycin 17.7%, 31.8% sulfamethxazole trimethoprim, and chloramphenicol 9.0%, 2.5% clindamycin, and for vancomycin showed no resistance.

Conclusions: The analysis reflected greater recovery of S. pneumoniae in children under five years, mainly from 0 to 12 months (39.8%), compared with over 60 years (9.7%). Penicillin and ceftriaxone remain high percentage of sensitivity (96%). Showed increased antimicrobial resistance: sulfamethxazole trimethoprim (31.8%) and erythromycin (17.7%). Serotypes associated with multidrug resistance were 6A, 6B, 19A, 19F in children under 5 years and 19F, 6B, 23F at other ages.
DISTRIBUTION OF SEROTYPES AND TRANSPOSONS AMONG MACROLIDE-RESISTANT STREPTOCOCCUS PNEUMONIAE ISOLATES IN COLOMBIA

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Background: Resistance to macrolide antibiotics is increasing in clinical isolates of Streptococcus pneumoniae and has been associated with the presence of transposons. The aim of this study was to determine the distribution of serotypes and transposons among macrolide-resistant invasive isolates of S. pneumoniae in Colombia from 1994 to 2009.

Methods: A total of 156 macrolide-resistant S. pneumoniae isolates were analyzed. The isolates had epidemiological data, serotyping and antimicrobial susceptibility patterns. The phenotypes were tested by erythromycin-clindamycin double-disk test. We investigated the presence of transposons by several multiplex PCR to identify the genes: \textit{ermB}, \textit{mefA}, \textit{mefE}, \textit{tetM}, \textit{Cat}, \textit{Aph3-III}, \textit{int-Tn916}, \textit{xis-Tn916}, \textit{trpA-Tn917}, \textit{trpR-Tn917} and \textit{int-Tn5252}.

Results: The prevalence of resistance to macrolides was 4.4%. The 61.8%, 2.5% and 35.7% of isolates exhibited cMLSB, iMLSB, and M phenotypes, respectively. Multiresistance was observed in 62% of these strains. The most frequent serotypes of erythromycin-resistant isolates were 6B (33.1%), 6A (17.3%), 14 (15.3%), 19A (9.5%), and 19F (7.6%). The most prevalent genetic elements for erythromycin resistance were the \textit{mega} element (23.7%) associated to serotype 6A, \textit{Tn5253} (23.7%) mostly carried in serotype 6B isolates, \textit{Tn3872} (10.9%) mainly detected in capsular type 6B and 14 and \textit{Tn1545} (8.3%) in serotype 19A. Other seven transposons (33.4%) were associated with different serotypes.

Conclusions: The majority of erythromycin resistance isolates of S. pneumoniae in Colombia had the cMLSB phenotype and was associated with the presence of transposons, which carries multiple resistance determinants to other antibiotics. Moreover, isolates with M phenotype carry the gene \textit{mefE} in the \textit{mega} element.
Background and aims: AOM is the most common disease caused by Spn and one of the most frequent diagnoses in children <2 y.o. Macrolide-resistant Spn emerged in Argentina in 1995 and represent 26% in invasive infections in <5 y.o. Our aim was to determine the prevalence of ermB (ribosomal methylase) and mefA (efflux pump) genes in macrolide resistant SPN isolates from AOM and to determine their genetic relatedness.

Methods: Serotypes were determined by Quellung, susceptibility was determined by disc diffusion and MIC by agar dilution (CLSI), genotypes by PCR and genetic relatedness by Smal PFGE and MLST.

Results: From a total of 324 immunocompetent children with a first episode of AOM that came to our hospital between 05/2009 and 08/2010, 126 (39%) Spn were isolated. Of them 26 (20.6%) were erythromycin-resistant (ERY-R). Serotypes of ERY-R Spn were: 14 (46.2%), 6A (23.1%), 19F (7.7%), 9V (7.7%) and 6B, 19A, 33F and non-typable (3.8% each one). Percentage of non-susceptibility was: penicillin V (MIC≥0.12µg/ml) 65.4%, trimethoprim-sulfamethoxazole 57.7%, tetracycline 50% and penicillin G (≥4µg/ml), cefotaxime (≥2µg/ml), chloramphenicol, ofloxacin and vancomycin 0%. Twenty (76.9%) ERY-R Spn carried the mefA gene (MIC range µg/ml) 4-64), 5 (19.2%) the ermB gene (1024->1024) and 1 (3.9%) ermB+mefA genes (1024). A total of 10 clonal types were identified, 77% (20/26) were related to 5 clones: Sweden15A-25/ST63(27%), clone B/ST473(23%), England14-9/ST9(15%), Spain9V-3/ST156(8%) and Poland6B-20/ST315(4%).

Conclusions: Among Spn from AOM, 20.6% were ERY-R, mainly due to the presence of MefA efflux pump. Dissemination of ERY-R Spn strains was related to at least four international clones.
EARLY NASOPHARYNGEAL CARRIAGE OF STREPTOCOCCUS PNEUMONIAE IN BRAZILIAN CHILDREN

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Background and aims: Introduction of Pneumococcal Conjugate Vaccine 10-valent into the routine immunization programs is justified by the pneumococcal disease burden. However, the epidemiology of pneumococcal carriage varies from geographically distinct regions. In this study, we determine the pneumococcal carriage in a special group of Brazilian infants prior to receiving the first PCV-10 dose.

Methods: A group of infants were enrolled from June to September 2011 at the Reference Center of Special Immuno-Biological (CRIE) at Salvador, Bahia, Brazil. These included HIV-exposed uninfected or infected, and others immunological disorders. Nasopharyngeal swabs to detect pneumococcal colonization were taken prior to the first dose of PCV-10 and transported in STGG medium. The pneumococcal detection was performed by lytA-specific real-time PCR and conventional methods. Serotyping was performed using the Multiplex PCR method.

Results: A total of 30 children aging from one to 17 months were enrolled in the cohort study. The age median was 2 months, 53.3% were male, and 66.7% were black. The most prevalent comorbidities were HIV-exposed (63.3%) followed by cardiovascular disease (10%). Seventy-one percent of STGG-NP specimens were positive for both, real-time lytA-targeted PCR and culture. The overall prevalence of pneumococcal carriage in the baseline was 23.3%(7/30). Among these, 3(42.8%) had capsular serotype determined. The most common serogroups were: 23F(28%) and 19F(14%). Carriage of vaccine type pneumococci was identified in 42.8% of the infants.

Conclusion: This preliminary result suggests an early exposure of children to vaccine pneumococcal serotypes, which may promote a suboptimally protection against invasive pneumococcal diseases.

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MOLECULAR CHARACTERIZATION OF PNEUMOCOCCAL SEROTYPE 19F FROM CARRIAGE AND INVASIVE DISEASE
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Background and aims: *Streptococcus pneumoniae* serotype 19F is one of the most common serotype causing invasive disease in children < 5 years old. In addition, it has been associated with penicillin resistance. In this study we determine whether *S. pneumoniae* serotype 19F isolated from the nasopharynx are representative of data from patients with invasive disease.

Materials and methods: Sixty-six serotype 19F pneumococci isolated from invasive disease in Salvador during the last 15 years, and 18 isolates from nasopharyngeal (NP) carriage among children were characterized by antibiotic susceptibility, Pulsed-Field Gel Electrophoresis and Multi-Locus Sequencing Typing.

Results: The prevalence of penicillin nonsusceptible (MIC > 0.125 µg/ml) strains in invasive disease and NP carriage was 19% and 66%, respectively. Decreased susceptibility to co-trimoxazole was identified in 65% of invasive isolates and within 94% of NP carriage strains. Up to twelve different genotypes were observed among invasive isolates and six among carriage isolates. Five (27%) carriage isolates were ST 177, the most frequent ST identified within invasive isolates (16 of 66) and over identified across the whole period.

Conclusion: These results shown that the Portugal 19F-21 clone is predominantly represented among invasive and carriage isolates in Salvador, Brazil.
ACTIVE SURVEILLANCE FOR COMMUNITY-ACQUIRED PNEUMONIA AND INVASIVE PNEUMOCOCCAL DISEASE IN HOSPITALIZED CANADIAN ADULTS: BURDEN OF DISEASE, HEALTHCARE UTILIZATION AND OUTCOMES

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Background: Active surveillance for community acquired pneumonia (CAP) and invasive pneumococcal disease (IPD) in adults is critical to establish burden of disease and healthcare utilization to inform adult immunization policy and facilitate program evaluation.

Methods: The Public Health Agency of Canada/Canadian Institutes of Health Research (PCIRN) Serious Outcomes Surveillance (SOS) Network comprises 8 adult hospitals and ~5500 beds in 6 provinces. We conducted active surveillance for CAP and IPD from 12/1/10 - 9/30/11.

Results: 864 cases of CAP were enrolled; mean age 67.4y (17-102y); 39.7% >75y. 46% were female and 93.5% had ≥ 1 comorbidities; 29% had received pneumococcal vaccine (unknown in 32%). Mean length of stay (LOS) was 12d (1-250d), 17% required admission to ICU and 12% were ventilated. Among 345 patients ≥ 65y, mean frail scale scores increased from 4.2 at baseline to 4.6 at 30d (p< .0001). 30d all-cause mortality was 8%. 70% of patients had ≥1 blood culture and 37% had ≥1 sputum culture. \textit{S. pneumoniae} was confirmed in 7%. Pneumococcal urinary antigen and molecular typing results will be presented. 44 cases of IPD were enrolled; mean age 59y, 52% female. Mean LOS 22d (1-372d). 29.5% were admitted to ICU and 25% required ventilation. 30d mortality was 14%.

Conclusions: The PCIRN SOS Network provides critical infrastructure for assessment of CAP and IPD epidemiology in Canada and will allow assessment of vaccine effectiveness as new adult immunization programs are introduced. CAP and IPD are associated with considerable morbidity, mortality, and healthcare utilization. Improved prevention of pneumococcal disease is needed.
BURDEN OF PNEUMONIAE AND PNEUMOCOCCAL DISEASES IN REFERRAL HOSPITAL, NEPAL

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Background and aims: Acute respiratory tract infection is one of the major killers of under 5 years children and invasive Pneumococci are important pathogens for childhood pneumonia. The aims of this study was to find out the burden of Pneumococcal diseases in referral hospital setting.

Materials and methods: 6545 children, 0 to > 60 months, suspected bacterial pneumonia, sepsis and meningitis were enrolled in this study. Blood and cerebrospinal fluid culture and antigen detection for CSF was performed. Identification of *Streptococcus pneumoniae*, antimicrobial susceptibility testing was done by following standard methods and sero typing was done by the quelling reaction.

Results: All together 65 *Streptococcus pneumoniae* were isolated and serotyped. Of them 44 and 23 isolates were detected from blood and CSF sample (2 isolates were detected in both CSF and blood sample). The most common serotypes were serotype 1 (23.07%) followed by serotype 5 (15.38%) and serotype 2 (6.15%). The study showed that 29 (44.61%) isolates were resistant to cotrimoxazole, 1 (1.58%) isolate was resistant to Penicillin. The 3 (4.61%) isolates were resistant to erythromycin and 1 each (1.58%) isolate was resistant to chloramphenicol and cefotaxime.

Conclusions: The common serotype 1, 5 and 4 need to be incorporated in pneumococcal vaccine to immunize children in Nepal. Alarming level of drug resistance demands revision of pneumococcal disease treatment policy in Nepal.

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CARRIAGE ACQUISITION AND CLEARANCE RATES AND COMPETITIVE ABILITY FOR PNEUMOCOCCAL SEROTYPES: APPLICATION OF A MARKOV TRANSITION MODEL

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Background: Many biological and epidemiological properties of Streptococcus pneumoniae depend on the capsular serotype, of which >90 are known. Animal studies have suggested that carriage induces an acquired immune response that reduces duration of colonization in a fashion that is not serotype-specific.

Methods: We studied pneumococcal nasopharyngeal carriage longitudinally in Kenyan children 3-59 months, following up positive swabs at days 1,2,4,8,…,32,60,90,… until two swabs negative for the original serotype were obtained. As previously reported, 2840 children were swabbed at baseline, of whom 1868 (66%) were positive. Data were analyzed using a Markov transition model with 29 states (uncolonized, colonized with each of the 27 most common serotypes, and colonized with other serotype).

Results: Point estimates of type-specific acquisition rates ranged from 0.00025/d (type 1) to 0.0031/d (19F). Estimated time to clearance (inverse of type-specific clearance rate) ranged from 28d (type 20) to 123d (6A). For the serotype most resistant to competition (19F), acquisition of other serotypes was 52% (38-63%) less likely than in an uncolonized host. Fitness components (duration, acquisition rate, lack of susceptibility to competition) were positively correlated with each other and with baseline prevalence, and were associated with biological properties previously shown to associate with serotype. Duration of carriage declined with age.

Conclusions: These findings provide epidemiological evidence for variation in fitness components of pneumococcal serotypes, for the positive correlation in these components, and for the decline in duration of colonization with age.
Poster No 130

NASOPHARYNGEAL COLONIZATION BY STREPTOCOCCUS PNEUMONIAE AMONG NORTH INDIAN CHILDREN: ASSESSING CARRIAGE, ANTIMICROBIAL SUSCEPTIBILITY AND SEROTYPE DISTRIBUTION

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Background and Aim: Nasopharyngeal colonization provides a convenient niche for Streptococcus pneumoniae to cause lower respiratory tract infections. The knowledge of current serotypes circulating in a community and their antimicrobial susceptibility is crucial for effective therapeutic strategies; however, there is lack of information in this regard. Hence, a cross-sectional study was done in north Indian children to evaluate the carriage, prevalent serotypes and antimicrobial susceptibility.

Methods: Nasopharyngeal swabs were collected from 575 healthy children aged < 5 years residing in the urban, rural and slum areas of Chandigarh, North India. S. pneumoniae isolates were identified by gram staining, optochin sensitivity and bile solubility test and characterized by multiplex PCR based on the cps gene cluster. Further, S. pneumoniae were screened for antimicrobial susceptibility by Kirby Bauer disc diffusion method using CLSI guidelines.

Results: Significant nasopharyngeal carriage rate (49.6%) of S. pneumoniae was observed. Serotypes 6A/B/C, 11A/D, 10A, 15B/C, 15A/F, 7C/B, 10F/C, 14, 9N/L, 23A, 23F, 19A, 13, 5, 22F/A were found prevalent in descending order. All S. pneumoniae isolates were sensitive for amoxicillin, cefotaxime and vancomycin with variable resistance towards chloramphenicol, tetracycline, erythromycin, co-trimoxazole. About 53% isolates showed complete susceptibility towards penicillin and 7.4% isolates emerged as multidrug resistant.

Conclusions: This study highlights the considerable carriage rate of S. pneumoniae showing increasing antibiotic resistance as well as the presence of non-vaccine serotypes in the community which require continuous monitoring for the management of pneumococcal diseases.
MOLECULAR EPIDEMIOLOGY OF SEROTYPE 19A STREPTOCOCCUS PNEUMONIAE ISOLATED FROM CHILDREN IN BEIJING, 1997-2006


Background: Despite the prevalence of Streptococcus pneumoniae serotype 19A, the molecular characteristics of this serotype are yet to be fully elucidated. The aim of this study was therefore to determine the homology of the serotype 19A in China.

Methods: Pulsed-field gel electrophoresis and multilocus sequence typing were done to these forty nine serotype 19A isolates to investigate the relationship between the strains prevalent in Beijing and other regions.

Results: From 1997 to 2006, the percentage of serotype 19A isolates increased. The susceptibility rate to penicillin and amoxicillin decreased and the resistance rate to cefuroxime increased. ST320 was the most prevalent ST, followed by ST3546. There were six new STs identified in our study. The serotype 19A strains were classified into six different pulsedfield gel electrophoresis (PFGE) patterns. ST320, which was associated with two different PFGE patterns (A and D), accounted for 32 isolates, and ST3546, which was associated with two PFGE patterns (B and E), accounted for eight isolates.

Conclusions: From 2003 onwards, ST320 was the most common ST and the rate of resistance to cefuroxime increased significantly. Further long-term surveys of Streptococcus pneumoniae serotype 19A are required to monitor ST prevalence and antimicrobial resistance in this important human pathogen.
MOLECULAR EPIDEMIOLOGY OF INVASIVE STREPTOCOCCUS PNEUMONIAE STRAINS RESISTANT TO PENICILLIN AND ISOLATED FROM CHILDREN ≤ 5 YEARS OLD IN VENEZUELA

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Background and aims: To determine the serotypes distribution, resistance patterns and the genetic diversity of invasive Streptococcus pneumoniae strains, isolated during the years 2000-2005 and causing invasive disease in children ≤ 5 years old in Venezuela.

Methods: S. pneumoniae strains were serotyped by the Quellung method. Susceptibility was determined by the agar diffusion and broth microdilution method. Pulse field gel electrophoresis (PFGE) was used to determine genetic relatedness of strains resistant to penicillin.

Results: 417 strains of S. pneumoniae were analyzed. The most common serotypes were 14(36.9%), 6B(12.9%), 5(8.9%), 1(6.2%) and 19A(6.2%) corresponding to 71% of the isolates. 37 strains (8.9%) showed resistant to penicillin, of which 30 strains were isolated from cerebrospinal fluid. 45.2% of the strains also showed resistance to Trimetoprim/sulfamethoxazole, 30.9% to erythromycin and 9.3% to chloramphenicol. Low (1.7%) or no resistance was found for cefotaxime and vancomycin. 37 isolates, resistant to penicillin, were typed and 16 genotypes were found with 10 unique patterns and 6 clusters with more than 83% similarity, represented by 2-5 isolates. Analysis of the two largest clusters showed one cluster with 4 strains of serotype 14 and a strain of serogroup 9, all with the same resistance pattern (SXT-P) and a cluster of 4 strains of serotype 6B with a similar multidrug resistance pattern. (CC-C-E-TE-SXT-P/CC-C-E-SXT-P).

Conclusions: PFGE typing revealed the existence of small possible outbreaks of strains resistant to penicillin. The classification of S. pneumoniae strains into clonal groups can be helpful for future control measurements of resistance.
Poster No 133

USING PCR TO ESTIMATE THE BURDEN OF PNEUMOCOCCI DISEASE IN CHILDREN REPORTING WITH INVASIVE BACTERIAL INFECTION IN KUMASI-GHANA

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Background and aims: Pneumococci disease is the leading cause of childhood illness worldwide. Bacterial meningitis infection is a common cause of mortality in children presenting with invasive bacterial disease in developing countries. A departmental surveillance program has been established with the aim of understanding the epidemiology of the disease in children in the geographic area.

Methods: Children aged birth to 12 years reporting with acute severe illness, with suspected bacterial meningitis, were evaluated for invasive bacterial disease. Clinical evaluation and laboratory evaluation were conducted and information entered onto database system. Cerebrospinal fluid was obtained from all children with suspected meningitis reporting to the emergency unit. Samples were analyzed using PCR.

Results: Between January and December 2010 a total of 576 cases were examined using PCR technique. *Streptococci Pneumoniae* was the leading cause of infection in children 12%. *Neisseria Meningitis* and *Heamophilus Influenzae* were 4.1% and 2.4% respectively. Out of the total cases of 69 *Streptococci Pneumoniae*, over 50% of the children were less than 12 months. Children less that one month old were 8.7%.

Conclusions: *Streptococci Pneumoniae* is an important cause of invasive bacterial disease in children under five years. Infants under one year of age are the most vulnerable in this study population. Efforts to reduced the burden of the disease in this age category with the introduction of the pneumococci conjugate vaccine will contribute immensely to the reduction of the disease in the populations.
Poster No 134

PNEUMOCOCCAL MENINGITIS IN THE DISTRICT OF TONE, TOGO, 2010-2011


Background: Neisseria meningitis (Nm) and Streptococcus pneumoniae are the leading causes of acute bacterial meningitis among the total population of the African meningitis belt. Togo introduced Haemophilus influenzae type b (Hib) conjugate vaccine in 2008.

Methods: From May 2010 to April 2011, we enrolled all patients treated for suspected bacterial meningitis in health centers of Tône district, northern Togo, which are seen in 5 sites. Diagnosis was confirmed through culture and polymerase chain reaction (PCR) testing on cerebrospinal fluid.

Results: Of 129 included suspected meningitis cases 47 (36%) were confirmed as pneumococcal, six (5%) as meningococcal (all serogroup W135) and three (2%) as Hib meningitis. The monthly incidence rate of pneumococcal meningitis varied from 0 to 4/100 000 inhabitants, peaking between January and April. Serotype 1 accounted for 64% (25/39) of confirmed pneumococcal cases and was predominant in all age groups except in < 1-year-old children. Serotypes included in the 13- or 10-valent pneumococcal conjugate vaccine caused 90% (35/39) and 87% (34/39) of pneumococcal meningitis, including serotypes 1, 3, 5, 7F, 14, and 23F. The case-fatality ratio for pneumococcal meningitis was 33%.

Conclusions: Pneumococcus was the major cause of acute bacterial meningitis during a period in which no major meningococcal epidemic occurred. Serotype 1 caused most pneumococcal meningitis among all age groups except infants. This surveillance also provides evidence for low meningococcal serogroup A incidence during the meningitis season 2010/11, in the absence of meningococcal serogroup A conjugate vaccine.
Background and aims: Streptococcus pneumoniae is a leading cause of bacterial invasive diseases community acquired in Cuba. The aim of this study was to determine the serotype distribution and antimicrobial susceptibility of invasive S. pneumoniae isolates in the country over a time period prior to the introduction of the pneumococcal vaccine.

Methods: Conventional methods were used to study the distribution of the serotypes/serogroups of 211 strains of S. pneumoniae isolated from cases of pneumonia and meningitis from all provinces of the country between 2008 and June 2011. Potential coverage by conjugate vaccines was evaluated.

Results: The most common serotypes were: 14 (15.1%), 6B (14.2%) and 23F (11.8%). Serogroup/serotype coverage of the 10- and 13-valent conjugate vaccines was only of the 64.4% and 79.1%, respectively. Overall, 78 (36.9%) of the isolates were resistant to penicillin, 6 (2.8%) to ceftriaxone, 42 (19.9%) to erythromycin, 8 (3.8%) to cloramphenicol, 36 (17.1%) to trimethoprim-sulfamethoxasole and 4 (1.9%) to vancomycin Among the penicillin resistant isolates, 69 were isolated from cerebrospinal fluid and penicillin resistance was mostly associated with S. pneumoniae serotypes 14 (16.6%) and 6B (16.6%).

Conclusions: The serotype distribution suggests that the incorporation of the 13-valent conjugate vaccine into the routine immunization program of Cuba has the most potential prevent the highest burden of invasive pneumococcal disease. The findings from this study also provided guidance for the clinical use of antimicrobial agents. However, long-term surveillance is needed in view of the spread and antimicrobial resistance of other serotypes not contained into13-valent pneumococcal conjugate vaccine.
A LONGITUDINAL STUDY OF PNEUMOCOCCAL CARRIAGE IN A COHORT OF INFANTS AND THEIR MOTHERS LIVING ON THE THAILAND-BURMA BORDER

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Background and aims: Almost a third of the world's children live in Southeast Asia, but there is a paucity of data on pneumococcal disease in the region. We nested a pneumococcal carriage study within a longitudinal pneumonia aetiology/epidemiology study in a cohort of Burmese/Karen refugee children. The aims of the carriage study were to define the prevalence, serotype distribution, and dynamics of pneumococcal colonisation in this population.

Methods: 234 mother-infant pairs were followed from birth for 24 months. Nasopharyngeal swabs were taken from mother and infant at monthly follow-up visits. These swabs were cultured using the WHO protocol and pneumococcal isolates were serotyped by latex agglutination.

Results: 8,386 swabs were collected (median: 23 per individual) and 4,396 pneumococci were isolated. Cross-sectional carriage prevalence was 24.0% in mothers and 76.3% in infants. 94.4% infants were colonised by pneumococcus at least once, with a median age at first acquisition of 45.5 days. Infants carried a median of five serotypes in the first two years of life, compared with a median of two serotypes in mothers. Eight serotypes/groups (19F, 23F, non-typeable, 6B, 14, 6A, 15B/C, and 6C) accounted for 67.0% of isolates from infants. 29.6% of pneumococcal isolates from mothers were non-typeable. PCV13 serotypes accounted for 55.8% of pneumococci from infants, compared with 27.5% from mothers.

Conclusions: Pneumococcal carriage prevalence was high and colonisation by non-typeable pneumococci was unexpectedly common. This baseline data will allow changes in pneumococcal carriage characteristics to be monitored as PCV use becomes more widespread in the region.
STREPTOCOCCUS PNEUMONIAE CARRIAGE IN THE NASOPHARYNX (NP) AND/OR OROPHARYNX (OP) OF COSTARICAN CHILDREN WITH OTITIS MEDIA BEFORE PCV-7 VACCINATION

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Background and aims: The NP/OP is the main Streptococcus pneumonia (SP) reservoir. It is important to analyze the SP serotypes carriage to evaluate antimicrobial dynamics as a surrogate for potential vaccine coverage. The aim of this study was to analyze the NP/OP SP serotype distribution and antimicrobial susceptibility in children with otitis media (OM) before PCV7 universalization in Costa Rica.

Methods: Between years 2002-2006, NP/OP samples were obtained from 641 children with OM. SP serotyping was performed and antimicrobial susceptibility was determined.

Results: A total of 386 SP isolates were recovered from 376 (59%) children (59% < 24 months of age). The distribution of the SP serotypes in children < 24 months of age versus children > 24 months of age, respectively were: 1 (0.55% vs 0.8%), 3 (8.5% vs 8.2%), 4 (1.4% vs 2.2%), 5 (0% vs 0.8%), 6A (4.3% vs 4.5%), 6B (13.7% vs 10.4%), 7F (0.5% vs 0%), 9V (1.4% vs 3.7%), 14 (10.4% vs 14.2%), 18C (1.9% vs 1.5%), 19A (1.4% vs 0.8%), 19F (21.8% vs 21.6%), 23F (6.2% vs 12.7%). MDR and penicillin resistant isolates were distributed as follows: PCV 7 57%, PCV 10 60% and PCV 13 62%. PCV potential coverage was: PCV 7: 60.2%, PCV 10: 61.4% and PCV 13: 75.2%.

Conclusions: SP was isolated from the NP/OP in 59% of children with OM. PCV 13 offers the highest potential vaccine coverage including antimicrobial resistant serotypes.
Poster No 138

SEROTYPE 5 PNEUMOCOCCAL ACUTE PURULENT PERICARDITIS (APP) AS A COMPLICATION OF EMPYEMA AND SEPSIS IN A COSTA RICAN (CR) CHILD

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Background: Streptococcus pneumoniae (SPN) is an uncommon cause of pediatric APP, and even more, that caused by non-PCV7 serotypes. No previous serotype 5 pericarditis cases have been reported in Latin America.

Case: A 17-month-old aboriginal boy, with 1 dose of PCV7 at 4 months, was transferred to the only pediatric hospital of CR with a 2-day history of fever, cough, abdominal distension and irritability. On admission, he had diaphoresis, tachycardia, tachypnea, nasal flaring, grunting, retractions, crepitations, decreased left breath sounds and hepatomegaly. Chest radiograph evidenced bilateral pneumonia, a left pleural effusion, and cardiomegaly. CBC revealed anemia, leukocytosis and bandemia; CRP was 202 mg/L. Empiric intravenous cefotaxime was started. He developed respiratory failure, required mechanical ventilation (6 days), and PICU management (2 weeks). A pericardial rub was heard, an EKG revealed ST segment elevation and an echocardiogram documented a pericardial effusion. On pericardiocentesis, 80 cc of fluid were aspirated (10 leukocytes/mm³ and Gram (+) diplococci), and a pigtail catheter was inserted (for 8 days). A left chest tube was inserted for 16 days, pleural fluid revealed an exudate with Gram (+) diplococci, and vancomycin was added. His respiratory condition worsened, he was switched to HFOV for 3 days, required inotropic support, and intrapericardial streptokinase. Blood, pleural, and pericardial fluid cultures grew penicillin/cefotaxime susceptible SPN serotype 5. He was switched to penicillin (total of 3 weeks of intravenous antibiotics) and recovered completely.

Conclusions: This case illustrates the importance of continuous pneumococcal serotype circulation surveillance and the need for expanding vaccine serotype coverage.
ETIOLOGY OF PNEUMONIA WITH PLEURAL EFFUSION AMONG CHILDREN IN THE DOMINICAN REPUBLIC

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Background and aims: Pleural effusion is a serious complication of pneumonia, and Streptococcus pneumoniae is a leading cause of pediatric pneumonia with effusion. We describe the etiology of pediatric pneumonia with effusion among children in the Dominican Republic prior to the introduction of pneumococcal conjugate vaccines (PCV).

Methods: From 7/2009 to 6/2011, we aimed to enroll all children < 15 years old admitted with pneumonia and significant pleural effusion to Robert Reid Cabral Children's Hospital, a large referral hospital in the capital city. Pleural fluid was tested by culture and PCR for Streptococcal species, respiratory syncytial virus (RSV) and rhinovirus.

Results: Among 120 cases, the median age was 31 months (range 1 week to 14 years) and 81 (68.1%) were male. Pleural fluid culture was performed for all cases; PCR testing was performed on 111 (92.5%). Etiology was determined for 85 (70.8%) cases, including 61 S. pneumoniae (50.8%), 19 Staphylococcus aureus (15.8%), 1 S. pneumoniae/ S. aureus co-infection (0.8%), 2 Streptococcus pyogenes (1.7%), 1 Streptococcus mitis (0.8 %) and 1 Candida spp. (0.8%). No RSV or rhinovirus was detected. Multiplex PCR serotyped 60 (98.3%) of pneumococcal cases, the most prevalent serotypes were 14 (n=20), 1 (n=13), 3 (n=12) and 6 A/B (n=6). Serotype coverage of 10- and 13-valent PCVs would be 71% and 95%.

Conclusions: More than half of cases of pneumonia with effusion among children in the Dominican Republic are caused by S. pneumoniae. Introduction of PCV10 or PCV13 could reduce the burden by 36-49%.
Poster No 140

RISK FACTORS WHICH PREDICT CEFTRIAXONE NON-SUSCEPTIBILITY OF STREPTOCOCCUS PNEUMONIAE: ANALYSIS OF SOUTH AFRICAN NATIONAL SURVEILLANCE DATA (2003-2010)

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Background: Current recommended South African empiric meningitis treatment is third-generation cephalosporin monotherapy. Monitoring pneumococcal susceptibility is important to inform treatment guidelines.

Methods: We conducted an analytical cross-sectional study from national laboratory-based surveillance data (2003-2010). Data from 24 enhanced surveillance sites for 2009-2010 were used for risk factor analysis.

Results: From 36679 IPD cases (2003-2010), 9217 random isolates were tested for ceftriaxone susceptibility using broth microdilution: 733 (8.0%) were ceftriaxone non-susceptible, with no proportional change from 2003 (32/451, 7.1%) to 2010 (225/2859, 7.9%), p=0.64. Non-susceptibility prevalence was higher amongst < 5 years [341/2631, 13.0% (intermediate 280/2631, 10.6%; resistant 61/2631, 2.3%)] vs ≥5 years [398/6586, 6.0%; (intermediate 331/6586, 4.9%; resistant 67/6586, 1.0%)] (OR=1.30, 95% CI: 1.12-1.50). Amongst children < 5 years, 93.1% (283/304) and 95.4% (290/304) of non-susceptible isolates were serotypes in PCV-7 and PCV-13 respectively, while in patients ≥5 years this was 73.8% (279/378) and 90.0% (340/378). Ceftriaxone non-susceptibility prevalence was similar among patients with (84/1014, 8.3%) and without meningitis (127/1723, 7.4%) (OR=0.88, 95% CI:0.66-1.17).

On multivariable analysis, controlling for province, ceftriaxone non-susceptibility risk factors were age [< 1 (54/447, 12.1%; OR=2.11, 95% CI:1.25-3.56) and 1–4 years (55/415, 13.3%; OR=1.80, 95% CI:1.07-3.02)] compared to 15–44 years (52/1182, 4.4%); vaccine-serotype [210/1290 (16.3%) vs 12/1558 (0.8%); OR=16.22, 95% CI:8.62-30.5] and recent β-lactam use [23/154 (14.9%) vs 129/1770 (7.3%); OR=2.19, 95% CI:1.20-3.99].

Conclusion: Adequate ceftriaxone doses treat intermediately resistant strains. Additional vancomycin should be considered in high risk individuals and those not responding to empiric therapy. Pneumococcal vaccine use may change prevalence of non-susceptibility.
CAPSULAR SEROTYPES AND GENOTYPES ASSOCIATED WITH INVASIVE PNEUMOCOCCAL DISEASE IN MANHIÇA, SOUTHERN MOZAMBIQUE

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Background and aims: *Streptococcus pneumoniae* is an important cause of child morbidity and mortality in Manhiça District, Southern Mozambique, where surveillance for pediatric pneumonia and invasive pneumococcal disease (IPD) was established in 2001. We aimed to determine serotypes and genotypes among isolates from 2008 to 2010 to provide information on serotype coverage before introduction of pneumococcal conjugate vaccines (PCV) and to identify circulating clonal types.

Methods: *S. pneumoniae* were isolated from blood (n=182), and CSF (n=13) from children < 15 years admitted to Manhiça district hospital between 2008 and 2010. Isolates were serotyped by PCR and Quellung. Multilocus sequence typing (MLST) was performed on all 195 isolates.

Results: Among 29 different serotypes identified, 127 (65.1%) were serotypes included in PCV10 and 162 (83%) in PCV13. Prevalent serotypes were 5 (15.4%), 6A (13.3%), 1 (10.8%), 6B (8.2%), 14 (7.2%), 4 (5.6%) and 23F (5.1%). Extensive clonal diversity was observed, with 79 MLST types (STs), including 15 new STs. Most or all isolates within each PCV13 serotype were represented by one major clonal complex, with STs 289, 5073, 217, 2909, 4902, 5410 and 802 each accounting for the majority of isolates within serotypes 5, 6A, 6B, 14, 4 and 23F, respectively.

Conclusions: Most pediatric IPD in rural Mozambique could be prevented by PCV10 or PCV13. The majority of isolates represent a small sub-set of clones, some that are unique to this region. Continued IPD surveillance is important to encourage PCV introduction and to evaluate effectiveness and monitor serotype distribution after implementation.
THE EFFECT OF INFLUENZA ON INVASIVE PNEUMOCOCCAL DISEASE VARIES WITH AGE AND SEROTYPE

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Background: Influenza affects host susceptibility to pneumococcus. We sought to quantify the impact of influenza on the incidence of different serotypes using a large Danish IPD database covering three decades.

Methods: Weekly rates of invasive pneumococcal disease (IPD) were obtained from the Danish National Laboratory Surveillance System, and influenza-like illness (ILI) data were collected from Danish sentinel surveillance, Statens Serum Institut, 1977-2007. We fit Poisson regression models for each age group, with predictors for seasonality and secular changes, ILI activity, and an interaction term for serotype and ILI.

Results: Influenza had little impact on IPD incidence among 0-4 year olds (-0.7% of IPD attributable to influenza, 95% CI: -9.3-6.4) but accounted for 13.9% (95% CI: 3.8-23.3%) of IPD in the 15-24 year olds, 4.0% among the 5-14 year olds (95% CI: -8.3-14.4%), 6.5% (95% CI: 2.5-11.8%) in the 25-64 year olds and 6.2% (95% CI: 3.2-10.7%) in the 65+ population. Among the 25-64 year olds, serotypes 1, 3, 7F, and 14 significantly increased during influenza periods, and 5, 6B, 8, and 19F also tended to increase. Among 5-24 years olds, serotypes 1, 5, 7F, 8, 9N and 14 tended to increase with influenza, while serotypes 4 and 19A significantly increased with influenza among the elderly. There was no relationship between the serotype-specific effect of influenza and invasiveness, carriage prevalence, or case-fatality rate.

Conclusions: Our data suggest that influenza circulation is associated with a significant increase in the rate of IPD, with the magnitude of the effect varying with both age and serotype.
Poster No 143

PNEUMOCOCCAL DISEASES IN CHINA, OUR RECENT STUDIES

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We review the major contributions to our understanding of pneumococcal diseases among children in hinterland of China. The data demonstrated that *S. pneumoniae* is an important pathogen and death of pyogenic meningitis, pneumonia, and other infectious diseases in children. The distribution of serotypes showed great diversity in several studies in various cities during different years. The penicillin nonsusceptibility rates, although variable, demonstrated an increase over time in China. The prevalence of resistance to erythromycin, trimethoprim-sulfamethoxazole or tetracyclines was reported to be over 80%. A total of 202 paraffin-embedded lung autopsy tissues of children aged 1 month to 5 years old who died of CAP were selected at random from the Beijing Children's Hospital. Conventional PCR, Southern blotting and ISPCR were used to detect *S. pneumoniae* in paraffin-embedded lung tissue of a mouse pneumonia model and in 202 autopsy samples from fatal childhood CAP cases, 1 month to 5 years old, between 1953-2002. We found a combined total of 116/202 (57.4%) samples were found to be positive by both methods, comprising 66/116 (56.9%) samples collected in 1953-1969 and 50/86 (58.1%) from 1980-2002. There is evidence that *S. pneumoniae* was an important cause of fatal childhood CAP in China, as elsewhere. Recently a total of 171 *Streptococcus pneumoniae* isolates causing invasive disease were isolated from Chinese children. The serotype distribution and antimicrobial resistance were tested. The results suggested that the 7-valent pneumococcal conjugate vaccine has a preventive effect among children and that there should be long-term surveillance for serotype 19A.
Poster No 144

SEROTYPING AND MOLECULAR EPIDEMIOLOGY OF S. PNEUMONIAE ISOLATED FROM CHINESE CHILDREN PRIOR TO THE INTRODUCTION OF 7-VALENT PNEUMOCOCCAL CONJUGATE VACCINE

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Background and aims: To explore the serotype distribution and molecular epidemiology of pneumococci before introduction of PCV7, a case-control study was conducted in Suzhou, China.

Methods: Nasopharyngeal swabs or aspirates were collected from the cases admitted with acute respiratory infectious diseases (ARI) and the controls for surgical procedures. Serotyping by Quellung reaction and/or multiplex PCR. Antibiotics susceptibility was tested by Kirby-Bauer disk diffusion or E-test. The phenotype and genotype of macrolide-resistant-S.-pneumoniae (MRSP) isolates were determined by dual-disk test and PCR, respectively. MLST was applied to all MRSP strains.

Results: From March 2006 to March 2007, 60 pneumococcal strains were isolated from 453 ARI cases while 72 strains from 394 control children. The most common serotypes of pneumococci isolated from cases were 19F, 19A and 14 and from controls were 19F, 23F, 18C, accounting for 66.7% vs 45.2% of the strains, respectively. The non-susceptible rates of penicillin, cefuroxime, ceftriaxone, erythromycin, clindamycin and tetracycline were higher in cases isolates than that in control isolates (p< 0.05). Among the 48 MRSP cases isolates, 89.6% strains' phenotypes were cMLSb, 8.3% were iMLSb and 2.1% were M type. ermB gene was detected in 47.9% isolates, mefE in 2.1% and both in 50% isolates. There were 31 STs found in the 48 MRSP strains by MLST. The most common clone-complex was CC271 and CC876. 41.6 % isolates were identical to or derived from Taiwan 19F-14. The PMEN clone strains have higher resistant rate in penicillin and cefuroxime than other isolates (P< 0.05).

Conclusion: The most MRSP strains were derived from PMEM14 in China.
INDIRECT, POPULATION IMPACT OF UNIVERSAL PCV7 VACCINATION OF CHILDREN ON THE INCIDENCE OF PNEUMONIA MORBIDITY IN KIELCE, POLAND

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Background and aims: In 2006, the City of Kielce, Poland introduced mandatory PCV7 vaccination programme for children < 2 years of age. The aim of the study was an analysis of indirect, population effects of PCV7 on pneumonia incidence rate in the 5-year follow-up period.

Methods: PCV7 vaccinations were carried out according to 2 + 1 scheme. The compliance rate of vaccinations amounted to approx. 99%. The following age groups were analysed: 0-1, 0-29, 30-49, 50-65 and 65+. Cochran-Armitage test investigated the significance of the observed trend in pneumonia morbidity. The significance of deviations from a linear trend was also tested. In addition, the importance of the trend (in case of deviations from linearity) was confirmed by Mantel test.

Results: The highest decrease in pneumonia morbidity in 2006-2010 was observed for children below 2 years of age: 82.6% (2005 - 25/1000; 2010 - 4.34/1000). In the whole group aged 0 - 29 a decrease amounted to 48.0% followed by 45.0% in the 65+ age group. Lower declines, although of statistical significance (p< 0.05) were observed for other groups as well: 16.5% in the 30-49 years and 40.0% in the 50-64 years group. (Fig. 1).

Conclusions: The results clearly indicate statistical significance of the population effectiveness of the PCV7 vaccine programme in 2+1 schedule applied in Kielce, Poland.
EXPANSION OF SEROTYPES CAUSING INVASIVE PNEUMOCOCCAL DISEASE AMONG UTAH CHILDREN THROUGH THE PCV7 ERA

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Background: Introduction of PCV7 led to dramatic decreases in PCV7 serotype invasive pneumococcal disease (IPD) in the United States, while changing pneumococcal population dynamics via herd protection and serotype replacement.

Objective: Assess the population structure of pneumococcal serotypes causing IPD in Utah children before and after introduction of PCV7.

Methods: IPD cases from 1997-2010 were identified through laboratory-based surveillance. We performed pneumococcal serotyping on isolates obtained from children < 18 years with IPD.

Results: We observed a 74% decrease in PCV7-serotype IPD and a 105% increase in non-PCV7-serotype disease following introduction of PCV7. Among 128 children during the pre-PCV7 period (1997-2000), 23 serotypes were identified, most commonly 14 (17%), 19F (11%), and 6B (9%). Following PCV7 (2001-2010), 42 different serotypes were identified from 383 children. Serotypes 7F (19%), 19A (16%), and 1 (8%) were the most common; however, the contribution of individual serotypes fluctuated over time. As well, there was expansion in the number of individual serotypes among cases of pneumonia (9 vs. 32), meningitis (11 vs. 24), and bacteremia (22 vs. 40).

Conclusions: Our data suggest that PCV7 resulted in selective pressure that reduced PCV7-serotypes and was associated with dynamic changes to pneumococcal ecology. The ecologic niches vacated by PCV7-serotypes unmasked a significant number of pneumococcal serotypes with invasive potential. New serotypes have become predominant causes of IPD in children and changes continue indicating perhaps competition between the fittest serotypes. PCV13 will result in further changes. Continued surveillance will be essential to detect IPD from previously rare serotypes.
INVASIVE PNEUMOCOCCAL DISEASE IN UTAH INFANTS YOUNGER THAN 90 DAYS (1997-2010)

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Background: PCV7 has been associated with significant changes in invasive pneumococcal disease (IPD) in U.S. children. Few data exist describing the impact of PVC7 in infants < 90 days, who are too young to be fully immunized. Our aim is to describe changes in the epidemiology of IPD in infants < 90 days cared for at Primary Children's Medical Center.

Methods: We identified all S. pneumoniae sterile site isolates in children < 18 years from 1997-2010. We evaluated demographics and clinical data, during 1997-2000 (pre-vaccine) and 2001-2010. Serotyping was performed using the capsular swelling method.

Results: Thirty-six of 513 (7%) children with IPD were < 90 days old; 9/128 (7%) during 1997-2000 and 27/385 (7%) during 2001-2010 (p=NS). Most were male (58%) and < 60 days (81%). Three (8%) infants died and 44% required intensive care. Bacteremia without focus (56%) was the most common clinical syndrome in 1997-2000 and serotype 4 (22%) was most often isolated. In 2001-2010, meningitis (44%) and serotypes 7F (44%) are the most common presentation and serotype isolated, respectively. Infections by PCV7-serotypes decreased from 44% to 11% in 1997-2000 and 2001-2010 respectively (p =0.049). From 2001-2010, PCV13-serotypes accounted for 70% of infections.

Conclusion: Among children with IPD, there has been no change in the proportion of infants < 90 days in 2001-2010 compared to 1997-2000. Infections by non-PCV7-serotypes and meningitis are the most common form of IPD during the PCV7 period. These findings have implications for vaccine policy and warrant continuing surveillance in the PCV13 era.
Poster No 148

EFFECTIVENESS OF THE 13 VALENT PNEUMOCOCCAL CONJUGATE VACCINE AGAINST IPD IN ENGLAND AND WALES


Background and aims: In April 2010 PCV13 replaced PCV7 in the UK infant immunisation programme with a 2,4,13m schedule. This study provides estimates of the effectiveness (VE) of PCV13 against invasive pneumococcal disease (IPD) for the additional 6 serotypes in PCV13 plus 6C.

Methods: The Health Protection Agency introduced enhanced IPD surveillance prior to PCV introduction and obtains additional clinical and vaccination histories for laboratory confirmed cases in vaccine-eligible children. The indirect cohort method, where non-vaccine-type cases serve as controls is used to estimate VE. Adjustment is made for age and period. The impact of differential serotype replacement by vaccination status on this methodology is investigated.

Results: By September 2011 a total of 303 serotyped IPD cases eligible for PCV13 had been identified of whom 140 had vaccine serotypes. Adjusted VE for one, two doses under one year of age and one dose over a year of age was 55% (95% CI: 11 to 72), 80% (38 to 84) and 73% (37 to 88) respectively. Effectiveness of the full 2+1 schedule could not yet be assessed. Serotype specific VE for at least one dose for serotypes 1, 3, 7F and 19A was 65% (-22 to 90), 26% (-94 to 72), 84% (56 to 94) and 75% (42 to 89) respectively. Differential replacement was estimated to lead to a maximum 5% overestimate of VE.

Conclusions: PCV13 has high effectiveness against the additional serotypes covered by PCV13 and is expected to have a large impact on disease caused by these serotypes.
PNEUMOCOCCUS CARRIAGE 7 YEARS AFTER IMPLEMENTATION OF VACCINATION PROGRAM IN A POPULATION WITH VERY HIGH AND LONG-LASTING COVERAGE, ITALY

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Background and aims: To evaluate how the 7-valent pneumococcal vaccine (PCV7) programme and the very high vaccination coverage reached for over 4 years affected the prevalence of S. pneumoniae serotypes in the paediatric population and to evaluate demographic, behavioural and risk factors for carriage in the post-vaccination era, a cross-sectional study on nasopharyngeal carriage was performed.

Methods: 669 children under the age of 5, representative of the open population, were enrolled by cluster sampling. High sensitive techniques for detection of multi-serotype carriage were used.

Results: Of the enrolled children, 97.8% were compliant with the recommended PCV7 vaccination schedule. Post-stratification carriage prevalence adjusted for age was 50.1% and 78% of carriers were colonized by more than one serotype. The prevalence of carriage increased with age from 22% in the first year of life, to 48.6% in the second and to 60% in the 25-59 month age group. Post-stratification prevalence of any of the PCV7, PCV10 or PCV13 serotypes was 10.3%, 20.3% and 27.5%, respectively. PCV7 serotypes were mainly represented by serotype 4. Among the serotypes included in recently available vaccines, serotypes 5 and 19A showed a higher prevalence. A multivariate analysis showed that age, the presence of child siblings at home and day care attendance covariates were strongly associated with carriage.

Conclusions: Very high coverage in the last years has led to high prevalence of non-PCV7 serotypes carriage. Serotypes 5 and 19A, two among the most prevalent serotypes showing the highest invasion ability, are included in PCV13 composition.
FLUORQUINOLONE-RESISTANCE (FQ-R) IN STREPTOCOCCUS PNEUMONIAE. EVOLUTION, SEROTYPES AND GENOTYPES, OVER A TWENTY-YEAR PERIOD IN A SPANISH HOSPITAL (1991-2010)

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Background and aims: Fluoroquinolones are used to treat several infections including community-acquired pneumonia. This study aimed to analyze trends in FQ-R over a 20-year period, and to describe the serotypes and genotypes associated with FQ-R.

Methods: All 8081 consecutive pneumocci isolated at our laboratory (1991-2010) were tested for ciprofloxacin (Cip) and levofloxacin susceptibility by microdilution method. Isolates with Cip-MIC≥4µg/ml (CipR) were selected for serotyping (Quellung/PCR, n=257), genotyping (PFGE/MLST, n=210) and analysis of QRDRs (sequencing/PCR-RFLP, n=200).

Results: A total of 267 CipR pneumococci were isolated from 216 patients (3.3%). The 63% of episodes occurred in people over 65. Rates of CipR increased from 0.9% in 1991-1992 to 5.1.0 % in 2009-2010 (p< 0.01). The most frequent serotypes were 9V (n=29), 19F (n=24) and 23F (n=27) and non-typable pneumococci (n=29). PCV7 serotypes decreased from 53% to 28% (1991-2000 vs 2001-2010; p< 0.01). The most frequent genotypes were: CC81 (n=29), CC156 (n=28) and CC63 (n=17). 138 isolates had high level CipR with MICs ≥16µg/ml (HLCipR) and 129 isolates had low level CipR with MICs of 4-8µg/ml (LLCipR). All HLCipR isolates had two (ParC/GyrA or ParE/GyrA) or three (ParC/ParE/GyrA) changes at QRDRs. The 95% of LLCipR had single changes in ParC (S79 or D83). The 93% of LLCipR isolates with ParC changes had levofloxacin MICs ≤2µg/ml considered susceptible.

Conclusions: Although the rate of CipR has increased, it remains low. LLCipR strains with ParC-changes are usually levofloxacin susceptible. Serotype and genotype distributions of CipR strains changed after PCV7 introduction for children.
Poster No 151

**19F PERSISTS IN TOP 3 CARRIAGE SEROTYPES IN REMOTE ABORIGINAL COMMUNITIES IN NORTHERN TERRITORY OF AUSTRALIA**

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**Background:** Indigenous children in remote communities are at risk of Invasive Pneumococcal Disease (IPD) and acute otitis media with perforation (AOMwiP). The schedule of Prevenar (PCV7) at 2,4,6 months and Pneumovax (23PPV) booster at 18 months of age began in July 2001, and rates of IPD fell dramatically.

**Methods:** Between 2001 and early 2010, nasopharyngeal swabs (NPS) were collected from 2464 Aboriginal children between 0 and 8 years of age, living in 30 remote communities and enrolled in various research studies. NPSs were transported, stored and processed according to published methods.

**Results:**

<table>
<thead>
<tr>
<th>Year</th>
<th>No. children</th>
<th>Spn carriage (%)</th>
<th>19F carried (% children)</th>
<th>23F carried (% children)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996-2000 (pre PCV7)</td>
<td>108</td>
<td>78</td>
<td>7.4</td>
<td>12</td>
</tr>
<tr>
<td>2000 (pre PCV7)</td>
<td>89</td>
<td>90</td>
<td>2.2</td>
<td>9</td>
</tr>
<tr>
<td>2001 (pre PCV7)</td>
<td>119</td>
<td>82</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>2001-2004 (post PCV7)</td>
<td>77</td>
<td>72</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>2003,2005 (post PCV7)</td>
<td>1720</td>
<td>80</td>
<td>4.3</td>
<td>2.7</td>
</tr>
<tr>
<td>2008-2010 (post PCV7)</td>
<td>351</td>
<td>73</td>
<td>8</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*Carriage of 19F and 23F pre and post PCV7*

**Conclusion:** The proportion of children with 19F carriage, but not 23F, has returned to pre-vaccine levels. Serotype 19F is still one of the top 3 serotypes found in this population. Synflorix (PHiD-CV10) at 2,4,6, 18 months commenced in October 2009. Ongoing surveillance may indicate whether Prevenar7 and Synflorix vary in efficacy for serotypes common to both, such as 19F.
NASOPHARYNGEAL CARRIAGE OF NON-TYPEABLE HAEMOPHILUS INFLUENZAE IN AUSTRALIAN INDIGENOUS CHILDREN FOLLOWING VACCINATION WITH PHID-CV10


Introduction: In October 2009, PHID-CV10 replaced PCV7 on the childhood vaccination schedule in the Northern Territory of Australia and this was the only jurisdiction to do so. NTHi carriage is up to 80% for Indigenous children in this region. Whether the Haemophilus protein D component of PHID-CV10 affords protection against NTHi carriage and associated disease is unclear. Here we perform a unique but preliminary assessment of the effectiveness of PHID-CV10 on NTHi carriage.

Methods: Nasopharyngeal swabs were collected at 7 months of age from 178 Indigenous children as part of a larger trial conducted in Darwin and remote communities from 2006-2011. 32 children completed a 3 dose regimen (2, 4 and 6 months) of PHID-CV10 at least 14 days prior to swab collection and 115 children did not receive any PHID-CV10 (complete and partial PCV7 (n=114) or no PCV7 (n=1)). NTHi carriage status was determined using standard microbiological methods.

Results: 48/115 (41.7%) of children who did not receive PHID-CV10 were positive for NTHi carriage compared to 14/32 (43.8%) in PHID-CV10 vaccinated children (RD 2.0% [-17.4%, 21.4%]; p=0.84). Swabs were collected at a median of 34 [15-91] days after the final vaccination.

Discussion: Our study suggests NTHi carriage has not substantially reduced following the introduction of a 3 dose primary series of PHID-CV10 in this population. Whilst a relatively small sample size, the confidence interval suggests that any reduction in NTHi risk is likely to be marginal (<17%). Impact following a 4th booster dose of PHID-CV10 remains a possibility.
INVASIVE PNEUMOCOCCAL DISEASE (IPD) IN ALASKA CHILDREN: IMPACT OF THE 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV13)

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Background: Alaska Native (AN) children have experienced high rates of IPD. After statewide introduction of PCV7 in 2001, rates of IPD declined; however, non-PCV7 vaccine type IPD started increasing in 2004. In March 2010, PCV13 was introduced statewide in Alaska. We evaluated the impact of PCV13 on IPD in children, 18 months after introduction.

Methods: Pneumococcal sterile site isolates, reported through state-wide surveillance, were serotyped using standard methods. Vaccine coverage was evaluated from electronic medical records at AN medical facilities. We defined a pre-vaccine time period April 2006-March 2009 and post-vaccine time period April 2010-September 2011. April 2009-March 2010 was excluded because PCV13 was introduced pre-licensure in one high-risk region in 2009.

Results: Among Alaska children < 5, PCV13 serotypes comprised 68% of IPD in the pre-vaccine time period and 46% in the post-vaccine time period. 94.8% of AN children 19-35 months of age received ≥3 doses of PCV13 during the post-vaccine period. Among Alaska children < 5 years, IPD rates decreased from 59.4 (pre) to 17.3 (post) per 100,000/yr (p< .001); PCV13 serotype IPD decreased from 38.2 to 6.9 (p< .001). Among AN children < 5, IPD rates decreased from 141.0 to 40.2 (p< .001); PCV13 serotype IPD decreased from 84.2 to 20.1 (p< .001); non-PCV13 serotype IPD decreased from 46.3 to 12.1 (p=.02).

Conclusions: Within one year of introduction of PCV13, PCV13-serotype IPD rates decreased >75% in Alaska children < 5 years old. A decrease was noted in non-PCV13 disease among AN children, similar to patterns seen after introduction of PCV7.
EFFICACY OF PCV13 IN PREVENTION OF AOM AND NP COLONIZATION IN CHILDREN: FIRST YEAR OF DATA FROM THE US

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Background and Aims: This ongoing study will evaluate the effectiveness of PCV13 in reducing AOM caused by the 13 serotypes in PCV13 and track the impact on NP colonization in children.

Methods: 60 children aged ≥6 mos. were enrolled in Rochester NY (Legacy Pediatrics [Legacy]) commencing Oct 2010; the data of this report involves subjects through Sept 2011. Children had NP cultures at age 6, 9, 12, 15, and 18 mos; at the time of AOM; and 1 mo later. Middle ear fluid was obtained by tympanocentesis from every subject at the occurrence of each AOM episode. Legacy has collected prospective data from children since 1995 using these same methods and techniques. For comparison to the first year of the PCV13 era, data from 58 children prospectively enrolled from 1 Oct 2007-30 Sept 2009 were selected.

Results: Demographics and AOM/NP carriage risk factors were similar between the study groups. Among PCV13 vaccinees 1 of 15 children that developed AOM had Spn (serotype 11) compared to 15 of 23 PCV7 vaccinees (p< 0.001). PCV13 serotypes among PCV13 vaccinees causing AOM was 0/1 vs. 7 (all 19A)/15. Otopathogen distribution of AOM in PCV13 vaccinees showed a trend for increases in nontypeable H. influenzae (p=0.08). Significant decreases in NP carriage of Spn during healthy visits (p< 0.01) of PCV13 serotypes occurred in PCV13 vaccinees compared to PCV7 vaccinees.

Conclusion: Early data suggests that PCV13 is effective in preventing AOM and NP carriage of serotypes contained in the vaccine.
SEROTYPES AND ANTIBIOTIC SUSCEPTIBILITY OF \textit{STREPTOCOCUS PNEUMONIAE} CAUSING ACUTE OTITIS MEDIA IN TAIWANESE CHILDREN

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**Background and aims:** To monitor continuing shifts in the strains of \textit{Streptococcus pneumoniae} that causes AOM, with particular attention to capsular serotypes and antibiotic susceptibility, following the introduction of a pneumococcal 7-valent conjugate vaccine in Taiwan.

**Methods:** Prospective cohort study using tympanocentesis to identify \textit{S. pneumoniae} strains that caused AOM in children in early period from December 2009 to October 2010 and late period from November 2010 to September 2011. Demographic data on \textit{S. pneumoniae} causing AOM were prospectively collected for the determination of serotypes and antibiotic susceptibility.

**Results:** Among children in whom AOM was diagnosed, tympanocentesis yielded 52 cases of \textit{S. pneumoniae} infection. The most frequent serotypes were 19A (31, 59.6%) and 19F (10, 19.2%). Patients with AOM caused by serotype 19A were younger (33.4 ± 15.4 vs. 54.2 ± 43.2 month-old, P=0.045). The rate of serotype 19A increased from 38.9% to 70.6% (P=0.027). Serotypes included in PCV7 decreased from 50.0% to 20.5%. Among these strains, 26.7% were penicillin susceptible, 51.1% were intermediate, and 17.8% were fully resistant, and serotype 19A represented 66.7% of the fully resistant strains. The proportion of penicillin fully resistant increased from 16.7% to 33.3%. Five strains of penicillin and cefotaxime- nonsusceptible serotype 19 A were isolated in later period.

**Conclusions:** Our results reflect that \textit{S. pneumoniae} serotype 19 A emerged in Taiwan as an otopathogen is increased in resistant to penicillin. Shifts in serotype distribution and increases in penicillin nonsusceptibility among pneumococcal isolates from children with AOM underscore the continuing surveillance.
MATHEMATICAL MODELLING LONG-TERM EFFECTS OF REPLACING PREVNAR 7 WITH PREVNAR 13 ON INVASIVE PNEUMOCOCCAL DISEASES IN ENGLAND AND WALES

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Background and aims: Prevnar 7, the 7-valent pneumococcal conjugate vaccine (PCV), was replaced by Prevnar 13, the 13-valent equivalent, from April 2010 in the UK. Mathematical models have been developed to estimate long-term effects of two scenarios about the use of PCV13 in England and Wales: ceasing PCV7 on April 2010 or replacing PCV7 with PCV13.

Methods: A compartmental deterministic model was used to estimate parameters governing transmission of infection and competition between different groups of pneumococcal serotypes. An individual based model using estimated parameters was developed to capture the effects of using PCV7 and PCV13 at different levels of coverage by dose. We explored a number of alternative models representing the increasing trend in invasive pneumococcal disease cases prior to PCV7 introduction, as well as different levels of coexistence between serotypes included in PCV13 but not in PCV7, against non-PCV13 serotypes.

Results: Replacing PCV7 with PCV13 is predicted to reduce the overall number of invasive pneumococcal disease (IPD) cases in all scenarios considered. Stopping PCV vaccination altogether is predicted to increase the burden of IPD until it rebounds to the levels in the pre-PCV7 era.

Conclusion: The model suggests that in the long-term, introducing PCV13 in England and Wales may cause IPD cases due to the 6 additional serotypes in PCV13 to be eliminated. However, the overall reduction in IPD will be less dramatic, since there is likely to be an increase in invasive pneumococcal disease caused by non-PCV 13 serotypes.
PNEUMOCOCCAL NASOPHARYNGEAL CARRIAGE IN PRE-SCHOOL CHILDREN, PARENTS AND OLDER ADULTS DURING THE INTRODUCTION OF THE 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE

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Background and aims: In the UK, PCV7 was introduced in 2006 for children under 2 years of age in a 3 dose schedule; 2, 4, 12 months of age and was found to be highly effective against vaccine-types, but serotype replacement reduced the overall impact against pneumococcal disease. In April 2010 PCV13 replaced PCV7 in the routine infant schedule without a catch-up campaign. We assessed the prevalence and serotype distribution of nasopharyngeal carriage in 3 generations prior to widespread use of PCV13.

Methods: Children who received 3 doses of PCV7 (aged 21 - 55 months), their parents and a cohort of community-dwelling adults aged 65 and over were recruited. A swab was taken in STGG media and cultured within 8 hours. Pneumococci were cultured, serotyped using the Quellung reaction and genotyped using multilocus sequence typing.

Results: S.pneumoniae was recovered from 46% (270/592) of children, 12% (12/100) of parents and 6.2% (37/600) of older adults. PCV13 vaccine-types were found in 20% of children (56/270). The 10 most common serotypes among children, in descending order, were: 19A, 11A, 23B, 6C, nontypeables, 3, 22F, 23A, 35F and 21. 4/12 serotypes in parents and 3/37 in older adults were vaccine-types.

Conclusions: 18 months after the launch of PCV13 in the UK, vaccine-type pneumococci were still circulating among preschool children although most isolates were non-vaccine types. Carriage rates in parents and older adults were lower. Further studies will allow monitoring of the impact of the vaccine once PCV13 immunity is fully established in early childhood.
SYSTEMATIC REVIEW OF PNEUMOCOCCAL CONJUGATE VACCINE (PCV) DOSING Schedules ON INDIRECT EFFECTS AMONG OLDER CHILDREN AND ADULTS

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Background and aims: To aid country decision making for pneumococcal conjugate vaccine (PCV) use in national immunization programs, we summarized the indirect effects of infant PCV schedules among older children and adults.

Methods: We systematically reviewed English literature published from 1994-2011 on the effects of infant PCV use on vaccine-type (VT) nasopharyngeal carriage, VT invasive pneumococcal disease (IPD), and syndromic pneumonia in unimmunized older children and adults.

Results: Of 10,205 citations reviewed, we identified 14 IPD, 5 carriage, and 9 pneumonia primary, analyzable studies. Nineteen (66%) had information on 3 primary doses plus booster (3+1), 5 (17%) on 3+0, 4 (14%) on 2+1, and 1 (3%) on 2+0. Most (93%) used PCV7.

No studies directly compared IPD or pneumonia indirect effects by infant PCV schedules; one study compared VT-carriage of 2+0 vs. 2+1 infant schedules and found no indirect effect of either. Of two controlled trials (3+0, 3+1) and two observational studies (3+1, 3+neumococcal polysaccharide vaccine booster) assessing VT-carriage, only 3+1 schedules showed significant indirect effects. All 14 IPD observational studies (2+1, 3+0, 3+1) demonstrated VT-IPD incidence reductions in adults < 65 years. In 9 observational studies (2+1, 3+0, 3+1) of pneumonia effectiveness, only 3+1 schedules showed significant indirect impact.

Conclusions: Studies of 3+1 infant PCV schedules demonstrated significant indirect effects on VT-carriage, VT-IPD and pneumonia. For 2+1 and 3+0 schedules, only an indirect effect on VT-IPD was seen. However, data paucity, especially of head-to-head and PCV10 and PCV13 studies, limits a fuller understanding of schedule impact on indirect effects.
SYSTEMATIC REVIEW OF PNEUMOCOCCAL CONJUGATE VACCINE DOSING SCHEDULES ON CLINICAL OUTCOMES AMONG YOUNG CHILDREN

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Background and aims: Pneumococcal conjugate vaccines (PCV) are rapidly being introduced into national immunization programs. To aid decision-making regarding the optimal dosing schedule, we summarized the effect of PCV schedule on clinical outcomes.

Methods: A systematic review of English literature published from 1994-2011 was conducted on the effects of PCV dosing schedules on vaccine-type (VT) nasopharyngeal carriage, VT invasive pneumococcal disease (IPD), and syndromic pneumonia among young children.

Results: Of 10,205 citations reviewed, we identified 26 carriage, 48 IPD, and 45 pneumonia analyzable studies. Of these, 84 evaluated 3 primary doses plus a booster ("3+1"), 27 "3+0", 15 "2+1", and 4 "2+0"; 90% described PCV7. Eleven studies directly compared schedules: 7 showed no significant differences; one described more 6B IPD cases with 2 primary doses compared to 3 in a "3+1" population; one carriage controlled trial showed greater reduction with "2+1" than "2+0"; and 2 (1 carriage controlled trial and 1 syndromic pneumonia case-control study) described greater short-term benefit of 3 primary doses compared to 2. Discerning quantitative differences between schedules was difficult when comparing across studies; programs using "3+1", "3+0", and "2+1" all showed significant reductions in IPD and pneumonia, although most programs also used catch-up campaigns.

Conclusions: The available literature demonstrates benefits for 3+1, 3+0, and 2+1 schedules. While some differences in effect were identified between schedules, these may not be significant in settings of a mature program with herd effects or when introduced with a catch-up campaign. More data are needed on these schedules using PCV10 and PCV13.
A LENGTHY WIDESPREAD OUTBREAK OF SEROTYPE-1 INVASIVE PNEUMOCOCCAL DISEASE SHOWS PROTECTION WITH THE 10-VALENT CONJUGATE VACCINE AND INCREASED PNEUMONIA COMPLICATIONS

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Background/aims: Outbreaks of invasive pneumococcal disease (IPD) due to serotype-1 have been well documented globally, predominantly in closed settings. The Central Australian (CA) region has exceedingly high rates of IPD however since 1994 only sporadic cases of serotype-1 disease have been reported. In October 2010 a rise in serotype-1 IPD was observed, initially in school-aged Indigenous children. New cases continue to be reported through 2011.

Pneumococcal vaccines have been in use in the CA Indigenous population for many years. The 10-valent conjugate vaccine replaced the 7-valent vaccine for infants in late 2009, with vaccine coverage greater than 85%. In those groups recommended for 23-valent vaccine, over 50% of people have had at least 1 dose.

This study describes the clinical and epidemiological features of this serotype-1 outbreak.

Methods: A laboratory-based surveillance system reports all cases of IPD. Routine and enhanced data collection since 1994 was obtained from case interviews, medical records and the regional immunisation register. Queensland Health performed the serotyping and molecular sequencing.

Results. The first 12 months of the outbreak showed a 65% (95%CI 48 to 77) increase of total IPD compared to the previous 12 months. The median age of serotype-1 cases was 11.5 years (range 3-61 years). An increase in empyema was identified. No cases occurred in children vaccinated with a 10-valent vaccine.

Conclusions: The 10-valent vaccine provides individual protection against serotype 1 disease in the outbreak setting. Complications of pneumonia in serotype-1 IPD were higher compared to other serotypes in the previous 2 years.
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PNEUMOCOCCAL COLONIZATION BEFORE AND AFTER INTRODUCTION OF 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV13) IN A POPULATION WITH HIGH DISEASE RATES, ALASKA

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Background: Invasive pneumococcal disease rates in Alaska Native (AN) children declined after conjugate vaccine (PCV7) introduction, then increased due to non-vaccine serotypes. PCV13 was introduced in one Alaska region in January 2009 and statewide in March 2010; IPD rates in children < 5 years have since fallen by >70%. We assessed nasopharyngeal (NP) pneumococcal carriage before and after PCV13 introduction.

Methods: NP pneumococcal carriage surveys were done from 2008-2011. Carriage among persons of all ages in 8 AN villages in 3 regions (population 4375) was determined each spring. Carriage among children < 5 years (AN and non-Native) was determined at urban pediatric clinics each winter. Vaccine uptake was obtained from individual medical records.

Results: Overall pneumococcal NP colonization was unchanged following PCV13 use among all-ages in rural AN (33% among a mean of 3163 persons/year) and urban children < 5 years (36%, mean 450/year). PCV13-serotype carriage declined in rural children < 5 years (26% of carriers (2008-10) to 10% in 2011, p < 0.001), but not significantly in urban children (22% to 15%, p = 0.06). In children < 5 years, we saw significant decreases in carriage of serotypes 7F and 19A. Where PCV13 was introduced first, significant decreases in PCV13 serotype carriage was observed in all age classes. Among AN children 19-35 months old in the study regions, 96% have received ≥ 3 PCV doses.

Conclusions: PCV13 use led to decreases in vaccine-type carriage among vaccinated children and their close contacts soon after introduction.
HIGH CARRIAGE OF NON-7-VALENT CONJUGATE VACCINE SEROTYPES IN WESTERN AUSTRALIAN ABORIGINAL CHILDREN AND ADULTS AFTER 10 YEARS OF CHILDHOOD IMMUNISATION

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Background and Aims: Australian Aboriginal people continue to have high rates of invasive pneumococcal disease (IPD). The 7-valent pneumococcal conjugate vaccine (7vPCV) was given in a 2-4-6 month schedule from 2001, and replaced with 13vPCV in July 2011. Monitoring pneumococcal carriage complements IPD surveillance in Western Australia (WA). Visits to Aboriginal communities are used to raise awareness of IPD and vaccination.

Methods: From 2008, nasopharyngeal swabs were collected opportunistically from Aboriginal people of all ages living in urban, rural and remote regions of WA. Swabs were cultured using selective media. Isolated pneumococci were serotyped using the Quellung reaction and tested for antimicrobial susceptibility.

Results: 1360 swabs were cultured. Pneumococci were carried in 47% of children < 6 months of age, 78% of children aged 6 months-2 years, and 33% of people ≥5 years of age. Of 42 serotypes identified, the most common were 19A, 16F and 6A in children < 5 years, and 15B, 6C and 34 in older people. 84% were non-7vPCV serotypes. Serotypes 1 and 12F were rarely carried, despite causing recent IPD outbreaks in WA. Complete penicillin resistance was found in 7% of 19A isolates, and intermediate resistance in over 50% of 33D, 9V and 19F isolates.

Conclusions: The 7vPCV immunisation program has almost eliminated carriage of 7vPCV serotypes. Some 13vPCV serotypes are now circulating in Aboriginal communities, but a high proportion are not covered by any conjugate vaccine. Ongoing surveillance of carriage is needed to monitor circulating serotypes and antimicrobial susceptibility.
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INCREASE IN INVASIVE PNEUMOCOCCAL DISEASE AMONG THE WESTERN AUSTRALIAN ABORIGINAL POPULATION DUE TO NON-VACCINE SEROTYPES, IN PARTICULAR SEROTYPE 1

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**Background:** The seven-valent pneumococcal conjugate vaccine (PCV7) program for Aboriginal children commenced in mid-2001 and reduced rates of invasive pneumococcal disease (IPD) caused by PCV7 serotypes (VTs). Recently, increasing IPD caused by non-PCV7 serotypes (NVTs) has been reported.

**Methods:** Descriptive analysis of IPD notifications among Aboriginal people in Western Australia (WA), 2001 to 2011.

**Results:** From 2001 to 2009, total Aboriginal IPD notifications fluctuated between 22 and 53 cases/year (33-75/100,000 person-years), VT IPD rates decreased from 30 to 3/100,000 while NVT IPD rates increased from 38 to 50/100,000. Aboriginal total IPD notifications increased 3-fold from 34 cases in 2009 (46/100,000) to 106 cases (140/000,000) in the period January to September 2011, the highest recorded in WA. NVTs accounted for most of the increase. Between the years 2001-2002 to 2011, annual NVT IPD rates increased 17-fold in those aged 5-14 years (6/100,000 to 97/100,000) and 9-fold in those aged < 5 years (29/100,000 to 248/100,000) and 15-29 years (11/100,00 to 102/100,000).

In 2011, serotype 1 was the most common serotype among Aboriginal people (42 cases; 41%) and cases were widely distributed across WA. It emerged in 2010 (15 cases) after being absent during 2007-2008. Other emerging serotypes included 8, 12F, and 19A. Only serotypes 1 and 19A are contained in the new 13-valent pneumococcal conjugate vaccine.

**Conclusions:** The emergence of NVTs, especially serotype 1, and recent record IPD rates in Aboriginal Western Australians are of concern. Continued surveillance is essential to monitor the impact of new generation pneumococcal conjugate vaccines.
EMERGING HIGH-LEVEL CEFOTAXIME-RESISTANT STREPTOCOCCUS PNEUMONIAE OF SEROTYPE 19F IN HONG KONG

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Background and Aims: Hong Kong introduced universal pneumococcal immunization with PCV-7 protein conjugate vaccine in September 2009. Childhood pneumococcal surveillance study and monitoring of antimicrobial susceptibilities of Streptococcus pneumoniae was conducted in a teaching hospital in Hong Kong before and after the vaccination program. Antibiotic susceptibilities, serotypes, molecular characterization and resistance determinants of the cephalosporin-resistant Streptococcus pneumoniae were further studied.

Methods: The antibiotic susceptibilities of S. pneumoniae were determined by microbroth dilution method according to CLSI and serotype distribution was determined by agglutination and PCR methods according to protocols from CDC website. Potential clonal relationships among penicillin- and/or cefotaxime-resistant isolates were studied by PFGE and MLST. Resistance mechanism(s) to cefotaxime was further elucidated through characterization of known mechanisms, including analysis of major penicillin binding protein (pbp) genes, 1a, 2b, 2x and other PBP-independent pathways.

Results: All S. pneumoniae isolates were susceptible to respiratory fluoroquinolones and linezolid. The prevalence of penicillin-nonsusceptible isolates was 9.4% whilst that for cefotaxime resistance (MIC ≥2.0 µg/ml) was 14.9% for the period 2008-10. Further elucidation on representative strains indicate a clone belonging to a single-locus variant of Taiwan-19F-14 (ST271) with cefotaxime MICs ≤32 µg/ml. Analysis of amino acid substitutions in β-lactam-resistance determinants of PBP1a, 2b, and 2x were identical in strains with MICs ranging 1-32 µg/ml.

Conclusions: A β-lactam-resistant pneumococcal clone belonging to SLV of Taiwan-19F-14 (ST271) with high-level cefotaxime resistance is identified. Established resistance mechanisms due to mutations in pbp genes do not fully explain the cephalosporin-resistance in S. pneumoniae.
MONITORING THE EFFECTIVENESS OF FINNISH NATIONAL VACCINATION PROGRAMME (NVP) OF THE 10-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV10)

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Background and aims: Finland introduced PCV10 into NVP for infants in September 2010 using a 2+1-schedule (3, 5 and 12 months) without catch-up vaccinations. THL monitors the effectiveness of NVP using national, population-based health registers and reference laboratory data. An annual birth cohort in Finland is approximately 60,000.

Methods: National Infectious Disease Register is used for monitoring changes in the incidence and serotype-distribution of invasive pneumococcal disease (IPD). Data for 2004-2008 were used as baseline, and compared with September 2010 to August 2011, following NVP-implementation. January 2009 to August 2010 was excluded since >30,000 infants received PCV10 as part of a blinded clinical trial during this time. IPD rates were stratified by serotype (vaccine-type, vaccine-related, other) and age (0-11 months, 12-23 months, 24-59 months).

Results: Comparing the baseline with post-NVP implementation, overall IPD rates per 100,000 persons/year among children 0-11, 12-23 and 24-59 months were 45.5 vs 22.9, 81.1 vs 65.8, and 21.3 vs 20.6, respectively. Among children < 1 years, the PCV10, PCV10-related, and other serotype-rates for pre- and post-NVP were 33.8 vs 18.0, 5.5 vs 0.0 and 4.8 vs 3.3, respectively. Among children who had received ≥1 doses of PCV10 as part of NVP, 5 cases of IPD were identified: 3 were vaccine serotypes; all of which occurred at age < 6 months.

Conclusions: Reduction in the IPD incidence among infants was observed within one year of NVP implementation. Continued surveillance will determine potential indirect effects among unvaccinated persons and vaccine-induced changes in the IPD serotype distribution.
Background and aims: Increasing rates of antimicrobial resistance (AMR) have been seen among invasive pneumococcal disease (IPD) cases in various populations. We aimed to describe AMR before and after PCV7 introduction.

Methods: We compared active surveillance IPD isolates from Navajo < 5-year-old (yo) children in the pre-PCV7 (1995-1997) and late-routine-PCV7 (2007-2009) eras. Serotyping was conducted by Quellung reaction and PCR for serotypes 6A and 6C. Antimicrobial susceptibility was determined by broth microdilution for 121 (63.7%) 1995-1997 and 72 (92.3%) 2007-2009 available isolates (CLSI 2011 guidelines). We imputed missing data for rate calculations.

Results: Comparing 1995-1997 to 2007-2009, < 5-yo IPD rate decreased from 222 to 110 cases/100,000 person-years (P<0.001). The rate of penicillin, erythromycin and meropenem resistance increased from 2 to 11 (P=0.03), 4 to 14 (P=0.03) and 0 to 8 (P=0.02) cases/100,000, respectively. The 19A IPD incidence increased from 7 to 21/100,000 (P=0.02), and the contribution of serotype 19A to antimicrobial resistance increased. The incidence rate (cases/100,000) of < 5-yo resistant 19A isolates increased significantly in 2007-2009 compared with 1995-1997 for penicillin (11 vs 0; P=0.002), erythromycin (11 vs 0; P=0.002), tetracycline (12 vs 0; P=0.001), meropenem (8 vs 0; P=0.02) and trimethoprim-sulfamethoxazole (14 vs 2; P=0.009). Excluding 19A isolates among non-PCV7 type IPD cases, the rates of resistance did not change between the two periods for any antimicrobial.

Conclusions: Increasing rates of antimicrobial resistance, driven by 19A have been observed among Navajo < 5-yo children. Sustained surveillance of AMR is therefore important in the PCV13 era.
Background/aims: We previously reported serotype-specific trends in pneumococcal NP colonization soon after PCV7 introduction in 2002 (Kellner et al. PIDJ 2008;27:526). Our current aim is to describe longer term trends after PCV7 and early trends after PCV13 introduction in 2010.

Methods: The Calgary Area Streptococcus pneumoniae Epidemiology Research (CASPER) team conducted 9 point-prevalence surveys of pneumococcal NP colonization in healthy children, aged 12 and 18 months and 4.5 years, bi-annually from 2003-05 (previously reported) and annually in 2006, 2010 and 2011. We determined colonization by PCV7 and PCV13 (less PCV7) serotypes, and by serotypes in neither vaccine.

Results: Over 9 surveys, 5743 children were enrolled and 997 (17.4%) were colonized with SP. From 2003-6, the colonization rate was 19.0% versus 12.3% in 2010-11 (P< 0.0001).

The most common non-vaccine serogroups/serotypes in 2010-11 included 11A, 15, 22F, 23 and 35. Serotype 19A decreased from 18.2% of all serotypes in 2010 to 7.1% in 2011 after PCV13 introduction.

Conclusions: Serotype-specific pneumococcal colonization in healthy children has profoundly changed since the introduction of PCV7 and PCV13 vaccines. Additionally, it is not clear if the recent decline in proportion of healthy children colonized with any pneumococcus is related to vaccine use.
INVASIVE PNEUMOCOCCAL DISEASE IN CHILDREN BEFORE AND AFTER THE INTRODUCTION OF PNEUMOCOCCAL CONJUGATE VACCINE IN NORTHERN GREECE: A HOSPITAL EXPERIENCE

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Aim: The aim of this study is to describe the epidemiology of invasive pneumococcal disease (IPD) in children before and after the introduction of pneumococcal conjugate vaccine (PCV) in Northern Greece (2005).

Methods: The medical records of pediatric patients with IPD that were hospitalized in the Hospital for Infectious Diseases in Thessaloniki in the period 1997-2011, were retrospectively studied.

Results: 69 children with IPD were divided into two groups, before and after the introduction of PCV: n₁=43 (1997-2004), n₂=26 (2005-2011). There was no difference in the size of these two groups (p=0.054), in the distribution of age (p=0.071) and sex (p=0.336). Streptococcus pneumoniae was isolated either from the cerebrospinal fluid (n=27), or from the blood (n=26), or from both samples (n=16). The frequencies per year and site of infection are seen in the graph. 11 of the children in the post vaccination era were immunized against pneumococcus (9/11 partially immunized), while 14 were not (1 with unknown status) (p=0.556).

Conclusions: Although the actual incidence of IPD in our area has not significantly changed, there seems to be a trend for lower incidence after the introduction of PCV. As the majority of affected children were not fully immunized, the improvement of immunization coverage of all children should be a priority for healthcare providers.
UPS AND DOWNS OF INVASIVE PNEUMOCOCCAL DISEASE (IPD) IN AUSTRALIA

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Background: National IPD surveillance data is available from 2002. A 7-valent pneumococcal conjugate vaccine (7vPCV) program for Indigenous children commenced mid 2001 (3+0 at 2,4,6 months with at-risk children getting 23-valent polysaccharide pneumococcal vaccine[23vPPV] at 18-24 months). Universal 7vPCV was introduced in 2005 (3+0 at 2,4,6 months). 23vPPV adult programs have been ongoing since the 1990s. Surveillance aims to analyse disease trends in confirmed data to 2008 and preliminary data through 2011 by age-groups, Indigenous-status, serotypes, vaccine introduction, vaccine failures and drug susceptibility.

Methods: Information collected via laboratory-based reporting and enhanced data gathered via jurisdictions through the EIPDSWG.

Results: By 2008, 4 years into universal 7vPCV program, vaccine serotype IPD decreased in all non-Indigenous age-groups compared to 2002 (95% in < 5 year olds, 74% in ≥65 year olds). However, non-7vPCV serotype IPD increased by 170% in < 5 year olds with 5-fold rise in 19A and similar 19A increases in the elderly. In the same period 7vPCV serotype IPD decreased in Indigenous < 5 year olds by 77% while non-7vPCV serotypes increased by 76% but without notable 19A increases. IPD increased in Indigenous adults ≥50 years with similar increases in 23vPPV and non-23vPPV serotypes. Preliminary data from 2010-2011 show increases in total Australian IPD cases when compared with 5-year monthly averages with serotypes 19A(20%), 7F(8%), 1(7%), 3(6%), 6C(5%) and 22F(5%) causing the most disease.

Conclusions: While gains have been made in reduction of 7vPCV IPD, non-7vPCV disease has increased with 19A being most notable serotype except in the Indigenous population.
DOWN AND UPS OF INVASIVE PNEUMOCOCCAL DISEASE (IPD) AMID VACCINE INTRODUCTIONS IN THE INDIGENOUS POPULATION OF THE NORTHERN TERRITORY, AUSTRALIA

V. Krause, H. Cook, Darwin, NT, Australia

Background/Aims: Indigenous Australians in the Northern Territory (NT) are burdened with exceedingly high rates of IPD. A 7-valent pneumococcal conjugate vaccine (7vPCV) program for Indigenous children was introduced mid-2001 (7vPCV 3+0 at 2,4,6 months with a 23-valent polysaccharide pneumococcal vaccine [23vPPV] at 18-24 months) recognising only 56% of IPD cases were caused by 7-valent serotypes. 10vPCV was introduced in October 2009 and 13vPCV in October 2011. 23vPPV adult programs have been promoted since the1990s. IPD NT-wide surveillance aims to analyse trends by age-groups, regions, Indigenous-status, risk factors, serotypes, vaccine introduction, vaccine failures and drug susceptibility.

Methods: A descriptive study of information collected via laboratory-based reporting of all NT IPD cases from 1994 with data gathered via case interviews, medical records and immunisation register. Queensland Health provides serotyping.

Results: Overall IPD in Indigenous < 5 year olds reduced by 54% from pre-7vPCV years, 1994-2000, to post-7vPCV years, 2002-2011, (288/100,000 to 131/100,000) with 7vPCV serotype IPD reducing by 93% and non-7vPCV IPD increasing by 15%. Overall IPD rates in Indigenous people ≥15 year olds in these years did not change, with 7vPCV IPD decreasing by 49% and non-7vPCV IPD increasing by 23%. A serotype-1 outbreak starting mid-2010 in older Indigenous children contributed to non-7vPCV IPD but no increase in 19A disease or antibiotic resistance emerged. 7vPCV failures are rare and no 10vPCV failures were reported.

Conclusions: While expected gains in reducing 7vPCV IPD were made in the targeted group with the 3+0 and 23vPPV schedule more is required to control IPD overall.
Pneumococcal conjugate vaccine was first introduced in the National Childhood Vaccination program in April 2011. In recent years about 7% of invasive pneumococci in Iceland and almost 40% of strains in the respiratory tract have had decreased penicillin susceptibility (PNSP). Our aim was to investigate serotype distribution and antimicrobial resistance of pneumococci in children before the introduction of nationwide vaccination.

Nasopharyngeal samples were collected in March 2009 (n=516), 2010 (n=446) and 2011 (n=420) from children attending 15 day care centres representing the Reykjavik capital area. Susceptibility testing was by disk diffusion (CLSI methods and criteria) and the E-test. All isolates were serotyped.

The carriage rate was 72%, 66% and 58% for the respective years and the PNSP prevalence about 10% for all years (non-capsulated strains excluded). PNSP were dominated by serotype 19F accounting for 71%, 83% and 100% respectively and the majority were multi-resistant with identical antimicrobial susceptibility pattern. Great fluctuation in serotype prevalence was seen between the years. PCV-10 would cover 46%, 52% and 33% of serotypes found 2009, 2010 and 2011 respectively and PCV-13 72%, 72% and 60%.

The prevalence of PNSP was much lower than in isolates from children with respiratory tract infections. All PNSP isolated in the study would be covered by PCV-10. Fewer PCV serotypes were found in the year 2011 compared to the years before.
ISPPD-8
Poster Shift 1: Monday, March 12, 2012 – Tuesday, March 13, 2012

Poster No 172

SEROTYPES IN INVASIVE PNEUMOCOCCAL INFECTIONS IN ICELAND 10 YEARS PRECEDING THE INTRODUCTION OF CONJUGATED VACCINES

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Iceland introduced pneumococcal conjugate vaccine (Synflorix®) in the National Childhood Vaccination program in April 2011. Our aim was to analyse the incidence and serotype prevalence in invasive infections before commencing the vaccinations.

All invasive pneumococcal infections in Iceland are recorded at the Department of Microbiology, Landspitali University Hospital. Information about age, type of infection and serotypes was gathered for the years 2001 to 2010. The possible serotype coverage of the two available vaccines (PCV-10, PCV-13) was calculated according to age groups. Information about deaths was obtained from the Icelandic National Registry and information about the population from Statistics Iceland (http://www.statice.is/).

A total of 448 invasive pneumococcal infections were diagnosed in Iceland during the last 10 years, giving an annual incidence of 15/100,000. The incidence was 85/100,000 in < 2 years old and 51/100,000 in patients >65 years old. Serotype coverage for all ages by the PCV-10 and PCV-13 vaccines was 72% and 85% respectively. For serotypes causing meningitis (n=27; 6%) the coverage was 56% and 78% respectively. During the study period 50 patient died (11%) within one month of the diagnosis and the coverage for pneumococci from fatal infections was 66% and 86% respectively. The serotype prevalence was different for the different age groups. The PCV coverage was highest in the youngest age groups (< 2 years), 84% for PCV-10 and 95% for PCV-13.

Information about vaccine coverage was obtained for the whole population giving an important baseline in Iceland before general vaccination and cost benefit calculations.
DETECTION OF PNEUMOCOCCAL SEROTYPE 1 CARRIAGE DURING SEROTYPE 1 INVASIVE PNEUMOCOCCAL DISEASE OUTBREAK IN CENTRAL AUSTRALIA, 2010-2011

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Background and aims: During October 2010 and continuing into late-2011, an outbreak of serotype 1 invasive pneumococcal disease (IPD) occurred in Central Australia. As part of the public health response, surveillance of serotype 1 carriage was conducted to determine epidemiological features of asymptomatic carriage that potentially could be driving the outbreak.

Methods: In March 2011, patients and their accompanying children or carers presenting at Alice Springs Hospital Emergency Department (ED) consented to nasopharyngeal swabs (NPS) and a brief demographic questionnaire. Standard culture methods with selection of 4 colonies per NPS, and serotype 1-specific real-time PCR were used.

Results: Of the 130 people swabbed, 16.2% of NPS samples were pneumococcal positive by culture with carriage highest in the under 10 year old age group. Presenters had mean age 37 years (SD±20.5years); 25% < 10 years of age and 21% from remote communities. 4 of 130 (3%) NPS were positive for serotype 1 by real-time PCR; 3 were also culture-positive. Serotype 1 had an atypical morphology (small and rough); no co-colonising serotypes were found. All serotype 1 carriers were well children 5 to 7 years of age from remote communities (~10% children < 10 years serotype 1 carriers). By MLST, serotype 1 isolates were ST306, as were the IPD isolates associated with this outbreak.

Conclusions: During an outbreak of serotype 1 ST306 IPD, carriage of the outbreak strain was detected in four of 130 (3%) NPS tested. All carriers were from healthy children 5 to 7 years of age.
Poster No 174

NATIONWIDE SURVEILLANCE OF INVASIVE PNEUMOCOCCAL DISEASE IN DENMARK FOUR YEARS AFTER INTRODUCTION OF PNEUMOCOCCAL CONJUGATE-VACCINE IN THE CHILDHOOD IMMUNISATION PROGRAMME

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Background and aims: In October 2007 pneumococcal conjugate vaccine PCV7 was introduced in the Danish childhood immunisation programme. In May 2010 use of PCV13 started. Three years after PCV-introduction a significant decrease of invasive pneumococcal disease (IPD) due to PCV7-vaccine-types (PCV7-VT) in the whole population and a minor but significant increase of nonPCV7-VT, mainly the six additional types covered by PCV13 (PCV13nonPCV7-VT), was reported. We present preliminary results on serotype distribution four years after PCV-introduction.

Methods: Invasive pneumococcal isolates were serotyped. Data from 2011 (January-September) is based on 651 isolates (24 from children < 5years).

Results: The proportion of PCV7-VT among all invasive isolates decreased from 39% pre-PCV7 (years 2000-2007) to 8% in 2011. The proportion of IPD due to PCV13nonPCV7-VT was 32% pre-PCV7 and increased to 47% in 2011. Among children < 5years the relative proportion of PCV7-VT decreased from 63% pre-PCV7 to 4% in 2011. In contrast the proportion of PCV13nonPCV7-VT was 63% in 2011 compared to 27% pre-PCV7, however the total number of IPD cases was reduced by two-thirds. In 2011 the predominant serotype was serotype 1 constituting 21% of all IPD isolates and 41% of isolates from children. None of the children experiencing serotype 1 IPD were PCV13 vaccinated.

Conclusions: IPD due to PCV7-VT in both children and the whole population continued to decrease in 2011. Although IPD was reduced by two-thirds in children compared to pre-PCV, the relative proportion of PCV13nonPCV7-VT IPD was higher in 2011 than pre-PCV with serotype 1 as the predominant serotype.
ACUTE OTITIS MEDIA WITH PERFORATION (AOMWIP) AND CHRONIC SUPPURATIVE OTITIS MEDIA (CSOM) IN AUSTRALIAN INDIGENOUS CHILDREN

A.J. Leach, C. Wigger, P. Morris, Darwin, NT, Australia

Background and aims: In Australia, only the Northern Territory (NT) childhood vaccination schedule switched from PCV7 to PHiD-CV (October 1st 2009). The NT then switched to Prevenar13 on October 1st 2011. Elsewhere, the schedule switched from PCV7 to Prevenar13 on July 1st 2011. Ongoing surveillance aims to capture shifts in disease and carriage prevalence.

Methods: Indigenous children 0 to 6 years of age and living in remote communities were eligible for ear assessments and nasal (and ear discharge where appropriate) swab collection.

Results: To date, PCV7-era and early PHiD-CV-era data included 376 and 197 child assessments. At least 3 doses of PCV7 had been given to 94% and 40%, and PHiD-CV to 0% and 40%, respectively. Mean age at assessment was 17 months and 18 months respectively. Less than 10% children had no OM; 33% and 41% had OME; 40% and 38% had AOM without perforation, 7% and 6% had AOM with perforation, and 19% and 15% had chronic suppurative OM (26% and 21% having AOMwIP or CSOM). Pneumococcal and NTHi carriage were above 70% in PCV7 and early-Synflorix eras. For 80 children having 3 doses PHiD-CV and an assessment on average 3 months after their 3rd dose, 40% had OME, 35% had AOM and 16% had AOMwIP or CSOM.

Conclusions: Preliminary analyses are encouraging with a possible reduction in AOMwIP and CSOM in PHiD-CV vaccinated children. An RCT (PREV-IX_COMBO) is underway to directly compare these vaccines and a 4-dose combination schedule commencing at one month of age.
IMPACT OF PCV13 VACCINE ON PNEUMOCOCCAL CARRIAGE IN MASSACHUSETTS CHILDREN

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Background: Surveillance of nasopharyngeal (NP) carriage of Streptococcus pneumoniae (SP) following introduction of PCV13 can assess its direct and indirect impact on colonization and identify potential replacement serotypes.

Methods: NP surveillance is ongoing among children < 60 months old attending primary care in Massachusetts. NP cultures are processed by routine microbiologic methods; isolates of SP are serotyped using Quellung reaction. Minimum inhibitory concentrations (MIC) for 5 antibiotics are determined using Etest®.

Results: During the first 15 months (7.2010 to 9.2011) SP was recovered from 507/2149 (23.6%) children. 65 (3%) carried one of the six PCV13 serotypes not found in PCV7: 19A (n=43), 3 (n=12), 6A (n=6) and 7F (n=4). Twenty (47%) 19A isolates were resistant to ≥1 antibiotic; 16/20 were resistant to ≥3 antibiotics. During Oct-Dec 2010 the age-adjusted prevalence of PCV13 serotypes peaked with 4.8 per 100 children colonized. In this quarter, the prevalence of PCV13 carriage was 12.3 per 100 [5.6/100-18.9/100] among under- or un-immunized children and 2.2 per 100 [0/100-5.2/100] among “fully” immunized children. 428/507 (84%) of colonized children carried a non-vaccine serotype (NVST); 15BC (n=73), 11A (n=55), 6C (n=41), 21 (n=29) and 35B (n=28) were 5 most prevalent serotypes.

Conclusion: Carriage of the 6 PCV13 serotypes was lower among immunized children in the first winter following its introduction; multi-antibiotic resistant 19A persists in Massachusetts’ children.
LONG-TERM IMPACT OF 7V PCV ON INVASIVE PNEUMOCOCCAL DISEASE IN GREATER SYDNEY, AUSTRALIA

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Background: All sterile site Streptococcus pneumoniae isolates have been serotyped in a single reference laboratory in the greater Sydney region (population > 5 M) since 1997. 7 valent conjugate pneumococcal vaccine (7vPCV) was funded only for children at high risk of IPD from 2001, and for all children from 2005, using a ‘3+0’ schedule (doses scheduled at 2, 4, and 6 months without booster).

Methods: Incidence of IPD by age group and serotype was compared for baseline (pre 2004) with the most recent 2 years (2008-9) using the incidence rate ratio (IRR).

Results: There were 2529 IPD cases < 5 yrs and 4099 cases in > 5 yrs; vaccine coverage increased rapidly from 2.5% to > 85% within 12 months of universal funding. In < 5 year olds, IPD incidence decreased from 69 to 20 per 100,000, with an IRR for all IPD of 0.3 (0.05 for vaccine type (VT) and 2.4 for non-vaccine type (NVT)). IRR for meningitis (164 cases, 0.31) was similar to other focal disease (462 cases, 0.45) and unlocalised bacteraemia (1277 cases, 0.22). In > 5 year olds, IPD incidence decreased from 12.3 to 8.3, IRR was 0.67 (0.2 VT and 1.06 NVT) and similar across age groups. Serotype replacement dominated by 19 A (IRR post vs pre of 4 to 5.5)

Conclusions: The data, with long stable baseline pre 7vPCV, demonstrate similar outcomes to other countries using 3+1 or 2+1 schedules. Australia moved to 13vPCV in mid 2011 in its National Immunisation Program.
WHY IS IPD INCREASING IN INDIGENOUS AUSTRALIAN ADULTS?
R. Menzies, H. Wang, Sydney, NSW, Australia

Background and aims: IPD rates remain high in Indigenous Australian adults despite national funding of 23vPPV for Indigenous adults since 1999, and 7vPCV for Indigenous children since 2001. A recent study found rates actually increasing in one state. This study examines national data and includes vaccination status, to investigate whether the vaccine has been effective in this population, known to have high rates of chronic disease.

Methods: National IPD notifications were available from 2002 to 2010. One of eight jurisdictions was excluded due to incomplete data in critical fields. IPD numbers were adjusted for missing data on Indigenous status, serotype and vaccination status, so IPD trends do not reflect changes in data completeness.

Results: IPD notifications increased by 29% from 2002-03 to 2009-10 in Indigenous people aged ≥15 years, the greatest increases were in those aged ≥50 years (109% increase). 7vPCV-type IPD decreased by 63%, 23v-non7v type increased by 88% and non-23vPPV type increased by 9%. The proportion of 23vPPV-type IPD cases that had been vaccinated in the previous 5 years decreased from 30% in 2002-03 to 21% in 2008-10, and for non-23vPPV-type cases from 60% to 44%.

Conclusions: Vaccine failures have decreased in number over time, and they are more prevalent in non-vaccine type IPD. This is consistent with a 23vPPV vaccine that is effective but for which there is low vaccination coverage. There is some evidence of herd immunity from childhood conjugate vaccination but an increase in non-7vPCV disease has resulted in an overall increase in IPD.
IMPACT OF 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV13) ON INVASIVE PNEUMOCOCCAL DISEASE (IPD), U.S. 2010-11

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Background: U.S. introduction of PCV13 began in March 2010. We evaluated routine use of PCV13 on rates of IPD.

Methods: IPD cases (isolation from sterile sites) were identified through 10 Active Bacterial Core surveillance (ABCs) sites. Isolates were serotyped at reference laboratories. We compared quarterly rates of IPD (cases per 100,000) in 2010 and the first quarter of 2011 with baseline rates from 2006-08 (excluding 2009 pandemic). Because of multiple comparisons, we considered P< 0.0025 as significant.

Results: During 2006-2008 and 2010 through quarter 1 of 2011 (Q12011), ABCs identified 937 and 296 cases of IPD, respectively, among < 2 year-olds; 87% had serotyping results. Overall and PCV13 IPD rates were lower in the fourth quarter of 2010 (Q42010) and Q12011 compared with baseline (Table). Statistically significant reductions in IPD rates due to PCV13 serotypes 7F (-86%) and 19A (-87%) were observed during Q42010 and Q12011, respectively.

<table>
<thead>
<tr>
<th>Serotypes All</th>
<th>Serotypes PCV13</th>
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</thead>
<tbody>
<tr>
<td>Quarter/Year</td>
<td>Comparison (Year)</td>
</tr>
<tr>
<td>Jan-Mar</td>
<td>43.4</td>
</tr>
<tr>
<td>Apr-Jun</td>
<td>37.1</td>
</tr>
<tr>
<td>Jul-Sep</td>
<td>22.0</td>
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<tr>
<td>Oct-Dec</td>
<td>40.3</td>
</tr>
<tr>
<td>Jan-Mar</td>
<td>19.9</td>
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*P<0.0025

[Rates of IPD (cases/100,000) among children <2yrs]

Conclusions: These findings are consistent with early impact of PCV13 on IPD among young children, driven largely by serotypes 19A and 7F.
MODELLING AS TOOL IN SETTING EVALUATION CRITERIA FOR TENDER SPECIFICATIONS FOR PNEUMOCOCCAL CONJUGATE VACCINE (PCV) IN NATIONAL IMMUNIZATION PROGRAMME (NIP)

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Background and aims: Finland introduced PCV10 into NIP in 9/2010. In 2011, a suggestion for specifications were prepared by THL for the National Advisory Board's (NAB) review to set the tender criteria for the choice of PCV from 9/2012 onwards. By 2011, data on replacement phenomenon after post PCV-introduction on large scale were widely available.

Methods: Updates on IPD incidence and pneumococcal serotypes from the National Infectious Disease Register were obtained. A mathematical model on long-term predictions of replacement, based on complete replacement of vaccine-type carriage by non-vaccine types and stable IPD case-to-carriage ratios was utilized to calculate expected replacement in IPD. In addition to this model, a scenario of 'no replacement in IPD' was considered. In subsequent cost-effectiveness analysis (CEA), costs were estimated from the health care provider perspective. CEA did not address non-invasive disease.

Results

In an incremental CEA (PCV13 vs. PCV10), the NIP effectiveness with the 'replacement' or 'no replacement' scenarios did not differ significantly. NAB ensured that decision be made on conservative assumptions by using the replacement-scenario with an acceptable cost-effectiveness ratio of 10000€ per incremental QALY. These settings determined the acceptable price difference between PCV13 and PCV10. The final tender criteria included a quality criterion, based on the difference in the number of vaccine-serotypes, which yielded equal total points at the acceptable price difference.

Conclusions: When candidate vaccines have differential expected effectiveness, tender specification need to include price and quality criteria. Incremental CEA is a necessary tool for synthesizing evidence for setting tender criteria.
UPTAKE OF PNEUMOCOCCAL POLYSACCHARIDE VACCINATION AMONG WORKING-AGE ADULTS WITH UNDERLYING MEDICAL CONDITIONS, UNITED STATES 2009

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Background and aims: U.S. Advisory Committee on Immunization Practices (ACIP) recommends pneumococcal polysaccharide vaccine (PPSV23) for non-elderly adults with certain medical conditions. In 2008, ACIP added asthma and cigarette smoking as PPSV23 indications. The 13-valent pneumococcal conjugate vaccine (PCV13) is currently under licensure review for use in adults ≥50 years. To assess the feasibility of adult PCV13 program and identify potential strategies to improve coverage, we estimated national PPSV23 uptake among non-elderly adults.

Methods: To identify factors independently associated with receiving PPSV23, we used data from 2009 National Health Interview Survey, multivariable logistic regression and predictive marginal analyses.

Results: In 2009, 35.2 million adults 18-64 years (18.6%) had established PPSV23 indications; adding asthma and smoking increased high-risk population to 71.6 million (37.9%). Overall PPSV23 coverage was 26.1% for established indications and 17.4% for all indications; coverage among persons 50-64 years was significantly higher compared with 18-49 years overall (34.6% vs. 16.7%; p< 0.001), and for all specific indications, except cancer. For asthmatics or smokers without other indication, coverage was 12.3% and 8.5%, respectively. Among persons with established indications, older age, white race, unemployment, more physician visits, hospitalization, having regular physician and health insurance were independently associated with PPSV23 receipt.

Conclusions: PPSV23 uptake varies substantially by age and indication, but remains low with about 59 million unvaccinated, high-risk working-age adults. As infrastructure and resources to support adult vaccination are limited, achieving sufficient coverage with adult PCV13 is uncertain. Effective strategies are needed to improve pneumococcal vaccination uptake among at-risk groups.
Poster No 182

SELECTION OF OPTIMAL SEROTYPE COMPOSITIONS FOR PNEUMOCOCCAL CONJUGATE VACCINATION UNDER SEROTYPE REPLACEMENT

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Background: The potential adverse effects of serotype replacement are a major concern when implementing pneumococcal conjugate vaccines in routine programmes. We present a quantitative tool to investigate the implications of serotype replacement on the net effectiveness of vaccination against invasive pneumococcal disease (IPD) and to guide in the selection of optimal vaccine serotype compositions.

Methods: Assuming complete elimination of vaccine-type carriage, its full replacement by non-vaccine-type carriage and stable case-to-carrier ratios (probability of IPD per carriage episode), we project post-vaccination IPD incidences for currently available vaccine serotype compositions (7-, 10- and 13-valent vaccines). We apply a sequential algorithm to search for the optimal serotype composition and assess the robustness of inferences to uncertainties in data and assumptions about carriage and IPD.

Results: The use of existing pneumococcal conjugate vaccines in Finland is expected to decrease the IPD incidence among the target age group of children < 5 years of age. However, replacement may entirely erode the benefits of reduction in vaccine-type IPD among the elderly. In general, more optimal compositions than the currently available ones exist. Some serotypes with low case-to-carrier ratios should not be included in the vaccine.

Conclusions: Reliable pre-intervention data on age- and serotype-specific incidence of carriage and on case-to-carrier ratios are essential in predicting the net effectiveness of a vaccination programme. The serotype composition of currently available pneumococcal vaccines may not be optimal. The effectiveness of vaccination could be improved by including new serotypes in the vaccine or by excluding existing vaccine serotypes.
Early Impact of PCV13 on Nasopharyngeal Colonization among Vaccinated American Indian Children and Unvaccinated Household Members

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Background and aims: 13-valent pneumococcal conjugate vaccine (PCV13) replaced PCV7 in March 2010 among Navajo and White Mountain Apache (N/WMA) communities. We evaluated PCV13 impact on serotype-specific NP colonization from January 2010-March 2012.

Methods: A NP swab was collected from N/WMA of all ages enrolled into this prospective, community-based, cross-sectional study. Pneumococci were isolated by culture following broth enrichment and serotyped (Quellung). Analyses compare NP colonization prevalence before and after PCV13 introduction.

Results: Between January 2010-September 2011, 5164 participants enrolled (<2y,N=1551; 2-7y,N=998; 8-17y,N=471; 18-49y,N=1994; 50+y,N=150). Of swabs tested through June 2011, 35% (1347/3875) had ≥1 pneumococcus (<2y,53%; 2-7y,54%; 8-17y,30%; 18-49y,12%; 50+y,11%). Of positive swabs, 15% were PCV13 vaccine-type [VT] (201/1347) with a decreasing prevalence over time for <18y. Comparing January-April 2010 (PCV7 era) and 2011 (PCV13 era) for <2y and 2-7y, PCV13-VT significantly decreased in both groups (<2y: 10%(2010)-2.8%(2011), p=0.0003; 2-7y: 10.4%(2010)-4.9%(2011), p=0.04) particularly for 19A (<2y: 7.2%(2010)-1.3%(2011), p=0.0003; 2-7y: 5.5%(2010)-1.5%(2011), p=0.03). Vaccine-associated 6C colonization has not significantly decreased (<2y: p=0.2, 2-7y: p=0.6); however, a single 6C was isolated among 111 pneumococci from children <8y between May-June 2011. Some common serotypes in 2011 are similar across ages.

<table>
<thead>
<tr>
<th>Age group</th>
<th>&lt;2 years</th>
<th>2-7 years</th>
<th>8-17 years</th>
<th>18-49 years</th>
<th>50+ years</th>
</tr>
</thead>
</table>

[Five most common serotypes by age group in 2011]

Conclusions: Pneumococcal colonization is common among N/WMA children <8y in the PCV13 era. PCV13-VT colonization (19A) has decreased among children <8y. A decreasing trend of 6C colonization among <2y is emerging.
EFFECT OF THE SEVENVALENT VACCINE ON QUANTITATIVE BACTERIAL LOAD OF PNEUMOCOCCAL NASOPHARYNGEAL CARRIAGE

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Background: Pneumococcal conjugate vaccination has lead to widespread herd protection in non-vaccinated populations due to impact on carriage. We assessed effect of conjugate vaccine (PCV7-CRM) against nasopharyngeal carriage using semi-quantitative assessment of bacterial load.

Methods: 1662 children recruited into FinOM Trial were vaccinated at 2,4,6 and 12 months with PCV7-CRM or control vaccine during 1995-99. At scheduled check-ups at 12 and 18 months, nasopharyngeal swab (NPS) was taken, immediately plated on chocolate and gentamicin blood agar plates, incubated overnight and sent for bacteriological identification and serotyping of *Streptococcus pneumoniae*. Quantitative assessment was performed semi-quantitatively on the primary culture plates. Number of colonies on the plate with most abundant growth was evaluated using a three-point scale: +(1-20 colonies); ++(21-99); +++(>=100).

Results: NPS was successfully obtained from 98% of children at 12 and 96% at 18 months of age. The semi-quantitative assessment showed lower number of bacterial colonies in the PCV7-CRM recipients at 18 months. The PCV7-CRM reduced the vaccine-type carriage prevalence 17% (95%CI -9-37) at 12 and 40% (95%CI 22-54) at 18 months of age. Using semi-quantitative cut-points for high-density (+++ carriage produced higher point estimates for vaccine effect (33% (95%CI 0-55) at 12 and 61% (95%CI 42-74) at 18 months of age).

Conclusions: There was a quantitative impact on culture-positive samples even with crude and non-standardized semi-quantitative methods used. Thus, PCV7-CRM not only reduced the prevalence of vaccine-type carriage but also the bacterial load in the remaining carriers. This probably further lowers transmission in populations contributing to herd protection.
IMPACT OF PCV13 ON ANTIBIOTIC-RESISTANT STREPTOCOCCUS PNEUMONIAE COLONIZATION IN MASSACHUSETTS, USA

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Background: PCV7 use caused an initial decline in antibiotic-resistant S. pneumoniae colonization in Massachusetts, followed by a rise, primarily due to serotype 19A. We sought to evaluate rates of antibiotic resistance in pneumococcal isolates after PCV13 use began in April 2010 in Massachusetts.

Methods: Children 0-7y seen by healthcare providers in 8 Massachusetts communities between Oct 2010 and Mar 2011 were eligible. Pneumococcal isolates were serotyped and classified as PCV7 (4, 6B, 9V, 14, 18C, 19F, 23F), PCV13-additional (1, 3, 5, 6A, 7F, 19A), or non-PCV13 serotypes. E-test MICs and multilocus sequence types (MLST) were determined. Pneumococcal isolates obtained in 2010-11 (post-PCV13) were compared to isolates obtained pre-PCV13 (2006-07 and 2008-09 combined) using generalized linear mixed models to account for clustering by community.

Results: Among 1,057 children swabbed, non-significant increases in antibiotic resistance were noted in the post vs. pre-PCV13 era for penicillin (7.1% vs. 4.8%), ceftriaxone (10.9% vs. 7.5%), erythromycin (28.3% vs. 24.2%), clindamycin (10.6% vs. 8.6%), and vancomycin (0.6% vs. 0.2%). Though 19A colonization decreased among children overall (4.4% vs. 3.7%), it constituted 96% of penicillin-resistant and 68% of ceftriaxone-resistant isolates. Using MLST, ST320 (46%) was most common among 19A isolates in 2011, followed by ST695 (18%) and ST199 (13%).

Conclusions: During the first year of PCV13 use in Massachusetts, no reduction in antibiotic resistance was observed among pneumococcal isolates in the nasopharynx. In fact, a non-statistically significant increase was identified despite a declining trend for 19A colonization.
INVASIVE PNEUMOCOCCAL DISEASE (IPD) SURVEILLANCE IN MASSACHUSETTS: EVOLVING IMPACT OF PCV13 IN CHILDREN < 2 YEARS OF AGE

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Introduction: Following the success of 7-valent conjugate vaccine (PCV7), serotypes 19A and 7F emerged causing ~60% of residual IPD in MA between 2007 and 2010. PCV13 with six additional serotypes(1,3,5,6A,7F,19A) replaced PCV7 for the birth cohort in April 2010; in addition a single ‘catch-up’ dose was recommended for children 14-59 months.

Methods: Enhanced IPD surveillance is ongoing in MA since 2001. All IPD cases in children < 18 years of age and available Streptococcus pneumoniae(SP) isolates are submitted to MDPH and parents/physicians are interviewed for case data including vaccine history.Available isolates are confirmed as SP and serotyped by Quelling.

Results: The incidence of IPD fell to 14.3 per 10^5 children < 5 years in the first year following PCV13 introduction compared to mean for the prior 8 and 3 years respectively (20.3 and 21.7/10^5).The incidence of IPD due to 19A and 7F among children < 2 years was 9.7 and 1.9/10^5 respectively; comparable to those observed during the 3 prior years (10.8 and 4.6/10^5 respectively).In 15 months since introduction of PCV13, serotype 19A and 7F combined were recovered from 49% of cases in < 2 and 71% of cases 2 to 5 years. Among 39 vaccine serotype cases, 28 had received 0 doses; only 4 had received age appropriate dosing.

Conclusion: Serotypes 19A and 7F remain the dominant cause of IPD 15 months following introduction of PCV13; early experience suggests the incidence and proportion of IPD cases due to these serotypes have begun to decline in children < 2 years.
IMPACT OF PCV13 ON STREPTOCOCCUS PNEUMONIAE COLONIZATION IN MASSACHUSETTS, USA

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Background: In April 2010, 13-valent pneumococcal conjugate vaccine (PCV13) replaced PCV7 in Massachusetts. Prevalence of pneumococcal colonization by serotype was evaluated in serial cross-sectional surveillance.

Methods: Children 0–< 7y seen by healthcare providers in 8 Massachusetts communities between Oct 2010–Mar 2011 received nasopharyngeal swabs, and pneumococcal isolates were serotyped by Quellung and classified as PCV7 (4, 6B, 9V, 14, 18C, 19F, 23F), PCV13-additional (1, 3, 5, 6A, 7F, 19A), or non-PCV13 serotypes. Vaccination status was obtained by chart review. Isolates from the 2010-11 season (post-PCV13) were compared to isolates obtained pre-PCV13 (2006-07+2008-09 combined) using generalized linear mixed models to account for clustering by community.

Results: 1,057 swabs were obtained from children < 2y (48%) and 2–< 7y (52%). Pneumococcal colonization was similar in the pre vs. post-PCV13 era (29% vs. 32%, p=0.13), however, fewer children were colonized with PCV13-additional serotypes (6.2% vs. 4.9%, p=0.16) and more were colonized with non-PCV13 serotypes (22.4% vs. 26.5%, p=0.012). The most common serotypes were 15B/C (5.1%), 19A (3.6%) and 6C (2.8%) in 2010-11, with significant increases noted for serotypes 15B/C (p=0.007), 21 (p< 0.001) and 3 (p=0.03). Children < 2y were more likely up-to-date for PCV13 (85%) compared to children 2–< 7y (30%); greater declines in carriage with PCV13 serotypes were noted in < 2y (26% vs. 15%, p=0.007) vs. 2–< 7y (21% vs. 19%, p=0.61).

Conclusions: During the first year of PCV13 use in Massachusetts, PCV13 serotype carriage declined in children < 2y. Herd effects are not yet evident in older children.
CONSEQUENCES AND IMPACT OF THE VACCINATION: MONITORING OF ANTIBIOTIC RESISTANCE AND SEROTYPE DISTRIBUTION OF STRAINS \textit{STREPTOCoccus PNEUMONIAE} IN SLOVAKIA

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\textbf{Background and aims:} In 2010, \textit{Streptococcus pneumoniae} (pneumococcus) was responsible for more than 80\% cases of acute otitis media (AOM) among children in Slovakia. The aim of this study is to assess the distribution of serotypes and antibiotics resistance among pneumococcal strains causing AOM among children under 5 years old. This surveillance is submitting results of pneumococcal distribution and antibiotics resistance before and after introduction of pneumococcal conjugate vaccines into the immunization schedules in Slovakia.

\textbf{Methods:} If AOM was diagnosed, middle ear fluid was aspirated for cultivation of bacterial pathogens. Cultivation of clinical samples in laboratory followed standard procedures and susceptibility testing was performed according to EUCAST and CLSI guidelines. Pneumococcal strains were serotyped using latex agglutination and Quellung reaction.

\textbf{Results:} Serotypes 23F (29.5\%), 14 (24.6\%) and 18C (13.1\%) were most common among AOM isolates in 2006. Results of most frequent serotypes obtained in 2010 are as follows: 3 (21.53\%), 19F (13.84\%) and 23F (13.84\%). Trends in resistance to antibiotics point to decreasing resistance to of 13.1\% in 2006 to 3.1\% in 2010. During the years was confirmed high resistance to macrolides repeatedly: 47.5\% (2006), 40.9\% (2009) and 49.2\% (2011).

\textbf{Conclusion:} The overall coverage of serotypes contained in PCV7 causing AOM decreased from 88.5\% in 2006 to 53.84\% in 2010. PCV13 included 87.69\% strains coverage in 2010. The most important was the expansion of the serotype 3, as well as the detection of serotypes 18A (6.2 \%) and 18F (4.6 \%), which haven't appeared in Slovakia so far.
FIRST IMPACT DATA OF 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV13) ON INVASIVE PNEUMOCOCCAL DISEASE IN CHILDREN IN MADRID, 2010-2011 (HERACLES STUDY)

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Background and aims: Systematic use of PCV13 for children aged < 2 years started in Madrid (approx. 6 million inhabitants) in June 2010 (2+1 schedule), with flawless transition from PCV7 that was introduced as NIP in November 2006 (3+1 schedule). The HERACLES study aimed to assess changes in the incidence rate of invasive pneumococcal disease (IPD) in hospitalized children (< 15 years) before and after PCV13 implementation in Madrid.

Methods: A prospective, laboratory-confirmed (culture and/or PCR) IPD surveillance study was performed from May 2007 to April 2011 in Madrid, in all hospitals with Paediatric department (27 centres). The incidence of IPD (cases per 100,000) from May 2010-April 2011 was compared to May 2007-April 2010 (pre-PCV13) by age group.

Results: A total of 115 IPD cases were identified from May 2010-April 2011 compared with 499 pre-PCV13 [163 cases: May 2007-April 2008; 167 cases: May 2008-April 2009; 169 cases: May 2009-April 2010]. Table shows incidence rates per age groups before and after PCV13.

<table>
<thead>
<tr>
<th>Age group</th>
<th>May 2007-April 2010</th>
<th>May 2010-April 2011</th>
<th>Decrease %</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2y</td>
<td>43.96</td>
<td>24.92</td>
<td>43.31</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>≥ 2y - &lt; 5y</td>
<td>29.00</td>
<td>22.72</td>
<td>21.64</td>
<td>NS</td>
</tr>
<tr>
<td>≥ 5y - &lt; 15y</td>
<td>6.25</td>
<td>4.21</td>
<td>32.69</td>
<td>NS</td>
</tr>
<tr>
<td>&lt;15y</td>
<td>17.09</td>
<td>11.34</td>
<td>33.62</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Conclusions: A decline in the IPD incidence rate for children up to 15 years of age was observed in Madrid 11 months after PCV13 introduction, mainly due to the impact on the young children (< 2 years).
SEROTYPE DISTRIBUTION OF INVASIVE PNEUMOCOCCAL DISEASE CASES AFTER THE INTRODUCTION OF 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV13) IN CHILDREN IN MADRID

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Background and aims: Systematic use of PCV13 for children aged < 2 years started in Madrid in June 2010. HERACLES study aimed to assess changes of serotypes causing invasive pneumococcal disease (IPD) in hospitalized children (< 15 years) before and after PCV13 implementation.

Methods: A prospective, laboratory-confirmed (culture and/or PCR) IPD study was performed from May 2007 to April 2011 in Madrid. All isolates (for serotyping) and culture-negative pleural/cerebrospinal fluids were sent to the reference laboratory for pneumolysin and autolysin genes PCR analysis.

Results: Table shows serotype distribution by study period [N]. Rates (cases per 100,000) of IPD due to PCV13-ST significant decreased after PCV13 introduction [from 13.49: May 2007-April 2010 to 8.78: May 2010-April 2011; 34.94%, p< 0.001].

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<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>50</td>
<td>54</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>8</td>
<td>11</td>
<td>8</td>
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<tr>
<td>5</td>
<td>34</td>
<td>17</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>6A</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>7F</td>
<td>14</td>
<td>14</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>19A</td>
<td>23</td>
<td>39</td>
<td>48</td>
<td>28</td>
</tr>
<tr>
<td>PCV7</td>
<td>9</td>
<td>6</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Other/non-typeable isolates</td>
<td>24/14</td>
<td>30/3</td>
<td>20/14</td>
<td>20/6</td>
</tr>
<tr>
<td>TOTAL</td>
<td>163</td>
<td>167</td>
<td>169</td>
<td>115</td>
</tr>
</tbody>
</table>

Conclusions: Despite the recent introduction of PCV13 in Madrid, early effects on IPD in children < 15 years due to PCV13-ST have been observed, particularly for serotypes 19A and 1.
LONG-TERM EFFECT OF 10-VALENT PNEUMOCOCCAL NON-TYPEABLE HAEMOPHILUS INFLUENZAE PROTEIN D CONJUGATE VACCINE (PHID-CV) ON NASOPHARYNGEAL BACTERIAL CARRIAGE IN CZECH CHILDREN


Background/aims: PHID-CV has been previously shown to reduce nasopharyngeal carriage (NPC) of vaccine pneumococcal serotypes (VTs) in the second year of life in Panamanian (COMPAS/NCT00466947 [randomised study]) and Czech children (study 014/NCT00496015 [partially randomised study]). There was also a trend towards increased carriage of non-VTs in study 014. This follow-up study (study 042/NCT00950833) investigated the long-term effect of PHID-CV on NPC in the latter study.

Methods: Children in a PHID-CV group (primed aged 3,4,5 months and boosted aged 12-15 months) and Control group (no pneumococcal vaccine, age-matched at booster to receive 1 dose of MenACWY-TT) were followed-up for NPC assessment at 2 time-points (average age 38 and 43 months). Swabs were cultured for pathogen identification; Streptococcus pneumoniae (Spn) and Haemophilus influenzae (Hi) isolates were (sero)typed; Hi/non-typeable Hi (NTHi) were discriminated from H. haemolyticus by PCR.

Results: The NPC according-to-protocol cohort comprised 216 and 210 children in the PHID-CV and Control groups, respectively. NPC of VTs was significantly reduced in the PHID-CV group, while there were trends towards reduced pneumococcal carriage (any Spn serotype) and increased carriage of non-vaccine, non-cross-reactive serotypes (Table). No consistent trend on impact on NTHi NPC was observed.

Conclusions: These observations indicate a long-term reduction of NPC of VTs following PHID-CV vaccination in the 3+1 schedule.

<table>
<thead>
<tr>
<th>Table 1. Impact of PHID-CV on nasopharyngeal carriage</th>
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<tbody>
<tr>
<td>Sampling time point</td>
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<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Pre-booster</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>12 mo post-booster</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>16–32 mo post-booster</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>25–38 mo post-booster</td>
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<td></td>
</tr>
</tbody>
</table>

no, months; VE, vaccine efficacy – estimated as [{1-relative risk}*100] with 95% confidence intervals (CI)
*p-value (two-sided conditional exact test <0.05 without any correction for multiplicity
†Data presented here only include the children who continued into the 042 follow-up study
Note: the study was not designed to demonstrate carriage differences between groups

[Table 1]
INVASIVE PNEUMOCOCCAL DISEASE IN PORTUGAL: SEROTYPE DISTRIBUTION AFTER SEVEN YEARS OF PCV7 INTRODUCTION


Background and aims: We have previously evaluated the effect of PCV7 in invasive pneumococcal infections (IPD) immediately after its introduction in Portugal. We showed that even a modest vaccine uptake (43%) can induce changes in serotype distribution, both in children and in adults. To evaluate the effect of increasing uptake (75%) and estimate the potential effect of PCV10 and PCV13 we characterized the pneumococcal population after 7 years of PCV7 introduction.

Methods: Between 2006 and 2008, 1451 isolates were recovered from IPD: 352 from children ≤17 years, 602 from adults 18-64 years and 497 from patients ≥ 65 years. Strains were characterized by serotyping and antimicrobial resistance profiling.

Results: PCV7 serotypes accounted for 17% and 18% of pediatric and adults IPD, respectively, representing a decline relative to the pre-vaccine and early post-vaccine period. The most common serotypes were 1, 7F and 19A in children and 3, 1, 7F and 19A in adults. Since PCV10 and PCV13 include these emergent serotypes, 46% and 71% of all IPD, respectively, could be prevented by these vaccines. PCV7 serotypes still accounted for 46% and 38% of penicillin non-susceptibility and macrolide resistance, respectively. PCV13, which includes serotype 19A, one of the most resistant serotypes, could prevent a higher proportion of IPD caused by resistant isolates (80% and 78%, respectively).

Conclusion: High PCV7 coverage resulted in a decline in IPD due to vaccine serotypes in all age groups. PCV13 has the potential to prevent a significant fraction of IPD also covering the majority of resistant isolates.
GENETIC DIVERSITY OF PNEUMOCOCCI RECOVERED FROM IPD IN PORTUGAL BEFORE PCV13 INTRODUCTION


Background and aims: PCV13 was introduced in Portugal in 2010 replacing PCV7 that was available since 2001. We have previously reported changes in serotype distribution of invasive pneumococci both in children and in adults after PCV7 introduction. Nevertheless little is known about the effect of the vaccine in the clonal structure. To evaluate the effect of PCV7 in pneumococcal clones we identified the major genetic lineages in circulation after 7 years of PCV7 use.

Methods: This study included 1451 pneumococcal isolates associated with IPD between 2006-2008 in Portugal. Strains were characterized by PFGE and MLST complementing serotyping and antimicrobial resistance data.

Results: The most frequent clones presented non-PCV7 serotypes 1, 7F, 3 and 19A and were mainly characterized by ST306, ST191, ST180 and ST276, respectively. These clones were found in all age groups although with different frequencies. The major clones of serotypes 1, 7F and 19A were already present in circulation before PCV7 introduction and their frequency has increased. The increasing 19A clone in Portugal was not the same reported in USA (ST320), suggesting geographical differences in pneumococcal clones. Non-susceptibility to penicillin was associated with clones 19A/ST276 and 14/ST156 while resistance to macrolides with 19A/ST256, 19A/ST193 and 14/ST9.

Conclusions: There was an expansion of pre-existing lineages presenting non-PCV7 serotypes after PCV7 introduction. Although the current major clones belonged to PCV13, minor clones associated with non-PCV13 serotypes were identified and may rise in frequency after introduction of this vaccine.
CO-MORBIDITIES IN CHILDREN WITH INVASIVE PNEUMOCOCCAL DISEASE FOLLOWING THE INTRODUCTION OF ROUTINE PNEUMOCOCCAL CONJUGATE VACCINATION IN ENGLAND AND WALES

M.P.E. Slack, S. Ladhani, P. Kaye, E. Miller, London, UK

Background and aims: PCV7 was added to the UK infant immunisation programme in September 2006, and replaced with PCV13 in April 2010. This study describes risk factors, clinical presentation and outcome of invasive pneumococcal disease (IPD) in children eligible for vaccination.

Methods: The Health Protection Agency (HPA) initiated enhanced national IPD surveillance following PCV7 introduction, obtaining clinical and vaccination histories for all laboratory-confirmed cases from clinicians.

Results: 1,577 children aged ≤5 years had IPD over the 43-month surveillance period. 90.1% (1432/1577) isolates were serotyped. PCV7-IPD cases were less likely to have been vaccinated than other serotypes (97/293 [33.1%] vs. 882/1140 [77.4%]; $\chi^2=211; P<0.0001$), while vaccinated cases developing PCV7-IPD were more likely to have co-morbidities (23/97 [23.7%] vs. 23/196 [11.7%]; $\chi^2=7.0; P=0.008$]. Compared with PCV7-IPD (47/295, 15.9%), co-morbidities were less common in IPD cases caused by the extra 6 PCV13 serotypes (73/672, 9.8%; $\chi^2=7.5; P=0.006$) but more common in non-PCV13 IPD cases (88/400, 22.0%; $\chi^2=4.0; P=0.046$). Pneumococcal-attributable case fatality was 4.8% (75/1,577 cases) and higher in those with co-morbidities (20/224 [8.9%] vs. 55/1353 [4.1%]; $\chi^2=10.0; P=0.002$), but comparable for PCV7-IPD (16/295, 5.4%), the 6 extra PCV13 serotypes (27/746, 3.6%) and non-PCV7-IPD (24/395 6.1%).

Conclusions: Following PCV7 introduction, PCV7-IPD cases occurred mainly in unvaccinated children or in vaccinated children with co-morbidities. The higher proportion of co-morbidities among IPD cases caused by non-PCV13 serotypes suggests these serotypes might be less virulent and more likely to infect vulnerable children, who may benefit from added protection with higher valency vaccines.
**Poster No 195**

**PNEUMOCOCCAL DISEASE IN CHILDREN: CHANGING SEROTYPES AND ANTIMICROBIAL RESISTANCE IN A TERTIARY HOSPITAL IN GREECE (2002-2011)**


**Background and aims:** To describe serotype distribution and antimicrobial susceptibilities of *S. pneumoniae* isolates causing invasive pneumococcal disease (IPD) or acute otitis media (AOM) in childhood.

**Methods:** The study was conducted in a tertiary pediatric hospital in Athens, between 2002-2011. Pneumococcal isolates were analyzed by epidemiological year and compared for the periods pre- and post-PCV7 implementation in the National Immunization Programme. Serotyping was performed by latex agglutination and Quellung reaction using antisera. Susceptibilities to antimicrobials were determined by E-test and interpreted by the CLSI criteria.

**Results:** A total of 579 isolates (IPD: 282 and AOM: 297) were collected; 263 pre-PCV7 (7/2002-6/2006), 253 post-PCV7 (7/2006-6/2010) and 63 post-PCV13 (7/2010-6/2011). Before PCV7, 74.9% of isolates were covered by PCV7; the commonest serotype for IPD was 14 (31.4%) and for AOM 19F (50.0%). Post-PCV7 coverage for PCV7, PCV10 and PCV13 was 27.3%, 44.3% and 79.8% respectively. Serotypes 7F (23.6%) and 19A (13.2%) were most prevalent for IPD and 19A (26.5%) and 19F (22.4%) for AOM. One year after PCV13 the burden from 19A remains substantial for both IPD (26.1%) and AOM (25.0%). Penicillin resistance (≥2 μg/mL) was observed in 11.8% and 2.0% of IPD and in 30.0% and 12.0% of AOM isolates in the pre- and post-PCV7 periods respectively. Resistance to penicillin for serotype 19F decreased from 11% pre-PCV7 to 3.7% post-PCV7, whereas for 19A increased from 3.7% post-PCV7 to 4.9% post-PCV13.

**Conclusions:** Following PCV7 introduction, IPD and AOM are caused mainly by serotypes 7F and 19A both included in PCV13.
Poster No 196

THE IMPACT OF 7-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV7) ON INVASIVE PNEUMOCOCCAL DISEASE (IPD): A LITERATURE REVIEW

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Background and aims: Prevenar (PCV7) is licensed for the prevention of pneumococcal diseases. This review aimed to evaluate the impact of PCV7 on pneumococcal diseases in industrialised countries.

Methods: A literature review was performed to assess the effectiveness and impact of PCV7 on vaccine type (VT) and all-type IPD in vaccine eligible and overall populations.

Results: A total of 1007 publications (2000-2011) identified, 429 articles short-listed and 81 articles (15 countries) included. The vaccine effectiveness on VT-IPD was 75-96% for ≥1 dose and 90-96% for complete schedule. In children < 2 years old, VT-IPD decreased from 10% (Australia) to 99% (US) (median 80%). Similarly, all-type IPD decreased from 2 to 85% (median 61%). In the overall population, VT-IPD decreased from 1 to 94% (median 65%) and all-type IPD from 10 to 53% (median 34%). A higher decrease was observed in the US reflecting higher pre-vaccination incidence. For all-type IPD, >30% reduction was observed only one year after introduction in Europe and >60% reduction was observed in the US within 2 years of PCV7 introduction which has been sustained.

Conclusions: Substantial reductions in VT and all-type IPD was observed in vaccine-eligible and overall populations after PCV7 introduction. The impact was influenced by pre-vaccination disease epidemiology, time since introduction, vaccine coverage evolution and variability in surveillance systems over the time.
THE IMPACT OF 7-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV7) ON PNEUMONIA AND MORTALITY: A LITERATURE REVIEW

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Background and aims: Prevenar (PCV7) is licensed for the prevention of pneumococcal diseases. This review aimed to evaluate the impact of PCV7 on pneumococcal diseases in industrialised countries.

Methods: A literature review was performed to assess the effectiveness and impact of PCV7 on pneumococcal and all-cause pneumonia and mortality in vaccine eligible and overall populations.

Results: A total of 1007 publications (2000 to 2011) identified, 429 articles short-listed and 81 articles included. Twelve studies described the impact on pneumococcal pneumonia (4) and all-cause pneumonia (8).

A decline in pneumococcal pneumonia was observed in all age groups. In children < 2 years old, 57 to 71% and 15 to 68% (median 37%) reduction was observed for pneumococcal and all-cause pneumonia, respectively. The highest impact was observed for hospitalisation (vs. outpatient visits), during earlier post-vaccination periods (vs. late periods), in younger children (< 2 years) (vs. 2-4 years) in the US. Mortality due to all-type IPD declined in all ages, ranging from 33 to 63% in children and 17 to 50% (median 34%) in older ages.

Conclusions: A decline in pneumonia and all-type IPD related mortality was observed, however, the impact varied greatly by age and case definition. The data was further confounded by the low specificity of outcomes.
EPIDEMIOLOGY OF S. PNEUMONIAE MENINGITIS FOLLOWING THE INTRODUCTION OF PCV7 IN GREECE: A 10-YEAR DATA ANALYSIS (2000-2010)


Background: S. pneumoniae is a common cause of bacterial meningitis in Greece. PCV7 was introduced for children < 5 yrs old in 2006 but was fully reimbursed in 2008. Aim of this study was to describe the epidemiology of pneumococcal meningitis (SPM) following introduction of conjugate vaccine.

Methods: Data of notified cases were reviewed retrospectively and were compared between three periods: A (pre-vaccine 2000-2005), B (transient 2006-2008) and C (post-vaccine 2009-2010). Three mPCR assays were performed; one genus specific and two for serotype identification.

Results: Of 521 SPM cases notified, 475 (91.2%) were confirmed by culture (n=185, 38.9%) and/or PCR (n=376, 61.1%). Overall annual incidence was estimated at 0.54/100,000 and by age group as follows: 2.52 (0-4 yrs), 0.58 (5-17yrs), 0.3 (18-49 yrs), 0.58 (50-59 yrs) and 0.52 (≥60 yrs). No change in mean incidence was noted between different time periods. A significant decrease was observed among the 0-4 yr olds: 3.17 vs 2.65 vs 1.76/100,000 for A, B and C respectively (p< 0.05). An increased trend was noted among ≥60 year olds (p=NS).

Most prevalent serotypes in period A: 14 (50%) followed by 23F (13.4%) and 6 (12.3%), in B: 3 (29.3%), 19F (26.8%) and 6 (21.9%) while in C there was further increase of 3 (33.3%) followed by 23 F (16.6%) and 19A (12.5%).

Conclusions: Incidence of SPM decreased significantly among younger children after the introduction of PCV7. Replacement with non-vaccine serotypes such as 3, and 19A highlight the need of prevention with new vaccines.
EFFECTS OF IMMUNIZATION WITH HIGHER VALENT PNEUMOCOCCAL CONJUGATE VACCINES IN GERMAN CHILDREN ON NUMBERS OF REPORTED IPD CASES

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Background and aims: A general recommendation for vaccination with pneumococcal conjugate vaccine was issued for German children < 2y in July 2006. In 2009, two higher valent PCVs were licenced in Germany: PCV10 in April 2009, PCV13 in December 2009. In this study, we present data on cases of IPD sent in for serotyping in the seven years following the start of PCV-vaccination, focusing on the effect on the new serotypes in PCV10 and PCV13.

Methods: Pneumococcal isolates recovered from children with invasive pneumococcal diseases were sent to the National Reference Center for Streptococci. Identification of the isolates was confirmed and serotyping was performed using the Neufeld Quellung reaction.

Results: In 2010-2011, IPD cases in children < 2y caused by PCV7 serotypes had decreased by more than 90%, while cases caused by non-PCV7 serotypes almost doubled. Of particular interest, among the six new serotypes, less cases caused by serotypes 1,3,6A and 7F were reported from 2010-2011 (n=39) as compared to 2009-2010 (n=21). Cases caused by serotype 19A were slightly higher (n=20 vs. n=22). Serotype 5 is still very rare. From July-September 2011 only one case of 19A was observed, compared to 7 in July-September 2010.

Conclusions: Seven years after the general vaccination recommendation reported cases caused by PCV7 serotypes have almost disappeared. Two years after the introduction of higher valent PCVs first effects are visible, with less reported cases among children < 2y due to serotypes 1,3,6A and 7F, and a possible decrease in serotype 19A.
INVASIVE PNEUMOCOCCAL DISEASE AND HERD PROTECTION EFFECTS AMONG RISK GROUPS IN ENGLAND


Background and aims: To inform national policy making on the use of the 13-valent pneumococcal vaccine among risk groups we estimated the increased risk of invasive pneumococcal disease (IPD) outcomes among clinical risk groups. Three years of post 7 valent pneumococcal conjugate vaccine data was included to investigate the herd protection effects.

Method: Over 22,000 IPD patients in England (March 2002-March 2009 - aged 2 and over) were linked to their hospitalisation records based on personal identifiers. The prevalence of risk factors in these patients was compared to the prevalence of risk factors in the general population.

Results: Children aged 2-15 years are 11.7 (95% CI 10.2-13.3) times more likely to develop IPD compared to children without risk factors, while adults (16-64years) were 7.6 (7.3-7.9) and adults 65+ years were 2.7 (2.6-2.8) times more likely. The most important risk factors that predict IPD are chronic liver disease, immunosuppression, chronic respiratory diseases and asthma. There was an increased risk of death among the patients with risk factors, with an odds of 2.4 (1.2-5.1) for children, 3.9 (3.4-4.4) for adults and 1.2 (1.1-1.3) for the age group 65+ compared to patients without any risk factors. Herd protection effects due to introduction of the 7-valent vaccine were identical in both patient groups.

Conclusions: There is a marked increased risk for IPD among risk groups, justifying a targeted vaccination approach, although the benefits of such an approach can be reduced due to herd immunity effects caused by the childhood vaccination programme.
HOW LARGE REALLY IS THE IMPACT OF PNEUMOCOCCAL CONJUGATE VACCINATION (PCV) ON OTITIS MEDIA (OM)? A SYSTEMATIC REVIEW


Background and aims: PCV studies suggest variable impacts on OM. We reviewed PCV efficacy/effectiveness studies on all-cause OM episodes/visits to better quantify impact.

Methods: A PubMed-based systematic search identified articles assessing PCV efficacy/effectiveness. Pre-PCV and post-PCV rate changes were calculated as the difference between first study year and last pre-PCV year estimates and last pre-PCV year and last study year estimates, respectively.

Results: 7 clinical trials and 8 observational studies were examined. Estimated efficacy against all-cause OM episodes/visits was 0%-9% in randomised trials and 17%-23% in non-randomised trials. With exception of one outlier, observational studies (Table) show declining baseline rates before PCV (-15%; range, -24% to +14%) with continuation after PCV (-18%; range -48% to +7%).

Conclusions: PCV7 provides moderate protection against OM but other factors have significantly contributed to the recent decline in OM incidence. Methodology of observational studies should be improved to more adequately assess true vaccine impact.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Database</th>
<th>Age (yrs)</th>
<th>N (x1000)</th>
<th>Comparison (pre)</th>
<th>Comparison (post)</th>
<th>Baseline rate (per 1000 person-years)</th>
<th>Pre-PCV decrease (%)</th>
<th>Post-PCV decrease (%)</th>
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<tr>
<td>Grijalva (US) Pediatrics 2006</td>
<td>NAMCS/ NHAMCS</td>
<td>&lt;2</td>
<td>--</td>
<td>94-95 vs. 98-99</td>
<td>98-99 vs. 02-03</td>
<td>1415</td>
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<td>12</td>
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<td>Zhou (US) Pediatrics 2008</td>
<td>Employer insurance</td>
<td>&lt;2</td>
<td>20-153</td>
<td>97 vs. 99</td>
<td>99 vs. 04</td>
<td>2073</td>
<td>-14</td>
<td>48</td>
</tr>
<tr>
<td>Grijalva (US) JAMA 2009</td>
<td>NAMCS/ NHAMCS</td>
<td>&lt;5</td>
<td>--</td>
<td>95-96 vs. 99-00</td>
<td>99-00 vs 05-06</td>
<td>950</td>
<td>23</td>
<td>13</td>
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<tr>
<td>De Wals (Can.) PIDJ 2009</td>
<td>Physician claims</td>
<td>&lt;5</td>
<td>25-26</td>
<td>00 vs. 07</td>
<td>587</td>
<td>--</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Singleton (US) PIDJ 2009</td>
<td>Indian Health service</td>
<td>&lt;5</td>
<td>775</td>
<td>94 vs. 96</td>
<td>96 vs. 03-05</td>
<td>1380 per 1000 children</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Sox (US) Pediatrics 2008</td>
<td>Physician claims(private)</td>
<td>≤12</td>
<td>--</td>
<td>96 vs. 99</td>
<td>99 vs. 04</td>
<td>385</td>
<td>22</td>
<td>37</td>
</tr>
</tbody>
</table>

AMCS, National Ambulatory Medical Care Survey; NHAMCS, National Hospital Ambulatory Medical Care Survey; NY: New York; TN, Tennessee

[Table]
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SEROTYPE SPECIFIC BURDEN OF INVASIVE PNEUMOCOCCAL DISEASE IN ADULTS IN BELGIUM

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Background and aims: Surveillance of pneumococcal infections is well established since 1996 in Belgium. In 2009 the Adult IPD Survey was started to obtain data on serotypes and clinical presentation of invasive disease and to assess the potential impact of adult conjugate vaccination. Data were analyzed for serotype specific burden of disease in adults of 50 years of age and more over a 2 year period.

Methods: Prospective surveillance of Invasive Pneumococcal Disease (IPD) in hospitalized adults. The IPD clinical presentation, complications and mortality were documented. Pneumococcal isolates were mailed to the National Reference Laboratory for capsular typing and susceptibility testing.

Results: In 2009 and 2010, 833 patients of 50 years old or more were included and 759 isolates serotyped. A similar serotype distribution was observed for both years. 

ST19A was present in 11.3 % of isolates followed by ST3 (11.1%), ST7F (10.8%), ST1 (9.6%), ST12F (8.8%), ST22F (6.2%), ST8 (4%), ST6A (3.7%), ST5 (2.8%), ST14 (2.4%), ST4 (2.4%), ST11A (2.2%), ST33F (1.7%), ST10A (1.6%). There were significant differences in age distribution with ST1 higher in younger patients (50-64 years) and ST3 in older patients (75 years or more). ST3 was highest in invasive pneumonia, while ST1 was predominant in empyema. ST 3 was associated with the highest number of deaths followed by ST19A and ST7F. Penicillin non susceptibility (MIC >0.06 mg/L) was present in 16.1% of ST19A isolates.

Conclusions: An adult pneumococcal conjugate vaccine program can influence the serotype-specific burden of IPD in adults.
EFFICACY OF 10-VALENT PNEUMOCOCCAL NON-TYPEABLE H. FLUENZAE PROTEIN-D CONJUGATE VACCINE (PHID-CV) AGAINST CARRIAGE AND AOM: DESIGN OF A CLUSTER-RANDOMISED TRIAL

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Background/aims: To evaluate vaccine efficacy against acute otitis media (AOM) and respiratory tract infections (RTIs), impact on nasopharyngeal bacterial carriage, safety/reactogenicity and immunogenicity of various PHID-CV schedules.

Methods: This Phase III study is nested in a larger, cluster-randomised, double-blind vaccine effectiveness study in Finland. Clusters were randomised to PHID-CV or control (hepatitis A or B vaccine, depending on age) arms with 2- or 3-dose primary schedules, followed by a booster, in infants aged 6 weeks to 6 months at enrolment. Children aged 7-11 months and ≥12 months will receive 3-dose (2+1) and 2-dose catch-up schedules, respectively (Table). In the nested study, blood samples (immunogenicity subset) and nasopharyngeal swabs (all children) will be taken at scheduled visits (Table). AOM data will be collected via parental reporting of physician-diagnosed episodes by telephone/questionnaire; RTI surveillance will be performed in a subset of children in the Turku area. Safety/reactogenicity and immunogenicity will be assessed.

Results: To date, >6000 children have been enrolled; of whom >1600 constitute the immunogenicity subset and >5000 were aged < 6 months at enrolment. Nasopharyngeal swabs have been taken from >90% of children.

Conclusions: This study should provide valuable data on efficacy against AOM and impact on nasopharyngeal carriage in children receiving different PHID-CV schedules.

| Table. Visit schedule by age group |

<table>
<thead>
<tr>
<th>Age at first vaccination and schedule</th>
<th>M0</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M6</th>
<th>M7</th>
<th>M8</th>
<th>M9</th>
<th>M11</th>
<th>M16</th>
<th>M18</th>
</tr>
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<tbody>
<tr>
<td>6 weeks–6 months</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>BS</td>
<td>V</td>
<td>BS</td>
<td>BS</td>
<td>NP</td>
<td>BS</td>
<td>BS</td>
<td>NP</td>
</tr>
<tr>
<td>3-dose primary schedule + booster</td>
<td>NP*</td>
<td>NP</td>
<td>NP</td>
<td>BS</td>
<td>NP</td>
<td>BS</td>
<td>BS</td>
<td>NP</td>
<td>BS</td>
<td>BS</td>
<td>NP</td>
</tr>
<tr>
<td>6 weeks–6 months</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>BS</td>
<td>V</td>
<td>BS</td>
<td>BS</td>
<td>NP</td>
<td>BS</td>
<td>BS</td>
<td>NP</td>
</tr>
<tr>
<td>2-dose primary schedule + booster</td>
<td>NP*</td>
<td>NP</td>
<td>NP</td>
<td>BS</td>
<td>NP</td>
<td>BS</td>
<td>BS</td>
<td>NP</td>
<td>BS</td>
<td>BS</td>
<td>NP</td>
</tr>
<tr>
<td>7–11 months</td>
<td>V</td>
<td>V</td>
<td>BS</td>
<td>NP</td>
<td>V</td>
<td>BS</td>
<td>BS</td>
<td>NP</td>
<td>BS</td>
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<td>NP</td>
</tr>
<tr>
<td>2-dose primary schedule + booster</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>BS</td>
<td>NP</td>
<td>BS</td>
<td>BS</td>
<td>NP</td>
<td>BS</td>
<td>BS</td>
<td>NP</td>
</tr>
<tr>
<td>≥12 months</td>
<td>V</td>
<td>BS</td>
<td>BS</td>
<td>BS</td>
<td>V</td>
<td>BS</td>
<td>BS</td>
<td>NP</td>
<td>BS</td>
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</tr>
<tr>
<td>2-dose catch-up schedule</td>
<td>NP</td>
<td>BS</td>
<td>BS</td>
<td>BS</td>
<td>NP</td>
<td>BS</td>
<td>BS</td>
<td>NP</td>
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<td>NP</td>
</tr>
</tbody>
</table>

BS, blood sample (immunogenicity subset only); M, study month; NP, nasopharyngeal swab; V, vaccination.

* Nasopharyngeal swabs taken in immunogenicity subset only. At M0, subjects in the ≥12 months group were 12–18 months old.
CLONAL DIVERSITY IN INVASIVE PNEUMOCOCCAL DISEASE FOLLOWING INTRODUCTION OF PCV7 IN NORWAY

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Background and aims: To assess the impact of routine childhood immunisation with the seven-valent pneumococcal conjugate vaccine (PCV7) on pneumococcal population biology, the molecular epidemiology of invasive pneumococcal disease (IPD) isolates was studied in pre- and post-PCV7 samples. PCV7 was introduced in the Norwegian Childhood Immunisation Programme in 2006.

Methods: Strains of S. pneumoniae isolated from cases of IPD are referred to the Norwegian Institute of Public Health for serotyping and antibiotic susceptibility testing. Multilocus sequence typing (MLST) was applied to consecutive samples from IPD cases in the first half of 2005, 2007 and 2009. Population diversity was assessed using Simpson's diversity index.

Results: In total, 1574 isolates were analysed by MLST; 561 from 2005, 551 from 2007 and 462 from 2009. A shift from vaccine serotypes (VT) to non-vaccine serotypes (NVT) was observed. The population diversity increased from 2005 to 2007. The rank order of dominating clones was unchanged, but the number of isolates in major clones belonging to VTs decreased, while the number of rare STs increased. From 2007 to 2009 the diversity decreased slightly; the number of isolates decreased, and the rank order of ST frequency changed. Among the most rapidly expanding clones were ST433 (serotype 22F), ST180 (3), ST199 (15B/C and 19A), ST1176 (7F) and ST393 (38 and NT).

Conclusions: A significant shift in the pneumococcal population structure was observed in 2007, with emergence of STs occurring in small numbers. In 2009, expansion of NVT clones and a concomitant decline of dominating VT clones was evident.
IMPACT OF 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE ON A POPULATION AT HIGH RISK FOR PNEUMOCOCCAL DISEASE

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Background and aims: Routine infant pneumococcal conjugate vaccine (PCV7) was introduced on the Navajo Nation in late 2000, which reduced rates of vaccine type (VT) disease. 13-valent PCV (PCV13) replaced PCV7 in April 2010. We aimed to describe all-age IPD epidemiology in the PCV13 routine use era.

Methods: Active, laboratory, population-based surveillance for IPD was conducted; a case was defined as pneumococcus cultured from a normally sterile site in a Navajo person. Isolates were serotyped by Quellung; 6A and 6C were distinguished by PCR. IHS User Population denominators were used for rate calculations. IPD cases from 2007-09 and Sept 1, 2010 - Aug 31, 2011 were compared.

Results: We identified 370 IPD cases in 2007-09 and 78 IPD cases in Sept 1, 2010-Aug 31, 2011, a 37% reduction in average annual cases. The annual rate of PCV13-VT disease in children < 2 yo fell from 102 to 10 cases/100,000; (P=0.001). Among those < 2 yo, rates of serotypes 7F (50 vs 0 cases/100,000 per year; P=0.014) and 19A (38 vs 0 cases/100,000 per year; P=0.035) fell significantly; a non-significant reduction in 6C cases occurred (4 vs 0 cases/100,000 per year; P=0.72). VT-IPD rates in adults were unchanged. The NVT-IPD rate remained unchanged in all age-groups except in 40-< 65yo, which declined by 48% (P=0.01).

Conclusions: VT-IPD rates among children declined significantly in the era of PCV13 use; indirect effects in adults have not occurred. With only a single year of PCV13 use, causal inferences must be made with caution.
EVALUATION OF CHARACTERISTICS OF NAVAJO CHILDREN WITH VACCINE TYPE DISEASE IN AN ERA OF WIDESPREAD PNEUMOCOCCAL CONJUGATE VACCINE USE

R.C. Weatherholtz¹, L.R. Grant¹, H.T. Kassa¹, S.E. O'Brien¹, C. Donaldson¹, J. Dallas¹, J.J. Campbell¹, M. Harker-Jones², K. Rudolph¹, R. Reid¹, M. Santosham¹, K.L. O'Brien¹,² Baltimore, MD, Anchorage, AK, USA

Background and aims: Pneumococcal conjugate vaccine (PCV7 and PCV13) were introduced on the Navajo Nation in 2000 and 2010, respectively reducing vaccine type (VT) disease rates. VT invasive pneumococcal disease (IPD) is still identified in children < 5 years old. We characterized VT-IPD + serotype 6A cases among Navajo children in the PCV routine use era.

Methods: IPD cases defined as pneumococcus cultured from a normally sterile site in a Navajo person, were identified by active, laboratory, population-based surveillance. Isolates were serotyped by Quellung and PCR for types 6A and 6C. IPD events and PCV status (doses prior to IPD) were characterized by chart review; underlying conditions were noted beginning in May 2001.

Results: From 2001-2011, we identified 24 VT-IPD cases among children < 5yo; 11 were < 1yo, 7 1-2 yo and 6 2-5yo. 17 (68%) were under-immunized for age; 11 (86%) received ≥1 PCV dose and 3 (21%) received 3 doses prior to the IPD episode. 1 child had an underlying condition (FAS). The serotypes and syndromes causing disease were 6B (n=5), 6A and 9V (n=4), 4 and 14 (n=3), 18C and 19F (n=2), and 23F (n=1); pneumonia (n=9), bacteremia without a source (n=9), and meningitis (n=3).

Conclusions: VT-IPD continues to occur in Navajo children < 5yo during routine PCV use. Most cases occurred in children with ≥1 PCV dose and who had no known underlying conditions predisposing them to disease. High PCV coverage is important for continued protection of VT-IPD among these children.
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COST-EFFECTIVENESS OF 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE CATCH-UP PROGRAM FOR CHILDREN UNDER 3 YEARS OF AGE IN AUSTRALIA

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Background and aims: 13-valent pneumococcal conjugate vaccine (PCV-13) replaced 7-valent pneumococcal conjugate vaccine (PCV-7) on the National Immunisation Program in Australia for routine vaccination of infants (3+0 course) in July 2011. PCV-13 contains serotype 19A which causes most invasive pneumococcal disease (IPD) in children under 3 years. Clinical data show a single dose of PCV-13 following routine PCV-7 vaccination has similar immunogenicity to routine PCV-13 vaccination. We evaluated the cost-effectiveness of a single "catch-up" dose of PCV-13 in children under 3 years who have completed vaccination with PCV-7 compared with routine infant vaccination.

Methods: Age-specific incidence of IPD, pneumonia and otitis media was sourced from Australian data. Using a state-transition model, cases of pneumococcal disease avoided with PCV-13 catch-up dose compared to no catch-up dose over 5 years were estimated. Cases were transformed to costs and quality adjusted life years (QALYs). Vaccine effectiveness was based on PCV-7 data adjusted for incremental serotype coverage. Incremental herd effect was applied to unvaccinated individuals for IPD.

Results: The catch-up program avoids 11,907 cases, saving 1,740 QALYs at a discounted cost per QALY of AU$15,466. Estimates were most sensitive to vaccine effectiveness against inpatient pneumonia and QALY losses, resulting in a cost per QALY < AU$32,000.

Conclusions: In children under 3 years who have completed vaccination with PCV-7 a catch-up dose of PCV-13 is cost-effective compared to no catch-up dose. Implementation of the program will provide direct benefit to vaccinated children and may hasten herd protection to unvaccinated individuals, particularly against serotype 19A.
PHYSICIAN PERCEPTIONS, ATTITUDES, AND PRACTICES REGARDING PNEUMOCOCCAL VACCINATION OF YOUNGER ADULTS WITH DIABETES IN THE UNITED STATES

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Background and aims: Pneumococcal vaccination is recommended for adults with diabetes younger than age 65 in the United States; however, vaccination rates remain low. This study aims to examine, from the physician’s perspective, vaccination coverage rates as well as drivers and barriers to vaccination in this population.

Methods: A cross-sectional self-administered online survey of U.S. nationally representative internal medicine and family practice physicians was conducted in 2010. The survey explores physicians’ perceptions of factors that may impact vaccination, including healthcare system, physician attitudes, and patient and physician characteristics. Descriptive and bivariate regression analyses were applied to analyze physician responses. Stepwise linear regression analyses were conducted to assess factors associated with vaccination coverage rates among younger adults with diabetes.

Results: A total of 309 interviewed physicians estimated that among their patients with diabetes 18 to 64 years of age, 61% of those 50 to 64 years old, and only 38.4% of those 18 to 49 years old have previously received the pneumococcal vaccine. "Physician’s belief that vaccination of this population is important" and "having a vaccination protocol" were significant drivers to pneumococcal vaccination, while "physicians' belief that vaccination for these patients is often not reimbursed by insurance" was significantly negatively associated with pneumococcal vaccination (all P< 0.05).

Conclusion: Our study suggests that to improve pneumococcal vaccination rates among adults with diabetes younger than 65 years, from the physician’s perspective, the most important areas to target include physician education on burden of disease, implementation of vaccine protocols, and vaccination reimbursement for these patients.
COST-EFFECTIVENESS ANALYSIS OF VARIOUS VACCINATION STRATEGIES TO PREVENT PNEUMOCOCCAL DISEASE AMONG ADULTS IN THE UNITED STATES

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Background and aims: Decision-makers are considering the 13-valent pneumococcal conjugate vaccine (PCV13) for prevention of pneumococcal disease in adults. In the United States, 23-valent pneumococcal polysaccharide vaccine (PPVS23) is recommended for adults 65 years of age and older as well as younger individuals with certain conditions. No known available data compare the effectiveness of the two vaccines. This study aims to explore the cost-effectiveness of various vaccination and re-vaccination strategies for preventing pneumococcal disease in adults 50 years of age and older.

Methods: A static cohort model was developed that incorporated costs, health outcomes, and quality-adjusted life-year (QALY) losses associated with invasive pneumococcal disease and non-bacteremic pneumococcal pneumonia. Vaccine effectiveness estimates were based on expert opinion generated through Delphi panels together with published literature. Eight vaccination and re-vaccination strategies as well as the no-vaccination baseline strategy were compared over the remaining lifetime of a 50-year-old cohort. Vaccine dose costs were assumed to be $46 for PPSV23 and $114 for PCV13.

Results: The base-case result indicates that a vaccination strategy that extends the current standard of care- PPSV23 vaccination for all healthy adults at age 65, and younger individuals with chronic conditions - supplemented with targeted PCV13 vaccination of adults with immunocompromising conditions dominated all other strategies. The incremental cost effectiveness ratio vs. no vaccination for this strategy is $8,900 per QALY saved.

Conclusions: The most efficient vaccination strategy in this analysis extends the current standard of care for adults 50 years of age and older, and would be considered cost-effective.
IMPACT OF CHILDREN UNIVERSAL PNEUMOCOCCAL VACCINATION (PCV7V/PCV13V) ON HOSPITALIZATIONS FOR PNEUMONIA AND PNEUMOCOCCAL INVASIVE DISEASE IN THE BRITISH HOSPITAL, URUGUAY


Background: In March 2008 Uruguay included PCV7 in the routine vaccination program (2+1 schedule). In April 2010 PCV7 was replaced by PCV13.

Objective: to assess the impact of this strategy in hospitalization for pneumonia and pneumococcal invasive diseases (PID).

Methods: Annual rates per 10,000 discharges are described in the period before PCV7 vaccination (2006 -2007) the year of vaccine implementation (2008), and after implementation (2009-2010). Annual rates for 2011 will be available.

Results: The percentage of reduction comparing median pre-vaccination rates 2006-2007 vs. 2009-2010 in children was -53.1% for pneumonia in < 15 years of age and -59.7% in < 2 years old. For PID the percentage of reduction was -65% in adults and -79.5% in children younger than 15 years of age. 14 different serotypes were isolated in adults. Serotypes 7F, 1, 12F and 5 were isolated more frequently. PCV13 would cover 87% of adults serotypes. 10 different serotypes were isolated in children. Prior 2008 the most frequent serotypes were 1, 14, and 5. In 2009-2010 only three children were hospitalized for PID. 2 pneumonia with bacteremia, 1 with serotype 9V was 4 years old and non immunized and 1 with serotype 24F. 1child with serotype 6B meningitis had 2 doses of PCV7, he was hospitalized again in 2011 with meningitis due to serotype 19A, fully immunized, spinal fluid fistula was diagnosis and repaired.

Results: There was a rapid reduction in discharge rates for pneumonia and PID in children and also in adults probably due to herd effect.
EARLY IMPACT OF 10-VALENT CONJUGATE-PNEUMOCOCCAL VACCINE ON COMMUNITY-ACQUIRED-PNEUMONIA AND PNEUMOCOCCAL CARRIAGE AFTER VACCINATION IN BRAZIL: A CROSS-SECTIONAL POPULATION-BASED STUDY

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Background and aims: The 10-valent conjugate pneumococcal vaccine (PCV-10) was introduced into the National Immunization Program of Brazil in March 2010. We evaluated the impact of vaccination on community-acquired pneumonia (CAP) and nasopharyngeal carriage (NPC) in infants, 6 to 9 months after the introduction of PCV-10 in a major city of Brazil.

Methods: A population-based study was undertaken in Goiânia (1,200,000 inhabs), Brazil, from December 2010 to March 2011. A random sample of children aged 7-18 months (n=1,291) was selected from the National Information System on Live Births. Nasopharyngeal swabs (placed into STGG), demographic and clinical data were collected at home visits. Children with history of CAP before complete vaccination were identified and checked against Hospitalization Information System and the attending outpatient department. Vaccination status was confirmed by immunization card. Pneumococcal carriage was ascertained by lytA-targeted real-time-PCR and capsular types by multiplex-PCR. Effectiveness of PCV-10 vaccination was assessed by 1-OR considering non-vaccinated, and vaccinated with complete or incomplete schedules, using multiple-logistic regression models.

Results: NPC prevalence was 55.9%. The effectiveness of PCV-10 on pneumococcal NPC for incompletely and completely vaccinated children was 26% (95%CI: 0-50%) and 36% (95%CI: 12-53%), respectively, adjusted by age, day-care attendance, and socioeconomic variables. The PCV-10 effectiveness for vaccine-types was 39.4% (95%CI: 16.7-55.9%). For CAP, the effectiveness for completely vaccinated children was 40% (95%CI: 1.4-63%). Lower bacterial loads (higher Ct values) correlated with higher number of vaccine doses (p= 0.035).

Conclusion: We found that PCV-10 was protecting Brazilian infants against CAP and NC soon after introduction into the routine schedule.

Financial support: CNPq-IATS(#573826/2008-0;306096/2010-2); FAPEG-GO (PRONEX/07-2009); FUNAPE-GO(#34131).
PNEUMOCOCCAL VACCINE INTRODUCTION IN BRAZIL: A PRELIMINARY IMPACT ANALYSIS

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Background and aims: Pneumococcal conjugate vaccine impact demonstration is still lacking in developing countries. \textit{Haemophilus influenzae}-protein D 10-valent conjugate vaccine (PHiD-CV) was introduced progressively into the National Immunization program in Brazil during the period of March-September 2010. We assessed the early impact of PHiD-CV vaccination in all age-groups in selected cities in Brazil.

Methods: We conducted an interrupted time-series analysis using secondary data from the Brazilian Hospitalization Information System (SIH) during the period of January 2005-July 2011. All individuals hospitalized with pneumonia were identified considering specific ICD-10 codes for pneumonia (J12-J18) as the primary discharge diagnosis. Duplicates were excluded. Hospitalization rates were calculated considering denominators from national population census. Capital cities of 6 Brazilian states were selected based on SIH data quality. Trends of monthly pneumonia rates for the pre-vaccine period (January 2005-February 2010) were compared with post vaccination period (September 2010-June 2011). Rates were stratified by age groups.

Results: Preliminary results indicate a significant decrease in pneumonia hospitalization rates post PHiD-CV, in particular in younger than 2 year of age. Rate reduction in children younger than 2 years of age in Belo Horizonte, one of the 6 cities evaluated, was 31\% (p=0.0039) (Figure 1), significantly higher than the one observed in other age groups. This was not observed in the comparator groups.

Conclusions: Despite its recent introduction, our data suggest a significant impact of PHiD-CV vaccination in reducing pneumonia hospitalizations in children. Prospective analysis considering a longer time period and other municipalities are ongoing.
USE OF MOBILE TECHNOLOGY FOR PNEUMONIA SURVEILLANCE TO ASSESS THE IMPACT OF PNEUMOCOCCAL VACCINATION: E-PNEUMO SOFTWARE PROTOTYPE FOR SMARTPHONES

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Introduction: Use of mobile technology in developing countries has been demonstrated effective, mostly for collection and reporting of notifiable diseases data. Assessment of vaccination impact using this technology has not been reported. We present preliminary results of an open-source data management software (e-Pneumo) used for pneumonia surveillance in Brazil, where pneumococcal vaccine was introduced in 2010.

Methods: e-Pneumo was developed for individual data collection, transmittal, and preliminary analysis. The application for mobile data collection uses the Java technology, available in most mobile phone. Processing and data analysis utilize “Java Enterprise Edition” and database “PostgreSQL” with Linux operational system in cloud. e-Pneumo is being used to evaluate pneumococcal vaccine impact in Goiânia (1,200,000 inhabitants), Brazil. Individual data for all hospitalized children are collected. We evaluated its ease of use, learning, timeliness, and data accuracy, assessing 279 cases. Data completeness and mistakes was assessed for admission diagnosis, age, and place of residence.

Results: This technology was easily used and twelve nurses were trained in data collection in a 3-hour session. Individual data was transmitted real-time with high accuracy. Immunization card and chest X-ray high-resolution images were directly embedded into the data files. When compared to manual data records, data collected by e-Pneumo presented lower higher accuracy for the variables considered (p = 0.000). All trained collectors refer e-Pneumo is easy and friendly technology.

Conclusions: e-Pneumo is effective for use in epidemiological field studies. Adaptable for other data collection activities, this technology has the potential to minimize data losses, enhancing quality at lower costs.
THE IMPACT OF PRE-AND POST-NATAL EXPOSURE TO PNEUMOCOCCAL CONJUGATE VACCINES ON NASOPHARYNGEAL MICROBIAL ECOLOGY AND PNEUMOCOCCAL CARRIAGE

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Background and aims: The impact of the 7-Valent Pneumococcal Conjugate Vaccine (PCV-7) on nasopharyngeal microbial ecology remains poorly characterized. This study addresses the hypothesis that community-wide vaccination influences the early development of the nasopharyngeal microbiome.

Methods: Twelve infants from villages with widespread use of PCV-7 and vaccinated at two, three and four months belonged to the early vaccination (EV) group. Ten infants from PCV-7 naïve villages and vaccinated after eight months belonged to the late vaccination (LV) group. Nasopharyngeal swabs were collected at regular intervals from 0-12 months (n=17 swabs/infant). Bar-coded 454-pyrosequencing of the 16S rRNA gene and pneumococcal culture were conducted.

Results: Overall pneumococcal carriage was 87% and 80% among EV and LV infants respectively. Within the first three months, carriage of vaccine serotypes rose above 50% among LV neonates but were not detected amongst EV neonates prior to vaccination. Streptococcus, Moraxella, Haemophilus, Pseudomonas, Staphylococcus and Corynebacterium made up at least 80% of the microbiome. The relative abundance of Streptococcus was significantly negatively correlated to Pseudomonas (OR 0.7; p< 0.01) and Staphylococcus (OR 0.5; p=0.02). Overall, the relative distribution of microbes over time was more turbulent among LV infants, the relative abundance of several genera including Streptococcus and Lactococcus changed significantly (p<0.01) among LV but not EV infants under 6 months.

Conclusions: Early exposure to PCV-7 not only alters the carriage of PCV-7 serotypes but also appears to have an effect on the ecological balance of microbes among infants with important implications for the long-term effectiveness of PCV-7.
SEROPREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF STREPTOCOCCUS PNEUMONIAE IN INVASIVE DISEASE IN GUANAJUATO, MEXICO

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Background: Invasive pneumococcal diseases (IPD) have been reported as major public health problems in US and Europe. However, data on Latin America are limited.

Objective: To determine pneumococcal serotype distribution and antimicrobial susceptibility in patients of Guanajuato, Mexico.

Methods: We conducted a prospective, epidemiological, multi-center, study in 3 hospitals in Guanajuato, Mexico. Serotyping and antimicrobial susceptibility were done using the Quellung reaction and broth microdilution according to CLSI at the INSP as part of the SIREVA network in Mexico.

Results: From December 2008 to October 2011, we had 44 patients with Meningitis accounted for 15% of episodes and 85% were pneumonia and bacteremia. The most commonly isolated S.pneumoniae serotypes were 19A (16%), 19F (11.5%), 6A (9%), 6B, 9V and 35B (7%). In meningitis 2/6 (33%), and in pneumonia-bacteremia 30/38 (79%) were susceptible to penicillin. All strains were susceptible to cefotaxime.

Conclusion: The most frequent isolated S.pneumoniae serotype 19A and the 6A were not covered in the 7-valent pneumococcal conjugate vaccine, or 10-valent, and now covered with the introduction of 13-valent pneumococcal conjugate vaccine in our country. The susceptibility to penicillin allow consider their use in pneumonia in base to epidemiological surveillance in every country. S.pneumoniae represent important target for vaccination strategies to reduce invasive disease in Mexican children. Pneumococcal Conjugate Vaccine (PCV-13) was shown to reduce invasive disease caused by vaccine serotypes >90% effective against invasive disease.
Background: *Streptococcus pneumoniae* Serotype 5 is amongst the most common serotypes causing invasive pneumococcal disease (IPD) in The Gambia. The introduction of the 13-valent pneumococcal conjugate vaccine (PCV-13) into the routine vaccination schedule in The Gambia in Jul-2011 is expected to lead to the reduction of serotype 5 IPD. However, the emergence of new clones that have modified their repertoire through serotype switching or recombination after vaccination with PCV-13 is a real threat. In order to monitor for such alterations a baseline population structure of serotype 5 pre PCV-13 was determined.

Methods: Fifty-nine consecutive invasive *S. pneumoniae* serotype 5 isolates were recovered from individuals of all age groups between Jan-2009 to Aug-2011 in a population based study in rural Gambia. Serotyping was done by latex agglutination and confirmed by serotype-specific PCR. Genotyping was undertaken using Multilocus sequence typing (MLST), software's SATA 9 and Bionumerics were used for Data analysis.

Results: MLST resolved all serotype 5 isolates into three sequence types (ST), namely ST 289, ST 3339 and ST 3404. ST 289 was identified as the major clonal complex. ST 3339 the prevalent genotype in 2009, declined and was replaced by ST 3404 in 2010. As compared to ST 3404, ST 3339 showed high resistant to tetracycline and oxacillin (P = < 0.001).

Conclusion: There has been the emergence of ST 3404 in The Gambia, prior to the introduction of the PCV-13. This provides important background data to assess the impact of PCV-13 into routine immunization in The Gambia.
PNEUMOCOCCAL INVASIVE DISEASE AMONG CHILDREN < 5 YEARS IN SAO PAULO AND UBERLANDIA BRAZIL IN A TRANSITION YEAR AFTER IMUNIZATION

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Background and aims: The aim of this study was to describe the distribution of serotypes associated with pneumococcal invasive disease (PID) among children younger than 5 years of age in the cities of Sao Paulo and Uberlandia, before and after the introduction, in mid 2010, of the 10V-pneumococcal conjugate vaccine (PCV-10) for children younger than 2 years in our National Immunization Program.

Methods: We performed a 6-year (2005 - 2010) hospital-based surveillance in Sao Paulo and Uberlandia, including all children < 5 years admitted due to PID, with isolation of S. pneumoniae. All pneumococcal isolates were serotyped.

Results: We have evaluated isolates of 135 patients admitted due to PID. During the pre-vaccine period (2005-2009) 71.3% of the PID cases were caused by PCV-10 serotypes and the prevalent serotypes were: 14 (52.1%), 6B (8.6%), 1, 3 and 23F (4.3% each). In 2010, considered a transition year, although coverage rates of the vaccine in children younger than 2 years were low (below 40%), we could observe a decreasing trend in the proportion of PID cases attributed to vaccine types (65%), especially to serotype 14 that represented 40% of the cases. This decreasing was more clear in S. Paulo maybe due to different uptakes of vaccines.

Conclusions: Based on our data, the 10V-pneumococcal conjugate vaccine would potentially prevent 71% of the PID cases among children < 5 years. Continued surveillance studies will be crucial to evaluate and confirm the early benefits of the vaccination on the disease rates and serotype dynamics in Brazil.
Poster No 218


Background and aims: Invasive pneumococcal disease (IPD) rates in children < 5 years old dropped 65% following introduction of 7-valent pneumococcal conjugate vaccine (PCV7) in early 2001; then increased due to replacement with non-vaccine serotypes. The proportion of persons colonized with pneumococci remained unchanged. We examine pneumococcal serotype diversity and invasiveness before and after PCV7.

Methods: We conduct lab-based state-wide surveillance for IPD throughout Alaska. Pneumococcal carriage surveys were conducted in 4 regions from 1998-2004 and 2008-2009. We calculated invasiveness ratios (IR) as the odds a serotype was isolated from IPD versus from a carriage survey participant. We used Simpson's index (D) to evaluate serotype diversity among carriage and IPD before and after PCV7 introduction.

Results: Among non-PCV7 serotypes, IRs did not change significantly between pre- and post-PCV7 introduction. Carriage diversity increased after vaccine introduction in urban children (pre-PCV7, D = 0.896, post-PCV7, D = 0.926, p = 0.01) rural children (D = 0.849 to 0.882, p = 0.02) and rural persons > 5 yrs old (D = 0.912 to 0.931, p < 0.01). State-wide IPD serotype diversity has declined in children (D = 0.848 to 0.759, p = 0.05) and persons > 5 years of age (D = 0.939 to 0.906, p < 0.01) since routine PCV7 use.

Conclusions: After nearly complete elimination of PCV7 serotypes, we observed decline in IPD diversity with an increase in diversity of carriage serotypes. Serotype replacement occurred in pneumococcal carriage, however, individual serotypes have not become more invasive since PCV7 introduction.
DISTRIBUTION OF PNEUMOCOCCAL SEROTYPES AFTER INTRODUCTION OF 10-VALENT CONJUGATE VACCINE: A STUDY INVOLVING ADULTS WITH INVASIVE DISEASE, PORTO ALEGRE, BRAZIL

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Background and aims: The incidence of invasive pneumococcal disease (IPD) was impacted by the use of the 7-valent conjugate vaccine. The distribution of the most prevalent serotypes was also affected, even among adults. In Brazil, a 10-valent pneumococcal conjugate vaccine (PCV10) is being used since 2010, as part of National Program of Immunization, and consequences of the implementation of this vaccine are yet to be determined. This study aimed to evaluate the distribution of pneumococcal serotypes among adults with IPD after the utilization of the PCV10.

Methods: Isolates from patients with IPD in Porto Alegre, Brazil, were obtained from January 2007-July 2010 (pre-PCV10), and August 2010-October 2011 (post-PCV10). All isolates were serotyped by sequential multiplex PCR.

Results: 136 isolates were obtained (94 pre-PCV10 and 42 post-PCV10). In the pre-PCV10 period, the most prevalent serotypes were 19F (9.57%), 23F (9.57%), 14 (7.45%), 4 (6.38%), 6A/B (5.32%), 9V (5.32%), 12F (5.32%), 5 (4.26%), and 7F (4.26%). In the Post-PCV10 period, serotypes 20 (19.0%), 23F (11.9%), 8 (9.52%), 12F (9.52%), 14 (7.14%), 3 (4.76%), and 6A/B (4.76%) were the most common. Overall, the proportion of vaccine serotypes declined (56.4% x 40.5%) and a significant increase in isolates of serotype 20 (a serotype not included in PCV-10) was observed post-PCV (OR=10.8, 95%CI=2.19-23.5).

Conclusions: The distribution of serotypes among IPD in adults was influenced by the use of PCV-10. The emergence of non-vaccine serotypes, such as serotype 20, must be subject of continuous surveillance.

Financial support: CNPq, FAPERGS
INVASIVE PNEUMOCOCCAL DISEASE FOLLOWING THE INTRODUCTION OF PNEUMOCOCCAL CONJUGATE VACCINES IN URUGUAY


Background: In March 2008, PCV7 was introduced into the national immunization program in Uruguay. In 2010, it was replaced by PCV13 using the same 2+1 schedule. National laboratory-based surveillance of invasive pneumococcal disease (IPD) among patients of all ages has been conducted since 1994. The results obtained since 2003, were analyzed to estimate changes in IPD caused by vaccine serotypes.

Methods: Laboratory records were examined searching for the serotypes of all IPD cases between 2003 and September 2011. The incidence of IPD caused by PCV7 serotypes, by the 6 additional serotypes in PCV13 and for non-vaccine serotypes was calculated for children < 2 years and 2-4 years old. The patients ≥ 5 years were analyzed as a group.

Results: A decrease in PCV7 related disease was seen among children < 2 years old, from a mean of 37/10^5 during the pre-vaccination period to 5, 4 and 1/10^5 in 2009, 2010 and 2011 respectively. For the second group the incidence changed from 7/10^5 to 5, 1 and 0/10^5 in the 3 years after vaccination. In 2010 and 2011, a reduction of 58% in PCV7 serotypes related IPD was observed among patients ≥ 5 years.

Conclusions: Decrease of PCV7 serotypes was observed in all ages. Although the time to evaluate changes in the incidence of the additional serotypes in PCV13 is very short, so far, a reduction of at least 50 % is seen in children < 2 years. A longer follow-up is needed to confirm this trend and detect herd immunity.
COST-EFFECTIVENESS OF CONJUGATED VS. POLYSACCHARIDE PNEUMOCOCCAL VACCINES IN ELDERLY COLOMBIAN POPULATION

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Background: PD is a major cause of morbidity and mortality in elderly. Immunisation has been implemented with PPV23, which effectiveness in this population has proven to be limited compared to younger. Conjugate vaccines are available, which despite its higher cost, may have a better immunogenicity profile.

Methods: An evaluation of cost-effectiveness of application of PPV23 and PCV-7 in the Colombian population over 60 years was performed to cost per dose of US$8 and US$64, respectively in 5-years time horizon. We conducted an exhaustive literature search to identify parameters of pneumococcal disease occurrence and vaccines’ effectiveness. A Markov model simulating the history of pneumococcal disease, including pneumonia and invasive disease, and construction of ICERs of different vaccination strategies from the Colombian Health System perspective were developed. Were conducted a sensitivity analysis.

Results: In the base case was estimated a cost-effectiveness of PPV23 strategy of US$1514 (RC -US$408-US$5404) per YLS. Moving from this strategy to PCV-7 have incremental cost of US$27,419 (RC US$7132-US$53,010) per additional YLS. In the sensitivity analysis the most important inputs were PCV-7 cost, effectiveness to prevent pneumonia, vaccination coverages, and pneumonia lethality.

Conclusion: Although there is evidence of effectiveness of PCV-7 in elderly, to consider changing PPV23 to PCV-7 requires a scheme cost less than US$17.8 and the effectiveness against noninvasive disease is at least as good as in children under 5 years.
Poster No 222

PNEUMOCOCCAL SEROTYPES CAUSING INVASIVE PNEUMOCOCCAL DISEASE AFTER PCV 7 VACCINATION IN TURKEY

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Background and aims: Streptococcus pneumoniae (pneumococcus) is the leading cause of severe pneumonia and other invasive diseases responsible for considerable morbidity and mortality in children across the world. Since November 2008, PCV 7 has been included in the National Immunization Programme (NIP) in Turkey for children aged < 2 years. In this study, we aimed to identify serotypes of pneumococcal strains causing invasive pneumococcal disease (IPD) in children in Turkey and emphasize the change in the serotypes before and after PCV 7 was included and PCV 13 was newly included in Turkey's NIP.

Method: The pneumococci were isolated from blood, cerebrospinal fluid and other body fluids of pediatric cases (1 month-17 years of age) with IPD and serotyping was performed.

Results: Streptococcus pneumoniae was isolated and serotyped in 258 samples between 2006 and 2011. After the vaccination with PCV 7 was included in our NIP (November 2008-2011) coverage rates for PCV 7, PCV 10 and PCV 13 vaccines were 39%, 44% and 59% respectively. Serotype 19A and 3 were the most frequent non-PCV 7 serotypes.

Conclusions: Our results indicates that the coverage rate of PCV 7 has decreased but the coverage rate of PCV 13 has increased with the widespread use of PCV 7 during two years after the inclusion of PCV 7 in our NIP. Our study appears to show a serotype replacement due to the national PCV7 vaccination in Turkey.
PNEUMOCOCCAL OTOMASTOIDITIS IN NORTHERN MEXICO: A RESULT OF 6 YEARS OF ACTIVE SURVEILLANCE

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Background and aims: Serotype replacement following heptavalent pneumococcal vaccine (PCV7) is currently a reality, however, little is known about this in pneumococcal otomastoiditis (POM). The aims of this study were to describe clinically and microbiologically all patients with POM.

Methods: Based on active surveillance from October/2005 - September/2011, a total of 12 computerized-tomography confirmed otomastoiditis (OM) cases were diagnosed. Cultures came from mastoid, cerebrospinal fluid (CSF), and/or blood. Following S. pneumoniae identification, serotyping was performed by the Quellung Reaction (Statens Institute®). Data was analyzed with Excel.

Results: From 12 OM cases, isolation was successful in 9, from which all were S. pneumoniae. Median age at admission was of 32 months (6 months - 15 years). Median of hospitalization days was of 10 (5-115). Isolation sites came from only mastoid - 2, both mastoid and blood -4, only blood -3, and only CSF -1. PCV-7 vaccination status: No immunization -5, incomplete immunization -3, and complete vaccination -1. Pneumococcal serotypes distribution was: 19A (3), 3 (2), 7F (2), 6A (1), and 18C (1). Six patients underwent mastoidectomy, three developed sequelae, and two had other pneumococcal infections (1-pneumonia, 1-meniningitis). All but 1 cases occurred after the universal implementation of PCV-7.

Conclusions: S. pneumoniae is the leading cause of OM in Tijuana, (the busiest frontier in the world). Non-PCV-7 serotypes caused 88.9% of all cases, being 19A the most predominant. One third of all patients developed sequelae, and two thirds underwent surgery. The 13-valent pneumococcal conjugated vaccine covers 100% of all serotypes associated with POM.
INVASIVE PNEUMOCOCCAL DISEASE: SEROTYPE 19A AND YOUNG AGE ARE ASSOCIATED WITH HIGHER MORBIDITY AND/OR MORTALITY IN NORTHERN MEXICO

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Background and aims: In Tijuana, Mexico, > 90% of all invasive pneumococcal diseases (IPD) have been replaced by serotypes not included in the heptavalent conjugated vaccine, mainly 19A, 7F and 3. The aims of this study were to examine whether serotype 19A and age < 2 years old were associated with longer hospitalization days, mortality, and incidence of meningitis.

Methods: Based on active surveillance, from October/2005 - September/2011, 38 patients with confirmed IPD were included. Once isolated, S. pneumoniae serotyping was performed by the Quellung Reaction (Statens Institute®). Vassar Stat® was used for statistical analysis, a p< 0.05 was interpreted as statistically significant.

Results:

Serotype 19A accounted for 21% of all S. pneumoniae isolates:

Mortality: Serotype 19A Serotypes non-19A p:
25% 6.7% =0.06

Meningitis: Serotype 19A Serotypes non-19A p:
50% 23.3% =0.07

Hospitalization days:
Serotype 19A Serotypes non-19A CI: p:
38.25±38.5 17.1±9.7 ±47.6 vs. ±4.9 =0.04

< 2 years old accounted for 34% of all S. pneumoniae isolates:

Mortality: < 2 years > 2 years p:
23% 4% =0.03

Meningitis: < 2 years > 2 years p:
38.5% 24% =0.14

Hospitalization days:
< 2 years > 2 years CI p:
16.6±12.1 23.4±23.9 ±10.3 vs. ±13.4 =0.13

Conclusions: Serotype 19A was associated with significant longer hospitalization, and with a trend in mortality and incidence of meningitis. Children < 2 years old had significant higher mortality, and moderate tendency for longer hospitalization and incidence of meningitis. Implementation of massive vaccination with the 13-valent pneumococcal conjugated vaccine is mandatory in our region.
EVALUATING THE EFFECTIVENESS OF CONJUGATED PNEUMOCOCCAL VACCINES IN COLOMBIA

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Introduction: This study aimed to evaluate pneumococcal vaccine effectiveness against hospitalization by all causes of ARI, meningitis and sepsis in children under two years old.

Methods: A retrospective cohort study was conducted in 2631 children < 2 yrs affiliated to one health insurance company in Colombia. Children were followed for at least six months after the last dose of heptavalent pneumococcal vaccine. Outcomes of interest were acute respiratory infections (bronchitis, bronchiolitis and pneumonia), meningitis and sepsis. Exposed children were defined as having at least 3 doses for those younger than 12 months or two doses for children over that age. Unexposed were considered those with zero doses. Association between vaccination and disease was evaluated using Cox proportional hazards models. Adjustment was done by sex, family income and previous event of hospitalization.

Results: During follow up 114 hospitalizations occurred in the unvaccinated group and 42 in the vaccinated. Vaccinated children had a significant reduction in hospitalization risk for all outcomes (HR 0,59 CI 95% 0,42 - 0,85). For meningitis, sepsis and pneumonia (excluding other causes of ARI) the reduction was slightly higher (HR 0,53 CI 95% 0,32 - 0,89). Male sex was also identified as a risk factor for hospitalization (HR 1,76 CI 95% 1,27 - 2,45).

Discussion: Our results show a significant risk reduction in clinical syndromes potentially associated to pneumococcal infection in children vaccinated with heptavalent pneumococcal vaccine confirming the benefits of using conjugate vaccines in a medium income developing country. Conjugate vaccines including more serotypes are recommended.
EFFECTIVENESS OF SEVEN-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV7) AGAINST INVASIVE PNEUMOCOCCAL DISEASE IN SOUTH AFRICA: A MATCHED CASE-CONTROL STUDY

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Background: South Africa introduced PCV7 in April 2009 using a three-dose schedule (6 and 14 weeks and 9 months) with no catch-up. We present preliminary analysis of the effectiveness of ≥2 PCV7 doses against vaccine-serotype invasive pneumococcal disease (IPD) and all IPD in HIV-infected and -uninfected children.

Methods: Invasive disease (pneumococcus isolated from a sterile site) was identified in children aged ≥ 16 weeks through national laboratory-based surveillance. Isolates were serotyped by Quellung or PCR. Four hospitalized controls, matched for age, HIV-status and hospital were selected for each case. We calculated effectiveness as 1 minus matched odds ratio for vaccination.

Results: We enrolled 133 HIV-uninfected cases and 535 controls and 83 HIV-infected cases and 254 controls from March 2010 through September 2011. Coverage with ≥2 doses was 65% (346/535 [71/346, 21% received ≥3 doses]) in HIV-uninfected and 67% (170/254 [52/170, 31% ≥3 doses]) in HIV-infected controls. Effectiveness of ≥2 doses against vaccine serotypes was 68% (95% CI: 4.89) in HIV-uninfected and -18% (95%CI: -303.65) in HIV-infected children. Effectiveness against all IPD was 46% (95% CI: -2.71) in HIV-uninfected and 26% (95% C1: -62.66) in HIV-infected children. In the subgroup aged 16-40 weeks effectiveness against vaccine serotypes was 79% (95% CI: 5.95) in HIV-uninfected and 51% (-440.96) in HIV-infected children and remained unchanged for all IPD.

Conclusions: Preliminary results indicate that PCV7 is protecting HIV-uninfected children in South Africa. A schedule including three primary doses, efficacious among HIV-infected children in an earlier South African trial, may be needed for HIV-infected children.
EVALUATING THE ECONOMIC IMPACT OF PNEUMOCOCCAL CONJUGATE VACCINES IN ECUADOR, HONDURAS AND PARAGUAY

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Background: Pneumococcal disease causes significant health and economic burden. Health and economic data help to establish the value of vaccination to reduce this burden.

Methods: An economic model was constructed to estimate the cost-effectiveness of the three available pneumococcal conjugate vaccines (PCV7, PCV10, PCV13) from the societal perspective in Ecuador, Honduras and Paraguay. Hypothetical birth cohorts were followed for a 20-year period in each country. Costs were expressed in 2010 US dollars. Sensitivity analyses were performed to assess the impact of the uncertainty on estimates.

Results: Low-income Honduras experienced the greatest health burden (351 DALYs/1,000 children) due to pneumococcal disease. Over the 20 years of vaccine program implementation, the healthcare costs of pneumococcal disease would range from US$13,428,396 to US$18,485,142. Vaccination would prevent more than 50% of pneumococcal cases and deaths. At a cost of US$16.90/dose, the cost per DALY averted compared to no vaccine ranged from US$1,002 (Honduras) to US$1,677 (Ecuador), US$796 (Honduras) to US$1,340 (Ecuador), US$691 (Honduras) to US$1,166 (Ecuador) for PCV7, PCV10 and PCV13 respectively. At a reduced price (US$7/dose), the cost per DALY averted ranged from US$418 (Honduras) to US$668 (Ecuador), US$327 (Honduras) to US$528 (Ecuador), US$281 (Honduras) to US$456 (Ecuador) for PCV7, PCV10, PCV13 respectively.

Conclusions: The cost-effectiveness of vaccination compared to other interventions directed at reducing pneumococcal mortality will depend on serotype coverage, vaccine price and ability of vaccination programs to reach children at risk of mortality in a timely manner. Broader approaches should be considered when evaluating the full benefits of vaccination.
RECURRENT INVASIVE PNEUMOCOCCAL DISEASE INFECTIONS IN CHILDREN ≤15 YEARS OLD, IN SOUTH AFRICA, 2003 - 2010

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Background/Aim: Recurrence of invasive pneumococcal disease (IPD) is uncommon. We describe recurrent IPD episodes.

Methods: IPD cases were identified through laboratory-based, national surveillance and were defined as identification of Streptococcus pneumoniae from a normally sterile site. Recurrence (RIPD) was defined as ≥2 episodes of IPD >21 days after initial confirmation. PCV7 was introduced into the Expanded Programme on Immunisation (EPI) in South Africa in 2009. We reviewed IPD cases in children ≤15 years from 2003 through 2010.

Results: 14,221 IPD episodes were reported in 13,795 individuals. Among patients with known outcome, 4530/5856 (77%) recovered from their initial IPD episode; extrapolating for all episodes, 10,950 were estimated to have recovered nationally. 367/10,950 (3%) experienced more than one episode: 45, 10, 3 and 1 children had a subsequent 3rd, 4th, 5th and 6th IPD episode, respectively. 429/685, 63% RIPD episodes were PCV7 serotypes (5796/10917, 53% - single IPD, p< 0.001). 239/793, 30% presented as meningitis compared to bacteraemia (4640/13428, 34% - single IPD, p=0.01). 325/399, 81% were HIV positive (vs 2399/3797, 63% - single IPD, p< 0.001). Case fatality ratio was 6.6% (30/452) for RIPD (vs 1209/4304, 28% - single IPD, p< 0.001). RIPD cases decreased from 5% (367/7293) in 2003-2008 (pre-vaccine introduction) to 4.1% (45/1138) in 2009-2010 (post-vaccine), p=0.32.

Conclusions: Recurrent IPD was associated with PCV7 serotypes, HIV and TB, but was less likely to present as meningitis or result in death. Ongoing surveillance will monitor further decreases in RIPD cases.
SAFETY/REACTOGENICITY AND IMMUNOGENICITY OF 2-DOSE CATCH-UP VACCINATION WITH 10-VALEN'T PNEUMOCOCCAL NON-TYPEABLE HAEMOPHILUS INFLUENZAE PROTEIN-D CONJUGATE VACCINE (PHID-CV) IN MALIAN CHILDREN

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Background/aims: To assess the safety/reactogenicity and immunogenicity of a 2-dose PHID-CV catch-up vaccination in the second year of life in Malian children.

Methods: In this phase III, open, single-centre study, 2 doses of PHID-CV were administered to children (at 15-21 and 17-23 months of age) who were previously vaccinated with 3 primary doses of DTPw-HBV/Hib and OPV vaccines, but no pneumococcal vaccine. Solicited local/general (within 4 days post-vaccination) and unsolicited symptoms (within 31 days post-vaccination) were recorded. Serious adverse events (SAEs) were recorded up to study end. Immune responses were assessed (pre-vaccination and 1 month post-dose 2) using GSK’s 22F-ELISA and OPA assays (pneumococcal serotypes), and ELISA (protein D).

Results: 69 children were included in the total vaccinated cohort and 59 in the according to-protocol cohort. Swelling (56.6%) and fever (27.9%) were the most commonly reported solicited local and general symptoms, respectively. The incidence of grade 3 solicited and unsolicited AEs was low (3 events reported for 3 children). No SAEs were reported. Robust immune responses were observed against each vaccine pneumococcal serotype (Table). Immune responses against cross-reactive serotypes 6A and 19A were also observed (Table). The post-dose-2 anti-protein-D antibody GMC was 839.3 EL.U/mL.

Conclusions: PHID-CV was well-tolerated and highly immunogenic when administered to Malian children as 2-dose catch-up vaccination in the second year of life.

¹Dicko, ESPID2011 (Abstract 853)

Percentages of children with anti-pneumococcal antibody concentrations ≥0.2 μg/mL (GSK’s 22F-ELISA) and OPA titres ≥8 after 2-dose catch-up vaccination with PHID-CV (ATP cohort for immunogenicity)

<table>
<thead>
<tr>
<th>Serotypes</th>
<th>n</th>
<th>% of children with antibody concentrations ≥0.2 μg/mL (95% CI)</th>
<th>n</th>
<th>% of children with OPA titres ≥8 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine serotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>57</td>
<td>100 (93.7–100)</td>
<td>57</td>
<td>96.5 (87.9–99.6)</td>
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<tr>
<td>4</td>
<td>57</td>
<td>100 (93.7–100)</td>
<td>55</td>
<td>100 (93.5–100)</td>
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<tr>
<td>5</td>
<td>57</td>
<td>100 (93.7–100)</td>
<td>57</td>
<td>94.7 (85.4–98.9)</td>
</tr>
<tr>
<td>6B</td>
<td>57</td>
<td>82.5 (70.1–91.3)</td>
<td>50</td>
<td>94.0 (83.5–98.7)</td>
</tr>
<tr>
<td>7F</td>
<td>57</td>
<td>100 (93.7–100)</td>
<td>57</td>
<td>100 (93.7–100)</td>
</tr>
<tr>
<td>9V</td>
<td>57</td>
<td>98.2 (90.6–100)</td>
<td>43</td>
<td>100 (91.8–100)</td>
</tr>
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<td>57</td>
<td>100 (93.7–100)</td>
<td>41</td>
<td>100 (91.4–100)</td>
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<tr>
<td>18C</td>
<td>57</td>
<td>100 (93.7–100)</td>
<td>38</td>
<td>97.4 (86.2–99.9)</td>
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<tr>
<td>19F</td>
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<td>94.7 (85.4–98.9)</td>
<td>47</td>
<td>89.4 (76.9–96.5)</td>
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<td>87.7 (76.3–94.9)</td>
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<tr>
<td>Cross-reactive serotypes</td>
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<td></td>
</tr>
<tr>
<td>6A</td>
<td>57</td>
<td>19.3 (10.0–31.9)</td>
<td>48</td>
<td>58.3 (43.2–72.4)</td>
</tr>
<tr>
<td>19A</td>
<td>57</td>
<td>86.0 (74.2–93.7)</td>
<td>54</td>
<td>83.3 (76.7–92.1)</td>
</tr>
</tbody>
</table>

n = number of available results; CI = confidence interval; OPA = opsonophagocytic activity
COST-EFFECTIVENESS ANALYSIS FOR PNEUMOCOCCAL NON-TYPEABLE HAEMOPHILUS INFLUENZAE PROTEIN D CONJUGATE VACCINE (PHID-CV) IN TURKEY

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Background and aim: While otitis media does not pose a large absolute threat in contrast to mortality, it is one of the most frequent medical conditions. The financial burden of otitis media on a society is well documented. The aim of this study was to estimate the incremental costs, health effects and cost-effectiveness of pneumococcal non-typeable Haemophilus influenzae protein D conjugate vaccine (PHID-CV), PCV7 and the currently used PCV13 in Turkey using a societal perspective.

Methods: We calculated the incremental costs, health effects and cost-effectiveness for PHID-CV vaccination, using a Markov model and performed sensitivity analysis for key parameters. Epidemiological and vaccine efficacy results, probabilities, costs, and quality-of-life values were derived from published/unpublished sources (including TR-AOM study) and the potential differing efficacy of the three vaccines against all cause otitis media was also taken into account.

Results: This analysis show that more deaths will be averted using the PCV13 and PHID-CV than PCV7 and the avoided deaths result from the higher serotype coverage. Our model predicts that PHID-CV saves more treatment costs and indirect costs for AOM than PCV13 and PCV7. The results also show that, assuming the same vaccine cost, the PHID-CV vaccine has a better cost-effectiveness ratio than the PCV7 and PCV13 with a value of per DALY averted.

Conclusion: As a result, in countries like Turkey where child mortality rates are relatively low, access to medical care and antibiotic consumption are high, the cost-effectiveness of PHID-CV may be driven largely by its impact on common infections with large economic consequences like otitis media.
**DISTRIBUTION OF SEROTYPES, ANTIMICROBIAL RESISTANCE AND GENOTYPES AMONG PNEUMOCOCCAL ISOLATES IN QATAR (2009-2010)**

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**Background and aims:** PCV7 was introduced into Qatar in August 2005 and PCV13 in November 2010. In this study we describe antimicrobial resistance patterns, serotype distribution and genotypes associated with disease in both children and adults post PCV7 introduction in 2009 and 2010.

**Methods:** We characterized 100 isolates from blood (n=71), CSF (n=7), BAL (n=5) and sputum (n=17) from 2009-2010. All isolates were subjected to serotyping by Quellung, antimicrobial susceptibility by broth microdilution and multilocus sequence typing (MLST).

**Results:** We identified 36 different serotypes with 8% (n=8) and 46% (n=46) included in PCV7 and PCV13 vaccines. The most prevalent serotypes were 19A (14%), 1 (10%), 8 (8%), 3 (7%) and 11A (6%). Non-susceptibility to penicillin, erythromycin and ceftriaxone was 10%, 26% and 6%. Genotyping by MLST revealed a heterogenous population of isolates with only 3 clonal groups comprising more than 4 isolates. These 3 genotypes accounted for 15% of isolates: ST558 (serotype 35B), ST306 (serotype 1) and ST172 (serotype 19A). Eight new alleles and 24 new sequence types (STs) were identified within this study population.

**Conclusions:** Data from this study in Qatar show a low prevalence of PCV7 serotypes five years after PCV7 introduction. An additional 38% of serotypes are covered by PCV13 primarily through targeting serotypes 19A and 1. MLST data suggests that the clonal structure is diverse, with few isolates belonging to any one genotype. Surveillance post-PCV13 introduction will assist in developing continued strategies to combat infections in children and adults in Qatar.
POPULATION STRUCTURE OF STREPTOCOCCUS PNEUMONIAE SEROTYPE 1 IN THE GAMBIA FROM 1995-2010: A DRAMATIC SHIFT FROM ST618 TO ST3081 LINEAGE


Background and aims: This study examines the population structure of invasive S. pneumoniae serotype 1 isolates obtained in the Gambia from 1995-2010, providing background data for monitoring the impact of PCV-13 on IPD in The Gambia.

Methods: Ninety-three consecutive S. pneumoniae serotype 1 isolates sent to the WHO regional reference laboratory for pneumococcal serotyping from the MRC clinics in The Gambia were collected during January 2004-December 2010. The isolates were genotyped by multilocus sequence typing (MLST). The present MLST data was compared with data from a previous study of invasive serotype 1 MLST data obtained between 1995 and 2003 to determine any temporary changes over time.

Results: Six different genotypes were obtained of which one was novel, with ST3081 being the most prevalent (74.6%), followed by ST618 (14%). All genotypes belonged to the ST217 hyper virulent clonal complex. Between 1995 and 2006, ST618 was the predominant and stable ST (70.5%). It began to decline in 2007 and by 2008 had disappeared completely in IPD. In contrast, ST3081 was first seen in 2007 and by 2010 was the predominant ST. Use of the minimum spanning tree showed a clear geographical clustering of the Gambian genotypes compared with those from other parts of Africa and Asia.

Conclusion: There has been a temporary change in the epidemiology of S. pneumoniae serotype 1 genotypes in The Gambia, prior to the introduction of the PCV-13. This provides important background data to assess the impact of introduction of PCV-13 into routine immunization in The Gambia.
EVIDENCE OF PNEUMOCOCCAL SEROTYPE 19A AND 15B REPLACEMENT FOLLOWING PCV-7 VACCINATION AMONG GAMBIAN INFANTS

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Background: The long term success of the 7-valent pneumococcal conjugate vaccine (PCV-7) is threatened by the emergence of serotype replacement and serotype switching. We investigated the emergence of serotype replacement among Gambian infants exposed to PCV-7 at different points during infancy.

Methods: 578 nasopharyngeal swabs were collected from thirty-four newborns at regular intervals from 0-12 months. Group 1 infants were recruited from PCV-7 naive communities and vaccinated with PCV-7 after 8 months. Groups 2 and 3 infants were vaccinated with PCV-7 at 2, 3 and 4 months; however, group 3 infants were recruited from villages where all the older children and adults had received one dose of PCV-7. Conventional microbiology methods were used for S. pneumoniae identification and capsular serotyping by latex agglutination.

Results: Overall pneumococcal carriage was 80%, 82% and 87% amongst groups 1, 2 and 3 infants respectively. The most prevalent serotypes among the infants were 23F, 6A/6B, 14, 13, 15A, and 19A. Overall prevalence of serotype 19A was 3%, 15% and 8% amongst group 1, 2 and 3 infants respectively. Serotype 19A occurred following vaccination of infants regardless of pre-existing immune pressure in highly vaccinated communities. Serotype 15B (1-5%) was found post-vaccination among group 2 and 3 infants only.

Conclusions: This study confirms the importance of serotype 19A as a replacement serotype following widespread use of PCV-7. Serotype 15B may also be important in serotype replacement in the Gambia.
GENOTYPIC CHARACTERIZATION OF PNEUMOCOCCAL SEROTYPE 19A CAUSING INVASIVE AND NON-INVASIVE DISEASES IN MEXICO (1993-2011)


Background and aims: Serotype 19A represents the most frequent pneumococcal serotype in Mexico since 2010. The aim of the study was to determine the sequence type (ST) of isolates causing invasive (IPD) and non-IPD in our country.

Methods: SIREVA II-Mexico network comprises 22 hospitals from which we analyzed 19A strains isolated since 1993. Serotyping was done using the Quellung reaction and antimicrobial susceptibility by broth microdilution according to CLSI guidelines. Pulse field gel electrophoresis (PFGE) was performed and representatives of each clone were characterized by MLST. Statistical analysis was done using SPSS v.16.

Results: A total of 193 IPD and non-IPD isolates serotype 19A was recovered in the period 1993-May 2011. Sixty nine strains were selected for PFGE. Three major clones and 4 unique patterns were analyzed by MLST. Clonal complex (CC) 320 was found since 1997 with 46 strains followed by ST 4154 (SLV of ST320) with 15 strains, ST229 with 4 strains and 3 other ST. Penicillin and erythromycin resistance was present in 41.3% of strains in ST320.

Conclusions: We provide the first background data on genotypic characteristics of serotype 19A prior and after introduction of heptavalent pneumococcal conjugate vaccine (PCV7) in Mexico. Ongoing surveillance is necessary to assessing the impact of the new vaccines.

Acknowledgements: We thank all participant institutions from the SIREVA II - México network. This project was supported in part by a grant from Pfizer. We acknowledge the use of the S. pneumoniae MLST database which is housed at Imperial College, London.
Poster No 235

PNEUMOCOCCAL SEROTYPES AND ANTIMICROBIAL SUSCEPTIBILITY IN MEXICAN CHILDREN AFTER INTRODUCTION OF THE HEPTAVALENT CONJUGATE VACCINE. SIREVA II (2000-MAY, 2011)

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Background and aims: Introduction of pneumococcal conjugate vaccines (PCV) has been gradual in Mexico. We present results of serotyping and antimicrobial susceptibility from the laboratory-based surveillance network SIREVA II-Mexico since 2000 in pediatric population.

Methods: SIREVA II-Mexico network comprises 24 hospitals and 2 national laboratories. Pneumococcal serotyping was done using the Quellung reaction and antimicrobial susceptibility by broth microdilution according to CLSI guidelines. Statistical analysis was done using SPSS v.16.

Results: A total of 1289 pneumococcal invasive isolates were recovered from hospitalized children. Fifty two percent were from < 2 years of age. The most frequent serotypes were 19F, 6B, 23F, 19A, 14 and 6A. Significant changes of serotype distribution were observed in the last two years analyzed with decreased of all serotypes included in PCV7 except 6B and increase of serotype 19A. Decreased susceptibility to penicillin was present in 29.1% of non-meningeal isolates and resistance was present in 64% of LCR strains.

Conclusions: Despite gradual introduction of PCV in Mexico, invasive pneumococcal serotype distribution has changed since 2009 with significant decreased of PCV7 serotypes and increase on serotype 19A which is the most frequent serotype in children since 2010. Considering that PCV7, PCV10 and PCV13 were still used in different populations in Mexico during the first semester of 2011 and PCV10 and PCV13 from then on, surveillance is necessary to identify emerging invasive serotypes and monitor antimicrobial susceptibilities.

Acknowledgements: We thank physicians, nurses and laboratory personnel from all participant institutions from the SIREVA II network in Mexico.
INDIRECT EFFECT OF 7-VALENT PNEUMOCOCCAL CONJUGATE VACCINE ON PNEUMOCOCCAL CARRIAGE IN NEWBORNS IN RURAL GAMBIA


Background: In the Gambia, acquisition of pneumococci commences very early in life, long before the recommended age for pneumococcal vaccination. Additional protection of unvaccinated individuals in this setting would be of significant public health benefit.

Methods: Twenty-one villages were randomised into 11 intervention ("vaccinated") and 10 control villages. A 7-valent pneumococcal conjugate vaccine (PCV7) was given to all subjects in vaccinated villages and to infants aged 2-30 months in control villages. Meningococcal C conjugate vaccine was given to all other subjects in control villages. Nasopharyngeal swabs were collected soon after birth from newborns (184 from vaccinated and 144 from control villages) and then weekly till the age of 8 weeks.

Results: The overall pneumococcal carriage rate during the first 8 weeks of life was similar in infants from control and vaccinated villages but fewer infants from vaccinated compared to control villages carried pneumococci of vaccine serotypes (VT) (16.9% [31/184] vs. 37.5% [54/144], p< 0.001). Concomitantly, carriage of non-vaccine serotypes (NVT) was higher in infants from vaccinated compared to those from control villages (80.9% [149/184] vs. 75.7% [109/144], p=0.246). Infants from vaccinated villages had a significantly lower hazard of acquiring VT than infants from control villages (0.39 [0.26 - 0.59], p< 0.001) and a higher but non significant hazard of acquiring NVT (1.2 [0.9 - 1.5], p=0.183).

Conclusion: Community vaccination with PCV7 was associated with indirect protection of unvaccinated infants against VT carriage in rural Gambia and with a small but non significant increase in NVT carriage.
LONG-TERM EFFECTS OF COMMUNITY-WIDE VACCINATION WITH PCV-7 ON PNEUMOCOCCAL NASOPHARYNGEAL CARRIAGE IN THE GAMBIA: CLUSTER-RANDOMIZED TRIAL

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Background: A village-randomized trial of PCV-7 showed a significant decrease of vaccine-type (VT) and a non-significant increase in non-vaccine-type (NVT) nasopharyngeal carriage of pneumococci two years after vaccination. Here, we report findings four years after vaccination.

Methods: PCV-7 was given to children below 30 months of age or born after vaccination started in all study villages. Older children and adults were randomized by village to receive PCV-7 (11 vaccinated villages) or meningococcal-serogroup-C conjugate vaccine (10 control villages). Sixteen rural villages participated in the long term evaluation. Genotyping was performed by multilocus sequence typing (MLST).

Results: Four years after vaccination, the prevalence of carriage with VT and NVT pneumococci was similar in control and vaccinated villages - 6.4% versus 3.9% (p=0.120) and 32.7 versus 29.8% (p=0.392) respectively. The prevalence of VT carriage was lower than at baseline in control and vaccinated villages [OR=0.18;95%CI(0.11,0.28) and OR=0.13;95%CI(0.07,0.21) respectively]. A slightly higher prevalence of serotype 6A was detected in control versus vaccinated villages (3.5% versus 1.6%; p=0.093). The prevalence of serotype 19A carriage was similar in control and vaccinated villages (2.9% versus 2.5%, p=0.779) and had not increased from baseline. The most prevalent serotype 19A clone before and after vaccination was Sequence Type 847 representing more than 50% of analyzed isolates. Genotyping data for serotype 6A will be presented.

Conclusions: Four years after PCV-7 vaccination, the effect of vaccination on carriage of VT pneumococci was similar in vaccinated and control villages and no significant serotype replacement was detected even for serotype 19A.
Poster No 238

MOLECULAR EPIDEMIOLOGY OF STREPTOCOCCUS PNEUMONIAE CAUSING INVASIVE DISEASE AFTER THE INTRODUCTION OF 10-VALENT CONJUGATE VACCINE

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Background and aims: The 10-valent pneumococcal conjugate vaccine (PCV10) was introduced in the Brazilian immunization program in May 2010. The aim of this study was to characterize the profile of invasive disease isolates after the introduction of PCV10.

Methods: From May 2010 through August 2011 laboratory-based surveillance for laboratory-confirmed IPD was done in Salvador, Brazil. Capsular serotyping was performed by multiplex-PCR and antimicrobial susceptibility was determined by microdilution method. Genetic relatedness of isolates was determined by PFGE and MLST.

Results: A total of 72 IPD cases were identified. The majority of cases were patients aged > 5 years (48/72; 67%). The most frequent serogroup were 14 (17%), 6A/B (12%), 19A (12%), 18 (10%), 3 (9%) and 19A, 12F, 7C (5% for each). Non-susceptibility to penicillin (PNS) was identified in 38% of the S. pneumoniae isolates and was associated with serogroup 14 (100%), 6A/B (80%) and 19A (75%). The estimated rate of coverage of the PCV10 was 67% among patients ≤ 5 years old and 81% among those infected with PNS isolates. Of the 67 isolates that were genotyped, 37 (55%) were distributed among 11 fingerprint patterns with two or more isolates whereas 30 (45%) had non-cluster patterns. The two predominant clonal groups clustered serotype 14 (ST 156; PNS) and serogroup 18 (ST 193; penicillin-susceptible) isolates.

Conclusions: IPD cases were more frequent in adults and the isolates showed a high clonal diversity. This study highlights the importance to keep monitoring IPD after implementation of PCV10 on the epidemiology behavior of pneumococcal disease.
DECREASE IN PILUS-1 AND INCREASE IN PILUS-2 PREVALENCE AMONG STREPTOCOCCUS PNEUMONIAE CLINICAL ISOLATES IN GERMANY: IMPACT OF THE PCV7

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The recent discovery of the virulence factors PavB, PsrP and pili, extending from the pneumococcal surface, opened a new window in the research field of pneumococci. PsrP and PavB have been identified as adhesins, while the role of both pili (Pilus-1 and Pilus-2) in pathogenesis has been suggested. After 5-years of use in Germany, there are not molecular epidemiology studies showing the impact of the 7-valent pneumococcal conjugate vaccine (PCV7) on pneumococcal disease incidence. Here, we aimed to use the genomic loci of these pneumococcal virulence factors as nucleic acid-based genotyping biomarkers for molecular epidemiology studies. We analyzed their distribution among 419 clinical isolates, divided in three groups: invasive (n=233), non-invasive (n=73) and carriers (n=113). Forty-three serotypes were represented in this study population, including those used in PCV7. Overall, 100% of clinical isolates were pavB-positive and 29.8% were psrP-positive, while 102 (32.2%) isolates were pilus-positive: 17.2% Pilus Islet-2 (PI-2), 13.4% Pilus Islet-1 (PI-1), and 1.6% for both islets. Serotypes 19F, 19A, 11A, 6A, 7F, 1, 9V were strongly related to pili, and several STs were representing the pilus-positive population in MLST analysis. Interestingly, the majority of pilus- and psrP-positive isolates were susceptible to different antibiotics. Nevertheless, our major finding was the pilus prevalence behaviour (PI-1 decreased, while PI-2 increased) among the S. pneumoniae clinical isolates from 2008 to 2011 in Germany. These data show the impact of the PCV7 vaccination on the distribution of pneumococcal pill and PsrP among S. pneumoniae clinical isolates in Germany and may support the potential of Pilus-1 and Pilus-2 constituents as vaccine candidates.
SEROTYPES AND SUSCEPTIBILITY OF STREPTOCOCCUS PNEUMONIAE STRAINS ISOLATED FROM CHILDREN IN MEXICO CITY BETWEEN 2007 TO 2011

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Background and aims: Streptococcus pneumoniae is an important cause of invasive infections in children younger than 5 years. In Mexico, since 2000, the 7-valent conjugate vaccine (7VCV) is applied in private practice and, since 2006, is universally applied.

After the pneumococcal vaccination with 7VCV, different studies report a change of pneumococcal serotypes associated with pneumococcal disease.

In Mexico, before the 7VCV application, the most frequent pneumococcal serotypes isolated in invasive disease were: 14, 6B and 23F.

Methods: Descriptive study in a cohort, who included all patients with invasive pneumococcal disease who were ingresed in a private pediatric hospital in Mexico City (Hospital Español) between January 2007 to April 2011.

Results: We found 8 pneumococcal isolations in 8 patients. The specific infection was: 1 meningitis, 1 brain abscess, 2 mastoiditis, and 4 complicated pneumonias. 4 patients were younger than 24 months. 1 patient didn't have any vaccine. The pneumococcal serotypes isolated were: in meningitis and brain abscess, 19 A; in mastoiditis 6B and; and for pneumonia, one 19A, two 1 and one 5. We found penicillin resistance in one 1 serotype. We didn't find cefuroxime, ceftriaxone or cefotaxime resistance.

Conclusions: The pneumococcal infections in our population from 2007-2011 after 7VCV were associated to non vaccinated serotypes in 88.9%. It is important to continue the epidemiological studies after the introduction of the new conjugated pneumococcal vaccines.
MIDDLE EAR FLUID STREPTOCOCCUS PNEUMONIAE SEROTYPES ISOLATED COSTA RICAN CHILDREN FOLLOWING PARTIAL INTRODUCTION OF THE 7-VALENT PNEUMOCOCCAL CONJUGATE VACCINE

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Background/Aim: To determine serotype prevalence, antibiotic resistance patterns and potential coverage by pneumococcal conjugate vaccines among Streptococcus pneumoniae causing otitis media (OM) in Costa Rican (CR) children after partial introduction of the pneumococcal 7-valent conjugated vaccine (PCV7).

Methods: Analysis of all S. pneumoniae isolates recovered from CR children with OM between years 1999 to 2003 (before PCV-7 usage) were compared against data obtained from 2004 to 2008 (PCV-7 partially introduced).

Results: Between periods 1999 - 2003 and 2004 -2008, a total of 184 and 218 middle ear fluid (MEF) S. pneumoniae were analyzed. A clinically important change in the prevalence of S. pneumoniae serotypes was observed for serotypes: 14: 4.2% to 20.6% (P = 0.00002); 19F: 53.2% to 18.3% (P < 0.05); 9V: 5% to 1.4% (P = 0.05); 11A: 0% to 4.1% (P = 0.01) and 3: 6.4% to 12.8% (P = 0.07). Antimicrobial non-susceptible distribution among the two study periods was: Penicillin: 34.3% to 20.7% (P = 0.006); TMP/SMX: 38.8% to 52.8% (P = 0.01). PCV-7, PCV-10 and PCV-13 potential coverage between the two periods was: 77.3% to 60.5% (P = 0.01); 78% to 61.5% (P = 0.001) and 88% to 80.1% (P = 0.00002).

Conclusions: After partial introduction of PCV-7 in Costa Rica, changes in the prevalence of certain S. pneumoniae MEF serotypes in CR children with OM were observed along with a reduction in the percentage of penicillin non-susceptible strains. PCV-13 offered the highest potential vaccine coverage (80.1%).
REDUCTIONS IN NASOPHARYNGEAL CARRIAGE OF STREPTOCOCCUS PNEUMONIAE AND HAEMOPHILUS INFLUENZAE 6 MONTHS AFTER INTRODUCTION OF PCV10 IN KILIFI, KENYA

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Background: 10-valent pneumococcal non-typeable Haemophilus influenzae protein-D conjugate vaccine (PCV10) was introduced into the routine infant vaccination schedule in Kenya in January 2011, with catch-up vaccination for under-5's in Kilifi District; vaccine coverage with ≥1 dose was ~70%. We aimed to provide population-level data on the impact of PCV10 on nasopharyngeal carriage of Streptococcus pneumoniae, Haemophilus influenzae, and Staphylococcus aureus.

Methods: We conducted cross-sectional nasopharyngeal carriage surveys among 500 randomly selected residents of Kilifi District. Bacteria were identified from nasopharyngeal swabs by standard methods. Pneumococcal serotyping was performed by Quellung reaction. Proportions colonized before and after PCV10 introduction were compared by the Chi-square test.

Results: Swabs were collected in 2009 (n=506), 2010 (n=512), and 2011 (n=504). Among all participants, PCV10-type carriage declined from 16% to 7% (p<0.001; Figure 1). Significant declines occurred in children <1: 44% to 10%, p<0.001; children 1-4 years: 29% to 16%, p=0.01; and children 5-9 years: 21% to 6%, p=0.02. Among children aged <10 years, H. influenzae carriage declined from 58% to 41% (p<0.001); S. aureus carriage remained stable (7% vs 6%, p=0.61).

Conclusions: Reduced carriage of vaccine-type pneumococci and H. influenzae was observed among vaccinated and unvaccinated children within 6 months of PCV10 introduction. Repeat surveys will assess the sustainability of this effect and possible indirect effects in adults.
MULTIPLE-SEROTYPE PNEUMOCOCCAL CARRIAGE IN AUSTRALIAN CHILDREN IN THE NORTHERN TERRITORY: AGE, POPULATION AND REMOTENESS

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Background and aims: Rates of multiple-serotype carriage vary widely between populations. Indigenous children living in remote Aboriginal communities (RACs) have 70-85% pneumococcal carriage and up to 20% multiple-serotype carriage. We investigated carriage in Indigenous and non-Indigenous children from urban and remote settings to determine associations between multiple-serotype carriage and age, population and remoteness.

Methods: In a longitudinal study in urban child care centres (CCCs) and a cross-sectional study in RACs and CCCs, one colony per positive nasal swab was serotyped, plus any with differing morphology. In a study of Pneumovax vaccination for urban and remote Indigenous mothers, four colonies per positive swab were serotyped, including any with differing morphology; this protocol is slightly more sensitive (Hare et al/2008).

Results: In total 7700 swabs from 3269 children were analysed: 5591 from 1463 children (90% non-Indigenous) attending CCCs, 1753 from 1654 remote Indigenous children, and 356 from 152 urban Indigenous children. Carriage was significantly higher (p< 0.05) in remote Indigenous children at all ages (78% overall) compared to urban Indigenous children and children attending CCCs (50%), and multiple-serotype carriage was also higher (10% of positive swabs versus 2.7%). Carriage and multiplicity were lower in children < 6 months old (60% and 2.7% respectively in RACs, 19% and 0% respectively in urban settings) but otherwise little affected by age (data not shown).

Conclusions: Higher rates of multiple-serotype carriage were found where overall carriage was higher; both likely contribute to increased risk of pneumococcal disease. Carriage was influenced more by remoteness than ethnicity.
WHO’S SLEEPING WITH WHOM? PNEUMOCOCCAL SEROTYPE CO-COLONIZATION IN AUSTRALIAN INDIGENOUS CHILDREN IN THE NORTHERN TERRITORY

K. Hare, H. Smith-Vaughan, A. Leach, Darwin, NT, Australia

Background and aims: Multiple-serotype carriage occurs more frequently in populations with high carriage (abstract A-428-0010-00487). We previously published multiple-serotype detection in 20% of 98 swabs from Indigenous children living in remote Aboriginal communities (RACs) after selection of colonies at random and based on colony morphology (Hare et al 2008). Here we report further data on multiple-serotype carriage in this population at high risk of colonization.

Methods: Indigenous children 0 to 6 years of age living in RACs in 2003 and 2005 had nasal swabs collected, transported and processed according to published methods. One colony per positive swab was selected for serotyping, plus any with differing morphology.

Results: Data were obtained from 1721 children; 91% had received ≥2 doses of PCV7. Pneumococcal carriage was 79% and 141 (10.4%) were co-colonized. Carriage serotypes 19A, 16F, 11A, 6C, 19F, 23B and 6A predominated; 16F, 33F and 19A were the predominant co-colonizers. The proportion of positive swabs with a second serotype varied by serotype; 29 of 195 (15%) 19A-positive swabs had a second serotype, 20% for 16F, 11A 14%, 6C 5%, 19F 24%, 23B 13%, 6A 31%, and 33F 57%. Overall, 44 serotypes and 33 co-colonizing types were found. In the 29 19A-positive swabs with a second serotype, 16 different co-colonizing types were found, and for 38 16F-positive swabs with co-colonization, 20 different serotypes were involved.

Conclusions: With few exceptions (e.g. 33F), co-colonizing serotypes are usually those most commonly carried. The diversity of co-colonization was high in this population and no particular pairings predominated.
DECLINE IN PEDIATRIC PNEUMONIA HOSPITALIZATIONS FOLLOWING PCV7 AND PCV13 INTRODUCTION IN URUGUAY

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Background: In Uruguay the incidence of pneumonia hospitalizations in children < 2 years prior to PCV vaccination was 2,407/10^5. PCV7 was incorporated to National Immunization Program in March 2008 in a 2+1 schedule plus a catch-up for the 2007 cohort. In 2010 PCV13 replaced PCV7 with same dosing schedule and catch-up for children up to 5 years of age.

Objective: To compare the impact of PCV7 and PCV13 on the incidence of pneumonia hospitalizations in children as assessed before and after vaccination.

Methods: We used the same methodology as that used in the baseline study. All hospitalized patients in whom a chest radiograph was performed to confirm pneumonia were eligible. Radiographs were interpreted using the WHO criteria. Clinical parameters, radiographic and microbiologic results and vaccination status were recorded.

Results: Vaccine compliance for PCV7 was 92%. The 2008 cohort vaccinated with 3 doses of PCV7 and observed during 2009, had an incidence of 1.261/10^5 person-year representing 41% reduction compared with the baseline. Post PCV7, a significant decline was seen in incidence of pneumonias hospitalizations caused by serotype 14. Hospitalization of pneumonia with pleural effusion increased from 5% (26/519) to 14.3% (11/77) following PCV7. Surveillance post PCV13 (2010) showed incidence reduction (1.065/10^5) in pneumonia hospitalizations and by June 2011, 69% decrease was registered.

Conclusion: Surveillance documented a significant impact of PCV7 using 2+1 schedule on hospitalization for pneumonia in children < 2. Recently data on PCV13 has demonstrated early effectiveness for pneumonia control.

Study supported: By a Wyeth/Pfizer grant.
ARE PNEUMOCOCCAL CONJUGATE VACCINES FOR DEVELOPING COUNTRIES LIKE INDIA?

R. Kishore Kumar, Perth, WA, Australia

Background and aims: Pneumococcal conjugate vaccination (PCV) became the norm in most developed countries. Developing countries are still slow to implement it as a mandatory vaccine. Hence it gave an opportunity to “compare” those got the PCV and those who didn’t?

Methods: PCV (Prevenar) was available in India since 2006 and given to those who “opted”. The study period was from Jan 2007 till June 2011, when we had seen 12,877 out patients for primary vaccinations. 2107 babies were fully vaccinated with 4 doses of Prevenar vaccine - accounting for 6321 OPD visits and 1986 babies were NOT vaccinated with PCV and 829 babies had received less than 2 doses of PCV - and there was no subsequent visits to our hospital.

Results: Babies who received 4 doses of PCV - had an average of 4 OPD visits for non-vaccination episodes and most of them due to feeding or other issues in the first 2 years and had less than 2 URTIs. Those who received NO prevenar had more than 7 OPD visits in the first 2 years and had a minimum of 4 to 5 URTIs in the first 2 years. There were 2 episodes of “pneumonia” in non-vaccinated group with none in the vaccinated group.

Conclusions: PCV has shown worldwide reduction in Pneumococcal diseases. This study clearly shows there are more benefits than just decreasing invasive pneumococcal disease. It may be of more benefit in developing countries like India where pneumococcal data is very sketchy.
INTRODUCTION OF PNEUMOCOCCAL CONJUGATE VACCINES 7 AND 13-VALENT IN THE MUNICIPALITY OF CAMPOS-RJ, CORRELATING WITH NO OCCURRENCE OF PNEUMOCOCCAL MENINGITIDIS


Background: Pneumococcal diseases are responsible by over one million of deaths in children under 5 years old in development countries. Pneumococcal invasive diseases, such as meningitis, is the outcome with the worriest mortality rate and sequelae. The main strategy to prevent this condition is vaccination. The municipality of Campos (Rio de Janeiro) is the first city in Brazil to introduce the pneumococcal conjugate vaccine 7-valent in children under one year old in 05/29/2009, until 10/24/2010, when it was replaced by the pneumococcal vaccine 13-valent.

Aims: Describe that, after the introduction of the pneumococcal vaccines, there wasn’t any case of meningitis by S. pneumoniae in 2010, according with data from the Ministry of Health Diseases Database system.

Methods: Vaccines were administered for free to all children that have born in Campos city, State of Rio de Janeiro, Brazil. Routine administration recommended the first three doses at age 3, 5, 7 months, with booster dose at 13 months age.

Results: After 19 months of vaccination, at least 6.440 children received 4 doses of vaccine, with 92% of coverage vaccination status (7.000 births/year). There was no one suspect or confirmed pneumococcal meningitis case in 2010, when compared with 2009, who was notified 5 cases, and 1 death.

Conclusions: The absence of pneumococcal meningitis cases in 2010 after the introduction of pneumococcal conjugated vaccines in the municipality of Campos-RJ (Brazil) is supported by international studies, and the occurrence of any case of meningitis in individuals not vaccinated might represent herd immunity presence.
ADVOCATING EFFECTIVELY FOR PNEUMOCOCCAL DISEASE PREVENTION - LESSONS LEARNED DURING FIVE YEARS OF PACE ADVOCACY

C. de Quadros¹, O. Levine². ¹Washington, DC, ²Baltimore, MD, USA

In 2006, the Sabin Vaccine Institute created the Pneumococcal Awareness Council of Experts (PACE), a group of 21 of the world’s leading experts on infectious diseases and vaccines, to raise awareness about pneumococcal disease and to advocate for its prevention. PACE has worked towards this through a range of methods, including hosting high profile events; placing commentaries in global news outlets; convening meetings with government officials; publishing studies on pneumococcal disease and its impact; and raising general awareness through participation in global efforts.

Since PACE’s inception, 67 countries have introduced pneumococcal vaccine into their national immunization programs, a number of them in part due to PACE advocacy efforts. Additionally, over 115 medical societies from 50 countries have endorsed PACE’s Call to Action; PACE has published editorials in twelve prominent scientific journals, and conducted and published two studies on pneumococcal disease in Africa; and PACE has convened more than 30 events in 21 countries to raise the issue of pneumococcal disease prevention.

Much work still remains to be done. PACE has identified key lessons learned and opportunities for future engagement that can be used to inform future efforts: In particular, our five years of advocacy work has underscored the need for reliable, evidence based data as critical to decision-making; supported the notion that independent and trusted voices make for the most credible spokespersons; that a targeted approach to advocacy is the best use of limited resources; and that efforts must be led from inside the country to be most effective.
COMPLICATED PNEUMONIA IN CHILDREN 0-14 YEARS OF AGE AFTER THE INTRODUCTION OF PNEUMOCOCCAL CONJUGATED VACCINES (PCV7/13). HOSPITAL PEREIRA ROSSELL-URUGUAY 1/1/2010-31/9/2011


Introduction: S. pneumoniae is the major cause of Community Acquired Pneumonia (CAP). Empyema and necrotizing pneumonia (NP) are frequent complications. In March 2008 Uruguay included PCV7 in the routine vaccination program (2+1 schedule), and replaced by PCV13 in 2010.

Objective: Analyze clinical presentation and aetiology of complicated pneumonia cases hospitalized between 1/1/2010 to 31/9/2011.

Methods: All hospitalized empyema and NP cases were included. Empyema was defined by International Classification of Diseases 10th Revision criteria (J86). PN was defined as CAP with multiple small lucencies or pneumatoceles at thorax radiograph, with at least one of following criteria: clinical worsening, reappearance of fever or fever >7 days, bronchopleural fistulae, white blood count>30.000 or < 5.000 mm³, protein C reactive >120mg/dL, lactic dehydrogenasa >2.500 UI in pleural fluid. Serotyping was performed at Servicio Nacional de Laboratorios de Salud Pública.

Results: Of 494 children hospitalized for CAP in this period 100 (20.2%) were complicated: 56 empyema, 7 PN, 37 with both complications. The median age was 30 months; the majority was healthy. The median hospitalization was 15 days; one third requires CTI. Five patients require surgical management. S.pneumoniae was isolated in 18 empyema cases and in 10 with both complications. The serotypes most frequent were: 3 (n=8), 1(n=7), 5 (n=3), 14 (n=3), 7F (n=2), 19A (2), 12F (n=1). A 16 months child with serotype 3 received 3 doses of PCV13; the other children with PCV13 or PCV7 serotypes were not vaccinated or had 1 dose.

Conclusion: Universal vaccination with PCV13 will reduce complicated pneumonia.
Poster No 250

THE IMPACT OF 7PCV 2 YEARS AFTER INTRODUCTION IN THE GAMBIA: POPULATION-BASED SURVEILLANCE AMONG ALL AGES

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Background and aims: PCVs have reduced IPD in affluent countries but the impact in developing countries is unclear. We measured the impact of PCV introduction in a rural area of The Gambia.

Methods: We conducted outpatient and inpatient surveillance for suspected pneumonia, sepsis and meningitis in health facilities throughout the Basse HDSS. Surveillance was conducted 12 months pre-7PCV and 24 months post. There was a limited catch-up campaign. After 20 months, 13PCV replaced 7PCV. Nurses screened patients with standardised criteria, referring to clinicians for standardised assessment and investigation.

Results: Vaccine-type IPD among those aged 2-23 months fell by 94%. Overall IPD was not significantly different pre- and post-PCV.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Year</th>
<th>2-23 mo</th>
<th>24-59 mo</th>
<th>&gt;=5 yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-7PCV</td>
<td></td>
<td>132 (74, 218)</td>
<td>12 (2, 45)</td>
<td>(0, 4)</td>
</tr>
<tr>
<td>Vaccine-type IPD</td>
<td></td>
<td>42 (14, 99)</td>
<td>35 (13, 75)</td>
<td>(0, 4)</td>
</tr>
<tr>
<td>Post Yr 1</td>
<td></td>
<td>8 (0, 46)</td>
<td>27 (9, 64)</td>
<td>(0, 3)</td>
</tr>
<tr>
<td>Post Yr 2</td>
<td></td>
<td>229 (150, 336)</td>
<td>87 (48, 146)</td>
<td>9 (4, 16)</td>
</tr>
<tr>
<td>Pre-7PCV</td>
<td></td>
<td>254 (171, 362)</td>
<td>115 (70, 178)</td>
<td>15 (9, 23)</td>
</tr>
<tr>
<td>Post Yr 1</td>
<td></td>
<td>316 (224, 434)</td>
<td>103 (62, 162)</td>
<td>14 (8, 21)</td>
</tr>
<tr>
<td>Post Yr 2</td>
<td></td>
<td>1438 (1226, 1676)</td>
<td>398 (307, 509)</td>
<td>70 (56, 86)</td>
</tr>
<tr>
<td>All IPD</td>
<td></td>
<td>1404 (1199, 1635)</td>
<td>380 (294, 484)</td>
<td>68 (54, 84)</td>
</tr>
<tr>
<td>X-ray Consolidation or Effusion</td>
<td></td>
<td>1962 (1720, 2229)</td>
<td>419 (330, 524)</td>
<td>57 (45, 71)</td>
</tr>
</tbody>
</table>

[Table. Disease Incidence (cases per 100,000 pyrs)]

Conclusions: Reductions in vaccine-type IPD were observed. The lack of an observed reduction in all IPD may be due to changes in serotype-specific incidence or surveillance sensitivity.

Acknowledgements: PneumoADIP, B&MGF, URR Regional Health Team, MoH, BHDSS.
EPIDEMIOLOGY OF PNEUMOCOCCAL NASOPHARYNGEAL COLONIZATION IN HIV-INFECTED (HIV+) AND HIV-UNINFECTED (HIV-) INFANTS VACCINATED WITH 7-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV)

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Aim: We studied the epidemiology of nasopharyngeal colonization by Streptococcus pneumoniae in HIV+ and HIV- infants vaccinated with three doses of PCV at 6, 10 and 14 weeks of age.

Methods: Nasopharyngeal swabs were done prior to each dose of PCV and one- and six-months later. Standard microbiologic methods were used and serotyping by Quellung method. The study included HIV+ infants with CD4+ ≥25% randomised to immediate antiretroviral treatment (ART) (HIV+/ART+: n=172) or deferred until clinically/ immunologically indicated (HIV+/ART-; n=77); and HIV- infants born to HIV- mothers (M-/I-; n=114) and HIV+ mothers (M+/I-; n=120).

Results: The prevalence of overall, vaccine-serotype+6A (VT) and non-vaccine serotype (NVT) colonization did not differ at any time-point between HIV+/ART+ and HIV+/ART-; or between M+/I- and M-/I- children. Consequently, we compared all HIV+ to HIV- children.

Following PCV dose-1, HIV+ children had a lower prevalence of NVT colonization throughout; and a lower prevalence of overall colonization until one-month post PCV dose-3. The prevalence of VT colonization was similar between HIV+ and HIV- infants throughout.

Conclusion: VT colonization was similar between HIV+ and HIV- children. The lower prevalence of overall and NVT colonization, however, may be due cotrimoxazole prophylaxis provided to HIV+ children protecting against colonization by NVT which usually are more susceptible to cotrimoxazole.
EFFECT OF HIV-EXPOSURE AND ANTIRETROVIRAL TREATMENT ON IMMUNE RESPONSES FOLLOWING ONE, TWO AND THREE DOSES OF PNEUMOCOCCAL CONJUGATE VACCINE (PCV)

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Background: The immunogenicity of a two-dose primary series of PCV has not been established in HIV+ children. We describe the antibody responses one-month following each of three doses of PCV administered at age 6, 10 and 14 weeks in HIV+ and HIV- children.

Methods: The study included HIV+ infants with CD4+ ≥25% randomised to immediate antiretroviral treatment (ART) (HIV+/ART+) or deferred ART until clinically or immunologically indicated (HIV+/ART-), a convenience sample of HIV+ children on ART with CD4+ < 25% (HIV+/< 25%); and HIV- infants born to HIV- (M-/I-) and HIV+ mothers (M+/I-).

Results: Serotype-specific antibody concentrations increased significantly following each sequential dose of PCV7 in all groups. The proportion of children with serotype specific antibody concentration ≥0.35ug/ml after each dose is tabulated.

| Study group | Post dose 1 4/6|9/14|13/C/19F/23F | Post dose 2 4/6|9/14|18/C/19F/23F | Post dose 3 4/6|9/14|18/C/19F/23F |
|-------------|----------------|----------------------------------|----------------|----------------------------------|----------------|----------------------------------|
| M-/I-       |                | M+/I-                            |                | H+/ART+                          |                | H+/CD4+<25%                      |                |                               |
| (n=117, 116, 114) | 58/26|49|74|60|82/37 | 92/37|64|97|97|97|97|97|98/82|99|95|99|95|95 | 98/82|99|95|99|95|95 |
| M+/I-       | 56/9|35|60|54/68|18 | 99/35|100|55|94|96|87 | 100/92|69|96|100|99|99 |                               |
| (n=122, 121, 120) |                |                |                | (n=156, 191, 172) |                |                |                               |
| H+/ART+     | 59/15|33|69|50|69|24 | 96/50|64|95|94|96|79 | 98/92|88|96|98|95 |                               |
| (n=156, 191, 172) |                |                |                |                               |                |                |                               |
| H+/CD4+<25% | 67|20|44|77|54|85|52 | 90|60|85|92|89|95|87 | 97|88|95|97|93|97|97 |                               |
| (n=11, 10, 8) |                |                |                |                               |                |                |                               |

1 n = number observations after the 1st, 2nd and 3rd dose of PCV. 2Proportion with serotype specific antibody concentrations ≥0.35ug/ml for respective serotypes.

Immune responses following each of 3 PCV doses ]

The proportion of children with antibody ≥0.35 ug/ml after the second dose of PCV7 was especially low for 6B. Generally, immune responses in HIV+ children were similar to M-/I- following the second dose of PCV.

Conclusion: PCV7 given at 6 and 10 weeks of age may not provide adequate protection to some common disease causing serotypes, irrespective of HIV-infection status.
SEROTYPE AND GENOTYPE DISTRIBUTIONS AMONG INVASIVE PNEUMOCOCCAL ISOLATES RECOVERED FROM PERUVIAN CHILDREN

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**Background and aims:** PCV7 was introduced into the national immunization program of Peru in 2009. The aim of this study was to determine serotype and genotype distributions among invasive strains from 2006 to 2010 to provide information on vaccine coverage and circulating clonal types, pre and post-vaccine introduction.

**Methods:** We characterized 131 strains from invasive pneumococcal disease (IPD) in children (< 16 yrs) from a passive surveillance study in 16 public and private hospitals in Lima (2006-2010). All isolates were serotyped by Quellung, and genotyped through multilocus sequence typing (MLST).

**Results:** We identified 27 serotypes with 65.6% (n=86) and 80.9% (n=106) included in PCV7 and PCV13, respectively. The prevalent serotypes were 14 (26%), 6B (19.8%), 19F (9.9%), 23F (6.1%), 19A (6.1%) and 5 (5.3%). For MLST, 59 different STs were observed, with 11 new alleles and 30 new STs identified. Seven MLST types (ST156, ST1421, ST90, ST5625, ST242, ST289 and ST81) accounted for 47.3% of the isolates. A large proportion of isolates were related to globally disseminated Pneumococcal Molecular Epidemiology Network clones in both pre-PCV(43.3%) and post-PCV(37%) periods and these are associated primarily with PCV13 serotypes.

**Conclusions:** Data from this study in Lima shows that a large proportion of IPD serotypes are covered by conjugate vaccines and that PCV13 would provide a substantially greater benefit than PCV7, primarily through targeting serotypes 5 and 19A. Although the majority of isolates characterized represent a small subset of well-characterized global clones, we identified many new sequence types currently restricted to this region.
ECONOMIC EVALUATION OF HEPTA- DECA- AND THIRTEEN-VALENT VACCINES TO PREVENT PNEUMOCOCCAL PNEUMONIA IN PERUVIAN CHILDREN UNDER FIVE YEARS OF AGE

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Aim: To evaluate the cost-effectiveness (C-E) of vaccination with pneumococcal conjugate vaccine 10-valent (VCP10) and 13-valent (VCP13) in preventing S. pneumoniae pneumonia in children under five years in Peru considering the vaccination with 7-valent (VCP7) as basic strategy.

Methods: We conducted a C-E study, which included the probabilistic and deterministic sensitivity analysis; the effectiveness measure used was the number of hospitalizations for pneumonia avoided.

Results: Starting from the Direct Effect of PV7, a ratio of pre- and post-vaccination incidences (in Uruguay surveillance) for pneumonia per PCV10 and PCV13 serotype was estimated at 0.48 and 0.17, respectively. Likewise, these values would correspond to a risk reduction of 52 and 83 percent, respectively.

The cohort of 100 children vaccinated with PCV7, which develop pneumonia would have a potential cost of S/.38,583. The cohort of 100 children vaccinated with PCV10 which develop pneumonia would have a potential cost of S/.33,608; and the cohort of 100 children vaccinated with PCV13 which develop pneumonia would have a potential cost of S/.35,047. The C-E analysis for the interventions with PCV7, PCV10 and PCV13 was estimated at S/.2,014, S/.956 and S/.456, respectively; and the PCV7 alternative was dominated versus those of PCV10 and PCV13. The incremental cost effectiveness ratio between VPC13 and VCP10 was S/.32.

Conclusion: Interventions VCP13 and VCP10 are more cost-effective than VCP7 strategy in preventing hospitalization for pneumonia. However, the VCP13 is more cost effective than VCP10.
COMPARISON OF VACCINE SEROTYPE DISTRIBUTION AMONG INVASIVE PNEUMOCOCCAL DISEASE STRAINS ISOLATED FROM CHILDREN BETWEEN 2006-2008 AND 2009-2011 IN LIMA, PERU

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Background and aims: PCV7 was introduced into the Peruvian national immunization program in 2009. The aim of this study was to compare the serotype distribution among invasive strains isolated before and during the initial period of PCV7 introduction in Lima.

Methods: We conducted a passive surveillance study of invasive pneumococcal disease (IPD) in children (< 18 yrs) from 16 public and private hospitals in Lima (2009-2011). We compared the current serotypes with 99 strains isolated from a previous similar passive surveillance study conducted between 2006-2008. All strains were serotyped by Quellung. We determined the antibiotic resistance rates to penicillin and erythromycin.

Results: During the second period 59 IPD strains were isolated; 44% in children < 2 yrs. The main diagnoses were pneumonia in 31 (53%) and meningitis in 13 (22%). We completed the serotyping in 57 strains; the most common were: 14(21%), 19F(16%), 23F(11%), 6B(9%) and 19A(9%). During 2006-2008 and 2009-2011 the distribution of vaccine serotypes was 67% and 60% for PCV7; 73% and 67% for PCV10; and 82% and 77% for PCV13, respectively. Resistance to penicillin by MIC among meningal strains was 36% and among non-meningal strains 3%; resistance to erythromycin was 39%.

Conclusions: Although the distribution of vaccine serotypes between both periods was not statistically different, there was a tendency for a decrease in the serotype coverage because of an increase in the non-vaccine serotypes in the second period, which may represent an initial impact of the vaccination program with PCV7 that needs to be monitored.
PREVALENCE OF INVASIVE PNEUMOCOCCAL DISEASE AND ITS ANTIMICROBIAL SUSCEPTIBILITY AMONG MEXICAN CHILDREN UNDER FIVE YEARS OF AGE

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Introduction: Streptococcus pneumoniae (pneumococcus) is one of the main causes of invasive disease (ID). Variation in terms of serotype distribution and resistance to antibiotics accounts for the fact that these diseases continue to be a public health problem in Mexico.

Objectives: To describe the prevalence of ID and its etiology. To determine the antibiotic resistance profile of pneumococcal- serotypes causative of ID among children in Mexico.

Methods: From March 2010 to June 2011, active surveillance of children > 28 days and < 60 months of age, with clinical suspicion of ID was carried out in 8 hospitals from 4 states in Mexico. At least one blood sample and samples of other sterile body fluids were taken from these patients. The isolated strains of pneumococcus were serotypified and their antimicrobial susceptibility determined.

Results: Five-hundred forty-five cases with ID were included; among those, pneumonia was the most prevalent (75.8%). Forty-two strains of pneumococcus were isolated, being serotype 19A the most prevalent (31%). The serotypes with the highest resistance percentages were 23B, the non-typifiable and 19A. Up to 59.5% of the cases of invasive pneumococcal disease (IPD) had received one or more doses of heptavalent pneumococcal conjugate vaccine (PCV7); only 21.4% of the cases of IPD were caused by serotypes contained in the PCV7.

Conclusions: Pneumococcus was the most prevalent isolated agent in ID cases. The higher prevalence of 19A and 6A suggests a serotype replacement secondary to the universalization of the PCV7 in our country.
SYSTEMATIC REVIEW ON THE EFFECTS OF DIFFERENT PNEUMOCOCCAL CONJUGATE VACCINE DOSING SCHEDULES ON IMMUNOGENICITY

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Background: Despite the breadth of studies demonstrating benefits of pneumococcal conjugate vaccine (PCV), uncertainty remains regarding the optimal PCV dosing schedule in children.

Methods: We conducted a literature review of PCV immunogenicity published from 1994-2011. Studies included for analysis evaluated ≥2 doses of 7-valent or higher product (excluding Aventis-Pasteur PCV11) administered to non-high risk children ≤6 months of age. Impact of PCV schedule on antibody concentration (GMC) 1 month post-dose against 6 serotypes was assessed using random effects linear regression, adjusted for product, DTaP/DTwP co-administration, lab method, age at first dose, and geographic region.

Results: We evaluated 23 2-primary-dose, 87 3-primary-dose, 13 “2+1” (i.e., 2-primary plus boost), and 46 “3+1” schedules. GMC was higher following 3-primary compared to 2-primary doses for all serotypes except serotype 1. GMC was significantly higher for all serotypes when dose3 was administered in the second year compared to ≤6 months of age.

Conclusions: While giving the third dose in the second year of life produces higher antibodies than if given in the first 6 months, the lower GMC between the primary series and booster may increase an individual’s risk of disease in that interval. In populations with high PCV coverage in infancy, this risk may be offset by indirect effects. Whether theoretical advantages of higher antibodies induced by giving the third dose in the second year of life, such as potential improvement for serotype 1 disease, longer duration of protection, or more rapid induction of herd effects, occur in practice needs to be evaluated.
Impact of Universal Pneumococcal Vaccination (PCV7/PCV13V) on Hospitalizations for Pneumonia and Meningitis in Children Hospital Pediátrico-Centro Hospitalario Pereira Rosell, Uruguay

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Background: In March 2008 Uruguay included PCV7 in the routine vaccination program (2+1 schedule). In April 2010 PCV7 was replaced by PCV13.

Objective: To assess the impact of this strategy in hospitalizations for pneumonia and pneumococcal meningitis (PM).

Methods: Annual rates per 10,000 discharges are described in the period before PCV7 vaccination (2005-2007), the year of vaccine implementation (2008), two years and 9 month after implementation.

Results: The percentage of reduction comparing median pre-vaccination rates 2005-2007 vs. 2010 was -67.2% for pneumonia, -61.4% for empyema and -52.5% for pneumococcal pneumonia (PP). Significant reduction was observed for pneumonia and PP by PCV7 serotypes (serotype 14 rate decreased from 26.6 to 2.5) in < 2 years of age. Between 1/1/2010 to 31/9/2011, 142 children were hospitalized for pneumonia and 22 for empyema. In 2011 S. pneumoniae was isolated from 14 children: serotype 3 (n=5), serotype 5 (n=2), serotype 7F (n=1), serotype 19A (n=1), non PCV13 serotypes (n=4) and one not yet typed. In 2011 none fully immunized children were hospitalized for PCV7 serotypes PP. Children with serotypes 1, 5, 19A and 7F were not immunized with PCV13. One fully immunized child was hospitalized for serotype 3 PP. In 2011 two children were hospitalized for PM: 2 months old with serotype 24A and 13 years old with serotype 3. Any vaccine serotype PM was hospitalized after 2008. Complete data 2011 will be available.

Conclusions: There was significant reduction in discharge rates for pneumonia and PM. PCV13 provides additional benefit in the prevention of these diseases.
INITIAL EFFECTS OF PCV7 ON S. PNEUMONIAE (SP) & S. AUREUS (SA) CARRIAGE IN A POPULATION-BASED STUDY; THE PALESTINIAN–ISRAELI COLLABORATIVE RESEARCH

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Background: To assess overall vaccine effects, a vaccinated population should be compared to similar, but geographically separated, nonvaccinated populations. This population-based study assesses PCV7 effects on SP and SA carriage.

Methods: We enrolled Palestinians subjects from a) Ramalla, Nabulus and Bethlehem living under Palestinian Authority’s (PA) health policy, where PCV7 has not been implemented and b) East Jerusalem (EJ) where PCV7 was implemented (7/2009). We performed cross-sectional surveys of SP and SA carriage in children< 5y and parents in 2009 and 2010. Clinical data were collected and SA/SP/serotype/antibiotic susceptibilities were identified.

Results: 621 and 347 pairs of children/parents were sampled from PA and EJ respectively in 2009 and 592 and 311 in 2010. Clinical data from both population groups were comparable. Total SP carriage did not vary between the two years. SP-carriage in children was 29% in EJ and 34.5% in PA. Only 4% and 3% of PA and EJ-parents were carriers. Serotypes included in PCV7 were replaced in EJ (from 45% to 16%) but not in PA. Non-PCV7 strains that increased included 15A, 15B/C, 11A and 6C. While child-SA carriage slightly increased in EJ (20% to 26%) (P=0.08) vs. PA (21% to 22%), a significant increase in parent-SA carriage was observed in EJ (26% to 34%) (p=0.03) but not in PA (31% to 30%).

Conclusions: This population-based study demonstrates early carriage serotype replacement by non-VT. SA colonization increased significantly in parents of the vaccinated population but not in the unvaccinated, a trend seen to a lesser extent in children.
EFFECT OF 10- AND 11-VALENT PNEUMOCOCCAL NON-TYPEABLE HAEMOPHILUS INFLUENZAE PROTEIN D CONJUGATE VACCINES (PHID-CV AND 11PN-PD) ON NASOPHARYNGEAL BACTERIAL CARRIAGE

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Background/aims: An objective of two double-blind studies, Clinical Otitis Media and Pneumonia Study (COMPAS), and Pneumococcal Otitis Efficacy Trial (POET) was to demonstrate efficacy of PHID-CV and 11Pn-PD (GlaxoSmithKline Biologicals) against acute otitis media (AOM). As AOM-causing pathogens usually also colonise the nasopharynx, both studies evaluated impact of vaccination on nasopharyngeal carriage of Streptococcus pneumoniae, Haemophilus influenzae (Hi) and other bacteria.

Methods: Children were randomised to receive hepatitis vaccine (control groups) or PHID-CV in COMPAS, 11Pn-PD in POET, co-administered with DTPa-combination vaccine, in 3-dose primary series in their first year of life, followed by a booster dose in their second year. Nasopharyngeal swabs were taken from maximum 1,693 and 371 subjects/timepoint in COMPAS and POET, respectively.

Results: Reduced carriage of vaccine pneumococcal serotypes (VT) was consistently observed from 1 month post-booster in both studies (Table). A commensurate increase in non-vaccine non-cross-reactive serotype (NVT) colonisation was not observed until 3 months post-PHID-CV-booster and 12 months post-11Pn-PD-booster. No effect on non-typeable Hi (NTHi) colonisation was detected pre-booster; decreases were observed at 1 month post-booster for both vaccines, but had disappeared by 9/12 months post-booster. No impact on Staphylococcus aureus colonisation was seen at any timepoint in either study.

Conclusions: Comparable trends of reduction in nasopharyngeal carriage of VT and NTHi were seen following vaccination with pneumococcal PD-conjugate vaccines in COMPAS and POET.

<table>
<thead>
<tr>
<th>COMPAS</th>
<th>POET</th>
</tr>
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<tbody>
<tr>
<td>PHID-CV %</td>
<td>Control %</td>
</tr>
<tr>
<td>6 months post-primary: aged 12-15 months</td>
<td>7 months post-primary: aged 12-15 months</td>
</tr>
<tr>
<td>VT: 13.7</td>
<td>16.1</td>
</tr>
<tr>
<td>NVT: 10.7</td>
<td>11.0</td>
</tr>
<tr>
<td>NTHi: 5.8</td>
<td>5.5</td>
</tr>
<tr>
<td>1 month post-booster: aged 16-19 months</td>
<td>1 month post-booster: aged 13-16 months</td>
</tr>
<tr>
<td>VT: 10.3</td>
<td>13.9</td>
</tr>
<tr>
<td>NVT: 11.0</td>
<td>11.5</td>
</tr>
<tr>
<td>NTHi: 3.7</td>
<td>4.8</td>
</tr>
<tr>
<td>3 months post-booster: aged 18-21 months</td>
<td>3 months post-booster: aged 15-18 months</td>
</tr>
<tr>
<td>VT: 10.3</td>
<td>14.3</td>
</tr>
<tr>
<td>NVT: 12.7</td>
<td>9.9</td>
</tr>
<tr>
<td>NTHi: 8.9</td>
<td>5.4</td>
</tr>
<tr>
<td>9 months post-booster: aged 24-27 months</td>
<td>12 months post-booster: aged 25-27 months</td>
</tr>
<tr>
<td>VT: 10.3</td>
<td>13.3</td>
</tr>
<tr>
<td>NVT: 11.5</td>
<td>9.4</td>
</tr>
<tr>
<td>NTHi: 5.0</td>
<td>4.9</td>
</tr>
</tbody>
</table>

NTHi: Non-typeable Haemophilus influenzae; NVT: non-vaccine non-cross-reactive serotypes; VE: vaccine efficacy estimated as 1 relative risk (VT, vaccine pneumococcal serotypes)

Table

Conclusions: Comparable trends of reduction in nasopharyngeal carriage of VT and NTHi were seen following vaccination with pneumococcal PD-conjugate vaccines in COMPAS and POET.
THE IMPACT OF INTRODUCING 10-VALENT PNEUMOCOCCAL CONJUGATE VACCINE IN KENYA ON INVASIVE PNEUMOCOCCAL DISEASE AMONG CHILDREN UNDER 5 YEARS

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Background: The Government of Kenya, with support from the GAVI Alliance, introduced 10-valent pneumococcal conjugate vaccine (PCV10) into routine infant immunization in January 2011 with a 2-dose catch-up for under-5’s in Kilifi District. Since 2008 GAVI has supported surveillance for all immunizations and for Invasive Pneumococcal Disease (IPD) among persons of all ages in Kilifi to monitor vaccine impact.

Methods: The Kilifi Health and Demographic Surveillance System (KHDSS) monitors vital status and migration of 260,000 people through 4-monthly household visits. Kilifi District Hospital is the only inpatient facility within KHDSS. Hospital admissions are linked to the KHDSS population register and investigated with blood cultures. Immunizations are recorded at 26 vaccine clinics and linked to the population register to calculate immunization coverage.

Results: Among children aged < 5 years in KHDSS, the coverage of one and two doses of PCV10 was 65.0% and 33.3% on September 30, 2011 and the incidence of vaccine-serotype IPD was 14.9/100,000 from March-October 2011. This compared to 50.8/100,000 between 2003-2010 (Incidence rate ratio=0.294, 95%CIs 0.094-0.700).

Conclusions: KHDSS is sensitive to changes in IPD incidence. Early observations illustrate a ~70% reduction in incidence since completing the first catch-up round. Accumulation of surveillance time will determine the sustainability of this effect, improve precision and allow analysis of indirect vaccine effects.

Funding: GAVI Alliance / Wellcome Trust.
3 OR 2 PRIMARY DOSES FOR PCV IN DEVELOPING COUNTRIES? EVIDENCE FROM A SYSTEMATIC REVIEW

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Background: Many developing countries are introducing pneumococcal conjugate vaccines (PCVs). Alternative vaccination schedules include 3 primary doses (3p+0), as recommended by the World Health Organization in 2007, and 2 primary doses plus a booster (2p+1). We summarized evidence about these schedules in a systematic review.

Methods: We searched 12 databases up to March 2010. We included randomized controlled trials (RCTs) and case-control studies comparing 2p+1 and 3p+0 schedules to each other or no PCV. We analyzed data about clinical outcomes, nasopharyngeal carriage and seropositivity (>0.35µg/ml) or geometric mean concentration (GMC).

Results: No RCTs directly compared clinical outcomes after 2p+1 and 3p+0 schedules. RCTs in Africa showed high efficacy of 3p+0 schedules against vaccine serotype (VT) invasive pneumococcal disease (71%, 95%CI 52, 82%). Clinical data about 2p+1 schedules were available only from one case-control study. RCTs showed less VT carriage after either 3p+0 or 2p+1 schedules compared to no vaccination, and suggested less VT carriage after 3p than 2p. Seropositivity data at 6 and 12 months favored 3p over 2p but differences were small except for serotypes 6B and 23F at 6 months. One month after boosting, GMC were substantially higher after 2p+1 than 3p+0 schedules. Differences were smaller six months later.

Conclusions: Both 3+0 and 2p+1 schedules are likely to provide acceptable levels of protection against disease. More clinical data are needed for schedule comparisons. Until then, scheduling decisions should be based on factors such as programmatic considerations, serotype distribution and burden of disease in the first year of life.
ESTIMATING SEROTYPE-SPECIFIC REPRODUCTIVE NUMBERS FROM A LONGITUDINAL STUDY OF STREPTOCOCCUS PNEUMONIAE CARRIAGE IN UNVACCINATED MOTHER-CHILD PAIRS

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**Background and aims:** The evolutionary dynamics of pneumococcus transmission within and across populations is a complex process. We explored the utility of mathematical models in understanding the influence of mother-child mixing patterns on overall trends of pneumococcus dynamics.

**Methods:** Pneumococcal-vaccine naive mother-child dyads had nasopharyngeal carriage swabs done at nine time points during the two years of follow-up. Pneumococci were cultured and serotyped by standard methods. To gain insight of nasopharyngeal transmission patterns, a mathematical model of the system was formulated and fitted to mother-child *S.pneumoniae* carriage data.

**Results:** The empirically-supported model showed that in children, the mean reproductive numbers (average number of new infections from one colonized individual) for the most prevalent pneumococcal serotypes (19F, 23F, 6B, 6A, 14, 19A) were within the range of 1 to 4. For the three serotypes (19F, 23F, 6B), child-to-child and child-to-mother transmissions accounted for 51-67% and 16-35% of all transmission, respectively. Carrying children accounted for 80-94% and 77-98% of new infections in children and mothers, respectively. Transmission of *S.pneumoniae* from mothers to children and between mothers was minimal. Mother-to-mother and mother-to-child reproduction numbers were estimated to be below the value 1, and accounted for 0-11% and 4-17% of all transmission, respectively. Significant differences in serotype-specific duration of carriage were observed between children (range of 40-105 days) and their mothers (range of 8-12 days).

**Conclusions:** Children were the major drivers of pneumococcal transmission in the population. The introduction of a pneumococcal vaccine is projected to have a modest herd effect on carriage.
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MOLECULAR EPIDEMIOLOGY OF NON-VACCINE PNEUMOCOCCAL SEROTYPES ISOLATED FROM MENINGITIS PATIENTS IN SALVADOR, BRASIL, PRIOR AND AFTER THE INTRODUCTION OF PCV10

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Background and aims: In May 2010, 10-valent pneumococcal conjugate vaccine (PCV10) was introduced in the Brazilian childhood vaccination program. However, replacement by non-vaccine type (NVT) serotypes can reduce vaccine benefits. We examined the distribution, antimicrobial susceptibility and genetic relationship among NVT pneumococcal serotypes isolated from meningitis patients during pre (January 2008 to May 2010) and post-vaccine (June 2010 to June 2011) periods.

Methods: Surveillance for pneumococcal meningitis was established at Reference Hospital of Infectious Diseases in Salvador, Brazil. The serotype was defined by multiplex PCR or Quellung reaction. Antimicrobial susceptibility was determined by E-test and MIC. Genetic relationship was studied by PFGE.

Results: A total of 131 cases of meningitis were identified from January-2008 to June-2011, with a median age of 24.2 years. The incidence rates of pneumococcal meningitis ranged from 1.26 (pre-vaccine period) to 0.8 (post-vaccine period) cases/100,000 persons for all age groups, with an overall case-fatality rate of 20.6% (27/131) for all patients. NVT serotypes accounted 42 (45.6%) isolates in the pre-vaccine period, with the prevalence of serotype 3 (n=6; 14.3%). In the post-vaccine period 11 (42.3%) NVT isolates were identified, of which the serotype 12F (n=2; 18.2%) was predominant. Non-susceptibility to penicillin was detected in both serotypes 9N and 12F (1.9%; 1/53 for each). PFGE analysis revealed 8 different clonal groups with three more frequent clones (45.8%; 11/24) persisting in both periods.

Conclusion: During the period of study, no obvious serotype replacement was observed.

Acknowledgement: This work was supported by CNPq.
EFFECTIVENESS OF 7-VALENT PNEUMOCOCCAL CONJUGATE VACCINE AGAINST INVASIVE PNEUMOCOCCAL DISEASES IN CHILDREN IN URUGUAY

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Montevideo, Uruguay, Atlanta, GA, Washington, WA, USA

Background and aims: The 7-valent pneumococcal conjugate vaccine (PCV7) was introduced in Uruguay in March 2008, using a 2-dose primary series (2, 4 months) plus booster (12 months) for children born on/after January 1 2008 and 2 catch-up doses (15, 17 months) for those born in 2007. In March 2010 the 13-valent conjugate vaccine (PCV13) replaced PCV7. Effectiveness against PCV7 vaccine-type invasive pneumococcal disease (VT-IPD) was evaluated.

Methods: We used pre-existing laboratory-based surveillance data and the national immunization registry that includes all children regardless of vaccine receipt to conduct a case-control study. VT-IPD cases age-eligible to receive ≥1 PCV7 dose were identified through laboratory records and matched (by date of birth +/- 1 month and neighborhood) to all eligible controls from the registry. PCV7 doses received >2 weeks before case child's culture date were assessed through the registry. Effectiveness was estimated as (1-OR)×100.

Results: Between April 2008 and February 2010, 44 VT-IPD cases in children < 5 years old were identified and 43 (98%) were found in the registry; 7 (16%) were age-eligible for ≥1 PCV dose and were matched to 637 controls. Two (28.6%) cases and 1 (14.3%) case and 269 (42.2%) controls had ≥2 doses (effectiveness 94.8% [95%CI: 43.1-99.5]).

Conclusion: Despite small case numbers, we demonstrated high PCV7 effectiveness against VT-IPD in Uruguay, a middle-income country using a 2-dose primary series plus booster with a catch-up campaign at introduction.

A similar assessment of PCV13 is planned.
THE ACCELERATED ROLLOUT OF PNEUMOCOCCAL CONJUGATE VACCINE: ITS IMPACT ON HEALTH AND GLOBAL EQUITY

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Background: Historically, it takes ~15 years for new vaccines to reach low income countries. This global rollout pattern favors those who can pay for vaccines over those who need them most. The GAVI Alliance and the Advanced Market Commitment for pneumococcal conjugate vaccines (PCV) help overcome financial barriers to uptake in low income countries.

Methods: To measure the impact of PCV rollout acceleration, longitudinal data on population, introduction timing, and vaccine coverage from the first 20 years of Hib vaccine use were compared to real and projected data for the first 20 years of PCV use. Regression analysis was used to determine the impact of country-level disease burden estimates and GNI to the timing and extent of vaccine access.

Results: As of 2011, PCV has been introduced in 31% of low income countries, reaching that threshold 7 years faster than Hib vaccines. New generation vaccines (PCV10/PCV13) reached 30% of low income countries 16 years faster. This acceleration could result in an additional 433 million children with access to the vaccine in the first 20 years of licensure compared to Hib vaccine’s rollout. Although PCV introduction was accelerated in some countries, a statistically significant, negative correlation was found between time to introduction and GNI per capita with no correlation to disease burden.

Conclusion: Pneumococcal vaccine introduction in low income countries has been accelerated compared to Hib vaccine, with a resulting large increase in vaccinated children. Continued efforts to overcome market factors and assure early access for new vaccines are needed.
THE ECONOMIC BENEFITS OF SCALING UP VACCINES AGAINST PNEUMONIA AND MENINGITIS IN MIDDLE INCOME COUNTRIES

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Background: Much of child health research focuses on the poorest countries, leaving middle-income countries with less evidence to assess the benefits of interventions. This study projects the potential economic benefit of scaling up Hib vaccine and pneumococcal conjugate vaccine (PCV) over the next ten years in middle-income countries.

Methods: We selected 14 non-GAVI eligible middle-income countries that were part of the Countdown to 2015. We estimated the impact of scaling-up Hib vaccine and PCV coverage to 90% by 2015 on child pneumonia and meningitis mortality using the Lives Saved Tool (LiST) [http://www.jhsph.edu/dept/ih/IIP/list/]. We then modeled the economic benefits accrued over the decade (2011-2020) by using a cost of illness analysis to project healthcare treatment costs averted and productivity savings, and by using a value of statistical life analysis to determine society's willingness to pay for such mortality benefits.

Results: By scaling up coverage of Hib vaccine and PCV, 14 key middle income countries could save $2.4 billion in treatment costs alone and gain $46 billion in productivity over the next decade by decreasing child deaths due to pneumonia and meningitis by 318,000. Using the value of statistical life methodology, we estimated that this reduction in the risk of mortality is worth $98 billion to the population affected.

Conclusion: While a majority of interventions and research focus on the world's poorest countries, it is important for stakeholders to realize the large impact that improving pneumonia immunization access in middle-income countries can have on health and economic outcomes.
**Poster No 268**

**EFFECT OF PNEUMOCOCCAL CONJUGATE VACCINE ON OTITIS MEDIA WITH OTORRHEA IN 0-6 MONTH BANGLADESH INFANTS: A RANDOMIZED CONTROLLED TRIAL**

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**Background:** Acute suppurative otitis media (ASOM = otitis media with otorrhea), is a common infant illness in low resource regions, with substantial burdens of hearing loss, chronic otorrhea, and meningitis. Conjugate pneumococcal vaccines have not been evaluated for ASOM in randomized trials. We assessed the effect of 7 valent pneumococcal conjugate (PCV7) vaccine on ASOM + otorrhea in infants up to 6 months.

**Methods:** 325 infants were randomized to 3 doses of PCV7 (Prevnar®) or Hib conjugate vaccine at 6, 10, 14 weeks. 164 infants received PCV7 and 161 infants received Hib and were observed for 914 and 909 child-months, respectively. Weekly home visits were done from birth till 6 months, to document episodes and duration of ASOM. We compared incidence, duration, and prevalence of ASOM.

**Results:** We observed 23 episodes of ASOM. The incidence of ASOM episodes was 1.9 and 2.2/100 child-months in the PCV7 and control groups respectively, RR = 0.86 (p = 0.79). The mean duration of ASOM episodes was 4.1 days (95%CI = 2.9 to 6.2) vs. 12.2 days (95%CI = 5.9 to 18.4) (p< 0.005) respectively, and prevalence (= days ASOM/100 child-months) was 8.1 vs. 26.7, RR = 0.30 (P < 0.0001).

**Conclusion:** In our S. Asian population, this unique RCT of PCV7 in infants showed a 70% reduction in the duration of ASOM episodes. If confirmed, this vaccine effect could be important to reduce hearing loss, suppurative complications, and associated learning disabilities associated with ASOM in low resource regions.
IDENTIFYING AND ADDRESSING LOGISTICAL BARRIERS TO PCV INTRODUCTION IN NIGERIA

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Background and aims: Nigeria has the highest pneumococcal disease burden in Africa, and routine immunization (RI) coverage is below regional averages and global coverage targets. Nigeria plans to add PCV to its RI schedule in 2013, but introducing PCV into an ineffective system may result in wastage, shortage, or other consequences. It is therefore crucial to identify and address those issues in the current RI system that will be relevant to PCV introduction.

Methods: We collected qualitative data via 11 focus groups and 117 key informant interviews with health workers and officials at the facility, local, state, and national level. Interviews took place across 8 Nigerian states to ensure geographic and sociocultural diversity. Respondents identified perceived barriers to immunization coverage, as well as potential solutions.

Results: Cold chain maintenance, cold chain capacity, and transport issues were mentioned in every state and at each government level. The greatest negative impact occurs when lack of storage interacts with inconsistent transportation in a rural setting, as in the low-coverage state of Taraba. Even high-coverage states such as Osun cited transportation issues, but the impact was lessened by low travel distances and sufficient cold storage capacity. Possible solutions include transportation contracts and cold chain maintenance training.

Conclusion: The widespread reporting of logistical difficulties indicates that these barriers remain a concern in Nigeria’s RI system. To ensure successful PCV introduction, it will be important to address logistical issues so that the system can absorb the demands of an additional vaccine without adverse consequences.
THE COSTS OF INTRODUCING PNEUMOCOCCAL CONJUGATE VACCINE INTO THE Gambian IMMUNISATION PROGRAMME


Background: The Gambia introduced PCV7 in August 2009 and switched to PCV13 in April 2011. In 2009, prior to PCV introduction, tetravalent vaccine (DPT-Hib) was also transitioned to pentavalent vaccine (DTP-Hib-HepB). The aim of this study was to estimate total incremental costs (TIC) to the EPI for 2009 and specifically to estimate TIC of introducing PCV from a government's perspective.

Methods: Costs data were collected on all aspects of the immunisation program through key informant interviews and surveys. Annualised costs were calculated in 2009 US$ and sensitivity analyses for vaccine price, wastage rate and discount rate were conducted.

Results: With a PCV price of US$7 per dose, 6% discount rate and WHO predicted wastage rates, the TIC of introducing PCV was US$1,647,074 or US$24.67 per fully immunised child (FIC). Vaccine costs and injection supplies accounted for 91% and 2% of TIC respectively.

TIC for the year 2009 were US$1,616,943 (US$24.22 per FIC). The costs were not sensitive to a lower discount rate of 3% but decreased to US$864,394 with a vaccine price of US$3.5. With co financing at US$0.2 per dose for PCV and US$0.15 for pentavalent, the TIC were US$184,979.

Conclusion: With PCV introduction, the total systems costs associated with PCV introduction were higher than the US$100,000 "vaccine introduction grant" supported by GAVI Alliance. The costs were offset by the introduction grant received for pentavalent introduction.

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Poster No 271

PNEUMOCOCCAL CO-COLONIZATION OF THE NASOPHARYNX IN THE PRE- AND PCV7 ERAS

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Background and aim: Understanding the epidemiology of pneumococcal co-colonization is important for monitoring vaccine effectiveness and the occurrence of horizontal gene transfer (HGT) between pneumococcal strains. We evaluated the impact of the seven-valent pneumococcal conjugate vaccine (PCV7) on pneumococcal co-colonization among Portuguese children.

Methods: Nasopharyngeal samples from children up to 6 years old yielding a pneumococcal culture were clustered into three groups: pre-vaccine era (n=173), unvaccinated children of the vaccine era (n=169), and vaccinated children (4 doses; n=150). Co-colonization, serotype identification, and relative quantification were detected by analysis of DNA of the total bacterial growth of the primary culture plate using the plyNCR-RFLP method and a molecular serotyping microarray.

Results: Co-colonization rates were significantly lower among vaccinated children (8.0%) than among unvaccinated children from the pre- and PCV7 eras (17.3% and 18.3%, respectively; p=0.004, Fisher's exact test). In the PCV7 era there were significantly less non-vaccine type (NVT) co-colonization events than expected based on the NVT distribution observed in the pre-PCV7 era (p=0.024).

Conclusions: Vaccination with PCV7 resulted in lower co-colonization rates due to an asymmetric distribution of NVTs found in single and co-colonized samples. We propose that some NVTs prevalent in the PCV7 era are more competitive than others. This result may have important implications since decreased co-colonization is expected to translate in decreased opportunities for HGT, which might represent a novel benefit of conjugate vaccines.

Poster No 272

TRENDS IN INVASIVE PNEUMOCOCCAL DISEASE AFTER 7-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV7) INTRODUCTION IN SOUTH AFRICA, 2005-2011

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Background: PCV7 was introduced in 2009 as 3 doses at 6, 14 and 36 weeks with no catch-up. In 2010, estimated coverage for 1 or 3 doses were 87% and 63%, respectively. New HIV Prevention-of-Mother-to-Child-Transmission guidelines were released in 2008. We report trends in invasive pneumococcal disease (IPD) pre- and post-introduction.

Methods: IPD was tracked through active, national surveillance; isolates were serotyped by Quellung. IPD rates and number of cases (January to August each year) during pre-vaccine years (average from 2005-2008) were compared to 2011 figures.

Results: IPD incidence (episodes per 100,000 population) in children < 2 years decreased from 24 to 9 (-61%, p< 0.001). Reductions occurred for both vaccine serotypes (VT) (337 to 61 cases, -82%) and non-vaccine serotypes (NVT) (231 to 133, -42%). Among children < 2 years (~70% with known HIV status), the reduction of VT (77 to 10, -87%) was similar to NVT(48 to 14, -71%) in HIV-infected children. A decrease of VT (44 to 24, -45%) and increase in NVT (33 to 58, 78%) was observed in HIV-uninfected children; serotype 19A increased from 6 to 16 cases. Children 2-4 years showed reductions of VT(101 to 42, -58%), with little change in NVT (70 to 64, -9%). Stratification by HIV demonstrated similar trends as for < 2 years.

Conclusions: IPD reductions among HIV-infected children < 2 years were likely mainly due to HIV prevention and treatment. Among HIV-uninfected children, VT decreased with some 19A replacement. Indirect effects may be reducing disease in older children.
EVALUATING THE EFFECT OF RESPIRATORY SYNCYTIAL VIRUS (RSV) AND INFLUENZA ACTIVITY ON ESTIMATES OF PNEUMOCOCCAL VACCINE IMPACT

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Introduction: Viral infections are associated with increased pneumococcal disease incidence, and year-to-year variations in viral activity could potentially bias population-based estimates of vaccine impact. We used statistical models to estimate the impact of RSV and influenza activity on the incidence of radiologically-confirmed alveolar pneumonia (RCAP) and used these models to quantify changes in disease incidence following the introduction of PCV7 in Israel.

Methods: We determined the weekly RCAP incidence among Jewish and Bedouin children aged < 6 months, 6-17 months, and 18-35 months in Southern Israel during the period 2004-2011. To estimate the effect of the vaccine, we fit a regression model with predictors for seasonality (dummy variable for month), RSV and influenza activity (% of positive swabs), and a dummy variable for vaccine period (baseline period: 2004/05-2007/08, vaccination period: 2008/09, 2009/10, 2010/11). Confidence intervals were calculated using a moving-block bootstrap.

Results: The inclusion of RSV and seasonal predictors significantly improved the fit of the model and influenced the estimates of vaccine impact, while influenza did not. Two years after PCV7 introduction, the estimated RCAP incidence among the < 6 month-olds declined by 26-47% compared to a pre-vaccination baseline assuming a constant effect of RSV. Likewise, estimated incidence declined by 8-30% among the 6-17 month olds and 19-24% among 18-35 month olds children. Removing RSV from the model substantially reduced estimates of vaccine effectiveness.

Conclusions: This model provides a framework for improving estimates of vaccine effectiveness which can be validated using data from upcoming seasons.
ADDRESSING FINANCIAL SUSTAINABILITY CHALLENGES FOR PCV INTRODUCTION AND ROUTINE IMMUNIZATION IN NIGERIA

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Background and aims: Nigeria, with the highest burden of pneumococcal disease in Africa, plans to introduce PCV in 2013. Financial sustainability (FS) is a concern and a pre-condition for GAVI support. We aimed to identify financial barriers, focusing on adequacy and reach of funds to every level of the immunization system.

Methods: Between May and July 2011, we collected qualitative data from 8 states in Nigeria, selected to be representative geographically and of high, low or greatly improved immunization performance. A total of 117 key informant interviews and 11 Focus Group Discussions were conducted with politicians, health officials, health workers, mothers and community leaders at the federal, state and local government and health facility levels.

Findings: Overall, immunization funding gap for 2012 was 19%. Although states had budget lines for immunization, funds were deemed inadequate in 4/8(50%) and delayed or not released in 7/8(86%) of states. Of the 2 lowest-coverage states, financing was a problem in one, but not in the other; same was observed in the 2 best-improved states. The establishment of pooled funds (basket funds) in one high-performing state and direct release of funds to health facilities in the 6 top-performing states appeared to have improved program delivery.

Conclusions: Presence of an immunization budget line (a key WHO/UNICEF indicator for FS) is an insufficient measure of FS; tracking timing of budget release should be considered an additional indicator. Innovative approaches such as basket funds should be explored as avenues to bridge funding gaps and facilitate funding flows.
SEROTYPE COVERAGE OF PNEUMOCOCCAL CONJUGATE VACCINE AND PENICILLIN SUSCEPTIBILITY OF S. PNEUMONIAE ISOLATES FROM INVASIVE DISEASES IN BRAZILIAN (SÃO PAULO) CHILDREN

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Background: In Brazil the 10-valent pneumococcal conjugate vaccine (PCV-10) was introduced into the National Immunization Program in April 2010. We conducted a study to identify the most common pneumococcal serotypes in children with invasive disease, correlate isolated serotypes with those included in conjugate vaccines, and ascertain the sensitivity to penicillin.

Methods: From January 2003 to October 2011, a retrospective study of children with a diagnosis of pneumococcal invasive disease was conducted at the Hospital Universitário da USP. Criteria for inclusion were: over 29 days and under 15 years, with isolation of pneumococcus from a normally sterile site.

Results: The study included 212 children. Of these, 150 cases of pneumonia, 31 bacteremia, 23 meningitis, 4 cellulitis, 3 pyoarthritis and 1 pericarditis. The most common serotypes were: for pneumonia 14 (40.2%), 5 (13.6%), 1 (12.9%), 6B (6.1%) and 19A (4.5%); for bacteremia were 14 (34.5%), 6B (24.1%), 12F (6.9%), 19F (6.9%) and 18C (6.9%); for meningitis were 14 (27.8%), 18C (11.1%), 10A (11.1%) and 19F (11.1%). The coverage rates of PCV10 and PCV13 in meningitis disease was 61.1% and 72.2% respectively. For non-meningeal disease was 84% and 94.1%. The sensitivity to penicillin in meningitis disease were: sensitive (MIC< 0.06µg/mL) in 95.2%, intermediate resistant (MIC=4µg/mL) in 4.8%. The geometric mean of MIC were: 0.090µg/mL for meningitis, 0.122µg/mL for bacteremia and 0.195µg/mL for pneumonia.

Conclusions: Our results confirm a significant potential impact of PCV-10 for non-meningeal disease but lower for meningitis disease. The susceptibility testing results show that penicillin is still the treatment of choice for non-meningeal pneumococcal disease.
ANALYSIS OF INVASIVE PNEUMONIA CAUSING STRAINS OF STREPTOCOCCUS PNEUMONIAE: SEROTYPES AND ANTIMICROBIAL SUSCEPTIBILITY


Background: In Brazil the 10-valent conjugate vaccine was introduced into National Immunization Program in April 2010. *S. pneumoniae* is the main causative agent of bacterial pneumonia. Periodic reassessment with monitoring of prevalent serotypes and patterns of resistance is required for better therapeutic guidance and definition of control strategies.

Methods: From January 2003 to October 2011, a retrospective study of hospitalized children with a diagnosis of pneumococcal pneumonia was conducted at the Hospital Universitário da USP. Criteria for inclusion were: over 29 days and under 15 years, radiological and clinical diagnosis of pneumonia, and isolation of *S. pneumoniae* in blood cultures and/or pleural effusion.

Results: The study included 150 children. The most common serotypes isolated were 14 (40.2%), 5 (13.6%), 1 (12.9%), 6B (6.1%), 19A (4.5%), 4 (3%), 6A (3%), and 3 (3.3%). The proportion of identified serotypes contained in 7-V, 10-V and 13-V conjugate vaccines was 56.8%, 85.6% and 96.2% respectively. Pneumococcal strains were sensitive to penicillin (MIC≤2µg/mL) in 93.9% and intermediate resistance (MIC=4µg/mL) in 6.1%. No strains were penicillin-resistant (MIC≥8µg/mL) according to CLSI-2008 standards. The geometric mean MIC in ≤24 months was 0.297µg/mL and >24 months was 0.096µg/mL. Tested isolates were highly sensitive to vancomycin, rifampicin, ceftriaxone, clindamycin, erythromycin and chloramphenicol.

Conclusions: Our results confirm a significant potential impact of conjugate vaccines, mainly 10-valent and 13-valent, on invasive pneumonia. Furthermore, susceptibility testing results show that penicillin is still the treatment of choice for invasive pneumonia in our setting.
COMPLICATED COMMUNITY-ACQUIRED PNEUMONIA CAUSED BY STREPTOCoccus PNEUMONIAE IN HOSPITALIZED BRAZILIAN (SÃO PAULO) CHILDREN

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Background: Increasing prevalence of pleural empyema complicating community-acquired pneumonia (CAP) is reported worldwide. Pleural empyema is reported in up to 28% of children hospitalized with CAP.

Methods: From January 2003 to October 2011, a retrospective study of hospitalized children with a diagnosis of pneumococcal pneumonia was conducted at the Hospital Universitário da USP. Criteria for inclusion were: over 29 days and under 15 years, radiological and clinical diagnosis of pneumonia, and isolation of *Streptococcus pneumoniae* in blood cultures and/or pleural effusion.

Results: The study included 150 children. Eighty-four were male (56%), median age was 23 months (52% ≤ 24 months). Eighty-eight (58.7%) of them had complications. The most common serotypes isolated were 14 (40.2%), 5 (13.6%), 1 (12.9%), 6B (6.1%) and 19A (4.5%). Emphyema occurred in 48.9% and uncomplicated pleural effusion in 40.9%. The median age of children with empyema was 32 months. The most common serotypes in empyema were 14 (35%), 5 (27.5%), 1 (12.5%), and 19A (7.5%). The coverage for complicated pneumonia in 10-valent and 13-valent conjugate vaccines was 86.8% and 96% respectively and for empyema was 90% and 97.5%. The geometric mean MIC for uncomplicated pneumonia was 0.189 µg/mL and complicated pneumonia was 0.165 µg/mL. Death occurred in 3 children.

Conclusions: The complications occurred in older children being more frequent emphyema and pleural effusion uncomplicated. The coverage for complicated pneumonia by PCV-10 and PCV-13 were excellent.
CURRENT USE AND FUTURE OPPORTUNITIES TO ACCELERATE ACCESS TO PNEUMOCOCCAL AND HAEMOPHILUS INFLUENZAE TYPE B CONJUGATE VACCINES AMONG CHINESE CHILDREN

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Background and aims: China accounts for an estimated 12-14% of Haemophilus influenzae type b (Hib) and pneumococcal cases occurring worldwide among children. Despite this burden, Hib and pneumococcal conjugate vaccines (PCV) are not part of the expanded program on immunization (EPI), although they can be purchased at some facilities.

Methods: To estimate current vaccine use, we reviewed the National Institute for the Control of Pharmaceutical and Biological Products data on vaccine supply from 2007-2010. The retail price of Hib and PCV vaccines in China was obtained from the National Price Administration Department.

Results: Hib vaccine doses supplied increased from approximately 14.5 million in 2007 to 24 million in 2010. We estimate that 44% of the birth cohort in 2010 (18 million) could have been vaccinated with a 3-dose Hib series. In 2010, 585,738 of PCV were supplied, which would cover 1% of the 2010 birth cohort with a 3-dose series. Cost for monovalent Hib vaccine in China in 2010 was $23.3 from multinational and $12.7-17.4 from domestic manufacturers. Cost of PCV was $127, available from only one multinational manufacturer.

Conclusions: Hib vaccine use is increasing, but its cost remains high, representing up to one-fifth of a working family’s annual income. PCV is even more expensive. Accelerating introduction of these new vaccines into China’s EPI requires an initiative that addresses gaps in the local evidence base for disease burden, a communication strategy for informing policy makers and the public, and a close relationship with China’s Ministry of Health to build a sustainable, affordable supply of vaccines and achieve optimal population coverage.
SEROTYPES AND PREVALENT FEATURES OF STREPTOCoccus PNEUMONiAE IN ‘CENTENARIO HOSPITAL MIGUEL HIDALGO’ AGUASCALIENTES, MEXiCO

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Background and objectives: Streptococcus pneumoniae is frequent cause of hospitalization and mortality. Have been reported variations of serotypes, frequency, organ affinity or age group and vaccines efficacy, we review it epidemiology for our hospital.

Methods: Retrospective, observational study. Period January 2005-December 2010, we reviewed S. pneumoniae isolates at our institution, patient’s age and sex, site, serotype, drug-susceptibility and mortality.

Results: 140 cultures were done, identified serotype and drug-susceptibility in 122 samples, mostly bronchial secretions (n=67, 54.9%) pneumonia was principal clinical form. Higher prevalence for males (n=72, 59%). Adults (20 years-old) were most affected (n=67, 54.9%) mainly over 60 years-old (19.6% of total); 48 pediatric cases (39.3%). Observed susceptibility to oxacillin/penicillin (74.8%), cefotaxime (79.1%), erythromycin (72.6%) and trimethoprim-sulfamethoxazole (8.6%) and 100% to vancomycin. Mortality 41.8% (n = 51), 60.7% men mostly adults, both over 60 years-old and working-age (20to59 years-old) each group 18 cases and only 15 deaths in pediatrics. Serotypes prevalent were 3 (n=15, 12.29%), 23F (n=12, 9.83%), 6B and 14 each 10 cases (8.19%), 6A (n=8, 6.55%), 19F (n=7, 5.73%) and 19A (n=6, 4.91%), followed by 5, 6 and 11A, each 4 cases (3.27%).

Conclusions: None vaccine available cover all serotypes identified: PulmovaX® 3, Prevnar7® 4 and Pneumo23® only 7 of 10 most common serotypes. Unlike Prevnar13® covers 8 most common serotypes. But some aren’t found in any vaccine: 6A, 23A, 6, 9A, 35B, 29, 34, 35A, 15C, 16, 22, 28A, 31, 35 and 9N.
Poster Shift 2
STREPTOCOCCUS PNEUMONIAE: EVOLUTION OF SEROTYPES AND ANTIBIOTIC SUSCEPTIBILITY IN A PEDIATRIC HOSPITAL OF MENDOZA ARGENTINA

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Background: Streptococcus pneumoniae is one of the main causes of children morbimortality in developing countries.

Objectives: Analyze serotypes distribution (St) of identified Spn between 1993 and 2010 and its resistance to penicillin and cephalosporin 3 (C3) according MIC of CLSI 2009.

Methods: Cohort study, retrospective-prospective. Based on Microbiology records, subjects with blood, CSF or other sterile fluid Spn isolates were evaluated.

Results: 494 isolates recovered from blood 309 (62.5%), CSF 148 (30%) and others 37 (7.5%). Clinical diagnostics: meningitis (n:148; 30%), pneumonia (n:121; 24.5%), pneumonia with pleural effusion (n:120; 24.3%), bacteraemia/sepsis (n: 92; 18.6%) and others (n:13; 2.6%). Mean age: 36.8 months; < 5 years 76% (377/494) and < 2 years 54% (267/494). 255 strains were typified (51.6%). Most frequent St: 14 (25.1%), 5 (15.3%), 1 (13.3%), 7F (5.5%), 18C (4.3%), 6B (4.3%), 19F (3.9%), 9V (3.9%), 6A (3.1%) and 19A (2.7%). Significant difference St 1 < 2 years vs. >2 years: OR 0.29 IC 95% (0.10-0.51) p < 0.001. Significant difference St 5 between meningitis and non-menigitis: OR 0.3 IC 95% (0.11-0.81) p = 0.0129. Resistance to penicillin: meningitis: 23.2% (16/69) and no meningitis 0% (0/165). Resistance to C3: 0%. St resistant to penicillin: 14 (7/53: 13.2%), 6B (3/8: 37.5%), 19F (2/8: 25%), 9V (1/9: 11.1%), 18C (1/10: 10%), 23F (1/2: 50%) and 23B (1/1: 100%).

Conclusions: Most frequent St: 14 and 5 in < 2 years and 1 and 14 in > 2 years. Most frequent clinical diagnostic: pneumonia with and without pleural effusion and in < 5 years. The non-meningeal cases were all sensitive to penicillin.
Poster No 2

EMERGENCE OF STREPTOCOCCUS PNEUMONIAE ANTIMICROBIAL RESISTANCE IN JEDDAH, SAUDI ARABIA (2007-2011)

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Background and aims: The available updated data on the drug-resistance from different populations is vital to recognize changes in disease patterns and to adjust the recommendations for empirical treatment. This will have immense implications on the local and global health. Therefore, this study was designed to evaluate the pattern of the drug and the multidrug-resistance of S. pneumoniae in Jeddah/Saudi Arabia, in 5 years period from 2007 to 2011.

Methods: A total of 758 isolates from respiratory tract, blood, fluids and swabs were collected from four major hospitals. These isolates were identified and confirmed positive by the standard microbiological techniques. Susceptibility testing was performed to penicillin and various other antibiotics. Minimum inhibitory concentration (MIC) was then determined using E-test. Multidrug resistance was defined as isolates resistant to the minimum three of the tested antibiotics.

Results: Data showed that prevalence of resistance to penicillin was (37%), (34%) to erythromycin, (24.5%) to trimethoprim/sulfamethoxazole, (4%) to ceftriaxone and (0.5%) to vancomycin. However, 19% of the isolates showed multi-drug resistance. Rare strains (susceptible to penicillin but resistant to ceftriaxone) were 2.9%.

Conclusion: Our study points to possible changing pattern of S. pneumoniae antibiotic resistance especially to vancomycin. Surprisingly, the antibiotic resistance pattern was extremely variable from one hospital to another. We also document an emergence of rare strains. However, continuous surveillance and epidemiological serotyping are required.

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PRE-VACCINATION PNEUMOCOCCAL CARRIAGE IN A NIGERIAN POPULATION: AN EMBARRASSMENT OF NOVEL SEQUENCE TYPES AND RICHNESS OF CLONAL DIVERSITY

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Background: Introduction of pneumococcal vaccines in Nigeria is a priority. However, country data on the burden of pneumococcal disease (IPD) is limited and coverage by available conjugate vaccines is unknown. This study was carried out to describe the pre vaccination epidemiology and population biology of pneumococcal carriage in Nigeria.

Methods: This was a cross sectional survey. Nasopharyngeal swabs (NPS) were obtained from a population sample in a peri-urban Nigerian setting. Demographic characteristics and risk factor data for carriage were obtained from all study participants. Pneumococci isolated were characterised by serotyping, antimicrobial susceptibility and Multi Locus Sequencing Typing.

Results: The prevalence of pneumococcal carriage was 52.5%. Carriage was higher in children compared to adults (67.4% vs. 26%), highest (~90%) in infants < 9 months. Serotypes 19F (18.6%) and 6A (14.4%) were predominant. Potential vaccine coverage was 43.8%, 45.0% and 62% for PCV-7, PCV-10 and PCV-13 respectively. There were 16 novel alleles, 72 different sequence types (STs) and 3 Sequence Types (280, 310 and 5543) were associated with isolates of more than one serotype indicative of serotype switching. Antimicrobial resistance was 93% and 84% for cotrimoxazole and tetracycline respectively. A third of isolates had intermediate resistance to penicillin. Young age was the only significant risk factor for carriage.

Conclusions: Pneumococcal carriage and serotype diversity is highly prevalent in Nigeria especially in infants. PCV-13 appears the obvious choice to reduce disease burden and prevalence of drug resistant pneumococci. Our findings provide sound baseline data for impact assessment following vaccine introduction in Nigeria.
Poster No 4

IDENTIFICATION AND CHARACTERISATION OF THE BACTERIA CAUSING ACUTE OTITIS MEDIA EPISODES IN YOUNG CHILDREN IN SAUDI ARABIA

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Background/aims: Acute otitis media (AOM) is one of the primary reasons for antibiotic use in children. Information regarding AOM aetiology is important for developing and implementing effective vaccines. This epidemiological, prospective study assessed aetiology and antimicrobial susceptibility of bacterial pathogens in Saudi children with AOM.

Methods: Children, aged 3-< 60 months, diagnosed with either an untreated AOM episode (no antibiotic therapy within 72 hours prior to enrolment) or a treatment-failure AOM episode (antibiotic therapy received within 48-72 hours prior to enrolment) and from whom a middle ear fluid (MEF) sample had been obtained were enrolled. MEF samples were collected by tympanocentesis or by sampling spontaneous otorrhoea (if perforation had occurred < 24 hours prior to visit), and cultured for bacterial characterisation and serotyping.

Results: Sixty-six children (median age 20 months) were enrolled from 2009-2011 (62 untreated episodes; 4 treatment-failure episodes); 21 were spontaneous otorrhoea episodes. Sixteen children had received ≥1 dose of pneumococcal vaccine. Study pathogens were detected in only 14/66 (21%) episodes (Haemophilus influenzae, Hi (8/66 [12%]), Streptococcus pneumoniae, Spn (6/66 [9%])). No Moraxella catarrhalis positive samples were detected. All Hi samples were non-typeable (NTHi). Pneumococcal serotypes detected in Spn-positive episodes were 7F (n=2), 23F (n=2), 19F (n=1) and 15F (n=1). Spn-isolates showed rare resistance to erythromycin and tetracycline, amongst others. Hi-isolates showed some resistance to antibiotics including tetracycline and ampicillin.

Conclusions: Despite the low bacterial detection rate, NTHi and Spn were important AOM pathogens in Saudi Arabia. Protection against these pathogens with effective vaccines may substantially reduce AOM.
SIMULTANEOUS NASOPHARYNGEAL CARRIAGE OF HI AND SPN IN 1 AND 4-YEARS OLD CHILDREN FROM SAO PAULO, SP, BRAZIL

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Background and aims: Invasive and non-invasive Hi and Spn diseases are associated to nasopharynx carriage (NPC). Since conjugate vaccines reduce NPC of vaccine serotypes, evaluating colonization rates is critical for assessing vaccination impact. Hib and 10-valent conjugate (PCV10) vaccines were introduced in Brazil respectively in 1999 and 2010. We analyzed Hi and Spn NPC rates in 1,540 children living in the city of São Paulo, aged 1<2y(n=965) and 4<5y(n=575), which received Hib-vaccine and not PCV10.

Methods: For Spn culture, nasopharyngeal samples in STGG (NP-STGG) were plated after broth enrichment. NP-STGG from Spn-negative cultures were submitted to lytA-targeted RT-PCR. For Hi, ompF2-, Van Ketel-, and cap-targeted conventional PCRs were performed, as also hpd-targeted RT-PCR to exclude Haemophilus other than Hi.

Results: Colonization rates by culture and RT-PCR in the 1<2y group, were 13%(Hi+/Spn-), 24%(Hi-/Spn+) and 31%(Hi+/Spn+); for 4<5y, the rates were 14%, 22% and 42%, respectively. Overall rates in 1<2y were 44%(Hi) and 55%(Spn), in the 4<5y, 55%(Hi) and 63%(Spn). The RT-PCR for the 749 Spn negative cultures detected 13% (n=105) yield over the 791 culture-positive children. RT-PCR enhanced the overall pneumococcal carriage from 51% by positive-culture to 58%.

Conclusions: Increased NPC rates in children simultaneously colonized by Spn and Hi are noteworthy. This study provides further information on the epidemiology of pneumococcal and Hi isolates, and is an important baseline for evaluating the impact of PCV10 introduction in Brazil.

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Poster No 6

PNEUMOCOCAL COMMUNITY-ACQUIRED PNEUMONIA (CAP) AND SEROTYPES IN PARAGUAY: IMPLICATIONS FOR CONJUGATE VACCINE INTRODUCTION

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Introduction: CAP constitutes the main cause of mortality in Paraguay in children below 5 years, being Streptococcus pneumoniae (Spn) the most frequent bacterial agent identified.

Objective: To characterize the clinical outcome of pediatric CAP caused by Spn and determine the factors affecting the treatment outcome, with emphasis in serotypes.

Material and methods: Observational study that included patients (pts) < 16 years old, hospitalized in the IMT between 2004-2010 with pneumococcal CAP. Demographic and clinical variables, antibiotic susceptibility, pneumococcal serotypes, antimicrobial therapy, and clinical outcome were measured.

Results: Of 580 patients with CAP hospitalized in the study period, 85 pts (14.6%) were cases of pneumococcal pneumonia, with a mean age (2.5±2 years) None of 85 Spn isolates were resistant to penicillin and/or cefotaxime. Pneumococcal serotypes 14 (29/85), 1 (14/85) and 5 (9/85) were the most prevalent serotypes causing disease, and accounted for 61% of the isolates. Only 2 isolates were serotypes 19A (2%). The serotypes 14 and 5 were predominant in children < 5 years (p=0.05). A total of 48 pts (53%) met the criteria for complicated pneumonia. There was no relationship between pneumococcal serotypes and the presence of complications. The overall mortality was 7% (6/85), but for serotype 7F the mortality was 40% (2/5) (p=0.05).

Conclusions: Pneumococcal pneumonia exhibit in our population high rate of complications and mortality, being serotype 14, 1 and 5 the most prevalent isolates (all included in the new pneumococcal vaccine formulations). The introduction of pneumococcal vaccine in the childhood immunization programm in Paraguay is highly priority.
IMPACT OF S PNEUMONIAE (SPN) IN THE SENTINEL SURVEILLANCE IN CHILDREN UNDER 5 YEARS OLD IN PARAGUAY

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Background: S. pneumoniae (Spn) became the principal causal agent isolated in Paraguay.

Aims: a) To obtain standardized data on bacterial pneumonia and meningitis, b) To identify patterns of Spn antimicrobial susceptibility.

Methods: Prospective, cross-sectional multicenter, between epidemiological weeks (EW) 11/2007 until 39/2011. Children under 5 years old, with suspected pneumonia or meningitis hospitalized were enrolled. The isolated were characterized in the National Public Health Laboratory.

Results: Pneumonia: 6% (250/4,500) were confirmed: Spn 68% (169/250); Non-b Haemophilus influenzae (non-b Hi) 2% (5/250), 3.6% (9/250) Hib, and the remaining 67 bacterial isolates (frequent was Staphylococcus aureus). Spn isolates: 63% (106/169) were characterized. Serotype 14 was most frequently [57% (60/106)], followed by 1 and 5 (more common in children under 2 years old). The total was sensitive to penicillin. Fatality rate for bacterial confirmed pneumonia was 23% (57/250).

Meningitis: 31% (102/326) were confirmed: 43% (44/102) had Spn, 15% (15/102) Neisseria meningitidis, and 12% (12/102) Hib. The last 31 was considered others (Streptococcus group B and BGN). Spn isolates: 70% (31/44) were characterized, the serotypes distributed equally with pneumonia. 80% (35/44) were sensitive to penicillin, and 20 % were resistant. Fatality rate for confirmed bacterial meningitis was 39% (40/102).

Conclusions: Hospitalization for pneumonia and meningitis in children under 5 years is common. Spn was usually sensitive to penicillin, but serotype 14 was associated with resistance. In children under 2 years increased serotypes 1 and 5. Hib isolation was low, and less than non-b Hi. The case fatality rate was higher in meningitis cases.
EPIDEMIOLOGY OF NASOPHARYNGEAL COLONIZATION BY STREPTOCOCCUS PNEUMONIAE AND HAEMOPHILUS INFLUENZAE IN CHILDREN WITH RESPIRATORY TRACT INFECTION

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Background and aims: Nasopharyngeal carriage is a dynamic process necessary in route to invasive pneumococcal or Haemophilus influenzae disease. The aim of this study was to determine nasopharyngeal carriage levels of S. pneumoniae (Spn) and H. influenzae (Hi) in children during respiratory tract infection (RTI).

Methods: A prospective survey was conducted among children less than 5 years of age with RTI to estimate carriage prevalence, identify risk factors, determine antibiotic susceptibility patterns and serotype and genotype distribution.

Results: A total of 48/168 (28.6%) and 28/168 (16.7%) children were colonized only by S. pneumoniae or H. influenzae, respectively. Co-colonization by both pathogens were detected among 36/168 (21.4%) children. Pneumonia was diagnosed in children colonized by Spn only (97.8%), Hi only (92.6%), and by both pathogens (88.2%). Non-susceptibility to penicillin was identified in 41.7% of the S. pneumoniae isolates. The most common pneumococcal serogroup/serotypes were 6 (18%), 14 (15%), 19F (11%), 15 (10%), 23F (7%) and 19A (6%). S. pneumoniae isolates of serotypes 14 and 19F had genotypes that were similar to those identified among strains obtained from invasive disease in Salvador. Nontypeable H. influenzae (NTHi) was the most frequent type of Hi identified (82%), followed by serotypes “e” (9%), “b” (5%), “a” (2%) and “f” (2%).

Conclusion: Most S. pneumoniae isolates were resistant to penicillin and had genotypes associated with invasive disease. The current pneumococcal 10-valent conjugate vaccine may substantially reduce the risk of severe pneumonia and other Spn infections.
TYPE DISTRIBUTION OF STREPTOCOCCUS PNEUMONIAE (SPN) SEROGROUP 6 IN BRAZIL SINCE 1977

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Background and aims: Studies have shown that the incidence of non-vaccine types of Spn serogroup 6 may increase after PCV administration. Considering their clinical and public health significance, we investigated the presence of serotypes 6C and 6D, and the trends for serogroup 6 among Spn isolated before PCV10 introduction into the Brazilian national immunization program, by March 2010.

Methods: Spn isolates have been received since 1977 as part of passive laboratory-based surveillance nationwide. Those previously serotyped as 6A, were examined by PCR, dot-blot using MAbs that distinguish 6A from 6C, and/or Quellung reaction including the factor antiserum 6d. Serotype 6D was investigated among 183 6B isolates from 2009-2010.

Results: Of the 636 available serotype 6A isolates, 170 (26.7%) were serotype 6C. Type 6C was detected as early as 1979 and identified among carriage (17/862; 2%), non-invasive (3/166; 1.8%), and invasive (150/13043; 1.2%) isolates. No serotype 6D strain has been detected yet. Among invasive isolates, serotype 6C accounted for 1.2%, 6A for 3.2% and 6B for 7.9%. Type 6C was more frequent among ≥18-yr-old patients (52.3%), compared to serotypes 6A (32.4%) and 6B (24.5%). Penicillin non-susceptibility was observed for respectively 6.9%, 8%, and 31.3% of serotypes 6A, 6C, and 6B isolates.

Conclusions: Despite the differences in their epidemiology, types of serogroup 6 remained rather stable along the years. These baseline data will help assessing the effects of PCV10 in the years following its introduction.

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POTENTIAL IMPACT OF PNEUMOCOCCAL CONJUGATE VACCINES ON NASOPHARYNGEAL COLONIZATION AND INVASIVE STREPTOCOCCUS PNEUMONIAE SEROTYPES ISOLATED FROM UNVACCINATED BRAZILIAN CHILDREN

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Background and aims: Nasopharyngeal (NP) colonization by Streptococcus pneumoniae precedes the pneumococcal diseases development. We compared the potential impact of pneumococcal conjugate vaccines (PCV) on carriage and invasive serotypes in children from Brazil, where 10-valent pneumococcal conjugate vaccine (PCV10) was introduced on March/2010.

Methods: NP isolates (N=791) were obtained from a survey conducted in 16 immunization rooms in São Paulo municipality during March-August/2010 including 1,540 unvaccinated children aged < 5years. Invasive isolates (IPD, N=173) were obtained from the municipality through the national pneumococcal laboratorial surveillance during 2008-2010, and were matched for age with NP isolates. The PCV10 and PCV13 potential impact (coverage) were assessed by using the vaccine-types from NP and IPD isolates determined by Quellung reaction.

Results: The frequencies of NP serotypes included in PCV10/PCV13 were 6B(13.3%), 19F(9.1%), 14(7.6%), 23F(7.6%), 18C(1.5%), 7F(1.3%), 4(0.8%), 9V(0.4%), 1(0.3%), 5(0.1%), 3(1%), 6A(11.6%) and 19A(2.1%). The IPD serotypes were 14(39.3%), 6B(14.5%), 19F(5.8%), 5(5.2%), 23F(4.6%), 4(0.6%), 9V(0.6%), 18C(2.3%), 7F(1.7%), 1(1.2%), 3(2.3%), 6A(4%) and 19A(6.4%). Serotype 6A was more frequent among NP isolates whereas 19A was more prevalent among IPD isolates. PCV10 coverage was significantly higher for IPD (75.5%) compared to NP (41.8%). The same was observed for PCV13 coverage for IPD (88.4%) and NP (56.6%) (p< 0.001).

Conclusions: Colonizing studies are important tool to evaluate vaccine-type decreasing and detect serotype replacement, but we should be cautious with its use to estimate the PCV impact on IPD serotypes.

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IMPACT OF 10-VALENT PNEUMOCOCCAL CONJUGATE VACCINE ROUTINE IMMUNIZATION SCHEDULE IN INCIDENCE OF PNEUMOCOCCAL MENINGITIS IN THE STATE OF SÃO PAULO


Background and aims: Pneumococcal meningitis presented in the State of São Paulo, from 2001 to 2009, little variability in incidence (1.5 to 0.9 / 100,000) and mortality (29.6% - 29.6%). In 2010 a 10-valent pneumococcal conjugate vaccine was introduced in routine immunization schedule for children under 2 years old. The aim was to evaluate the impact of vaccine introduction in incidence of pneumococcal meningitis.

Methods: Descriptive epidemiological analysis used SINAN database and included all confirmed cases of pneumococcal meningitis under 2 years old from 2001 to 2009 reported in the months from January to August in the State of São Paulo. Statistical analysis used the Chi-square test and p < 0.05.

Results: A total of 822 cases (mean 81.3 cases per year, range 69 to 115 cases) of pneumococcal meningitis in children under 2 years old from 2001 to 2009 were reported. Incidence rate in this period was 7.1 / 100,000 inhabitants. From January to August 2011, 39 cases of pneumococcal meningitis were reported in children under 2 years old. The incidence rate in 2011 was significantly lower (3.7 / 100,000 inhabitants, p< 0.01) than that observed in the previous years, with a percentage variance in incidence rate of 47.9%.

Conclusion: The introduction of a 10-valent pneumococcal conjugate vaccine caused a reduction in the incidence of pneumococcal meningitis in the State of São Paulo in children under 2 years of age. Probably this benefit will not only be observed in children, but also in the entire community in the coming years.
SEROTYPE DISTRIBUTION OF STREPTOCOCCUS PNEUMONIA CAUSING PARAPNEUMONIC EMPYEMA IN TURKEY

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Background and aims: Pneumonia remains a cause of considerable morbidity in childhood with either sustained or increased rates of complicated pneumonia, including parapneumonic empyema (PPE). S. pneumonia is the most common etiological cause of this invasive disease. In this study we aimed to specify the serotypes of S. pneumonia causing PPE in children that have not been vaccinated.

Methods: This study was conducted with 30 PCR + samples collected from 13 hospitals representing different regions of Turkey. Samples were collected via thoracentesis from patients aged 0 - 18 years. Samples were tested in a Bio-Plex multiplex antigen detection assay capable of detecting 14 serotypes/groups (1,3,4,5,6A,6B,7F/A,8,9V,14,18,19A,19F and 23F).

Results: Median age was 5 years (range=0.5-15 years). Fourteen of children were male, 9 were female. No gender information specified for the remaining 7 children. Serotype-1 was detected in 8 (26.7%); serotype-5 was detected in 7 (23.3%) and serotype-3 was detected in 4 (13.3%) of the samples. However 2 of these were mixed infections, with serotype-1 and serotype-5. The remainder comprised 43.3%; with serotypes s8 (n=3), s6B (n=2), s14 (n=2), s19F (n=2), s9V (n=1), s18 (n=1), s19A (1), s23F (1).

Conclusions: In conclusion, PPE is a serious health problem. The most common PPE isolates in the present study belonged to serotypes 1 and 5, which are covered by both 10 and 13-valent vaccines. Clinical and serotype surveillance studies are needed to follow the changing S. pneumonia serotypes causing PPE.
CARRIAGE OF PNEUMOCOCCI IN PERUVIAN CHILDREN PRIOR TO PCV7 VACCINE INTRODUCTION (2007-2009)

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Background and aims: The aims of this study were to determine the distributions of pneumococcal carriage serotypes and associated genotypes before the introduction of PCV7 into the routine childhood vaccination schedule in Peru (2009).

Methods: We collected nasopharyngeal swab samples from 2,123 healthy children 2 to 24 months of age in pediatric hospitals and medical centers in 7 cities in Peru (2007-2009). Available isolates (n=506) were serotyped by Quellung, and genotyped through multilocus sequence typing (MLST).

Results: We found a nasopharyngeal carriage frequency of 27% (573/2123). The most prevalent serotypes were those in PCV7, with 19F (19.6%), 6B (14.4%), 23F (8.7%) and 14 (6.9%) accounting for 49.6% (n=251) of the isolates. Serotypes 6A and 19A accounted for 7.3% of isolates. One hundred sixty-eight STs were observed among 42 different serotypes, with 36 new alleles and 108 new STs identified. We identified 2 serotype 6D isolates, which were both ST6148 and closely related to other serogroup 6 isolates from this study. Most isolates within each serotype were represented by one major clonal complex (CC) and 11 CCs (CC1421, CC156, CC242, CC81, CC646, CC5625, CC5636, CC3669, CC5676, CC5628 and CC1121) accounted for 47.6% of the isolates.

Conclusions: This study provides important baseline information for future follow-up studies in Peru to determine vaccine efficacy and serotype replacement in carriage due to the introduction of PCV7. Multilocus sequence typing of carriage isolates enhances our understanding of the pneumococcal population dynamics in this region.
Poster No 14

SEROTYPE AND GENOTYPE DISTRIBUTION OF NASOPHARYNGEAL ISOLATES OF STREPTOCOCCUS PNEUMONIAE IN RURAL AND URBAN CHILDREN IN NEPAL

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Background and aims: Whilst most developed countries have vaccination programmes based on infant pneumococcal conjugate vaccination, no such vaccine is licensed in Nepal. Here, we characterize the nature of circulating S. pneumoniae in Nepal by examining the serotype and genotype distribution of nasopharyngeal isolates.

Methods: Swabs were obtained from children aged 6 weeks to 24 months, resident in urban and rural Nepal between January and December 2009. Swabs were transported using two different methods, cultured and genotyped by multilocus sequence typing. Serotyping was by PCR using primers that allowed detection of 69 distinct serotypes. cpsA negative samples were considered to be nontypeable S. pneumoniae.

Results: 1101 children were enrolled of whom 574 were from an urban setting. S. pneumoniae was isolated from 320 of the rural samples (N = 527) and 334 of the urban. Though a small proportion of these isolates are yet to be characterized the following picture is emerging. The serogroups/types most commonly identified, in order of decreasing frequency, were: 6, NT, 15B/C, 14, 34, 23F, 11A, 19F, 35B, 17F, 18, 9V and 23. 33% of the isolates from both settings (216/654) were types contained in PCV10 and 36% (236/654) were PCV13 vaccine-types. Most serogroups were common to both settings except serogroup 18 and serotype 9V (largely found in the rural area) and serotypes 17F, 21,23A predominantly found in the urban. 51% of the isolates had novel sequence types.

Conclusion: S. pneumoniae isolates circulating in Nepali children appear to comprise serotypes not included in PCV10 or PCV13.
THE LEBANESE INTER-HOSPITAL PNEUMOCOCCAL SURVEILLANCE PROGRAM (LIPSP)

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Objectives: The aim of the study was to evaluate the serotype prevalence and antibiotic susceptibility of invasive pneumococcal disease (IPD) in Lebanon, a country in the MENA region where such data is lacking.

Methods: This is a six-year (2005-2011) prospective study involving 78 hospitals from all areas of Lebanon. Using active surveillance, 240 isolates were obtained from patients of all age groups with culture-proven IPD. Clinical information was collected. Isolates were tested for antibiotic susceptibility to penicillin, ceftriaxone, erythromycin, and serotyped using latex agglutination or PCR.

Results: Patients older than 60 years accounted for the majority of IPD cases (33%, n=78), followed by patients under 2 years (23%, n=55). Most samples were collected from the blood (78%, n=185). Mortality was 12% (n=28). Pneumonia was the most prevalent clinical presentation (48%, n=111). Among isolates, susceptibility to penicillin G was 56% (n=125), to Ceftriaxone 89% (n=177), and erythromycin 73% (n=166). Susceptibility to Levofloxacin was 100%. The most prevalent serotypes/serogroups were: 19F (12%, n=29), 6 (8%, n=20), 3 (7.5%, n=18), 1 (7%, n=16), 14 (7%, n=16), 19A (5%, n=12). Vaccine coverage was 40% (n=96) for PCV7, 53% (128) for PCV10, 66% (159) for PCV13 when all age groups were considered but was higher for children under 5 years of age.

Conclusion: The study underscores the importance of conducting surveillance studies to identify epidemiologic characteristics, antibiotic susceptibility, and serotype prevalence of IPD in order to raise awareness about vaccination strategies that help decrease the disease burden at the national level.
**Poster No 16**

**NASOPHARYNGEAL CARRIAGE OF STREPTOCOCCUS PNEUMONIAE AND OTHER RESPIRATORY PATHOGENS IN NIGERIAN CHILDREN BEFORE ROUTINE USE OF HIB AND PCV-13 VACCINES**

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**Background and aims:** There is limited data on the nasopharyngeal carriage of respiratory pathogens in Nigeria which has Africa's largest birth cohort. This study describes the carriage of *S. pneumoniae*, a major cause of childhood mortality and its co-occurrence with three other clinically significant respiratory pathogens among Nigerian children.

**Methods:** This was an add-on study to a community-based pneumococcal nasopharyngeal carriage study that utilized conventional microbiology techniques. Species-specific quantitative PCR was conducted for *S. pneumoniae*, Hib, *M. catarrhalis* and *S. aureus* on nasopharyngeal (NP) swabs collected from children < 5 years in Southwest Nigeria (n=209).

**Results:** The overall prevalence of *S. pneumoniae*, *M. catarrhalis*, Hib and *S. aureus* was 89.5% (95CI 85-94%), 82.8% (95CI 78-88%), 31.6% (95CI 25-38%) and 25.4% (95CI 19-31%) respectively. The highest bacterial loads were associated with Hib 1.2x10⁵ cells/ml of sample. The bacterial loads for *M. catarrhalis*, *S. pneumoniae* and *S. aureus* were 1x10³, 2.0x10³ and 6.9x10¹ cells/ml of sample respectively. Carriage of *S. pneumoniae* was more frequent among male than female children (OR 0.22; 95CI 0.08-0.63; p=0.005) adjusting for age. *S. pneumoniae* occurred with at least one other pathogen in nine out of ten NP swabs and appeared to have a strong positive correlation with Hib (OR 5.25; 95CI 1.16-23.62; p=0.031) adjusting for age and gender.

**Conclusions:** The high rates of carriage and bacterial loads of *S. pneumoniae* and Hib, offer strong support for the introduction of effective pneumococcal and Hib vaccines in Nigeria and these baseline data will be crucial for monitoring their impact.
Background: Pneumococcal Disease Surveillance in the West Africa Region (pneumoWAR) complements sentinel based surveillance for Paediatric Bacterial Meningitis network supported by WHO as part of regional efforts to strengthen Pneumococcal Disease surveillance in West Africa. PneumoWAR is supported by a Regional Reference Laboratory (RRL) at the Medical Research Council Unit, The Gambia. The RRL works closely with the Ministries of Health to (1) provide evidence for disease burden (2) generate Streptococcus pneumoniae serotypes (3) support advocacy for vaccine introduction (4) evaluate vaccine impact. This study aims to improve the diagnosis of meningitis caused by S. pneumoniae and characterize circulating serotypes prior to PCV-13 introduction.

Methods: Ninety nine (99) S. pneumoniae isolates and one thousand, four hundred and thirty-two (1432) culture negative cerebrospinal fluids (CSF) from 8 West African countries were collected from children under 5 years old from January 2010-December 2010. Latex agglutination and multiplex PCR were used to serotype the isolates and CSFs positives for S. pneumoniae respectively.

Results: A total of 200 (52%) PCV-13 vaccine serotypes and 183 (48%) non PCV-13 vaccine serotypes were identified. Serotypes 1 and 5 were the most common vaccine serotypes which accounted for 51 % of the total serotypes and serotypes 23F, 6A, 14, 4 and 18c accounted for additional 33% of the total serotypes.

Conclusion: This data represents baseline information on S. pneumoniae serotype distribution in the West Africa region for 2010 and will contribute to efforts to measure the impact of PCV-13 once introduced in West African countries.
Poster No 18

SEROPREVALENCE OF S. PNEUMONIAE IN OVER 5 YEARS AND VACCINATION COVERAGE. VENEZUELA, 1999-2010
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Objectives: To determine the distribution of serotypes of S. pneumoniae in patients > 5 years and coverage of vaccines available in Venezuela, 1999-2010.

Methods: A retrospective descriptive study; pneumococcal isolates were processed and recorded in SIREVA bulletins. Data analysis was performed using Epidat3.0® and Excel®, the data was tabulated, graphed and analyzed by age and theoretical coverage for pneumococcal conjugate vaccine 7, 10 and 13 valent (V), proportions with confidence intervals: 95% and test Z.

Results: There were 207 isolates, 190 (92%) were identified, representing 26 of the 93 known serotypes. The total theoretical vaccine coverage was 35.8% (CI :28.7-42, 8) for 7V, 67.4% (CI :60.4-74, 2) to 10V and 81.05% (CI :75.2-86, 8) to 13V, (Z-test; p = 0.0000)

The 5-14 years group (47%) corresponds to a 7V theoretical coverage of 33.3% (CI :23.0-43, 6), for 10V, 80% (CI :71.18-88, 80), for 13V, 88.8% (CI :81.8-95, 9), with significant Z test to compare 7V to 10V, non-significant with 10V to 13V

In the 15-59 years group, 7V theoretical coverage was 34.84% (CI :22.5-47, 1), for 10V, 59.09% (CI :46.4-71,7), and 13V 77, 27% (CI :66.4-8,14). When comparing 7V to 10V and 10V to 13V, Z-test; p=0.0000.

In ≥ 60 years, theoretical coverage for 7V and 10V was 45.83% (CI :23.8-67, 8) and 62.5% 13V (IC :41.0-83, 9) and non-significant.

Conclusions: The 13V vaccine coverage is higher for all age groups; these findings suggest its application in the high risks adults and older adult to prevent invasive pneumococcal disease.
Poster No 19

STREPTOCOCCUS PNEUMONIAE SEROTYPES CAUSING INVASIVE INFECTION IN HOSPITAL DE NIÑOS “DR. ORLANDO ALASSIA”, SANTA FE, ARGENTINA, 2000-2010

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Introduction: Streptococcus pneumoniae (SP) cause invasive infections with high morbidity and mortality in children. SP serotypes (ST) vary according to country, age, and over the years.

Objectives: To describe epidemiological and clinical characteristics of these infections and to relate some of these features with serotypes isolated.

Methods: Analysis of isolates of invasive infections in our hospital between 2000-2010. The samples were cultured on blood and chocolate agar. Serotyping and MICs of penicillin (Pen MIC) were made in the INEI “Dr. Malbrán”.

Results: We analized 183 isolates. 43% in girls and 57% in boys, 52% in < 2 years and 48% in > 2 years.

Diagnosis: pneumonia 49%, meningitis 18%, peritonitis 8%. 22% had underlying disease. Predominant ST in < 2 years was 14 while in > 2 years was ST 1. All of not meningeal isolates were Pen susceptible (S). In meningitis, 22 were Pen S, 11 were Pen resistant (R) and 3 strains were Cefotaxime intermediate S. ST 14 had the highest Pen R. The representativeness of ST found in < 2 years in the conjugate vaccines were 57.1% (7-valent), 73.8% (10-valent) and 84.5%. (13-valent). Mortality was 6.5%.

Conclusions: SP serotyping is important to know the local epidemiology of these severe infections and the representation of conjugate vaccines in order to detect changes that may emerge after its incorporation into the immunization schedule.
PNEUMOCOCAL MENINGITIS IN DOMINICAN CHILDREN: RESISTANCE AND SEROTYPES


**Background:** *Streptococcus pneumoniae* (*Spn*) is the leading cause of bacterial meningitis (BM) worldwide. Antimicrobial resistance is increasing and specific serotypes have been related to high mortality rates. A cross-sectional study was conducted to describe clinical characteristics, serotype distribution and antibiotic susceptibility in Dominican children.

**Methods:** From January 2008 to July 2011, 353 cases, children < 15 years of age with BM, were hospitalized at the largest Pediatric Hospital of the Dominican Republic (DR). Records were reviewed to collect demographics, clinical and laboratory data including susceptibility and serotyping. Potential vaccine coverage rates were calculated using number of serotypes identified and total number of cases.

**Results:** From the total population of BM (n=353), *Spn* was isolated from CSF in 68 (19.2%) patients. Children < 5 years of age were the most affected (90%/n=61) and with a higher death rate 23.5%/n=13. Resistance to penicillin and cefotaxime was 55.9%/n=38 and 7.4%/n=5, respectively. Most serotypes (86.7%/n=59) were identified. Serotypes 6B(25%) and 14(20.3%) were the most frequent and showed highest penicillin-resistance with 73.3% and 100%, respectively. Potential coverage rates were calculated to estimate percentage of cases prevented if PCV13 and PCV10 were included into the Dominican Vaccination Program. It was observed that the potential coverage for PCV13 and PCV10 should be 81% and 75.9%, respectively.

**Conclusion:** A high drug resistance to penicillin/cefotaxime was observed. Inclusion of *Spn* vaccines into the Dominican Vaccination Program is urgently needed to reduce disease burden and protect children from the most prevalent *Spn* drug-resistant strains serotypes in DR.
Poster No 21

MORTALITY BY INVASIVE STREPTOCOCUS NEUMONIAE DISEASE IN CHILDREN IN EL SALVADOR, 2000-2010

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Background: Invasive pneumococcal disease (IPD) presents with high mortality in children less than 5 years of age, particularly in countries where anti-pneumococcal immunization has not been implemented as part of the national immunization schedule. We report mortality by IPD in children admitted to Hospital Nacional de Niños Benjamin Bloom (HNNBB) in El Salvador, the tertiary care pediatric hospital and national referral center.

Methods: We retrospectively reviewed medical charts of children who died from culture proven IPD at HNNBB between January 2000 and March 2010. We describe the epidemiology, serogroups and sensitivity data of the isolated pneumococcus.

Results: 45 out of 147 children (30.6%) with IPD died. Twenty six (57.8 %) were male; median age was 18.4 months. Meningitis was the predominant IPD (75.5%). The average hospital stay for IPD was 6.3 days, with average ICU stay of 3.4 days. Serogroups 3 and 19 were most frequently isolated. Thirty free percent of serogroups 3, 14, and 19 were resistant to penicillin. We found no resistance to third generation cephalosporins.

Conclusions: IPD continues to produce high rates of mortality in children in El Salvador, with high resistance rates to first line antibiotics frequently used in resource limited countries.
Poster No 22

PNEUMOCOCCAL DISEASE IN OLDER CHILDREN AND ADULTS GLOBALLY: RESULTS FROM THE AGEDD PROJECT


Background: The AGEDD project aimed to identify invasive pneumococcal disease (IPD) burden data to develop modeled estimates of the burden of IPD in older children and adults and the distribution of serotypes globally.

Methods: We searched 16 databases of published and unpublished literature and abstracted numbers of pneumococcal cases, deaths, CFR, incidence, mortality rate or serotype data among persons >5 years for studies conducted between 1980-2009. Studies were assessed for quality.

Results: Of 2162 (12.6%) publications meeting inclusion criteria, most were from developed countries in Europe (n=897) and the Americas (n=622), and only 88 reported IPD incidence (median 5.3/100,000 population; range 0.3-298). Of studies from low and middle income countries with ≥40 lab-confirmed cases, 39 reported the proportion of pneumonia due to pneumococcus (median 34%; range 1-71%), and 58 reported the proportion of meningitis due to pneumococcus (median 15%; range 1-66%). Although few studies were from Africa (n=20), the proportion of pneumonia (median 56%, range 6-71%) and meningitis (median 33%, range 8-66%) due to pneumococcus was greatest in Africa.

Conclusion: Data on the incidence of IPD in older children and adults are available from all regions, but few were from low and middle income countries. Relatively more data are available for estimating the proportion of pneumonia and meningitis cases due to S. pneumoniae. These studies indicate that pneumococcus is likely responsible for a significant proportion of pneumonia in older children and adults and that these proportions are higher in Africa than in any other region.
EPIDEMIOLOGY OF THE HOSPITALIZATIONS DUE TO PNEUMOCOCCAL INFECTION IN GENERAL POPULATION IN SPAIN (2005-2009)

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Background and aims: This epidemiological survey was undertaken to estimate the burden of hospital admissions for Pneumococcal Disease in Spain during a five year period (2005-2009).

Methods: Retrospective survey reviewing data of the National Surveillance System for Hospital Data. Codes for pneumococcal infection were selected by using the 9th International Classification of Diseases. The annual incidence of hospital admissions, average length of hospitalization, mortality and case-fatality rate were calculated by using census-derived population estimates.

Results: A total of 131932 hospital discharges for pneumococcal infections (61.5% male) were reported during the study period. The average length of hospitalization was 12 days. The overall annual incidence was 59 cases per 100,000. A total of 12163 deaths were reported with a case-fatality rate of 9.22 %. Hospitalization rate due to pneumococcal pneumonia and septicaemia decreased with age in children and increased with age in adults, reaching 500 cases per 100,000 in persons aged 85 or more. The pneumococcal meningitis hospitalization rate was highest in children up to 5 years old. Case-fatality rate increased with age for all forms of pneumococcal infections. Annual cost of these hospitalizations for the National Health Care System was 160 M €.

Conclusions: Morbidity and mortality of pneumococcal infections are still high in young children and old adults in Spain. They impose an important cost to the Spanish National Health System. These data can contribute to evaluate the impact in hospitalizations of the specific immunization strategies which are being undertaken in Spain in children and old adults.
SURVEY OF BACTERIAL INFECTIONS IN PEDIATRIC POPULATION

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Introduction: Microbial pathogens discovered as etiological agents over the last 50 years continue to present a major threat to human health. Strengthening of laboratory and public health surveillance is of paramount importance for early detection and management of infectious diseases.

Materials and Methods: Retrospective observational study was done using questionnaires from 100 pediatricians and 34 microbiologists of medical colleges.

Results: Isolation of bacterial infection from sputum sample was high up to 81% followed by pleural fluid, C.S.F and blood culture. The preferred method for isolation was by culture and microscopy. Isolated bacteria from these samples included S pneumoniae (5.32%), S. aures (6.8%) and Pseudomonas (5.5%), in sputum samples, Streptococci (3.29%), Haemophilus Influenza B (Hib) (1%) and N. meningitis (0.14%) in blood culture samples. Children of age group 0-5 years were mostly affected by Hib infection. Blood culture and pleural fluid samples also showed other pathogens like Acinetobacter, Klebsiella, E. coli, Proteus and Salmonella. The investigation also revealed that Hib and S pneumoniae were isolated from other body fluids such as ear fluid, pus, peritoneal fluid, joints and pericardium.

Conclusion: Hib remains a significant cause of disease in the developing world followed by S pneumoniae. This report suggests the need of vaccinating a child against Hib and S.pneumoniae with specific comments on the urgency of inclusion of Hib in the national schedule.
PREVALENCE OF NASOPHARYNGEAL CARRIAGE FOR S. PNEUMONIAE IN ADULTS OF MEXICAN RURAL COMMUNITIES

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Background: Despite the availability of effective preventive vaccines, pneumococcal infections continue to cause excess mortality or morbidity in the world. The nasopharyngeal carriage is the step previous to invasive disease. There have been multiple studies on nasopharyngeal colonization in children, but there is little information in adults. The aim of this study was to determine the prevalence of nasopharyngeal colonization by S. pneumoniae (Spn) and distribution of serotypes in adults of Mexican rural communities.

Methods: Prevalence study in adults aged 50 or older in rural communities in the state of Queretaro. The sample size was 126 subjects. The study was conducted from December 2009 to July 2010. We invite people who came to health centers to participate in the study. Those who agreed to participate, nasopharyngeal and throat swabs for bacterial culture were collected and cultured according to standard procedures. Pneumococcus was serotyped by Quellung reaction.

Results: 236 individuals were included with nasopharyngeal and throat swab culture, of which 51 individuals were positive for Spn. The most frequent serotypes were 19A (11/51), 6A (7/51) and 6B (7/51). Two subjects had 2 serotypes spn. Only 13 of the S. pneumoniae were PCV7 serotype.

Conclusions: The nasopharyngeal colonization rate found is of the highest worldwide. New serotypes that are circulating have been reported to cause invasive disease in other parts of the world. We need to know properly the distribution of serotypes in invasive and noninvasive disease in our country to choose the best option for prevention.
PNEUMOCOCCAL PNEUMONIA AT AN ARGENTINIAN TERTIARY HOSPITAL

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Background and aims: Pneumococcal pneumonia (PP) is an endemic disease for all age groups. The aim of this study is to describe the frequency at a tertiary reference hospital.

Methods: Prospective direct observational study, period 2001-2010, of inpatients aged 0-5 years old (y) with confirmed PP/empyema. S. pneumoniae was isolated and identified by standard methods either from blood or pleural effusion at Microbiology Laboratory Hospital de Niños Sor M. Ludovica. Strains were studied for serotyping methods at Instituto Nacional de Enfermedades Infecciosas Carlos Malbran in Buenos Aires (SIREVA Project).

Results: One hundred and sixty seven cases were studied, PP 58.7% (n=98). Empyema was more frequent (75.4%) in children ≥2-5y and PP in those under 2y (80.6%). Twenty one serotypes were isolated from blood (n=102) and serotype 14 accounted for 29.4% followed by serotype 5 (6.9%). Ten serotypes were isolated from pleural effusion; serotype 1, 14 and 5 accounted for 27.7%, 26.2%, and 20%, respectively; other serotypes were 3, 6B,7F, 9V, 19A, 23B and 23F.

Conclusions: Passive surveillance is useful but not enough to know the impact of pneumococcal lower respiratory tract infections. The hospital had an average of 13,000 inpatients per year during this period. Different reasons must be considered: low number of isolations due to characteristics of the bacteria and clinical condition of children that were admitted to a tertiary hospital in many occasions after receiving antibiotic therapy. In order to measure the impact of 13V pneumococcal conjugate vaccine in national universal strategies for vaccination, surveillance would be improved.
Background and aims: The AGEDD project identified relevant data to estimate the burden of invasive pneumococcal disease (IPD) among older children and adults globally. Surveillance data published on national health agency websites is often identified by search engines like Google. We characterized the availability, advantages, and quality of this data as supplemental to traditional literature search sources.

Methods: We searched Google and known surveillance websites to identify national IPD data. Availability and quality of web data were compared to data obtained via literature search.

Results: IPD surveillance data were identified for 79 countries (n=13 Africa, n=27 Europe, n=38 Americas, and n=1 Western Pacific) from 32 websites; 23 of these countries had no data identified in the literature search of 3890 screened articles while 55 were identified in the literature search but not the web. Four sites contained “queryable” databases, five had downloadable tables and 23 were static resources such as PDFs. Websites contained IPD incidence data for 44 countries and serotype data for 49; only pneumococcal meningitis data were available for Africa. Websites’ data were more detailed, current and easy to abstract; however, methods were more ambiguous on websites than in peer-reviewed sources. Additionally, static web sources limited abstraction of multi-stratified incidence (i.e. syndrome stratified by age, etc.).

Conclusion: National health agency websites increased the amount of data available and made collection of IPD incidence data more efficient than abstracting from published literature. Improvements in data formats and availability on websites would facilitate improved data capture for disease burden estimates.
STUDY OF INVASIVE PNEUMOCOCCAL DISEASE IN CHILDREN

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The WHO estimates that 1 million deaths among children annually are due to pneumococcal infection, and most of these deaths occur in developing countries. *Streptococcus pneumoniae* is a leading cause of invasive pneumococcal disease among children worldwide. Pneumococcal infections cause meningitis, septicemia, and other focal infections that result from bloodstream infection, as well as pneumonia, which is a major acute respiratory tract infection and a leading cause of death among children in developing countries.

With these issues a study to understand a prevalence of Invasive Pneumococcal disease was undertaken in the department of Pediatrics at SMS Medical College, Jaipur between 01st Jan. 2009 to 31st Dec. 2010.

All children admitted in the Medical unit 6 with the following diagnosis Meningitis, Pneumonia, & Septicemia were included in the study. In edition 2 the routine investigation all children were subjected to Blood culture & CSF culture (children with Meningitis). During the study period there were 3812 hospital admissions, of these 482 children had pneumonia, 210 had meningitis, and 182 had septicemia. The age range of patients was 01 month to 14 years. The mean was 5.2 years, the male is to female ratio was 2.8:1. Pneumococcal isolate in cases of meningitis in the TSF was 6 & in the blood culture in 18 cases. 12 children with septicemia had Pneumococcal isolate and 12 children with Pneumonia were positive.

**Conclusion:** The study reveals the prevalence of Invasive Pneumococcal Disease is higher percentage of children who are not vaccinated with Pneumococcal Vaccine.
**Poster No 29**

**INVASIVE PNEUMOCOCCAL DISEASE IN PEDIATRICS, PERIOD: SEPTEMBER 2009-2011**


**Background and aims:** *Streptococcus pneumoniae* is the prevailing cause of pneumonia, meningitis and bacteremia in children. Invasive pneumococcal disease is a critical health problem in our country so our research's aims is to analyze demographic, clinical characteristics regarding evolution of infections caused by *Streptococcus pneumoniae* in our community and antibiotic sensibility pattern.

**Methods:** Descriptive, observational and retrospective research.

Retrospective analysis of medical history of patients from 1 to 15 years old where *Streptococcus pneumoniae* was detected in blood culture or sterile sites interned in our service from 1st September 2009 to 30th September 2011.

**Results:** 70 patients registered with *Streptococcus pneumoniae* infection during this period (immission rate 8.26‰). Data analyzed from 44 patients: 1 to 180 months old (median 23 months), males: 57 %.

Primary Bacteremia: 6/44; secondary bacteremia: 32/44. Location of infection: pneumonia: 17/44; pleuropulmonary suppuration 10/44; meningitis 5/44; sepsis 3/44; others 3/44.

Initial treatment: ceftriaxone 36/44; ampicillin 4/44; vancomycin 4/44. Final treatment: ampicillin 19/44. Favorable evolution 41/44; 3 patients died.

29 cases belonged to oxacillin-sensitive strains, 8 cases to non-sensitive strains where penicillin MIC was 0.125 µg/mL.

**Conclusions:** Invasive pneumococcal disease is an important cause of hospitalization, being pneumonia the more frequent location.

We stress the importance of continuous antibiotic sensitivity monitoring because in our research the highest percentage of strains are oxacillin-sensitive.

It is considered of fundamental importance the latest introduction of pneumococcal conjugate vaccine to the official vaccination schedule in children under 2 years old, being them the most affected group.
THE DETERMINATION OF GENE DIVERSITY BY RANDOMLY AMPLIFIED POLYMORPHIC DNA AMONG EPIDEMIOLOGICALLY RELATED STRAINS OF STREPTOCOCCUS PNEUMONIAE FROM NIGERIA

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Background and aims: From intervention and policy viewpoint, several studies have suggested diversity in the epidemiology of Streptococcus pneumoniae. This study determined gene diversity by randomly amplified polymorphic DNA (RAPD) among S. pneumoniae strains from Nigeria.

Methods: A total of 71 consented patients with severe malaria, asthma, SCA, sepsis, meningitis and sinusitis from 4 urban hospitals in Lagos were enrolled. These patients provided biologically samples that were screened for S. pneumoniae using conventional methods including serologically testing. The boiling-obtained chromosomal DNA samples subsequently submitted to PCR using a random 10-mer primer coupled with specific detection of haemolysin (lyt) and pneumolysin (ply) genes.

Results: A total 15 S pneumoniae isolates were recovered from sputum, blood and nasal swab samples of patients with severe malaria (n=2), SCA complicated by pneumonia (n=3), sepsis (n=1), meningitis (n=5) and asthma (n=1). Of the 15 isolates, 12 (80%) were typed by RAPD with all (100%) yielding reproducible fingerprints. A total of 6 RAPD types constituted by 1 - 4 isolates were identified, yielding a discriminatory power of 84.9%. The lyt and ply genes were detected in all and 8 typeable/1 non - RAPD strains respectively. Of the 12 RAPD types, 6 belonged to serotype 19F shared by 1 non-RAPD strain. All the isolates were penicillin resistant but were sensitive to augmentin (73.3%) and ofloxacin (100%).

Conclusion: Even for smaller number of isolates, RAPD represents a reliable and reproducible typing technique for epidemiologically related S. pneumoniae isolates from Nigeria.
BURDEN OF STREPTOCOCCUS PNEUMONIAE DISEASES IN MAINLAND CHINA

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Background and aims: Pneumonia is the leading cause of child mortality in mainland China, yet the etiology-specific burden remains unclear. To estimate the burden of pneumococcal disease in children and adults, we systematically reviewed the literature.

Methods: Studies published between 1980-2010 reporting pneumococcal disease (PD) incidence or mortality rates, case fatality ratio, the proportion of pneumonia and meningitis caused by pneumococci, distribution of syndromes caused by pneumococci or pneumococcal serotype data from mainland China were identified through systematic searches of the China National Knowledge Infrastructure (CNKI) and 16 other global/regional literature databases.

Results: Of 58 studies reporting on pneumococcal disease (PD) from mainland China, 36 (62%) were identified only by searching CNKI. One study reported the incidence of pneumococcal meningitis in children < 5 years (0.98/100,000) and 35 estimated the proportion of pneumonia or meningitis cases/deaths due to pneumococci. Among children age 0-15 years, pneumococci caused a median of 17% (range: 1-36%) of meningitis cases and 10% (range: 1-25%) of clinical pneumonia cases. Three studies reported pediatric invasive PD serotypes; the cumulative proportion for the 10 most common serotypes for mainland China approximated published regional estimates (~68%). Limitations included lack of epidemiologic data for lab identified cases, high rates of antibiotic pretreatment and reliance solely on culture methods.

Conclusions: Searching the Chinese literature is important to identify data on pneumococcal disease in mainland China. Despite limitations in the number of studies and their quality, etiology studies consistently demonstrate pneumococci as an important cause of meningitis and pneumonia in China.
A STUDY OF STREPTOCOCCUS PNEUMONIAE ISOLATES FROM CARRIER DAYCARE CHILDREN IN FORTALEZA, BRAZIL

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Background and aims: Streptococcus pneumoniae is part of the commensal flora of the upper respiratory tract, colonizing the nasopharyngeal niche, especially in children under 5 years of age sharing confined spaces, as daycare centers, a predisposing risk factor for invasive disease. The increased resistance of this microorganism to macrolides, lincosamides and sulfamethoxazole-trimethoprim has already been reported in different countries. The objective of the present study is to verify the number of daycare goers which are S. pneumoniae carriers and the resistance rates of these isolates against different antibiotics.

Methods: From March to August of 2011, 177 samples from nasopharynx of healthy daycare goers, between 12 and 58 months of age, were collected in Fortaleza. The pneumococcal identification was based on Gram stain, alpha-hemolysis, optochin susceptibility, and bile solubility. Also, the minimal inhibitory concentration was determined by the E-test method, according to the CLSI guidelines (2010).

Results: Ninety nine (56%) S. pneumoniae strains were isolated from the 177 swab samples. Of those 99 isolates these are the numbers for resistant strains: 24 (24.2%) to penicillin, 11 (11.1%) to clindamycin/erythromycin, 79 (79.8%) to sulfamethoxazole-trimethoprim and 6 (6.1%) were multiresistant. There was no resistance to either ceftriaxone or amoxicillin. These findings were compatible with other Brazilian studies.

Conclusion: There is a high carriage rate among the daycare children in Fortaleza, as well as high resistance amidst the isolates. An additional study identifying the serotypes for these isolates could further elucidate about the circulating serotypes.
ISPPD-8

Poster No 33

PNEUMOCOCCAL MENINGITIS IN ARGENTINIAN CHILDREN BEFORE THE RUTINE INTRODUCTION OF PNEUMOCOCCAL CONJUGATE VACCINE

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Background: Streptococcus pneumoniae meningitis is a leading cause of morbidity and mortality in pediatric patients (p). The aim was to identify epidemiological and clinical features, antibiotic susceptibility and outcome of pediatric p with pneumococcal meningitis, before the introduction of the vaccine in Argentina, approved in 2011.

Methods: Study population included children younger than 18 year-old with pneumococcal meningitis confirmed by culture during 12 years (1999-2010). Data were collected from the microbiology laboratory and p records admitted at HPG a 510-bed primary care and tertiary care referral Center.

Results: A total of 111 infants with pneumococcal meningitis were identified. There were 40 cases in 1999-2002, 35 in 2003-2006 and 36 in 2007-2010. The mean age was 7 months (r: 1-191). Most of them were immunocompetent 104p (94%) and 88p (82%) had no underlying disorder. Neurological compromise 80p and sepsis 59p were the most common clinical presentation (75% and 53% respectively) and 49p (44%) required Intensive care unit at admission. Another foci was present in 24p (22%), being half of them pneumonia. Cerebrospinal fluid and blood culture were positive in 103p and 88p respectively (93% and 79%). Fifteen percent of the organisms were resistant to penicillin, and 5% were resistant to cefotaxime, with decreasing of resistance through the years. Fifty-six p (50%) had complications and 11 p (10% ) died.

Conclusion: Knowledge of previous situation in pneumococcal meningitis is important to be able to compare the impact of routine immunization in our community.
LIVING THIRTY YEARS WITH THE ENEMY: PENICILLIN-NON-SUSCEPTIBLE PNEUMOCOCCI (PNSP) IN ARGENTINA

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Background and objectives: The first isolation of PNSP in Argentina occurred during 1981 at a pediatric hospital in La Plata. During the following 7 years, they remain in this hospital without spreading to other centers. The objective is to describe the evolution of PNSP in Argentina along thirty years (1981-2011).

Methods: Publications and congress abstracts presented from 1981 to 2011 have been reviewed. In different studies, penicillin susceptibility tests were performed by the oxacillin disk screening test, broth macrodilution, Etest and agar dilution. Serotyping was performed by the quellung method.

Results: In 1988 16% of PNSP were documented in other pediatric hospital of Buenos Aires, while in 1994 they were detected in adult infections. Later, it was observed an increase from 11.4% to 44.3% between 1993 and 1998. In one hospital percentages varied from 3.95% R and 9.9% I (1992-94) to 23.2% R and 11.9% I (1995) in invasive pneumococci. Then R isolates dropped to 14.5% (1997). Data from SIREVA II were similar for PNSP but 26% were I and 11% were R (2000 - 2005). Now, R percentages decreased to almost zero with I isolates between 30 and 40%. The same was observed in pneumococci from acute otitis media (from 15.1 R in 1999 to 0 in 2008-2009).

Conclusions: As in Argentina there was not any intervention that could explain the decrease of R pneumococci, we postulate that it may obey to changes in prevalence of particular clons with better fitness due to having minimal alterations in their PBPs.
FREQUENCY OF STREPTOCOCCUS PNEUMONIAE SEROTYPE 6C IN INVASIVE AND CARRIAGE ISOLATES IN COLOMBIA

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Background and aims: *Streptococcus pneumoniae* serotype 6C was described in 2007 and has been reported in invasive disease and nasopharyngeal carriage by several countries. The aim of this study was to determine the frequency, antibiotic susceptibility and molecular typing of *S. pneumoniae* serotype 6C recovered in Colombia during 1994 to 2011.

Methods: Isolates of *S. pneumoniae* serotype 6C were differentiated by serotype-specific PCR and Quellung reaction, the antibiotic susceptibilities were determined according to The Clinical and Laboratory Standards Institute. The Molecular typing was performed by pulsed-field gel electrophoresis (PFGE).

Results: Among 4048 invasive and 217 nasopharyngeal isolates, 48 (1.18%) and 6 (2.7%) were serotype 6C, respectively. The invasive isolates were collected from meningitis (43.7%), pneumonia (22.6%), sepsis (15.9%) and others diagnostics (9.43%). One isolate (2.1%) was non-susceptible to penicillin (MIC of ≥0.125 µg/ml), four (8.4%) to trimethoprim-sulfamethoxazole and four (8.4%) to tetracycline. Twenty-one invasive and five nasopharyngeal isolates were typed by PFGE, of these, twenty isolates presented association in four clusters: I (eight invasive and two nasopharyngeal isolates), II (four invasive and one nasopharyngeal isolates), III (two invasive and one nasopharyngeal isolates) and IV (one invasive and one nasopharyngeal isolates) and the other six were not associated.

Conclusions: This study showed the circulation of *S. pneumoniae* serotype 6C in Colombia. The isolates recovered from nasopharyngeal carriage and invasive presented genetic association. The results emphasize the importance of continuing with the laboratory surveillance to determine the dynamics of this serotype over time.
MOLECULAR CHARACTERIZATION OF STREPTOCOCCUS PNEUMONIAE INVASIVE SEROTYPE 19A ISOLATES IN COLOMBIA (1994-2010)

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Background: In recent years Streptococcus pneumoniae serotype 19A isolates has increased and is now one of the most common serotypes recovered from invasive disease. The aim of this study was to determine the molecular characterization of invasive penicillin non-susceptible Streptococcus pneumoniae serotype 19A, collected in Colombia between 1994 and 2010.

Methods: A total of 73 isolates of S. pneumoniae serotype 19A were analyzed, serotyping was done by Quellung reaction and antimicrobial susceptibility testing was performed according to The Clinical Laboratory Standards Institute. The genetic relationship of 46 isolates with susceptibility diminished to penicillin was determined by Pulsed-Field Gel Electrophoresis (PFGE) and selected strains were studied by multilocus sequence typing.

Results: Among the 73 isolates, 68.5% were obtained from children < 6 years old and the 45% were identified over the last two years. The isolates showed resistance to penicillin (34.2%), trimethoprim-sulfamethoxazole (46.6%), erythromycin (38.4%) and tetracycline (37.0%). By molecular typing, twelve multiresistant isolates were genetically related to ST276 a single-locus variant of the Denmark 14-ST230 clone, six isolates were associated with ST1118, three isolates resistant to penicillin, erythromycin and trimethoprim/sulfamethoxazole were associated with the Spain 9V-ST156 clone, three isolates were clustering to ST199 and two isolates with intermediate susceptibility to penicillin were genetically related to Colombia 23F-ST338 clone. By only PFGE, ten multiresistant isolates showed >89.8% of genetic similarity and the remaining patterns (n=10) had a single isolate.

Conclusions: The rise in penicillin-resistant serotype 19A invasive pneumococcal in Colombia was associated with the emergence and spread of capsular variants of worldwide-disseminated multiresistant clones.
Introduction: The World Health Organization (WHO) developed a standard definition of radiologically confirmed pneumonia in children < 5 years for use in pneumococcal vaccine trials. We evaluated these criteria in older age groups in the African meningitis belt, an area known for high incidence of pneumococcal meningitis throughout all ages.

Methods: From May 2010 to April 2011, we enrolled all patients admitted for clinical pneumonia (cough, tachy-/dyspnea) at five hospitals in Dapaong, northern Togo. We obtained two blood samples for culture and C-reactive protein (CRP) and evaluated chest radiographs. Endpoint pneumonia was defined as lobar consolidation or pleural effusion as agreed upon by two primary readers or one primary reader and an arbiter.

Results: Among 713 inclusions, at least one blood culture and a radiograph were performed in 636. The primary readers agreed on 80% of films. In age groups 0-4 yr (N=121), 5-29 yr (N=277) and ≥30 yr (N=238), pneumococci were isolated from blood cultures in 0%, 6.9% and 7.6%, endpoint pneumonia identified in 27.3%, 35.7% and 55.0% and CRP ≥40 mg/l found in 78.2%, 71.0% and 81.9%. Of persons 5-29 and 30 years of age with bacteremic pneumococcal pneumonia, 74% and100%, respectively, had endpoint pneumonia, and 89% and 100%, respectively, had elevated CRP.

Conclusions: Among ≥5-year-old pneumonia patients in the meningitis belt, endpoint pneumonia -as defined by the WHO process- had good sensitivity in identifying persons with confirmed bacteremic pneumococcal pneumonia. We will update these estimates with results from ongoing molecular analyses for pneumococci on blood samples.
INVASIVE PNEUMOCOCCAL DISEASES IN ADULTS (IPD): FINAL RESULTS OF A MULTICENTER STUDY IN ARGENTINA


Background: Pneumococcal disease is a leading cause of morbidity and mortality worldwide. There is limited data regarding IPD in adults in Latin America and particularly in Argentina. The aim of this study was to evaluate clinical, epidemiological and serotype characteristics of IPD in our country.

Methods: Prospective, multicenter and observational study of IPD episodes in people >18 yo.

Results: From January 2000 to January 2002, 218 S. pneumoniae strains were obtained from 25 centers in 11 provinces of Argentina; 207 (94.9%) were available for clinical analysis and 187 (85.8%) for clinical, microbiological and serotype evaluation. Mean age: 64 yo (range=18-98); male 58%. Besides the age ≥65 años, 89.8% patients had others risk factors for IPD (smoking 25%, COPD 25%, immunocompromised 20%, alcoholism 10%, diabetes 11.6%, renal diseases 5%, HIV 5%). More frequent clinical presentation: pneumonia (78%), bacteremia (27%), meningitis 7%. Mortality: 18.7% (global). Serotypes: 1, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 14, 19A, 23F. All the strains were sensitive to penicillin. Previous pneumococcal vaccination: 4.3%; none of them died.

Conclusions: IPD occurred principally in people with risk factors and most of them had no pneumococcal vaccine. The serotypes isolated were sensitive to penicillin and all of them are included in PPV-23 vaccine.
BURDEN OF HOSPITALIZED COMMUNITY ACQUIRED PNEUMONIA (CAP) IN ARGENTINA

F. Nacinovich, R. Ruttimann, P. Bonvehi, N. Giglio, G. Lopardo, D. Stamboulian, Buenos Aires, Argentina

**Background:** There are limited data about the burden of CAP in Latin America. The purpose of this study was to evaluate the rate of hospitalization due to CAP in Argentina, during influenza vaccination campaigns performed in persons >65 years.

**Methods:** During vaccination campaigns, demographic data and underlying diseases: cardiovascular (CVD), chronic pulmonary (CPD) and others high risk conditions (i.e: diabetes) of each subject who was immunized were obtained through interview before vaccine administration. In a case-control study, subjects were retrospectively evaluated for hospitalization due to CAP during previous influenza season (June-October;1995-1998).

**Results:** A total of 984,329 people >65 y were evaluated during the 4 years of the study. Rate of (CAP) hospitalization was:

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>POPULATION</td>
<td>187,988</td>
<td>248,299</td>
<td>274,224</td>
<td>273,818</td>
</tr>
<tr>
<td>N HOSPITALIZED (GLOBAL)</td>
<td>1629</td>
<td>3101</td>
<td>2848</td>
<td>3044</td>
</tr>
<tr>
<td>Incidence/10000/year</td>
<td>170</td>
<td>250</td>
<td>230</td>
<td>250</td>
</tr>
<tr>
<td>N HOSPITALIZED CAP WITH CVD</td>
<td>604</td>
<td>1011</td>
<td>954</td>
<td>852</td>
</tr>
<tr>
<td>Incidence/10000/year</td>
<td>300</td>
<td>490</td>
<td>400</td>
<td>410</td>
</tr>
<tr>
<td>N HOSPITALIZED CAP WITH CPD</td>
<td>953</td>
<td>1478</td>
<td>1385</td>
<td>1561</td>
</tr>
<tr>
<td>Incidence/10000/year</td>
<td>1000</td>
<td>1470</td>
<td>1320</td>
<td>1640</td>
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</tbody>
</table>

**Conclusions:** Burden of Hospitalized CAP is important in Argentina, particularly people with CVD and CPD had the highest incidence. Influenza and pneumococcal vaccination should be promoted in this population.
Poster No 40

INVASIVE PNEUMOCOCCAL DISEASE IN CHILDREN 1 - 17 YEARS OLD IN LVIV REGION (UKRAINE)

O. Nadraha, Lviv, Ukraine

Background and aims: Vaccination with PCV in Ukraine is available only for high risk of developing IPD patients and for some private hospitals.

Methods: A retrospective chart review was conducted of 62 patients (1-17 y.o.) with IPD. All patients were treated during 2006-2010 yy in Lviv Regional Infection Diseases Hospital.

Results: Annual incidence rate of IPD was 10.2 per 100,000. The median of age of patients was 3 years. 48 children were younger than 6 years. Bacteric pneumonia (33.3%), purulent meningitis (22.6%) and septicaemia (17.9%) were the most frequent diagnoses. Mortality rate was 6.2% for IPD and 11.3% for meningitis. Long-term effects were reported in 10.7% of the cases. Serotypes were isolated in 74.1%, and the leading serotype was 1 (21 cases), followed by serotypes 14 (16 cases) and 6B (9 cases).

Conclusions: The rate pneumococcal disease in west part of Ukraine is still height. Pre-vaccination data on disease severity and outcome of IPD are important to determine the impact of vaccination strategies. The pneumoccocal vaccine must be included in national vaccination program.
GLOBAL REVIEW OF THE DISTRIBUTION OF PNEUMOCOCCAL DISEASE BY AGE AND REGION: IMPLICATIONS FOR VACCINATION SCHEDULES

F. Russell¹, C. Sanderson², B. Temple³, A.M. Henao-Restrepo⁴, K. Mulholland¹,²,³, Pneumococcal Epidemiology Group, ¹Melbourne, VIC, Australia, ²London, UK, ³Darwin, NT, Australia, ⁴Geneva, Switzerland

Background and aims: To compare whether different PCV schedules differ in vaccine effectiveness, the distribution of pneumococcal disease needs to be understood. The aim is to determine whether the distribution of IPD, pneumococcal meningitis, WHO radiographic and hospitalised WHO clinical pneumonia in children aged 0-59m varies significantly between and within regions. The impact of different PCV schedules was estimated.

Methods: Sites were identified by a literature review and informal methods. The proportion of cases aged 6-11m, and 12-23m by region were compared, and national data were compared with regional averages. A model was constructed to estimate the proportional risk of disease by month. Using these curves as the basis, curves were overlaid to describe the impact of PCV.

Results: For IPD, ~20% of cases occur in infants aged < 6m, 50% in infants < 12m, and 75% in children < 24m. For pneumococcal meningitis the age distribution is: 40%, 65%, and 83%, respectively. The peak of pneumococcal meningitis occurs earlier than IPD. For WHO radiographic pneumonia, between 8.7% and 52.4% of cases occur in infants aged < 6m, and is similar for hospitalised WHO clinical pneumonia. Mortality is greatest in younger infants.

Conclusions: There are no major differences in the age distribution of disease. The disease peak occurs in early infancy. Many children will be unprotected if PCV is delayed, coverage is incomplete, and herd effects are negligible. Moreover, PCV given at 9m maybe too late to offer much protection, if 2 doses provides insufficient protection, and no herd immunity.
AGE DISTRIBUTION OF SEROTYPES 1, 6B, AND 23F INVASIVE PNEUMOCOCCAL DISEASE BY REGION: IMPLICATIONS FOR VACCINATION SCHEDULES

F. Russell¹, B. Temple², A.M. Henao-Restrepo³, K. Mulholland¹,²,⁴, Pneumococcal Epidemiology Group, ¹Melbourne, VIC, ²Darwin, NT, Australia, ³Geneva, Switzerland, ⁴London, UK

Background: The optimal age of when to administer the third PCV dose is unknown. Two or 3 doses have similar immunogenicity, except for serotypes 6B and 23F. Serotype 1 has no efficacy against serotype 1 disease, using the EPI schedule, but post booster shows improved functional activity. The serotype distribution of IPD is documented.

Methods: Sites were identified by a literature review and informal methods. The proportions of IPD due to serotypes 6B and 23F IPD in infants aged 6-11m, and serotype 1 in children aged 12-59m were compared within regions.

Results: The proportion of IPD due to serotype 6B in infants aged 2-11m ranged between 0-15%, 6-21%, 11% (one site), 15-17%, 0-2%, and 0-4% in AFR, AMR, EMR, EUR, SEAR, and WPR respectively. For serotype 23F, the proportion of IPD in infants aged 2-11m ranged between 8-11%, 4-11%, 6% (one site), 4-13%, 0-3%, and 0-8% in AFR, AMR, EMR, EUR, SEAR, and WPR respectively. For serotype 1, the proportion of IPD cases in children aged 12-59m ranged between 7-28%, 0-16%, 9% (one site), 2-7%, 15-52%, and 1-11% in AFR, AMR, EMR, EUR, SEAR, and WPR respectively.

Conclusions: There are no major differences in the distribution of IPD by selected serotypes within regions. The proportion of IPD due to serotype 1 in children 12-59m was higher in AFR and SEAR compared with other regions. Any direct benefit of one schedule over another at an individual level may be balanced by the benefits of herd immunity at a population level.
SEROTYPES OCCURRENCE AND ANTIMICROBIALS RESISTANCE OF STREPTOCOCCUS PNEUMONIAE STRAINS ISOLATED IN MINAS GERAIS, BRAZIL

D.R.A. Camargo, M.L. Samuel, C.D. Faria, M.A.A. Oliveira, Belo Horizonte, Brazil

**Background and aims:** Evaluate the occurrence of Streptococcus pneumoniae serotypes and antimicrobials susceptibility in order to produce a database to an interpretation of possible changes in occurrence of serotypes profile after 10 valent vaccine introduction in Minas Gerais.

**Methods:** Descriptive epidemiological study, refer to data analyses of 159 Streptococcus pneumoniae strains isolated from patients with invasive disease in Minas Gerais. The data were obtained using registers of Ezequiel Dias Foundation from 2006 to 2009. The strains were identified using identification classic methods. The antibiogram was made according to CLSI. The serotyping was performed at Adolfo Lutz Institute. The data were analysed on EPI INFO™ 3.5.3 program.

**Results:** 159 strains were isolated from cerebrospinal fluid (86.8%), blood (7.5%) and pleural fluid (5.7%). The frequency in male sex were 61% and high occurrence was observed in patients with less than 5 years old (43.4%). It was identified 33 serotypes: 14,3,19F,18C,6B,1,23F,6A/C,12F,4,6A,9V,7F,11A,9N,10A,16F,19A,5,8,13,29,34,38,11F,15B,15C,18B,23B,24F,28A,35B,7C), from these, 6 showed resistance to penicillin (14,23F,19F,6B,9V,23B) , 3 resistance to ceftriaxone (14, 23F, 6B) and 2 resistance intermediate to ceftriaxone (14 e 9V). 62.9% from strains were resistant to Trimetroprim-Sulfametoxazole; 6.3% to Tetracycline; 3.8% to Eritromycin; 3.1% to Chloramphenicol; 1.9% to Clindamycin. There wasn't resistance in vitro to Ofloxacin, Rifampin and Vancomycin.

**Conclusions:** Among the serotypes isolated, 65.4% were in PCV 10 and 58.5% in PCV7. The serotype 14, the most common isolated, showed the highest antimicrobial resistance profile. The understanding of Streptococcus pneumoniae occurrence in specific community is relevant to guide posterior studies of new vaccines formulations.
Background: S. pneumoniae (Spn) is a major cause of meningitis and pneumonia worldwide in children < 5 years and elderly. Since 2000, Dominican Republic (DR) has participated in the Meningitis and Pneumonia Regional Surveillance Network (SIREVALL) with 5 local hospitals. The study objective is to determine the trend of Spn serotype and antimicrobial resistance from 2000 to 2010, before the Expanded Program on Immunization (EPI) implementation.

Methods: Between 2000 and 2010, all Spn strains isolated from children and adults with Invasive Pneumococcal Disease (IPD) received at our National Reference laboratory were serotyped and evaluated for susceptibility to penicillin, cefotaxime, erythromycin and trimethoprim-sulfamethoxazole. Isolation, identification and serotyping followed SIREVA laboratory protocol, while antibiotic sensitivity and interpretation followed CLSI Guidelines 2011. Potential vaccine coverage rates were calculated using total of serotyped cases.

Results: Of 817 total Spn strains, 601 were from children < 5 years (69.9% < 12 months). Of those 552(91.5%) serotyped, 239(43.3%) were from meningitis and 313(56.7%) from other IPD. Serotypes 14, 6B, 23F and 6A were most frequent: 41.1%, 11.1%, 6.5% and 6.0%, respectively. Penicillin and cefotaxime resistance was 54.1% and 23.9% in meningitis, and 10.1 % and 1.3% in other IPD, respectively.

Conclusions: In children < 5 years, high penicillin and cefotaxime resistance was observed in Spn meningitis. Potential coverage rate of PCV10 and 13 is 74.0% and 84.5% in meningitis, and 78.9 and 91.7% in IPD, respectively. This high antibiotic resistance in meningitis prompts DR public health policy change to introduce PCV into the established EPI.
INCIDENCE OF INVASIVE PNEUMOCOCCAL DISEASE IN SOUTHWEST SWEDEN DURING 45 YEARS

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Background and aims: The incidence of invasive pneumococcal disease (IPD) changes geographically and over time due to socioeconomic differences, prevalence of diseases with increased susceptibility to IPD and occurrence of serotypes and clones with increased virulence. Besides, the documented incidence differs due to availability of hospital care and blood culture policies. In this study changes of pneumococcal meningitis and nonmenigitic IPD in a defined area in southwest Sweden during 45 years was followed.

Methods: Retrospective review of case records from all patients living in Gothenburg and 5 surrounding municipalities with isolates of pneumococci from blood, CSF, synovial, pleural, pericardial, peritoneal fluid.

Results: Totally, 2505 IPD episodes were identified. The incidence of pneumococcal meningitis remained stable during the 45 years, varying between 1.1 and 1.6 cases/100,000/year. There was a continuous increase in nonmenigotic infections from 4.1 - 8.9 - 11.8 cases/100,000/year during the first 7, the middle 7 and the last 6 years of the study. The increase in incidence of nonmeningitis IPD was mainly seen in the elderly while the incidence in infants remained stable. The proportion of patients with risk factors remained stable.

Conclusions: A continuous increase in incidence of nonmeningitic IPD was observed during 45 years.
STREPTOCOCCUS PNEUMONIAE ISOLATED IN PATIENTS PEDIÁTRICOS WITH MENINGITIS: FREQUENCY, ANTIMICROBIAL SUSCEPTIBILITY AND SEROTYPES ISOLATED

A.C. Villagra, M.L. Gonzalez, G. Delgado, N. Sarzano, San Miguel de Tucumán, Argentina

Streptococcus pneumoniae (Spn), cause diverse severe invasive infections like meningoencefalitis.

Objectives: To emphasize frequency, sensitivity to penicillin and ceftriaxona, but serotipos presents meningoencefalitis causes purulenta in pediátricos patients of the Hospital of Children.

Methodology: Descriptive and retrospective analysis of 286 isolated stocks of Spn of I eliminate cefalorraquídeo between January 1985 to July 2011. They were identified biochemically according to manual procedure. To fifty and three isolated ones in 2005 to the 2011 minimum inhibiting concentration (CIM) to penicillin and ceftriaxona by And-test was determined. Of the stocks sent to the National Institute of Infectious Diseases “Dr. Carlos Malbrán” between year 2005 to the 2008, twelve serotipos inquired.


Conclusions: From year 2000, Spn was the bacterium more frequently isolated in the meningitis purulentas, with a gradual increase of resistance to penicillin, single three stocks were resistant to ceftriaxona. To alert to the presence of serotipos causes of severe disease and death in small children like the 1, 5 and 7F.
Poster No 47

EARLY NEONATAL SEPSIS WITH MENINGITIS BECAUSE OF STREPTOCOCCUS PNEUMONIAE

A.P. Villagra¹, A. Soto¹, A. Fonts¹, V. Dip¹, C. Lopez¹, ²Tucuman, ²San Miguel de Tucuman, Argentina

Background and aims: Systemic bacterial infections in the neonate affect 1 to 5 1000 live births. The incidence of meningitis varies between 0.13-2.8/1000 term newborn. The predominant pathogens causing early-onset bacterial infections are group B streptococci and enteric bacilli. *Streptococcus Pneumoniae* is not common microorganism causing of sepsis and meningitis in the neonatal stage.

We present the case of a newborn that displayed typical clinical manifestations of sepsis and neonatal meningitis.

Method: Descriptive, retrospective of a clinical case.

Results: Maternal antecedents: low social-economic level, multiple pregnancy, only one prenatal control in this pregnancy, premature rupture of membranes. Maternal fever during the first 24 hours postpartum.

Female newborn; 37 weeks of gestacional age; weigh at birth: 2.400 grs.

Hospitable expenditure: 34 hours after birth.

Hospitable income after 56 hours of birth, she was in a bad general state, marmorean reticulated, in apnea and bradycardia, hypotension. She required mechanical respiratory assistance and expansion with physiological solution and inotropic at the income in Neonatal Intensive Care Unit.

Complementaries studies: White blood: 3.000/mm3; PCR: +.

Microbiologic techniques: Blood culture and lumbar puncture.

She starts antibiotic treatment with ampicillin and cefotaxime. Torpid evolution with persistent metabolic acidosis, hemodynamically descompensated with intropics. She had refractory cardio-respiratory heart attack to the advanced handling resurrections, passing away at 76 hours of birth. The results of microbiology were received after postmortem: cerebrospinal fluid + *Streptococcus pneumoniae*.

Conclusions: Early neonatal sepsis with meningitis by *Streptococcus pneumoniae* is infrequent because of its microbiologic rescue but it presents high morbidity-mortality.
OTITIS MEDIA INCIDENCE IN CHILDREN 0-5 YEARS OF AGE FROM GUADALAJARA, MEXICO

A. Villaseñor-Sierra1, J.J. Jáuregui-Lomeli1, R. Martínez-Ramírez2, R. DeAntonio-Suarez2, M.Y. Cervantes3, D. Kolhe4, R. Colindres5, E. Ortega-Barria6, Guadalajara, Mexico, 7Wavre, Belgium, 8México City, Mexico, 9Bangalore, India, 10Rio de Janeiro, Brazil

Background and aims: Acute Otitis Media (AOM) is one of the most frequent paediatric bacterial infections (60% children < 1 year have at least one episode of AOM in developed countries) and among the primary reasons for antibiotic prescriptions in paediatric outpatients. This study aimed to estimate the overall incidence of AOM in Guadalajara, Mexico, as data on AOM incidence are limited.

Methods: Randomly selected children (N=1401 screened) aged 0-5 years were enrolled from eight public sector medical centres affiliated to the Community Medicine Program of the Autonomous University of Guadalajara (PMC-UAG). AOM incidence (overall, by age-group and 7-valent pneumococcal conjugate vaccination status) was estimated retrospectively (by medical records review 12 months preceding enrolment, or since birth for children < 1 year) and prospectively (bi-monthly follow-up for one year). AOM was defined as cases diagnosed by the physician and recorded in a medical file, including cases reporting AOM-related signs and symptoms.

Results: 858 children were enrolled. Overall retrospective and prospective incidence of AOM diagnosed by a physician was 84.9 (95%CI: 65.4-108.5) and 119.5 (95%CI: 96.1-146.8) cases-per-1000 person-years, respectively. The table summarizes AOM incidence by age group and vaccination status.

Conclusions: AOM incidence in Guadalajara seems to be lower when compared developed countries suggesting possible differences and limitations in diagnostic procedures and access to the counter treatment, including antibiotics.

Table Retrospective and prospective AOM incidence by age group and vaccination status (ATP cohort)

<table>
<thead>
<tr>
<th>AOM incidence</th>
<th>Retrospective data (N=134)</th>
<th>Prospective data (N=835)</th>
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<tbody>
<tr>
<td></td>
<td>P=751.03014</td>
<td>P=751.74521</td>
</tr>
<tr>
<td>n</td>
<td>Episode in 95% CI</td>
<td>n</td>
</tr>
<tr>
<td>1000 person-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>64</td>
<td>119.5</td>
</tr>
<tr>
<td>65.4-108.5</td>
<td>(96.1-146.8)</td>
<td></td>
</tr>
<tr>
<td>Age group</td>
<td>0-&lt;3</td>
<td>38</td>
</tr>
<tr>
<td>(years)</td>
<td>82.51 (57.70-114.23)</td>
<td>44</td>
</tr>
<tr>
<td>58.74-117.77</td>
<td>(87.17-161.68)</td>
<td></td>
</tr>
<tr>
<td>1-&lt;6</td>
<td>21</td>
<td>88.40</td>
</tr>
<tr>
<td>53.22-153.02</td>
<td>(82.12-153.02)</td>
<td></td>
</tr>
<tr>
<td>Vaccination status by age</td>
<td>0-&lt;3</td>
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</tr>
<tr>
<td>54.55-134.70</td>
<td>(54.55-134.70)</td>
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</tr>
<tr>
<td>Unvaccinated</td>
<td>54</td>
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<tr>
<td>43.54-128.32</td>
<td>(46.10-178.80)</td>
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</tr>
<tr>
<td>3-&lt;6</td>
<td>12</td>
<td>37.48</td>
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<tr>
<td>20.95-208.22</td>
<td>(29.11-301.23)</td>
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</tbody>
</table>

AOM=acute otitis media; N=number of children included in each group; n=number of AOM episodes in 1000 person-years in each category; P=total number of person-years; CI=confidence interval; 0-<3 years=0 to 2 years, 1 months; 3-<6 years=3 to 5 years, 1 months

*Vaccinated with pneumococcal conjugate vaccine 7-valent.

[tableOMAS5]
HIGH PNEUMOCOCCAL CARRIAGE IN 3-5-YEAR-OLD PAPUA NEW GUINEAN CHILDREN FOLLOWING 9-MONTH PNEUMOVAX23 DOSE WITH OR WITHOUT PRIOR 7-VALENT CONJUGATE VACCINE

M. Yoannes¹, A. Michael¹, G. Saleu¹, C. Opa¹, A.R. Greenhill¹, P. Siba¹, P. Richmond², W. Pomat¹, D. Lehmann², ¹Goroka, Papua New Guinea, ²Perth, WA, Australia

Background: In Papua New Guinea, where the burden of pneumonia is high, there are high rates of pneumococcal carriage from a young age. Little data are available for older children. We conducted a neonatal pneumococcal conjugate vaccine (PCV) study and have recently conducted a follow-up study investigating pneumococcal antibody persistence in later childhood. Carriage data were collected as part of these studies.

Method: Infants were randomized to receive 7-valent PCV (7vPCV) at ages 0-1-2 or 1-2-3 months or no 7vPCV. All children received 23-valent pneumococcal polysaccharide vaccine (23vPPV) at 9 months of age. At 3-5 years of age these children and unvaccinated controls had pernasal swabs (PNS) collected prior to and 4 weeks after being given a challenge dose of 0.1 ml 23vPPV. PNS were cultured using standard methods and serotyped using the Quellung reaction.

Results: Pneumococcal isolation rates ranged from 21.9% at 1 week of age to 86.5% at 9 months, with an isolation rate of 76.4% at age 18 months of age. At age 3-5 years >84% of children carried pneumococcus both pre- and post-challenge, irrespective of prior immunisation status. Non-10vPCV and non-13vPCV serotypes accounted for 80% and 54% of pneumococci, respectively. 52 serotypes were isolated, the most common being 6B, 19A, 23F and 33.

Conclusions: Pneumococcal carriage rates remain high at ages 3-5 years irrespective of prior pneumococcal vaccination. A broad range of serotypes are carried in PNG children, the majority being non-conjugate vaccine types. The impact of vaccination on density of carriage requires further investigation.

Poster No 50

ECONOMIC BURDEN AND EPIDEMIOLOGY OF PNEUMONIA IN KOREAN ADULTS AGED ≥50YRS

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Background and aims: Despite increasing socio-economic burden of pneumonia and awareness of prevention, few studies have investigated the burden-of-illness of pneumonia in Korea. This study was performed to estimate the direct medical costs and epidemiology of pneumonia in older adults of Korea.

Methods: This was a multi-center, retrospective observational study. We collected data targeting for community-acquired pneumonia (CAP) patients (≥50 years) from 11 large hospitals. Only costs attributable to the treatment of pneumonia were estimated by reviewing resource utilization and epidemiology data (including distribution of pathogen, hospital length of stay (LOS), overall outcome) were also collected.

Results: A total number of 693 patients were included; average 70.1 aged, 57.3% male and average 1.2 CURB-65 scored. The pathogen was identified in the 32.9% (228 patients) and Streptococcus pneumoniae accounted for 22.4% (51 patients) of identified pathogens. The hospital mortality was 3.2% (especially, for S.pneumoniae was 5.9%) and average length of stay was 9 days. The mean total cost for the treatment of pneumonia was US dollar (USD) 1,782 (SD: USD 1,501). Compared to the cost of all caused pneumonia, that of Pneumococcal pneumonia was higher, USD 2,049 (SD: USD 1,919), but not statistically significant. Intensive care unit stay and longer LOS, treatment failure were also analyzed as risk factors for increased costs.

Conclusions: This study is significant in that it examined the real CAP costs by reviewing resource utilization in multi-centers of Korea. The burden of Pneumococcal pneumonia was high, and the prevention can be considered as effective strategy.
SEROTYPE DISTRIBUTION AND ANTIBIOTIC RESISTANCE OF 140 PNEUMOCOCCAL ISOLATES FROM PEDIATRIC PATIENTS WITH UPPER RESPIRATORY INFECTIONS IN BEIJING, 2010

S.-J. Yu, Y.-H. Yang, Beijing, China

In the present study, the serotype distribution and antibiotic resistance of S. pneumoniae from pediatric patients with upper respiratory infections in Beijing, 2010 were described. 140 pneumococcal isolates were obtained, and the prevailing five serotypes were 19F (18.6%), 23F (9.3%), 14 (9.3%), 15 (9.3%), and 6A (7.1%). The vaccine coverage of PCV7, PCV10, and PCV13 were 43.6%, 43.6%, and 60.0%, respectively. According to the CLSI 2010 criteria, 99.3% of the S. pneumoniae isolates were susceptible to penicillin. The resistance rates to erythromycin and azithromycin were 96.4% and 97.1%, respectively. Meanwhile, 64.3% (90/140) of all pneumococcal isolates were multidrug-resistant S. pneumoniae (MDRSP). PCV13 covered 68.9% (62/90) of MDRSP strains, whereas it was 47.8% (43/90) for PCV7. ErmB was the dominant macrolide-resistance gene, whereas 30.4% pneumococcal isolates expressed both ermB and mefA. No isolate expressed ermTR. The potential coverage of PCV13 is higher than PCV7 and PCV10 because high rates of serotypes 6A and 19A, and the conjugate vaccines could prevent the spread of MDRSP. S. pneumoniae is still sensitive to penicillin. The resistance rate of S. pneumoniae to macrolides is high and ermB is the dominant macrolide-resistance gene in China, so continued surveillance of the antimicrobial susceptibility of S. pneumoniae may be necessary.
THE NASOPHARYNGEAL MICROBIOTA IN RELATION TO 7-VALENT PNEUMOCOCCAL CONJUGATE VACCINATION: A RANDOMIZED CONTROLLED TRIAL IN HEALTHY CHILDREN IN THE NETHERLANDS

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Background and aims: Implementation of 7-valent pneumococcal conjugate vaccine (PCV-7) has shown to induce shifts in colonization towards non-vaccine serotypes and other potential pathogenic nasopharyngeal residents. In view of vaccine-effectiveness and development it is important to study these shifts in more detail by a metagenomic approach.

Methods: We characterized nasopharyngeal microbiota profiles of 100 vaccinated (PCV-7 at 2, 4 and 11 months) and 100 unvaccinated children participating in a randomised controlled trial in The Netherlands. Children were sampled at 12 and 24 months of age in the pre-PCV7 era. To avoid seasonal bias, we selected samples from winter only. Bacterial density of microbiota was determined by a universal Real-Time PCR of the 16S-rRNA gene. The microbiota were analyzed by GS-FLX-Titanium-Sequencing of 16S-rRNA gene amplicons spanning the V5-V7 regions.

Results: We found the overall presence of 13 phyla and 280 Operational taxonomic Units (OTU), with Moraxella, Haemophilus, Streptococcus, Dolosigranulum and Corynebacterium as predominant genera. PCV-7 vaccination was associated with an increase in bacterial density and OTU diversity, showing a significant increase in Veillonella, Prevotella, Actinomyces and other streptococcal OTUs at 12 but not at 24 months of age. Though not significant, we also observed a trend towards increased presence and abundance of Haemophilus and Staphylococcus in PCV-7 vaccinated children at 12 months of age.

Conclusions: We observed a temporary effect of PCV-7 on nasopharyngeal microbiota composition and density 1 month after a booster dose with PCV-7. Duration and implications of those effects for example regarding respiratory health deserve further studies.
ECONOMIC COST OF PEDIATRIC PNEUMOCOCCAL PNEUMONIA IN PATIENTS ADMITTED TO A CHILDREN'S HOSPITAL IN BARCELONA, SPAIN

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Objective: To evaluate changes in economic cost of pediatric pneumococcal pneumonia hospitalizations during a 10-year period.

Methods: Cost estimation was based on a retrospective study of all pneumococcal pneumonia episodes confirmed by culture in children ≤ 18 years admitted to a referral children's hospital (Sant Joan de Déu, Barcelona-Spain) during period Jan-2001/Dec-2010. Estimation considered DRG weight per episode and 2010 DRG-based hospital reimbursement rates. Analysis of cost included several epidemiological, microbiological and clinical variables.

Results: A total of 129 children were admitted with pneumococcal pneumonia. Mean age (years) was 3.7±3.1 SD, and 61% were male. Mean length of hospital stay (days) was 12.3±8.2 SD. Admission to PICU occurred in 16 patients, median PICU stay (days) was 2.5 (range 1-26). Median total hospital cost (Euros) was 6860 (range 1702-19370). Forty seven patients were admitted during 2001-2005 period and 82 during 2006-2010. Prior PCV7 vaccination increased from 4.1% during 2001-2005 to 41.3% during 2006-2010; P< .001. Non-PCV7 serotypes increased from 29.7% during 2001-2005 to 70.3% during 2006-2010; P< .001. Empyema rose from 55.3% during 2001-2005 to 68.3% during 2006-2010; P=0.14. Higher costs (Euros) were associated with admission period 2006-2010 vs. 2001-2005 (8557 vs. 3603; P< .001), presence of empyema vs. non-emphyema (8549 vs. 3617; P< .001) and admission to PICU vs. non-PICU stay (10993 vs. 6152; P=0.002).

Conclusion: In this study, DRG-based hospital cost of pediatric pneumococcal pneumonia increased within last years associated with a rise of complicated pneumonia. Wider-spectrum conjugate vaccines are needed to minimize cost of pneumococcal pneumonia.
10 YEARS OF PNEUMOCOCCAL CARRIAGE SURVEILLANCE IN CHILDREN: TRENDS BEFORE AND AFTER 7 THEN 13-VALENT PNEUMOCOCCAL CONJUZGATE VACCINES

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Background: Acompanioning 7-valent pneumococcal conjugate vaccine (PCV7) introduction, a 10-year surveillance network on pneumococcal nasopharyngeal (NP) carriage in children was set up in 2001 in France. PCV13 was introduced in June 2010 for children < 2 years.

Methods: A NP swab was obtained from children 6 to 24 months of age suffering from acute otitis media with fever ± otalgia. Demographics, medical history and physical examination findings were recorded.

Results: From 2001 to 2011, 90 paediatricians enrolled 6240 children (mean age 13.6 months), among them, 36.7% were in day care centre and 99% were PCV7 vaccinated before PCV13 introduction. During last study year (October 2010 to June 2011), among 598 children enrolled, 67.2% were PCV13 vaccinated (≥ 1 dose). Over the 10 study years, Sp carriage and PCV7 serotypes (ST) decreased from 71.2% to 61.9% and 44.5% to 2% respectively (p< .0001). In Year 10, in PCV13 vaccinated population, 19A and 7F carriage was lower vs non PCV13-vaccinated population, 9.7% vs 18.9%, p = 0.002 and 0.8% vs 3.6%, p= 0.01, respectively.

Conclusion: After a 9 years regular increase in ST 19A, 6C and 7F following the implementation of PCV7, a decrease was rapidly observed in year 10 in children vaccinated with PCV13. On the other hand, these 3 ST continued to increase in non PCV13 vaccinated children.
AGE-SPECIFIC INCIDENCE DYNAMICS OF CULTURE-PROVEN AOM (CX-AOM) POST PCV7 INTRODUCTION IN SOUTHERN ISRAEL


Background: AOM for which a culture is obtained is enriched with complicated AOM cases, causing much burden in the community. We assessed age specific incidence risk-ratio (IRR) of Cx-AOM < 36m in the 2 years post-PCV7 introduction (July/2009-June/2010) to the 4 years pre-PCV7 (July/2004-June/2008) in southern Israel.

Methods: Vaccine uptake (≥2 doses age 7-35m) was 0 before 2007; ~25% in July/2008-June/2009 (transitional year); ~60% and ~80% in the 2 post-PCV7 years. Population-at-risk, patient population and culture methods were previously described (Dagan, 49th IDSA, Abst1343). Reduction was considered significant when post- vs. pre-PCV7 IRR was < 1.0 and the 95% CI did not cross value of 1.0.

Results: Of 5409 episodes (ages < 6m 22%; 6-17m 55%; 18-35m 23%), 3570 (66%) were Cx (+), of which 2334 (65%) had ≥1 complicating factors (antibiotic Rx last month; >3 previous AOM episodes; >3 tympanocenteses). Post- vs. pre-PCV7 IRRs were the lowest with PCV7 serotypes (+6A) and highest for NTHi and Cx(-), but all showed significant reduction in children < 18m. However, for age group 18-35m, only pneumococcal AOM was significantly reduced.

Conclusions: This unique set of data enabled to analyze aged-specific IRR of Cx-AOM in the immediate 2 years following PCV7 introduction. A significant incidence reduction beyond disease directly caused by PCV7 serotypes was seen in children < 18m, but not 18-35m, consistent with AOM evolution and the short post-PCV7 studied period.
OPTIMIZING ASSESSMENTS OF LONG-TERM COST-EFFECTIVENESS OF DUTCH PEDIATRIC IMMUNIZATION WITH PCV13 USING DYNAMIC TRANSMISSION MODELING

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Background and aims: The 10-valent conjugated pneumococcal vaccine (PCV-10) is replacing the current PCV-7 program among infants in the Netherlands. Since uncertainties regarding long-term replacement disease and herd protection among non-vaccines remain, we develop a dynamic model to accurately predict long-term cost-effectiveness of the new program. The impact of several vaccine formulations (PCV-7, PCV-10, PCV-13) and/or schedules specific for the Netherlands is analyzed.

Methods: We build on an existing age-structured transmission dynamic model to predict the effect on pneumococcal carriage and the incidence of pneumococcal disease. We use nationally representative Dutch data: case-carrier ratios, hospitalization data and long-term effects of PCV-7 and possible updated vaccination programs are provided by the Netherlands Reference Laboratory for Bacterial Meningitis. Carriage data, vaccination coverage and costs are based on the most recent data available.

Results: Indirect effects of PCV programs are critical to evaluate the alternative vaccines PCV-10 and PCV-13 as compared to PCV-7. Various aspects play a role, the broader serotype coverage of PCV-13, differences of both vaccines in their impact on carrier rates, herd immunity, serotype replacement and serotype-specific seriousness of disease. Critical biological parameters strongly influence the benefits of alternative PCV programs and they need to be cautiously estimated.

Conclusions: Dynamic models are necessary to obtain credible estimates of the effects of herd immunity and serotype replacement on the long-term cost-effectiveness of respiratory vaccines such as PCV-10 or PCV-13. This paper contributes to shed light on the controversial aspects about pneumococcal vaccination.

Acknowledgement: We thank Pfizer that financed the project.
INVASIVE PNEUMOCOCCAL DISEASE IN TWO BIRTH COHORTS VACCINATED, RESPECTIVELY, WITH 2+1 PCV-7 OR PHID-CV-10 DOSES

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In Quebec, a 2+1-dose pneumococcal conjugate vaccine program was implemented in 2004. PCVC-7 was first used and PHID-CV-10 was introduced in the summer of 2009, to be replaced by PCV-13 in 2011. So far, there is no data on the effectiveness of PHID-CV-10 for preventing IPD.

IPD cases were identified by the provincial reference laboratory in children born in 2007-2010 and followed up to December 31 2010. Serotype identifications was performed and immunization status of patients was assessed. The main ecological comparison was between children born in June 2007-June 2008 (n=92997) and exposed to PCV-7, and those born in June 2009-June 2010 and exposed to PHID-CV-10 (n=95800). The observation periods in the two groups were similarly truncated to adjust for age (observation from 6 to 18 months of age) and seasonality.

There were 35 IPD cases in the PCV-7 group: one 19F (vaccinated 2 doses), one 6A (not vaccinated), three 7F, fifteen 19A and fifteen of other serotypes. There were 20 IPD cases in the PHID-CV-10 group: one 4 (not vaccinated), nine 19F (not vaccinated), nine 19A and nine of other serotypes. Overall, IPD incidence was 64.1/100 000 person-years in the first group and 35.3 in the second group (p< 0.05).

Results of ecological studies should be interpreted with care. During the study period, IPD cases caused by PCV-7 serotypes were infrequent in children < 5 year-old, whereas 7F and 19A were on the rise. PHIDCV introduction was associated with a reduced incidence of IPD cases in exposed children.
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NATIONAL LABORATORY SURVEILLANCE OF INVASIVE PNEUMOCOCCAL DISEASE IN CANADA, 2010

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Background: Pneumococcal conjugate vaccines (PCV) were effective in reducing invasive pneumococcal disease (IPD) in Canada however shifts in pneumococcal serotypes have occurred.

Methods: Serotypes were determined for 344 child (0-14 years), 909 adult (15-64 years) and 778 senior (≥65 years) isolates. Oxacillin 1 µg, erythromycin 15 µg, and clindamycin 2 µg disc susceptibilities were done on 545 isolates.

Results: Serotype 19A was most prevalent in children (36%, n=124) and seniors (17%, n=136) and 7F most common in adults (19%, n=170) (Figure 1). PCV7 serotypes represented 6% (n=20), the 10-valent vaccine (PHiD10) 24% (n=83) and PCV13 66% (n=226) of the paediatric pneumococci (Figure 2). The 23 valent vaccine accounted for 80% (n=730) of the adult and 69% (n=540) of senior strains. Twenty two percent (n=119) were oxacillin non-susceptible, 25% (n=138) were resistant to erythromycin and 13% (n=71) to clindamycin (Figure 3).

Conclusions:

Monitoring the distribution of IPD serotypes is important to assess the effectiveness of the newly introduced PCV13 vaccine.
Figure 2: Coverage of Serotypes by Available Vaccines in 2010

[Coverage of Serotypes by Available Vaccines]

Figure 3: Antimicrobial Susceptibilities of Pneumococci Isolated in 2010

[Antimicrobial Resistance of Pneumococci]
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PNEUMOCOCCAL (SP) NASOPHARYNGEAL CARRIAGE IN THE IMMEDIATE POST-PCV-13 ERA IN CHILDREN PRESENTING TO THE EMERGENCY DEPARTMENT IN ATLANTA, GEORGIA

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**Background:** In a 2009 pre-PCV13 nasopharyngeal (NP) carriage study in Atlanta, 31% of children < 5yrs were colonized with SP; 22% of isolates were PCV13 serotypes, predominantly 19A. We evaluated the early impact of PCV13 on NP carriage of SP in Atlanta after PCV13 introduction in March of 2010.

**Methods:** NP swabs were collected from children aged 6-59 months in an emergency department from July 2010-July 2011. After broth enrichment, samples were cultured for SP; isolates were serotyped and antimicrobial susceptibility performed. Clinical and immunization records were reviewed. Findings during time periods 1 (July-Dec 2010) and 2 (Jan-July 2011) were compared.

**Results:** A total of 673 children were enrolled; 196 (29%) were colonized with SP. Mean age of carriers was 27 months. SP carriage was higher among black children, children with asthma, and daycare attendees (p< 0.05). Commonly carried serotypes included 19A (20.4%), 15B/C (15.3%), 35B (11.2%), 6C (9.2%), and 11A (8.2%); 23.5% were PCV13 serotypes. Infants aged 6-23 months receiving ≥2 doses of PCV13 increased from 25.2% to 45.4% from period 1 to 2 (p< 0.001). Overall SP carriage rates were unchanged from period 1 to 2, but serotype 19A declined from 26.6% to 9.7% (P=0.0047) and PCV13 serotypes declined from 29.8% to 12.5% (P=0.0058). Nonsusceptibility (I+R) to ceftriaxone and penicillin declined from 23.4% to 7% (P=0.0034), and 25% to 12.5% (P=0.0363), respectively.

**Conclusions:** Post PCV13 introduction, carriage of PCV13 serotypes and antibiotic nonsusceptible SP has significantly declined in young children, primarily due to a decline in serotype 19A.
IMPACT OF PCV-7 ON NASOPHARYNGEAL CARRIAGE OF STREPTOCOCCUS PNEUMONIAE SEROTYPE 1 GENOTYPES IN HEALTHY GAMBIANS


Background and aims: Our study aimed to evaluate the prevalence of nasopharyngeal carriage of pneumococcal serotype 1 in the context of a vaccine trial using PCV-7 in the Gambia.

Methods: A cluster-randomized (by village) trial was conducted in rural Gambia. PCV-7 was given to children below age 30 months and those born during the trial. Villages were randomized (older children and adults) to receive PCV-7 (11 vaccinated villages) or meningococcal-serogroup-C conjugate vaccine (10 control villages). Nasopharyngeal swabs (NPS) were collected before vaccination (Dec 2003–May 2004) and up to 30 months after vaccination (stratified in three time periods: post-vaccination periods 1 to 3: July 2006–March 2007, April 2007–March 2008 and April 2008–Feb 2009). S.pneumoniae serotype-1 isolates were identified by latex agglutination and confirmed by PCR prior to genotyping by multilocus sequence typing (MLST).

Results: S.pneumoniae serotype-1 was recovered from 87 NPS (0.71% of 12,319 samples collected). Prevalence of serotype-1 was associated with the study period [0.47%, 0.23%, 1.5% and 0.61% (p<0.001) in pre-vaccination and period 1-3, respectively]. Prevalence of serotype-1 was similar in vaccinated and control villages (0.73% versus 0.68%; p=0.703). Of 4 different genotypes obtained, ST3081 was the most prevalent (4/87) in vaccinated and control villages, although only found from period 2 onwards; followed by ST618 (25/87) which disappeared in period 3.

Conclusions: Prevalence of pneumococcal serotype-1 carriage as well as the most common genotypes varied over the course of the study. Such changes were most likely unrelated to vaccination as differences between groups were not apparent. This study provides valuable baseline data for further evaluation nasopharyngeal carriage following introduction of PCV-13 in The Gambia.
THEORETICAL COVERAGE OF PNEUMOCOCCAL CONJUGATE VACCINES ON BACTEREMIC PNEUMOCOCCAL COMMUNITY-ACQUIRED PNEUMONIA IN CHILDREN AGED < 5 YEARS IN ITALY

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Background and aims: Pneumococcal community-acquired pneumonia (CAP) is an important cause of morbidity and mortality in children worldwide. Pneumococcal conjugate vaccines containing 10 and 13 serotypes have been marketed. Little is known on the impact that they could have on this disease. This study was planned to evaluate incidence of bacteremic pneumococcal CAP (BP-CAP) and associated serotypes in children.

Methods: All the children aged < 5 years hospitalized in the period 9/2008 - 3/2011 in 5 pediatric hospitals in 5 Italian Regions with radiographically-confirmed CAP were enrolled. To diagnose BP-CAP, blood culture and RT-PCR for the identification of the pneumococcal LytA and cpsA genes were performed. Serotyping for the serotypes included in PCV13 was also carried out.

Results: A total of 510 CAPs were enrolled. BP-CAP was identified in 73 cases (14.3%). They were more frequently associated with complications, including empyema (22.2%; p=0.007). Serotyping was tried in 66 cases. In 19 (28.8%) it was not possible, suggesting a serotype different from those evaluated. Serotype 19A was identified in 17 cases (25.8%), serotype 14 in 10 (15.1%), serotype 4 in 5 (7.6%), serotype 3 in 4 (6.1%) and serotypes 7F and 19F in 3 (4.6%). Other serotypes were present only in 1 case each. Theoretical coverage offered by PCV7 was 31.8%, by PCV10 37.9% and by PCV13 71.2%.

Conclusions: In Italy, BP-CAP is frequently associated with serotypes not included in PCV7 and PCV10. Only PCV13 seems to offer adequate guaranties of protection against BP-CAP of children aged < 5 years.
TEMPORAL SURVEILLANCE OF PNEUMOCOCCAL CARRIAGE IN A UK PAEDIATRIC POPULATION DURING CONJUGATE VACCINE IMPLEMENTATION

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Background and aims: The 7-valent pneumococcal conjugate vaccine (PCV-7) was added to the UK routine childhood vaccination schedule in 2006 and superseded by PCV-13 in 2010. Pneumococcal colonisation is a recognised precursor to disease. We sought to describe changes to pneumococcal carriage during PCV implementation.

Methods: Nasopharyngeal swabs were taken from children aged 4 years and under in a hospital outpatients department during winters 2006/7-2010/11. Streptococcus pneumoniae was isolated using conventional microbiological techniques and serotyped by PCR and Quellung reaction. Multi-locus sequence typing and whole genome sequencing were employed to genotype all isolates.

Results: 528 pneumococci were collected during the five year period. The mean carriage rate was 30.8%, (range 27.9-37.2%). PCV-13 vaccine types (VT) declined to < 26%, with a >170% corresponding increase in non-VT (NVT) carriage. Approximately forty sequence types (ST) were observed each year. Genotype distribution varied annually, with only 7% of STs occurring in all years. 20% of observed STs accounted for 65% of the isolates. ST199 was the single most common ST, observed in all years, associated predominantly with serotypes 19A (49%) and 15B/C (27%). Clonal expansion of genotypes associated with NVT was observed for multiple serotypes including 6C, 11A and 23B.

Conclusions: Pneumococcal carriage has remained stable but NVT now dominate. Carried genotypes are diverse and shift temporally. Clonal expansion has contributed to NVT increases. Genomic characterisation of NVT combined with surveillance is essential to evaluate vaccine impact. This study will continue to contribute to understanding of the relationship between carriage and disease.
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IMPACT OF PREVENAR 13 ON PNEUMOCOCCAL CARRIAGE IN A UK PAEDIATRIC POPULATION


Background and aims: In April 2010 the 13-valent pneumococcal conjugate vaccine (PCV-13) superseded PCV-7 in the UK routine childhood vaccination schedule. PCV-7 introduction significantly reduced the carriage of vaccine serotypes whilst non-vaccine serotypes increased in prevalence. We sought to quantify the impact of additional PCV-13 serotypes 1, 3, 5, 6A, 7F and 19A on pneumococcal carriage.

Methods: Nasopharyngeal swabs were taken from children aged 4 years and under in a hospital outpatients department during winters 2006/7 to 2010/11. Streptococcus pneumoniae was isolated using conventional microbiological techniques and serotyped by PCR and the Quellung reaction.

Results: Throughout the 5 year period: serotype 5 was not observed in carriage and a low annual prevalence of serotypes 1, 7F (both < 1%) and 3 (< 2%) were observed. A significant reduction in serotype 6A carriage was observed, 15% in 2006/7 to 3% in 2010/11 (p=0.0038). However, the decrease observed post PCV-13 implementation between 2009/10 and 2010/11 was not significant. The annual prevalence of serotype 19A ranged from 3%-11% in 2006/7 and 2009/10 respectively (p=0.058). PCV-13 serotypes accounted for 10% of carried pneumococci in 2010/11, of which 19A (5%) and 6A (3%) were predominant.

Conclusions: Six months after implementation, PCV-13 had not yet significantly impacted on the carriage of the six additional serotypes. Serotypes 1, 3, 5 and 7F are rarely carried in this population whilst 19A and 6A still account for 5% and 3% respectively of carried pneumococci. Continued surveillance will reveal any further impact of PCV-13 on the carried pneumococcal population.
ISPPD-8

Poster No 64

INCIDENCE RATES OF HOSPITALIZATION DUE TO ALL-CAUSE PNEUMONIA AND PNEUMOCOCCAL PNEUMONIA IN ADULTS IN AUSTRALIA FROM 1998-2008

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Background and aims: Pneumococcal disease remains an important cause of hospitalization in Australian adults. A retrospective burden of disease study was conducted to assess the incidence of hospitalization from all-cause pneumonia (PN) and pneumococcal pneumonia (PP) in adults aged ≥18 years of age from 1998-2008.

Methods: Adults hospitalized due to PN and PP from 1998-2008 were identified from a database of hospitalizations in Australia (Cognos) using International Classification of Disease-10 codes. The Australian Bureau of Statistics database provided population at risk information.

Results: From 1998 to 2008, the incidence of hospitalizations due to PP (ICD Code J13) decreased by 45% in adults aged 60-69 years (21.34/100,000 to 11.75/100,000); 68% in adults aged 70-79 years (44.45/100,000 to 14.34/100,000); and 64% in adults aged ≥80 years (73.97/100,000 to 26.55/100,000). The corresponding changes in PN (J12-J18) were a decrease of 18% in those aged 60-69 years, a decrease of 14% in those aged 70-79 years, and almost unchanged in those aged ≥80 years (0.31% reduction). Mean length of stay (LOS) in PP remained comparable over time and age groups, ranging from 8.28-10.79 days.

Conclusions: Hospitalization rates for PP declined markedly in adults aged ≥60 years over the 10-year period and may reflect the direct, and indirect, effects of the national pneumococcal vaccination programs for older Australians (23-valent pneumococcal polysaccharide vaccine) and children (7-valent pneumococcal conjugate vaccine), respectively. Significant pneumococcal disease burden remains in adults aged 60 years and older.
Poster No 65

CLINICAL AND ECONOMIC BURDEN OF HOSPITALIZATION DUE TO STREPTOCOCCUS PNEUMONIAE (SP) PNEUMONIA IN CANADA 2004 TO 2009

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Background and aims: Understanding the burden of illness associated with Streptococcus pneumoniae (SP) is critical to inform public health policy around vaccination programs. We conducted retrospective database analyses from 2004-2009 to quantify incidence, mortality, length of stay (LOS), and hospitalization costs of SP pneumonia in Canada (excluding Quebec).

Methods: Hospitalizations were identified from a national database using International Classification of Diseases-10 codes. Statistics Canada provided population at risk data. The Ontario Costing Database was used to estimate costs.

RESULTS: From 2004-2008, SP pneumonia incidence (cases/100,000 persons) in ages 0-4 years declined from 10.6/100,000 to 7.2/100,000 but increased to 9.1/100,000 in 2009; mortality rates remained similar (0.0 to 1.1%). In patients aged 0-17 years, LOS increased from 5.9 to 10.8 days with average total cost of Canadian $16,973 (2009). In adults >65 years incidence decreased from 21.3/100,000 to 13.0/100,000 over 2004-2009, mortality rates changed from 13.7% to 12.0%, and LOS decreased from 13.8 to 12.6 days with average total cost in >70 years decreasing from Canadian $16,934 to $14,370. Incidence and mortality rates (2004-2009) were highest in ages >80 (30.8-16.8/100,000 and 16.9-17.6%, respectively).

Conclusions: In ages 0-4 and >65 years, clinical and economic burden due to hospitalized SP pneumonia demonstrated notable reductions corresponding with introduction of pediatric conjugate pneumococcal vaccine. However, SP pneumonia burden remains high, particularly in very young and very old. Mortality, LOS, and cost varied greatly by age. Prevention of additional SP infection may result in substantial cost savings.
TRENDS IN INVASIVE PNEUMOCOCCAL DISEASE-ASSOCIATED HOSPITALIZATIONS IN MARYLAND: COMPARISON OF HOSPITAL DISCHARGE RECORDS AND LABORATORY-BASED SURVEILLANCE

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Background and aims: In the US, dramatic declines in invasive pneumococcal disease (IPD) among children under five (U5) after PCV7 introduction have been documented at the national level with multiple data sources. However, the impact of PCV7 introduction on IPD rates may be more heterogeneous when analyzed at the state or hospital level. This study aims to compare state- and hospital-specific rates of IPD in Maryland for 1995-2009 using laboratory-based Active Bacterial Core surveillance (ABCs) and hospital discharge data.

Methods: Annual hospital-specific IPD rates for U5 children were calculated from two data sources: Maryland ABCs and the State Inpatient Database (MD-SID), which includes ICD-9 coded discharge records for nearly all Maryland inpatient admissions. Cumulative incidence rates and rate ratios were calculated using census data with Poisson regression.

Results: By MD-SID, annual IPD hospitalizations were 57.9-70.5 per 100,000 U5 children for 1995-1999 and 49.8-63.3 per 100,000 in the post-vaccine period (2001-09). IPD rate reductions observed in MD-SID were consistent but more attenuated than rates observed with ABCs. Broken down by hospital, the number of annual U5 IPD cases was variable for both data sources (MD-SID median: 3, [range 0-59]; ABCs median: 1, [0-17]).

Conclusions: At the state level, IPD rate reductions observed in discharge records were similar to those observed in MD-ABCs data. When analyses were limited to a single hospital, annual IPD rates were variable and may not reflect the overall aggregate pattern. Caution should be used in drawing conclusions on the impact of PCV7 introduction from single hospital studies.
Poster No 67

PREVALENCE OF S. PNEUMONIAE TYPE I PILUS, BEFORE AND AFTER IMPLEMENTATION OF PCV7 IN ISRAEL


Background: Pilus proteins encoded by rrg genes are candidates for future protein-based vaccines. We assessed the prevalence of rrgC (carrier strains termed 'piliated') during 7y pre- and 1y post-PCV7 implementation in Israel.

Methods: During 2002-2005 and 2009 (Pre-PCV7) and in 2010 (post-PCV7), repeated cross-sectional surveys of nasopharyngeal S. pneumoniae (SP) carriage by children < 5y were conducted. Antibiotic susceptibility was determined by Vitek2; serotype by latex-agglutination test, Quellung reaction and Quantitative-MultiPlex-PCR. PCR for rrgC was performed on 962 samples randomly selected and evenly distributed throughout surveys.

Results: Of 6268 screened children, 45% were SP carriers in each survey. 91% of piliated strains were PCV7-VT. During 2003-2009, 24% of strains were piliated, reduced to 8.33% in 2010 (p=0.001). PCV7-VT decreased from 44% in the pre-PCV7 period to 16% post-PCV7. In contrast, non-PCV13-VT increased from 9% to 16%, though pilus carriage among these strains remained unchanged.

A small but significant decrease in pilus carriage (33% to 22%) was observed between 2002 and 2003. This was concomitant with a decrease in the normally penicillin-nonsusceptible and piliated 19F (15.5% of all isolates to 8.1%, p=0.007), following a judicious antibiotic control intervention. Notably, 77% of piliated vs. 43% of non-piliated PCV7-VT strains were penicillin-nonsusceptible (p< 0.0001).

Conclusions: In the pre-PCV7 period pilus carriage was mostly stable, specifically following the decline in 2003. The sharp reduction in pilus carriage in the post-PCV7 period is probably due to a decrease in PCV7-VT. It is yet to be seen whether piliated strains will resurge in Non-PCV13-VT in following years.
ADULT INVASIVE PNEUMOCOCCAL DISEASE (IPD) IN ISRAEL; FIRST TWO YEARS AFTER NATIONAL PCV7 IMMUNIZATION PROGRAM IMPLEMENTATION


Background: PCV7 was introduced as universal childhood vaccination in Israel on Jul2009 and PCV13 on Nov2010. Here we report data on adult IPD, 2 years post PCV7 implementation.

Methods: An ongoing nationwide active surveillance (all 27 laboratories performing blood cultures in Israel), providing all blood & CSF pneumococcal isolates from persons >18y initiated on Jul2009. Capture-recapture method assured reporting of >95% cases. All isolates were serotyped in one central laboratory. IPD outcome and Medical & vaccination history were recorded in 90%.

Results: 971 IPD cases were reported (annual incidence [1/100,000] of 9.17 and 10.16 in the two consecutive years respectively). Respective case fatality rates (CFRs) were 20% and 19.1%. Incidence and CFR increased with age and number of comorbidities. Incidence rate was significantly greater in the 2nd winter, 7.79/100,000 vs. 6.14/100,000 in 1st winter, p=0.004, with a non-significant decrease in summer months (3.02 to 2.48/100,000).

PCV7-serotypes were 27.4% of IPD cases in 1st year vs. 13.5% in 2nd y (p< 0.001); non-PCV13-strains were 32.9% and 39.3% (p=0.048). Increase in non-PCV13-strains was observed only among older patients (>50y, especially >64y). The seasonal distribution and patterns of IPD paralleled that of respiratory viruses and LRIs during the 2 years.

Conclusions: While overall annual incidence of IPD did not change, winter incidence increased with a significant increase of non-PCV13 serotypes almost exclusively in older patients. The similarity between the epidemiology of respiratory virus disease and IPD in adults suggests the need for adjustment for general winter morbidity when PCV effect on IPD is evaluated.
CLINICAL FEATURES AND OUTCOMES OF SEROTYPE 19A (ST19A) INVASIVE PNEUMOCOCCAL DISEASE (IPD) IN CALGARY, CANADA

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Background/aims: ST19A became an important cause of IPD during the last decade. This study’s purpose was to examine severity and outcome of ST19A IPD.

Methods: The Calgary Area S. pneumoniae Epidemiology Research (CASPER) study collects clinical and laboratory data on all IPD cases in Calgary. Descriptive and multivariate analysis was performed comparing ST19A and non-ST19A IPD cases. Linear and logistic regression models examined duration of appropriate IV antibiotic therapy and ICU admission, respectively. The models were adjusted for age, gender, and primary diagnosis.

Results: From 2000-2010, ST19A caused 60 (5%) of 1,231 IPD cases. Of patients with ST19A, 30.0% were < 5 years old, versus 11.9% with non-ST19A (P< 0.001). Meningitis was diagnosed in 31.9% of IPD cases < 5 years old; versus 11.6% of IPD ≥5 years old, resulting in an apparent association between ST19A and meningitis. There was no difference in mortality between ST19A or non-ST19A cases. Of ST19A isolates, 19.2% were penicillin non-susceptible, versus 0.3% of non-19A isolates (P< 0.001). Linear regression showed no difference in duration of IV antibiotics between ST19A and non-ST19A IPD. Logistic regression showed no difference in ICU admission between ST19A and non-ST19A IPD (OR:1.11 (0.6-2.1)). Meningitis was an independent risk factor for ICU admission (OR:8.67 (4.9-15.2)), but association of meningitis and ST19A was not significant after adjustment for age.

Conclusions: ST19A is more common in younger persons, and cases are more likely to be antibiotic resistant. ST19A is not independently associated with worse clinical course or duration of IV antibiotic therapy.
COST EFFECTIVENESS OF VACCINATING RISK GROUPS IN THE UK AGAINST INVASIVE PNEUMOCOCCAL DISEASE USING THE 13 VALENT CONJUGATE PNEUMOCOCCAL VACCINE


Aims: To estimate the cost effectiveness of vaccinating individuals at increased risk of IPD (due to chronic kidney disease, asplenia or a dysfunction of the spleen, HIV infection, compromised immune system (including HIV infection), chronic heart, liver or respiratory disease, or diabetes) with the 13-valent conjugated pneumococcal vaccine (PCV13).

Methods: We developed an economic cohort model comparing PCV13 with the current PPV23 programme.

Results: Increasing herd protection effects caused by the infant PCV13 programme will mean that the burden of disease preventable by targeting high risk groups will diminish in time. If a risk vaccination programme would be launched in 2012/2013, it could prevent 1,200 cases IPD corresponding to a total gain of 5900 life years or 5600 QALYs. The total costs of such a vaccinating programme would require 4.1 million doses, costing around £233 million. Partly, due to the herd effects, is unlikely to be considered cost effective for most risk groups, with the exception being patients with chronic liver disease.

Conclusion: As the time since the implantation of the infant PCV13 programme s an important factor countries need to make a fast decision, and realise that targeting high risk groups might become cost ineffective quickly after introduction.
MOLECULAR EPIDEMIOLOGY OF INVASIVE PNEUMOCOCCAL DISEASE (IPD) DUE TO SEROTYPE 19A IN ALASKA: 1986 - 2010

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Background: After the introduction of the 7-valent pneumococcal conjugate vaccine in Alaska, the incidence of non-vaccine serotypes, particularly serotype 19A (sero19A), increased. We studied the molecular epidemiology of IPD due to sero19A in Alaska.

Methods: IPD data were collected from 1986 - 2010 through state-wide laboratory-based surveillance. Isolates were serotyped by the Quellung reaction and MICs determined by broth microdilution. Sero19A isolates were genotyped by MLST.

Results: Among 3294 cases of IPD, 2926 (89%) isolates were available for serotyping, of which 233 (8%) were sero19A. Sero19A IPD incidence among all ages increased from 0.73 cases pre-PCV7 to 2.56 per 100,000/yr. post-PCV7 (p< 0.001). Among 231 19A isolates, eighteen sequence types (ST) were identified; ST199 and related STs (n=150, 65%) and ST172 (n=59, 26%) accounted for the majority of isolates. Pre-PCV7, 90.2% (55/61) of isolates were ST199; post-PCV7, 56% (95/170) were ST199 (p< 0.001). ST172 isolates increased from 3.3% (2/61) (pre-PCV7) to 33.5% (57/170) post-PCV7 (p< 0.001). ST320 isolates were first seen in 2007 and increased from 8% (2/25) to 28% (5/18) in 2010. Multidrug resistance (MDR; 3 or more classes of antibiotics) was most common among ST199, ST156, and ST320 isolates. IPD caused by ST320 isolates did not differ significantly with regards to clinical syndromes, age distributions or case fatality rates from IPD caused by other sero19A IPD.

Conclusions: The genetic structure of sero19A pneumococci has changed in the post-PCV7 era with significant increases in the proportion of ST172 and ST320 isolates, but no change in clinical illnesses.
TEMPORAL TRENDS OF SEROTYPES INCLUDED IN THE NOVEL 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV13) AMONG YOUNG CHILDREN FROM PORTUGAL

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Background and aim: In January 2010, PCV13 became available in Portugal. One-year after its introduction, we evaluated PCV13 use among children attending day-care centers in Oeiras, Portugal. The prevalence and temporal trends of vaccine-serotypes were determined. We also analyzed serotype 6C, for which PCV13 could confer cross-protection.

Methods: Nasopharyngeal swabs were obtained from 448 children aged 0-6 years. Pneumococci were isolated and serotyped. PCV13 use was evaluated by reviewing children’s immunization bulletins. Serotype proportions were compared to similar studies carried out since 2006.

Results: In 2011, the carriage rate was 63.4%; 70.6% and 19.0% of the participants had received at least one dose of PCV7 (available between 2001-2009) or PCV13, respectively. The table summarizes the results obtained.

<table>
<thead>
<tr>
<th>Year</th>
<th>Serotypes</th>
<th>PCV7</th>
<th>1, 5 and 7F</th>
<th>3</th>
<th>6A</th>
<th>6C</th>
<th>19A</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006 (n=391)</td>
<td>11.3%</td>
<td>6.4%</td>
<td>4.4%</td>
<td>12.0%</td>
<td>4.1%</td>
<td>7.7%</td>
<td></td>
</tr>
<tr>
<td>2007 (n=423)</td>
<td>11.1%</td>
<td>0.2%</td>
<td>1.2%</td>
<td>7.3%</td>
<td>5.4%</td>
<td>11.8%</td>
<td></td>
</tr>
<tr>
<td>2009 (n=369)</td>
<td>8.1%</td>
<td>1.3%</td>
<td>5.2%</td>
<td>1.9%</td>
<td>8.1%</td>
<td>14.6%</td>
<td></td>
</tr>
<tr>
<td>2010 (n=306)</td>
<td>9.2%</td>
<td>0.0%</td>
<td>3.6%</td>
<td>1.0%</td>
<td>17.6%</td>
<td>13.1%</td>
<td></td>
</tr>
<tr>
<td>2011 (n=284)</td>
<td>12.0%</td>
<td>0.4%</td>
<td>2.1%</td>
<td>2.8%</td>
<td>7.4%</td>
<td>5.3%</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions: A decline in serotype 6A was observed prior to PCV13 introduction. A considerable decrease of serotypes 19A and 6C was observed in 2011. Whether such decline is due to secular trends, PCV13 use, or both, is not clear. Further studies are needed to establish the impact of PCV13 in colonization and improve our understanding of secular trends.

HOSPITALIZATION RATES OF RESPIRATORY INFECTIONS AND OTITIS MEDIA BEFORE AND FOLLOWING THE INTRODUCTION OF PNEUMOCOCCAL CONJUGATE VACCINATION IN THE NETHERLANDS

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Background: Following the introduction of the heptavalent pneumococcal conjugate vaccine (PCV7) in the United States in 2000, hospitalization rates for pneumonia and otitis media (OM) in both young infants and adults have decreased. We evaluated the impact of PCV7 introduction for newborns in June 2006 on hospitalisations for respiratory infections (RTIs) in the Netherlands.

Methods: National hospital discharge rates of RTIs, before and after the introduction of PCV7 were assessed. Incidences 3 years after introduction (2008-2009) were compared to average incidences during the decade before introduction (1995-2006).

Results: Hospitalization rates of OM, bronchitis and all-cause pneumonia in children < 2 years of age had decreased by 35.2%, 31.6% and 10.7%, respectively, despite an increase in hospitalizations for influenza in 2008-2009. While the decline for OM in children < 2 years had started years before PCV7 introduction, the decline in all cause pneumonia was observed only after implementation.

No herd effects for RTIs were observed in individuals ≥ 5 years of age. Hospitalizations due to all-cause pneumonia in individuals ≥ 5 years increased, starting before introduction of PCV7.

Conclusions: Within the target population of children < 2 years of age, hospitalization rates of pneumonia and bronchitis have decreased substantially 3 years after PCV7 implementation. The decline in OM already had started prior to PCV7 introduction. As yet, no clear herd effects were seen in unvaccinated individuals ≥ 5 years of age. Future studies are required to evaluate the impact of PCV7 on common pneumococcal-related RTIs and expected herd-effects on longer term.
INVASIVE PNEUMOCOCCAL DISEASE IN THE NETHERLANDS FOUR YEARS AFTER INTRODUCTION OF THE SEVEN-VALENT PNEUMOCOCCAL CONJUGATE VACCINE

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Background and aims: In the Netherlands, the seven-valent pneumococcal conjugate vaccine (PCV7) was implemented in the national immunization program for all infants born after April 1st 2006 without catch-up. This study compares the incidence, patient- and disease characteristics of invasive pneumococcal disease (IPD), in the Netherlands before and after implementation of PCV7.

Methods: Annual culture-confirmed IPD cases were identified by nine sentinel laboratories (covering ~ 25% of the Dutch population) between June 2004-June 2010. Clinical characteristics were extracted from hospital records. IPD incidence and patient and disease characteristics in the post-implementation period (June 2008-June2010) were compared with the pre-implementation period (June 2004-June2006).

Results: Significant declines in overall IPD incidence were observed in children < 2 years of age (60%) and adults ≥ 65 years (13%). Increases in non-vaccine type (NVT) IPD incidence were observed in all age-groups but proved to be significant in age categories 50-64 years: 37% and ≥ 65 years: 25%. The proportion of immunocompromised IPD patients has increased since the introduction of PCV7. Despite this, the overall case-fatality of IPD decreased due to a lower case-fatality rate in NVT IPD cases.

Conclusions: In the Netherlands, the overall IPD incidence after introduction of PCV7 decreased in both vaccinated children and unvaccinated elderly, implying herd-immunity has taken place. However, increases in NVT IPD have reduced net vaccine benefits. Immunocompromised patients seem to benefit less from the effects of PCV7. The replacement of vaccine-type by NVT IPD has led to a lower case-fatality rate.
GLOBAL ETIOLOGY OF ACUTE OTITIS MEDIA (AOM): A META-ANALYSIS OF RECENT EPIDEMIOLOGY STUDIES

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Background/aims: AOM is an important cause of childhood morbidity, and S. pneumoniae (Spn) and non-typable H. influenzae (NTHi) are considered leading otopathogens. However, their relative importance by age and severity is unclear because differences in study populations, pneumococcal conjugate vaccine (PCV) use, and case definitions may affect comparisons between individual studies.

Methods: We performed a meta-analysis of prospective AOM etiology studies conducted between 2008-2011 with similar case definitions and middle ear fluid samples available from tympanocentesis or sampling of otorrhea. Heterogeneity was tested using Cochran's Q-test, and 1258 AOM episodes from 11 countries were pooled for weighted analysis using a Freeman-Tukey double-arcsine variance-stabilizing transformation.

Results: Heterogeneity between studies was significant, so results from pooled random effect models are presented. 54% (95% CI, 46-62%) of episodes were bacteria-pathogen positive (listed below), and 3% (95%CI, 1-4%) were co-infections.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>% S. pneumoniae [95% CI]</th>
<th>% H. influenzae [95% CI]</th>
<th>% M. catarrhalis [95% CI]</th>
<th>% S. pyogenes [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>1258</td>
<td>22 [17-28%]</td>
<td>29 [24-33%]</td>
<td>2 [1-4%]</td>
<td>3 [2-5%]</td>
</tr>
<tr>
<td>Aged 3-11 months</td>
<td>270</td>
<td>26 [20-32%]</td>
<td>34 [28-40%]</td>
<td>3 [1-5%]</td>
<td>3 [1-7%]</td>
</tr>
<tr>
<td>1st reported episode for children aged 3-11 months</td>
<td>120</td>
<td>25 [19-33%]</td>
<td>30 [22-39%]</td>
<td>5 [2-9%]</td>
<td>5 [1-12%]</td>
</tr>
<tr>
<td>Otorrhea</td>
<td>239</td>
<td>30 [21-39%]</td>
<td>30 [22-40%]</td>
<td>1 [0-3%]</td>
<td>11 [6-18%]</td>
</tr>
<tr>
<td>Recurrent episode (≥3 in past 6 months or ≥ 4 in past 12 months)</td>
<td>257</td>
<td>20 [15-25%]</td>
<td>37 [28-47%]</td>
<td>3 [2-8%]</td>
<td>4 [1-8%]</td>
</tr>
<tr>
<td>PCV7-unvaccinated (&lt;2 doses at any age or 0 doses in those aged ≥1 year)</td>
<td>718</td>
<td>24 [19-30%]</td>
<td>25 [20-31%]</td>
<td>3 [1-4%]</td>
<td>12 [5-20%]</td>
</tr>
</tbody>
</table>

In PCV7-vaccinated children, 19% (95% CI, 14-26%) of episodes were due to Spn and 26% (95% CI, 10-47%) of those were due to PCV7 serotypes. 92% (95%CI, 80-99%) of all Hi-episodes were non-typeable.

Conclusions: The contributions of Spn and Hi were in young children and across studies, but Hi was more frequently found in treatment-failure and recurrent episodes. Spn and NTHi remain important targets for vaccination.
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EARLY CHILDHOOD RESPIRATORY INFECTIONS BEFORE AND AFTER THE INTRODUCTION OF THE PCV7 VACCINE INTO THE NORWEGIAN CHILDHOOD IMMUNISATION PROGRAMME


Background and aims: The seven-valent pneumococcal conjugate vaccine (PCV7) was introduced into the Norwegian Childhood Immunisation Programme in July 2006. The impact of PCV7 on acute otitis media (AOM) and lower respiratory tract infections (LRTIs) in Norway is unknown due to lack of systematic surveillance. Between 1999 and 2008 pregnant women were recruited to The Norwegian Mother and Child Cohort Study (MoBa). The aim of the current study was to examine the incidence of AOM and LRTIs among children included in the MoBa study.

Methods: Maternal reports of AOM and LRTIs were obtained from children included in the MoBa study at ages 6, 18 and 36 months. Incidence proportions of AOM and LRTI were calculated by year of birth.

Results: The current study included 86,744, 64,614 and 49,862 children with disease status reported at 6, 18 and 36 months of age respectively. The incidence of AOM and LRTI was stable pre-PCV7. The incidence of AOM between 6 and 18 months of age decreased from 30.54% to 28.08%, and between 18 and 36 months of age 43.10% to 40.52% among children born before and after 2006. Furthermore, the incidence of LRTIs between 6 and 18 months of age decreased from 13.83% to 11.43%, and between 18 and 36 months of age decreased from 14.77% to 11.37% among children born before and after 2006.

Conclusions: The results showed an overall decline of early childhood respiratory infections among children born before and after the introduction of PCV7 into the Norwegian childhood immunisation programme.
MOLECULAR CHARACTERISATION OF INVASIVE STREPTOCOCCUS PNEUMONIAE DEMONSTRATING REDUCED SUSCEPTIBILITY TO PENICILLIN IN IRELAND

I. Vickers, D. O'Flanagan, M. Cafferkey, H. Humphreys, Dublin, Ireland

Background and aims: The purpose of the study was to determine the molecular epidemiology of invasive S. pneumoniae disease isolates associated with reduced susceptibility to penicillin (PNSP), following introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) to the Irish immunisation schedule.

Methods: S. pneumoniae isolates from blood and CSF were serotyped using multiplex PCR and slide co-agglutination. Antimicrobial susceptibility was assessed using the Etest method. Reduced susceptibility to penicillin was defined as MIC≥0.12mg/L. Multi-locus sequence typing (MLST) was performed using standard procedures.

Results: All PNSP isolates (n=107) recovered between January 2009 and December 2010 were analysed. Eleven PNSP serotypes and 35 PNSP sequence types (STs) were identified. The most common serotype was 14 (27% of isolates) followed by 9V (18%), 6B (15%) and 19A (13%). However, a decline in serotype 14 from 2009 to 2010 was observed. Overall, PCV7 coverage was 71% (80%; 2009 vs 60%; 2010), while overall PCV13 coverage was higher at 90%. Pneumococcal Molecular Epidemiology Network (PMEN) clones or their variants were represented by 86% of isolates. Spain9V-3 and variants accounted for 38% of isolates, while Spain9V-2 and Sweden19A-25 and their variants accounted for 14% and 12% of isolates, respectively.

Conclusions: Within two years of PCV7 vaccine introduction in Ireland PCV7 serotypes accounted for a moderately high proportion of PNSP. However, there has been a PCV7 serotype decline since vaccine introduction. It is also anticipated that the introduction of PCV13 in December 2010 will have a positive impact on efforts to reduce antimicrobial resistance in Ireland.
THE IMPORTANCE OF SEROTYPE FOR DISEASE SEVERITY AND OUTCOME OF INVASIVE PNEUMOCOCCAL DISEASE

J.M. Ahl, N. Littorin, I. Odenholt, F. Resman, K. Riesbeck, Malmö, Sweden

Background: *Streptococcus pneumoniae* is represented by more than 90 serotypes and there is a debate whether the serotype is an independent risk factor for severity of invasive pneumococcal disease (IPD). Our hypothesis was that serotypes differ in their capacity to cause severe sepsis.

Methods: We performed a retrospective cohort study in Southern Sweden based upon 551 patients that were hospitalized for IPD in the prevaccination era 2006-2008. The serotype, co-morbidity, and sepsis severity were determined. The ST were divided into three groups based upon their respective invasive potential (low, intermediate and high). Moreover, all serotypes were further compared to serotype 14 as reference.

Results: A significant difference in age and co-morbidity (p≤0.001) was found between the groups. The median age and percent of patients with underlying co-morbidities in the three groups were: low, 70 (0-101) years, 75% underlying co-morbidities; intermediate, 67 (0-97) years, 57% co-morbidities, and; high, 59 (0-95) years and 47% co-morbidities. We found no difference in sepsis severity between the groups. Patients with *S. pneumoniae* serotype 3 had a significantly higher co-morbidity (79%, p=0.04), higher mortality (29%, p=0.034), and worse sepsis severity (p=0.03) compared to serotype 14. The difference in sepsis severity was also significant after exclusion of all patients with co-morbidities.

Conclusion: Our results support the hypothesis that ST with a low and intermediate invasive potential mainly cause IPD in the elderly with defined co-morbidities and thus can be considered as opportunistic. ST3 gave significantly higher mortality and sepsis severity when using serotype 14 as reference.
**EVALUATION OF BIOFILM FORMATION AMONG *STREPTOCOCCUS PNEUMONIAE* SEROTYPES 19A AND 3**

M. Villar Vidal, O. Esnal Lasarte, M. Alonso Asencor, J.M. Marimon Ortíz de Zárate, E. Pérez-Trallero, Donostia, Spain

**Background:** Biofilm production is associated with chronic or recurrent infections. The aim of this work was to correlate the ability to form early biofilms and several variables such as type of infection, serotype or antibiotic resistance in two frequent *S. pneumoniae* serotypes: 3 and 19A.

**Methods:** Overall 159 *S. pneumoniae* isolates, 80 serotype 3 and 79 serotype 19A, from patients with acute exacerbations of COPD (AECOPD) and invasive pneumonia were tested. Quantification of biofilm production was performed using a static microtitre plate model and expressed as absorbance units at 570 nm after crystal violet staining. Inverted optical microscopy and Scanning Electron Microscopy (SEM) were used to visualize adhesion on polystyrene surface.

**Results:** Biofilm (absorbance > 0.100) was produced by 62/80 (77.5%) serotype 3 and 69/79 (87.3%) serotype 19A isolates. Mean biofilm formation of *S. pneumoniae* serotype 3 was 0.174 ± 0.06 (range 0.101-0.401) and that of serotype 19A 0.232 ± 0.098 (range 0.102-0.528) (p< 0.05). No difference in the ability to form early biofilms between isolates from invasive pneumonia and from AECOPD was seen. Serotype 19A erythromycin-susceptible isolates produced more biofilm (absorbance 0.255± 0.106) than erythromycin-resistant isolates (absorbance 0.201 ± 0.079) (p< 0.05).

**Conclusions:** In vitro biofilm formation could not be associated to the clinical origin of the isolates but was related to serotype and erythromycin susceptibility.

With the protocol used, SEM failed to identify biofilm structure possibly due to the dehydration and fixation pretreatments that damaged the adhered cells.
CEREBRAL OUTPUT OF THE CINC-1 AND DISRUPTION OF BLOOD-BRAIN BARRIER IN WISTAR RATS AFTER PNEUMOCOCCAL MENINGITIS INDUCTION


Introduction: Pneumococcal meningitis is the most severe and frequent infection of the central nervous system (CNS) with a mortality rate of up to 20% and adverse neurological outcome up to 50% of the survivors, being that the microorganism and host’s inflammatory response are responsible in cerebral complications. Moreover the blood-brain barrier (BBB) itself secretes cytokines and because of the bipolar nature of the BBB, these substances can be secreted into either the CNS compartment or in the blood, then patients with acute bacterial meningitis frequently develop sepsis.

Objectives: Therefore, the aim of this study was to evaluate the cytokine/chemokine levels by different vessels and BBB injury after pneumococcal meningitis induction.

Methods: Wistar rats were infected either 10 µL of sterile saline as a placebo or an equivalent volume of S. pneumoniae suspension to the concentration of the 5x10^6 cfu/mL. The BBB integrity was investigated using Evan’s blue dye. Also, blood from carotid artery and jugular vein was collected to perform TNF-α, IL-1β, IL-6 and CINC-1 analyzes by ELISA.

Results: CINC-1 levels were increased at 6h in arterial plasma and at 3 and 6h in jugular plasma. We observed the BBB breakdown between 12 and 24h in hippocampus and at 12 and 18h in the cortex after pneumococcal meningitis induction.

Conclusions: The increase in CINC-1 occurred prior to the breaking of the BBB. As CINC-1 is a neutrophil chemoattractant, it may be related to early events in pathophysiology of pneumococcal meningitis.
POSITIVE FEEDBACK LOOP REGULATION OF TYPE1 PILUS EXPRESSION
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Backgrounds and aims: The pneumococcal type 1 pilus, which is present in 25-30% of clinical isolates, has been associated with increased adherence, biofilm formation and inflammatory responses. Its function in pneumococcal pathogenicity remains elusive. More recently, we and others showed that pilus expression is bistable, allowing a clonal bacterial population to differentiate into two populations P- and P+ (non-expressing and expressing the pilus, respectively) and potentially adapt to various environments. The focus of this work has been to further analyze the regulation of the bistable expression of the pilus.

Methods: We re-evaluated the role of the negative regulators described in the literature prior to the discovery of the bistable expression of the pilus by isolating P- populations using antibody coupled with magnetic beads for each of these mutants and analyzing the population behavior. We then analyze the role of the RlrA transcription factor on the pilus expression by modulating its ectopic expression in strains with or without the endogenous locus.

Results: We were able to show that only the MerR and RrgA proteins act as negative regulators on the pilus expression. Additionally, the RlrA transcription factor was shown to act in a positive feedback loop, which is sufficient to explain the bistable phenotype of the pilus expression.

Conclusions: Fewer proteins than previously expected seem to act on the pilus bistable expression. We are now proceeding with in vivo studies in animal studies to evaluate how this virulence factor is expressed in the context of colonization and infection.
GALU GENE EXPRESSION IN STREPTOCOCCUS PNEUMONIAE

L. Bonofiglio¹, E.A. García López², M.E. Mollerach¹, ¹Ciudad Autónoma de Buenos Aires, Argentina, ²Madrid, Spain

The galU gene of Streptococcus pneumoniae (Spn) encodes an UDP-glucose pyrophosphorylase absolutely required for capsule biosynthesis. Since eukaryotic GalU appear to be completely unrelated to their prokaryotic counterparts, we postulate that GalU may be an appropriate target for the search of new drugs to control pathogenicity of Spn.

Comparison of completely Spn sequenced genomes showed that galU and gpdA are adjacent and in the same orientation. Using OperonDB software we found that both genes are cotranscribed in the same unit in Spn and 60 genomes belonging to firmicutes. Transcriptional terminator prediction using TranstermHP showed that no terminator is present between gpdA and galU; however, a terminator was detected downstream galU gene. A promoter prediction program identified 4 putative promoter regions, upstream of gdpA. DNA fragments containing these putative promoters were cloned in the promoter probe vector pHSE4. Promoter activity was detected in one of these constructions. The cloned fragment was screened for the presence of Shine Dalgarno (SD) like sequence, -35 and -10 boxes consensus sequences.

An extended −10 site T-TG-TATAAT that lacks a −35 site was detected, the putative transcription start site is a purine and the SD sequence found is exactly the consensus. The predicted promoter region is highly conserved in 23 Spn completely sequenced genomes. Expression of galU gene was explored by semiquantitative real time RT-PCR at different points of the growth curve, indicating that galU gene is expressed in exponential phase.

These results contribute to the knowledge of the expression mechanism of GalU enzyme.
C-TERMINAL DELETION OF THE STREPTOCOCCUS PNEUMONIAE GALU PROTEIN CAUSES A DECREASE IN CAPSULAR POLYSACCHARIDE PRODUCTION

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The GalU protein plays a key role in polysaccharide biosynthesis. To better understand the implication of this protein in capsule production, the \textit{galU} gene was interrupted by \textit{mariner} mutagenesis. The derivative mutant obtained of \textit{S. pneumoniae} R6 presented an insertion of Himar1 minitransposon in the last 101 nucleotides of the \textit{galU} gene. DNA obtained from this mutant was used to transform the encapsulated M23 strain (serotype 3). Transformants were analyzed by PCR mapping and sequencing. M23c4 mutant has a deletion of the 33 C-terminal amino acids in GalU relative to the full length protein. Agglutination with S3 antiserum was observed under phase contrast microscopy, although M23c4 has rough colony morphology. Polysaccharide extraction was performed from wild type and M23c4 mutant; semiquantitative double-diffusion experiments indicated that a less quantitative polysaccharide is produced in the mutant compared to wild type strain.

A model of the truncated protein was performed using Swiss model and Deep-Viewer 4.0 using \textit{E. coli} protein as template. This protein is a tetramer composed by two dimers in which the monomeric subunits are tightly intertwined. According to our model, the mutated GalU has lost the helix involved in the subunit-subunit interaction of the tight dimer, producing an altered quaternary structure of the protein. This modification could explain the decrease in polysaccharide production probably due to a lower enzymatic activity of GalU. These results contribute to the knowledge of GalU enzyme, which is considered a target for the search of new antipneumococcal drugs.
ASSOCIATION BETWEEN LOSS OF WCJE-MEDIATED CAPSULAR O-ACETYLATION AND STREPTOCOCCUS PNEUMONIAE SEROGROUP 11 INVASIVE DISEASE ISOLATES

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**Background:** There are strong associations of certain pneumococcal serotypes with invasive pneumococcal disease (IPD), while other serotypes rarely cause IPD. This suggests that serotype-specific capsule polysaccharide (cPS) properties may contribute to the propensity to cause IPD. Strains reactive with factor serum 11c but not 11b (11c+11b-) can be classified into serotypes 11A and 11E with antibodies that detect an O-acetate on the sixth carbon of cPS β-Galactose. This O-acetylation, characteristic of 11A cPS and absent in 11E cPS, is mediated by the O-acetyltransferase encoded by wcjE. Analysis of wcjE mutations in 11E IPD isolates reveals that each variant evolved independently, suggesting that 11E strains are not transmitted host to host, but arise de novo from 11A precursors. To examine this hypothesis, epidemiological analysis of serogroup 11 isolates was performed.

**Methods:** 382 11c+11b- isolates from carriage or IPD were analyzed for expression of wcjE-mediated epitope. wcjE mutations were confirmed by sequencing.

**Results:** Of 355 nasopharynx isolates, 2 contained 11E and the remainder contained 11A. Of 27 IPD isolates, 10 (37%) contained 11E and 17 contained 11A. No two 11E strains contained identical mutations to wcjE.

**Discussion:** Serotype 11E strains lack evidence of clonal transmission and appear significantly more likely to occur among IPD isolates compared to nasopharyngeal isolates (OR=103.82[21.08-511.32]). Thus, wcjE-mediated O-acetylation may biologically favor nasopharyngeal carriage and transmission, while loss of wcjE activity potentially predisposes for invasion or is selected for during invasion to deeper tissue. We describe the first specific cPS glycoepitope apparently associated with pneumococcal tissue specificity.
Background and aims: Macrolide-efflux encoded by mef(E)/mel is inducible by erythromycin and the antimicrobial peptide LL-37. Other macrolide resistance determinants are controlled by attenuation but this has not been confirmed for mef(E)/mel. LL-37 does not target ribosomes and therefore is unlikely to influence mef(E)/mel attenuation suggesting independent mechanisms for induction by erythromycin and LL-37. We used transcriptome analyses to define the pneumococcal genes induced by each to elucidate the mechanism(s) of induction of mef(E)/mel.

Methods: Two mef(E)/mel-containing pneumococcal isolates and their mef(E)/mel-deletion derivatives were exposed to erythromycin, spiramycin, LL-37 or placebo. Spiramycin, a mef(E)/mel non-inducing macrolide, was used to control for translational stress-induced genes. RNA was sequenced by Illumina RNA-Seq technology.

Results: 180 genes were up-regulated and 171 down-regulated (>3-fold) by erythromycin, 24% (44) of up-regulated genes were ribosomal or tRNA-related suggesting a translational stress response. The mef(E) and mel genes were the two highest erythromycin-induced genes, with up-regulation ~20x higher than other regulated genes. An operon (orfs3-6) of unknown function located on the mef-containing mobile element MEGA was also up-regulated (~3-fold) suggesting a role in macrolide resistance. LL-37 and spiramycin analysis is underway.

Conclusions: Comparison of erythromycin and LL-37-induced expression patterns will likely reveal regulation of mef(E)/mel by independent mechanisms.

This project was funded by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services under contract number HHSN272200900007C and grant number 5R01AI070829. Surveillance and isolate collection was funded by CDC as part of ABCs surveillance in the Emerging Infection Program.
PNEUMOCOCCAL GENOME EVOLUTION DURING CARRIAGE AT THE POPULATION SCALE

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Background: Despite being best known as the cause of several infectious syndromes, Streptococcus pneumoniae is a commensal commonly detected in carriage in the nasopharynx and understanding this harmless state is essential for understanding the evolution of the pathogen. A longitudinal pneumococcal carriage cohort study was conducted in the Maela refugee camp located on the Thai-Burmese border. Nasopharyngeal swabs were collected over the first 24 months of life from infants born between 2007-2010, as well as from approximately one-quarter of the mothers. A randomly selected collection of approximately ~3,000 isolates was sequenced, allowing a precise investigation of mutation and recombination and their role in factors such as antibiotic resistance.

Methods: A phylogenetic tree was constructed for whole genomes plus the multi-locus resistance encoding transposon Tn916, and its derivatives. Pair-wise distance between samples within a tree was used as a tool to investigate relationship within and between MLST lineages. Recombination within lineages was detected based on SNPs density, while inter-lineage recombination was determined by Bayesian Analysis of Population Structure (BAPS) and admixture analyses.

Results and conclusions: Of more than 170 MLST lineages detected in the camp, degrees of diversity within each lineage varied considerably. The most diverse STs, whose diversity is largely contributed by recombination, were found to be among non-encapsulated lineages (ST4133 and ST4136, p-value < 0.01), which could be due to their highly transformable nature. One of the recombination hotspots corresponds with Tn916. A phylogenetic tree of Tn916 tree showed that the element has been acquired and lost multiple times.
SPECIFIC AND NONSPECIFIC COMPONENTS OF ACQUIRED IMMUNITY TOGETHER PERMIT COEXISTENCE OF PNEUMOCOCCAL SEROTYPES

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Dramatic changes in the distributions of pneumococcal serotypes have followed the use of vaccines, though the general mechanisms accounting for observed patterns of serotype composition are incompletely understood. In particular, it is unclear why some serotypes are able to persist at all: they appear inferior in every measured dimension of fitness. Serotype-specific immune responses, which could promote diversity in principle, have been measured as relatively weak. Nonspecific responses are expected to intensify competition and thereby reduce diversity. We show that an acquired immune response not specific to the capsule and weak serotype-specific immunity are together sufficient to reproduce observed patterns of diversity. Serotype-specific immunity stabilizes serotype competition, and realistic forms of acquired nonspecific immunity reduce fitness differences in partially immune hosts. We also show that another proposed explanation for coexistence, mediation of serotype competition by a co-colonizing Gram-negative pathogen, can promote diversity but only under narrow conditions that are unlikely to be consistently present. Our findings help explain the persistence of pneumococcal diversity before and after vaccination and can be used to predict qualitative changes in carriage following the introduction of broader vaccines.
**Poster No 88**

**EFFECTS OF CIGARETTE SMOKE CONDENSATE ON BIOFILM PRODUCTION BY STREPTOCOCCUS PNEUMONIAE AND ON THE BIOACTIVITY OF PNEUMOLYSIN**

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**Background and aims:** It is well known that smokers suffer from repeated and persistent pneumococcal infections, when compared to non-smokers. It is possible that the pneumococcus forms biofilm in the lungs of these individuals, rendering them more susceptible to persistent infections. Pneumolysin is essential in disease progression, but also activates innate and adaptive immune responses.

**Methods:**

**Bacterial strains:** A clinical isolate of *S. pneumoniae*, strain 172, was used.

*Cigarette smoke condensate (CSC):* CSC was used at concentrations of 20 - 160 ug/ml. Approximately 26 mg of CSC is generated during the smoking of one cigarette.

*Measurement of biofilm formation and bacterial viability:* Bacteria were grown in 6 well tissue culture plates in tryptic soy broth for 16 hours following measurement of biofilm formation using crystal violet based spectrophotometric procedure. Planktonic bacterial viability was determined using colony forming procedures.

*Effect of CSC on pneumolysin:* A Ca\(^{2+}\) sensitive fluorescent dye, fura-2/AM, was used to determine the effect of untreated or condensate-treated pneumolysin on the pore-forming activity of the toxin.

**Results:** CSC was found to increase biofilm formation in a dose-dependent manner, without affecting viability. CSC also inhibited the pore-forming action of pneumolysin.

**Conclusions:** CSC could act as a stressor to the pneumococcus, thereby enhancing biofilm formation as a survival strategy; it also inhibits the functionality of pneumolysin, which may attenuate anti-pneumococcal host defences.
COMPARATIVE GENOMIC ANALYSIS OF EMERGING DRUG-RESISTANT STREPTOCOCCUS PNEUMONIAE 19A STRAINS ISOLATED FROM PATIENTS IN DIFFERENT COUNTRIES

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The increase of multi-drug resistant 19A strains post-PCV7 was of great concern to clinicians. Sequence type (ST) 320 has been shown as predominant among MDR-19A in Canada, Korea and USA. However what remains unclear is whether a single clone disseminated globally or whether MDR19A arose independently.

To elucidate the mechanism of MDR-19A dissemination, we sequenced and compared genomes of five representative 19A strains predominant in Canada, USA, Israel, Portugal and Korea. Whole-genome sequencing was performed with the Roche 454 FLX and the contigs were assembled using MAUVE.

Pairwise alignment of three 19A-ST320 genomes identified 37 recombinations (30 insertions with average 430 bp and 7 deletions). Most recombinations occur in the loci of capsule, phosphotransferase system, restriction modification system, prophage and surface proteins. One recombination event in USA-19A deleted one copy of mef that may affect resistance level to macrolide. Comparison of two 19A-ST193 genomes revealed 63 recombinations (average 3.4 kb). Portugal 19A-ST193 genome contains a 58kb ICE element carrying tetM and mef that Israel ST193 lacks. 31 SNPs were detected between Korea 19A-ST320 and Canada 19A-ST320 compared to 3123 SNPs between Canada 19A-ST320 and USA 19A-ST320 genome. Exceptional high genome similarity suggests Canadian 19A-ST320 might be a direct spread of Korean 19A-ST320 in recent years, while USA 19A-ST320 could derive from local pre-existing strains. Portugal MDR 19A-ST193 is divergent from Israel 19A-ST193 (8530 SNPs), suggesting they are not related to each other.

Taken together, evidence for both dissemination and independently arising of 19A is present in our genomic comparisons.
INVASIVENESS OF PAEDIATRIC SEROTYPES AND CLONES OF STREPTOCOCCUS PNEUMONIAE PRIOR TO ROUTINE USE OF PCV-13 IN THE GAMBIA

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Background: Streptococcus pneumoniae strains expressing certain capsular serotypes appear to be better able to cause invasive disease than others, and a small number of serotypes account for the majority of invasive disease. In addition, there is a substantial variation in the rank order of serotypes/genotypes that cause disease according to the geographic location. The objective of this study was to determine the odd ratios (ORs) of serotypes and genotypes of S. pneumoniae causing invasive disease in children in The Gambia.

Methods: Isolates (230) of S. pneumoniae from cases of invasive disease were compared to isolates (270) from nasopharyngeal carriage. All isolates were obtained from children under five years old during the period of 1996 to 2005. Serotyping was done using latex agglutination and then followed by genotyping using Multi-locus Sequence Typing.

Results: A total number of 48 serotypes and 157 sequence types (STs) were obtained. 76 novel STs were discovered. Serotypes 1, 5, 14 and 46 were significantly associated with invasive disease, while serotypes 19F, 6B, 15B and 35B were associated with carriage (ORs>1). ST63, ST273, ST618, ST3321, ST3404 and ST5509 were also significantly associated with invasive disease, while ST925 ST3407, ST3329, ST3337 and ST4036 were associated with carriage (ORs>1). S. pneumoniae clones were highly diverse in both invasive disease and carriage (Simpson's Index of diversity = 0.906). eburst analysis revealed 31 clonal complexes and 39 singletons.

Conclusions: These findings revealed the invasiveness of serotypes 1, 5, 14 and 46, as well as ST63 and ST3321 from Gambian children.
Poster No 91

COMPARATIVE PHYLOGENOMICS OF STREPTOCOCCUS PNEUMONIAE ISOLATED FROM INVASIVE DISEASE AND NASOPHARYNGEAL CARRIAGE FROM WEST AFRICANS

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Background and aims: In contrast to the developed world, the biology of Streptococcus pneumoniae in developing countries is poorly understood, though the majority of pneumococcal diseases occur in the developing world. The aim of the study was to investigate the pathogenicity of S. pneumoniae and other features related to the biology of the organism using isolates from West Africa.

Methods: We applied comparative phylogenomics (whole genome comparisons of microbes using DNA microarrays combined with Bayesian-based phylogenies) to investigate 58 S. pneumoniae invasive and carriage isolates. The strains covered eight different S. pneumoniae serotypes of varying invasive disease potential.

Results: The core genome of the isolates was estimated to be 38% and was mainly represented by gene functional categories associated with housekeeping functions. Comparison of the gene content of invasive and carriage isolates identified at least eighteen potential genes that may be important in virulence including various surface proteins, transport proteins, transcription factors and hypothetical proteins. Thirteen accessory regions were also identified and did not show any loci association with the eighteen virulence genes. A constructed phylogenetic tree of the isolates showed a high level of heterogeneity consistent with the frequent S. pneumoniae recombination. Despite this, a homogeneous clustering of all the serotype 1 strains was observed.

Conclusions: Virulence determinants that contribute to S. pneumoniae pathogenicity are likely to be distributed randomly throughout its genome rather than being clustered in dedicated loci or islands. Compared to other S. pneumoniae serotypes, serotype 1 appears most genetically uniform.
EFFECTS OF PNEUMOCOCCAL VACCINATION ON NASOPHARYNGEAL CARRIAGE OF S. PNEUMONIAE, M. CATARRHALIS, S. AUREUS, AND H. INFLUENZAE IN FIJIAN INFANTS

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Background and aims: Colonization of the nasopharynx by Streptococcus pneumoniae, Moraxella catarrhalis, Staphylococcus aureus, and Haemophilus influenzae can lead to diseases such as pneumonia and otitis media. The 7-valent pneumococcal conjugate vaccine (PCV7) effectively reduces carriage of vaccine-type S. pneumoniae but is associated with replacement by non-vaccine serotypes and may affect carriage of other respiratory pathogens.

Methods: We investigated the effects of PCV7 on nasopharyngeal bacterial carriage in Fijian infants participating in a vaccine trial: Group A received 3 doses of PCV7, Group B received 3 doses of PCV7 plus a 23-valent pneumococcal polysaccharide vaccine booster, and Group H were unvaccinated. We also examined co-colonization by multiple pathogens and the epidemiological characteristics of the study population. A real-time quantitative PCR assay for detecting the four respiratory pathogens listed above was used to compare the bacterial content of nasopharyngeal swabs five months after vaccination.

Results: Overall, pneumococcal vaccination did not affect carriage rates or densities of the four pathogens examined. Co-colonization was common, with 60.1% of children colonized by multiple species. Significant differences were observed between the two major ethnic groups: Indigenous Fijians had higher carriage rates of S. pneumoniae, M. catarrhalis, and H. influenzae than Fijians of Indian descent and were more likely to carry multiple species.

Conclusions: These differences in carriage between ethnic groups highlight the role that host factors play in nasopharyngeal biology. The high levels of colonization by multiple pathogens typically observed in developing countries should be considered when designing and monitoring new pneumococcal vaccine introduction.
Poster No 93

EFFECT OF STREPTOCOCCUS SALIVARIUS ON STREPTOCOCCUS PNEUMONIAE COLONISATION OF HUMAN EPIDERMOID LARYNGEAL CARCINOMA CELLS IN VITRO


Background and aims: Colonisation of nasopharynx is the initial step in the pathogenesis of pneumococcal diseases, including otitis media. Pneumococcal vaccines are less effective against otitis media compared to invasive diseases and have other disadvantages. Studies have shown that Streptococcus salivarius, a commercially available oral probiotic, has potential use as a probiotic of the nasopharynx. We investigated the effects of Streptococcus salivarius on Streptococcus pneumoniae colonisation of respiratory epithelial cells using an in vitro assay.

Methods: The impact of S. salivarius on S. pneumoniae colonisation was examined by using human epidermoid laryngeal carcinoma (HEp-2) cells. High (5 to 10 times more), medium (approximately equal) or low (5 to 10 times less) numbers of S. salivarius were added before, with, or after S. pneumoniae (pre-, co- and post-administration respectively). The percent colonization of S. pneumoniae was determined after 3 h incubation.

Results: There was a dose dependent inhibition of S. pneumoniae colonisation of HEp-2 cells by S. salivarius. This reduction was more pronounced with pre-administration, which significantly reduced pneumococcal colonisation at the high and medium S. salivarius doses, whilst co-administration was effective at the high dose only.

Conclusion: Our data suggest that S. salivarius may inhibit the nasopharyngeal colonisation of S. pneumoniae. Further in vivo experiments using a murine model are warranted to better understand the effects of S. salivarius and evaluate its potential clinical use as a probiotic to reduce otitis media in high-risk populations.
ISPPD-8

Poster No 94

PBP2B PARTICIPATES IN THE CONTROL OF SHAPE DETERMINATION AND CELL DIVISION MECHANISMS IN STREPTOCOCCUS PNEUMONIAE

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Background: Mutations in penicillin-binding proteins (PBPs) PBP1a, PBP2x, and PBP2b confer β-lactam resistance in S. pneumoniae. These enzymes are normally involved in cell wall synthesis. We described that a laboratory strain harboring pbp2b mutations, isolated from clinical strains and that conferred penicillin resistance, showed morphological abnormalities (rod-shaped cells) with an abnormal septum pattern, suggesting alterations in the cell division mechanism. These cell alterations were restored by the acquisition of pbp2x and pbp1a resistance-conferring mutations obtained from clinical strains, showing evidences of a compensatory evolution mechanism.

Aims: To analyze the cause by which the PBP2b mutant proteins altered the control of shape determination and its putative involvement in the cell division mechanism.

Methods/results: Analyzing PBP-tag protein fusions, we demonstrated that all these pbp mutations conferred an increased stability to their respective mutant proteins, suggesting that a higher half-life of PBP2x and PBP1a mutant proteins is necessary to compensate the increased half-life of the PBP2b mutant protein (PBP2b*) to restore a normal shape. In the pbp2b mutants, we demonstrated that the FtsZ and PBP2B* were delocalized, and that PBP2b* displayed a helix manner, similar to that showed by FtsZ in the same pbp2b mutants. By two-hybrid system assays, we detected a protein-protein interaction between FtsZ and PBP2b*. In addition, we found that PBP2b is able to interact with other proteins that belong to the streptococcal divisome.

Conclusions: We proposed that PBP2b participates in the control of shape determination and cell division mechanisms in S. pneumoniae by a direct interaction with FtsZ.
DIVERSITY WITHIN THE SEQUENCES OF THE CAPSULAR GENE LOCI OF *STREPTOCOCCUS PNEUMONIAE* SEROGROUP 6 AND 19

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**Background and aims:** The polysaccharides comprising the pneumococcal capsule are encoded by genes in the capsular locus. We investigated the genetic diversity of the capsular genes within serogroup 6 and 19 to explore a possible effect of vaccination on variation and distribution of these serotypes in the Netherlands.

**Methods:** The complete capsular gene locus was sequenced for 25 serogroup 6 isolates and 20 serogroup 19 isolates. If one or more genes varied in 10 or more base pairs from the reference sequence, it was designated as a capsular subtype. Specific PCRs and sequencing of highly variable capsular genes were performed on 184 serogroup 6 and 195 serogroup 19 isolates to identify capsular subtypes.

**Results:** Sequencing revealed 6, 3 and a single capsular subtype within serotypes 6A, 6B and 6C, respectively. The isolates of serotype 19A and 19F revealed 3 and 4 capsular subtypes, respectively. For serogroup 6, the genetic background seemed to be closely related to the capsular subtypes, but this was less pronounced for serogroup 19. The data suggest shifts in the occurrence of capsular subtypes of serotype 6A and 19A after introduction of the 7-valent pneumococcal vaccine.

**Conclusion:** There is considerable DNA sequence variation within the capsular genes of serogroup 6 and 19. The shifts in occurrence within the non-vaccine serotypes might indicate that they are filling the niche of the vaccine serotypes. Furthermore, certain variants may be less sensitive for the vaccine induced immunity.
**ISPPD-8**
**Poster Shift 2: Wednesday, March 14, 2012 – Thursday, March 15, 2012**

**Poster No 96**

**STREPTOCOCCUS PNEUMONIAE CLONES SELECTED BY THE 7-VALENT PNEUMOCOCCAL CONJUGATE VACCINE IN HEALTHY CHILDREN SHOW INVASIVE POTENTIAL IN MICE**

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**Background and aims:** In a previous epidemiological study, we identified the major drug-resistant pneumococcal clones colonizing Portuguese children immunized with the 7-valent pneumococcal conjugate vaccine (PCV7). As the prelude to virtually all pneumococcal disease is colonization, we aimed at characterizing the colonization and invasion potential of these clones.

**Methods:** The three major drug-resistant clones isolated from healthy PCV7-immunized children (6 months-6 years) exhibited serotypes 6A, 15A and 19A, with the corresponding sequence types (STs) ST2191, ST63 and ST276. To mimic the natural entry route, these clones were introduced intranasally (10µL, 10⁸ cfu/mouse) into CD1 mice. Survival, colonization and tissue invasion were then assessed throughout a 21-day period. The virulent D39 strain was used as control.

**Results:** All mice survived the intranasal challenge with no visible signs of disease. For the three clones, mice exhibited substantial colonization of the nasopharyngeal mucosa (~10⁴ cfu/mouse). In addition, these clones were isolated from adjacent tissues such as the olfactory bulbs (~10⁷ cfu/mouse), brain (~10⁸ cfu/mouse), lungs (~10⁷-10⁸ cfu/mouse) and the middle ear mucosa (~1-6 cfu/mouse). The control strain colonized mice less efficiently (~10² cfu/mouse) and, with the exception of the lungs (~10⁷ cfu/mouse), was found in adjacent tissues at lower numbers (~3 cfu/mouse).

**Conclusions:** PCV7-selected clones, namely 6A-ST2191, 15A-ST63 and 19A-ST276, are able to efficiently colonize the mouse nasopharynx resulting in invasion of adjacent tissues. These results underscore the importance of coupling epidemiological and animal studies to evaluate and predict vaccination impact.

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GENOMIC COMPARISON OF THE PATHOGEN STREPTOCOCCUS PNEUMONIAE WITH COMMENSAL SPECIES S. MITIS AND S. ORALIS

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Streptococcus mitis and S. oralis are commensal residents of the upper respiratory tract and belong to the Mitis group of viridans streptococci. Genomic comparative analyses were performed between three species to understand the evolution and pathogenicity of S. pneumoniae as well as for the optimal design of vaccines. Methods involved in silico comparison including the finished genomes of S. mitis B6 and S. oralis Uo5, both multiple antibiotic resistant strains expressing unusual high penicillin resistance levels, and comparative genomic hybridization on S. mitis and S. pneumoniae based oligonucleotide microarrays.

In silico comparison of finished genomes shows that at least 1220 genes are shared between all three streptococcal species, and almost 500 remain Uo5 specific. In addition to unusual gene clusters involved in choline metabolism, only five choline-binding proteins are present. The striking X-alignment of the S. mitis B6 genome when compared with S. pneumoniae is largely maintained in S. oralis Uo5.

Most proteins described as pneumococcal virulence factors are present in S. mitis B6 and S. oralis Uo5. Special gene clusters present in S. pneumoniae could be responsible for the modulation of the virulence potential of oral streptococci. The data confirm the assumption that S. pneumoniae originated from an ancient S. mitis clone. The absence of the three choline binding proteins PcpA, PspA and PspC, and three gene clusters containing the hyaluronidase gene, the ply and lytA island in most S. mitis and S. oralis confirm their importance for the pathogenicity potential of S. pneumoniae.
ACCIDENTAL EVOLUTION OF INVASIVENESS AMONG STREPTOCOCCUS PNEUMONIAE COLONIZING CHILDREN

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Background and aims: Streptococcus pneumoniae is a human commensal pathogen causing invasive diseases, but also a common colonizer of the nasopharynx. Pneumococci are easily transformed and may incorporate foreign DNA from other strains. To monitor genetic relationships among strains pulsed-field-gel-electrophoresis (PFGE) and sequenced based multi-locus sequence typing (MLST) are used.

In this study we compared invasive and carriage isolates from children coming from the same geographic area, Stockholm, during the same time period 1997-2004 aiming at finding bacterial factors important for disease development

Methods used: PFGE, MLST, whole-genome-sequencing, characterization of phage content using PCR and mitolysin assays, functional characterization in in vitro and in vivo models

Results: We demonstrate that within distinct genetic lineages, based on MLST, we can identify sub-clones with different patterns using PFGE. Three PFGE sub-clones differed in odds ratio of causing invasive disease in children; one had a considerably higher invasive disease potential than the other two. Using whole genome sequencing and genomic comparisons of two invasive and two carrier isolates it became apparent that number, type, and chromosomal location of prophages was a major cause for intra clonal variation though other large insertions/deletions also played a role. Intraclonal variation was seen in the three major virulence surface antigens PcpA, PspA, and PspC.

Conclusions: Our data suggest that the constantly ongoing selection for immune escape variants of pneumococci during carriage in children create antigenic variants that accidentally may affect the invasive disease potential in children by simultaneously affecting disease promoting properties of individual proteins.
DETECTION OF GENES ESSENTIAL FOR GROWTH OF RESPIRATORY PATHOGENS


Background: Respiratory tract infections are a leading cause of global mortality and morbidity. The WHO estimates that annually 4-5 million people die of pneumonia. *Streptococcus pneumoniae* is among the most important respiratory tract pathogens. Infection by and growth of *S. pneumoniae* is a complex process dependent on a number of essential pathways, of which some could form ideal candidate targets for drug design and/or vaccine development.

Methods: To identify microbial genes essential for growth of respiratory pathogens, we have used the Tn-Seq insertion knockout and sequencing strategy and developed a bioinformatics tool that allows rapid identification of disrupted genes. Genes underrepresented in the knockout library are likely essential for growth. To identify shared essential pathways in bacterial species we have used statistical analysis, pathway analysis and functional category enrichment methods.

Results: In *S. pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* we observed that ~10% of all genes is essential, similar to what has been found in knockout studies. These genes primarily encode functions involved in transcription, translation or replication, but also encode for hypothetical proteins orthologous between the tested species.

Conclusions: High throughput screening of essential genes is feasible using Tn-Seq. To this end, a large knockout library is preferred to decrease the number of false positives. Importantly, genes encoding for orthologous proteins in all three species have been found to be essential, including hypothetical proteins, which are likely to play important roles in critical cellular processes and might form novel candidate targets for drug design and/or vaccine development.
GENOME-WIDE IDENTIFICATION OF STREPTOCOCCUS PNEUMONIAE GENES ESSENTIAL FOR GROWTH AND SURVIVAL IN CO2-POOR ENVIRONMENTAL CONDITIONS.


Background and aims: The spread of S. pneumoniae to new human hosts is a prerequisite for the persistence of this respiratory tract pathogen in the community. Pneumococcal transmission has also contributed to the significant rise of antibiotic resistance and vaccine escape observed over the recent decades. Hence, knowledge on pneumococcal transmission factors could contribute to the development of novel strategies to treat and prevent pneumococcal disease.

Methods: To identify pneumococcal transmission factors, we exposed S. pneumoniae mutant libraries to conditions reflecting the environment encountered by the pathogen outside the host. Mutants that failed to survive under these conditions were identified by the Tn-seq next generation sequencing technology, and signify genes essential for S. pneumoniae transmission.

Results: Tn-seq analysis of a 40,000 CFU S. pneumoniae R6 mariner transposon mutant library exposed to CO2-poor (0.035%) environmental and CO2-rich (5%) host conditions reproducibly identified 45 mutants specifically attenuated for pneumococcal growth in the CO2-poor environment. Only 2 genes, encoding a carbonic anhydrase (PCA; spr0026) and a dihydrofolate synthase (FolC; spr0178), were represented by multiple unique mutants. Validated experiment with S. pneumoniae directed mutants confirmed the essential role of the pca and folC genes for growth in CO2-poor environmental conditions.

Conclusions: The identification of putative pneumococcal transmission factors identified in this study could aid the design of intervention strategies to prevent acquisition of novel pneumococcal strains by the human host. It is foreseen that application of Tn-seq to conditions that reflect other relevant aspects of human transmission will identify more pneumococcal transmission factors.
Poster No 101

CAPNOPHILIC STREPTOCOCCUS PNEUMONIAE ST162 AND ST344 STRAINS HAVE A POLYMORPHISM IN THE MURF CELL WALL LIGASE

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Background and aims: Although approximately 8% of all pneumococcal strains are capnophilic, i.e require CO₂-enriched growth conditions, the genetic cause of this CO₂-dependent growth behavior is unknown. In this study we examined the genetic background of naturally occurring capnophilic pneumococcal isolates.

Methods: We performed MLST analysis of 71 capnophilic S. pneumoniae carriage and invasive isolates for molecular epidemiology. The genetic trait responsible for S. pneumoniae capnophilic behavior in two genetic clusters was revealed by transposon and directed mutagenesis.

Results: Capnophilic pneumococci could be grouped in at least seven different genetic clusters, four of which are closely related to international antibiotic resistant PMEN strains. Transposon mutagenesis linked the CO₂-dependent growth defect of ST162 strains of the Spain9V-3 clonal complex to a genetic polymorphism in the murF cell wall ligase gene. This MurF V179A mutation also explained the capnophilic growth phenotype of the NorwayNT-42 related ST344 strains, but appeared to be lacking in the five other identified capnophilic genetic clusters. Finally, our experiments showed that acquisition of novel non-capnophilic murF variants by ST162 and ST344 strains is linked with acquisition of the penicillin binding protein 2B (pbp2B) gene by a hitchhiking effect.

Conclusions: We have revealed a novel functional polymorphism in the S. pneumoniae MurF protein that explains the CO₂-dependent growth restriction of a significant proportion of capnophilic pneumococcal strains. Variation in the MurF amino acid sequence of capnophilic strains by recombinalional events that are imposed by pneumococcal exposure to CO₂-poor environmental conditions could have major consequences for genome plasticity and evolution.
Poster No 102

REDUCTION OF OPAQUE PHASE VARIANT OF STREPTOCOCCUS PNEUMONIAE BY SUB-MIC LEVELS OF MACROLIDES

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Introduction: Streptococcus pneumoniae is a leading cause of acute otitis media (AOM). The pathogen undergoes spontaneous intra-strain phase variations in colony morphology depending on the capsular synthesis. Transparent variant is more efficient in colonizing the nasopharynx while the opaque variant exhibits greater virulence on systemic infections. However, there is little information about the pneumococcal phase variation after antimicrobial treatment. In this study, we evaluate the capsular phase variations of pneumococci treated by sub-MIC levels of macrolides.

Methods: S. pneumoniae L82016 strain, serotype 6B, were cultured with or without sub-MIC levels of clarithromycin. Pneumococcal cells at mid log phase growth were harvested and evaluated their growth, phase variation and amount of capsular polysaccharides. For evaluating pneumococcal phase variation, the serially diluted cell suspensions were plated on the Trypticase soy agar (TSA) plates with 6300 U/plate catalase. After the 16 h incubation at 37˚C with 5% CO₂ atmosphere, the pneumococcal phase variations were determined under the phase contrast microscopy.

Results: The sub-MIC levels of clarithromycin reduce the ratio of opaque phase variants without inhibition of pneumococcal growth. Sub-MIC levels of clarithromycin also reduced the amount of capsular polysaccharide of pneumococcal cells.

Conclusion: The current results suggest that sub-MIC level of clarithromycin reduce the opaque variant that is more highly adapted to middle ear rather than the transparent variant. The effect of clarithromycin against pneumococcal phase variation will suggest that clarithromycin would reduce the virulence of pneumococci.
MICROARRAY ANALYSIS OF GENETIC FACTORS RESPONSIBLE FOR A SUCCESSFUL CLONE CAUSING PNEUMOCOCCAL PNEUMONIA IN CHILDREN

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Background: The reasons why certain pneumococcal clones commonly associated with diseases remain not clear.

Methods: Serotype 14 sequence type (ST) 46 clone had been the largest one causing pneumococcal pneumonia in children in Taiwan before the introduction of 7-valent pneumococcal conjugate vaccine. We compared genomic variation between a strain (NTUH-p15) of serotype 14 ST46, and 3 non-clonal expansion pneumonic strains by microarray analysis to get insights into the mechanism of expansion of the specific clone in Taiwan.

Results: Microarray analysis revealed 7 clones that had at least 10-fold increases in DNA levels in the NTUH-p15 strain. PblB, a phage-encoded gene, was chosen for further study. The prevalence of pblB gene was significantly higher in pneumonic strains than in colonized strains (47/83 vs. 34/82; p=0.036). Comparison of the growth rate of the NTUH-P15 wild type strain and pblB mutant revealed lower growth for the pblB mutant. When the pblB mutant strain was complemented with the pblB gene, the growth rate could be restored. Introduction of the pblB gene into a pblB-negative serotype 19A ST320 strain, a globally emerging clone, facilitate the growth of pneumococci. In murine model of colonization and pneumonia, the pblB mutant was outcompeted by the NTUH-P15 wild type strain. Introduction of the pblB gene into the serotype 19A ST320 strain out-competed the serotype 19A wild type strain.

Discussion: The pblB gene promote the fitness of S. pneumoniae, thereby provide a competitive advantage for the pathogen in the ecological niche to successfully transmit and gain access to cause pneumonia.
PENICILLIN NON-SUSCEPTIBLE PNEUMOCOCCI IN ICELAND, 1995-2010

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PNSP were first identified in Iceland in 1988. Rapid increase followed by expansion of multi-resistant clone of serotype 6B (Spain6B-2) which peaked at 20% in 1993. Other serotypes/groups found at low rates 1988-1994 were 9, 14, 19 and 23. Our aim was to follow the temporal evolution of PNSP until vaccination started in 2011.

Our laboratory serves about 85% of the population. Information was collected on all pneumococci isolated from patients 1995-2010, invasive and non-invasive, (n=13,938). Penicillin MIC was measured for all oxacillin resistant isolates, PNSP were serotyped, PFGE done on selected isolates and MLST on selected PFGE clones.

In 1995 the PNSP rate was 18%, declining until 2000, when it was at its lowest at 11%, then increasing to 36% in 2010. Most strains were multi-resistant, thus resistance trends to erythromycin and tetracycline were similar. In 1995 68% of PNSP belonged to serotype 6B, declining to 5% in 2010. In 1995 8% of PNSP belonged to serogroup 19, declining to 1% in 1999, then increasing to 90% in 2010. Since 1999 most serogroup 19 isolates belong to two multi-resistant 19F clones (ST271 and ST1968).

At the beginning of the period the prevalence of a widespread multi-resistant clone of serotype 6B was at its peak then constantly declining. After 2000, when the prevalence of PNSP was lowest, a rapid increase in the prevalence of multi-resistant clones of serotype 19F occurred. The overall prevalence of PNSP in Iceland reflects the development in these serotypes.
PREVALENCE OF PILI IN PNEUMOCOCCI FROM CHILDREN ATTENDING DAY CARE CENTERS AND PATIENTS WITH INVASIVE DISEASES

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Pneumococcal pili are considered a potential virulence factor. Two major types of pili are coded for by pilus islets, PI-1 and PI-2. The aim was to assess their prevalence in pneumococci isolated from children attending day care centers (DCC) and patients with invasive diseases.

Nasopharyngeal isolates from children attending DCC in 2009 and 2010 (N=518) and all isolates from invasive diseases (n=223) during 1990-2009 were included. The presence of the islets was determined with PCR and pili belonging to PI-1 were grouped into clades I-III using clade-specific primers. PFGE was performed on all invasive isolates and selected carriage isolates. Mortality rate was calculated for deaths within four weeks of positive culture.

PI-1 was found in 33% of the nasopharyngeal isolates and 39% of the invasive isolates, and most often belonged to clade I. PI-1 was most prevalent in isolates of serotypes 4, 6B, 9V, 15 and 19F, both in carriage and invasive isolates. PI-2 was found in 9% of both nasopharyngeal and invasive isolates. In carriage isolates PI-2 was found in serotypes 6B, 11 and 19F and in invasive isolates in serotypes 1, 7F and 19F. PI-1 was found in only three of the ten most common invasive clones (26% of isolates), and PI-2 in two. Mortality for the first 4 weeks after diagnosis appeared unrelated to the presence of pili or the type of pili.

PI-1 was not as common in carriage as invasive disease. Pili were present in quarter of invasive isolates and not associated with higher mortality.
GENETIC DIVERSITY AMONGST MENINGITIS AND BACTEREMIA CAUSING PNEUMOCOCCI FROM MALAWI

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Background and aims: Pneumococci are highly recombinogenic nasopharyngeal commensals capable of invading a range of sterile sites, causing life threatening disease. The host and bacterial factors that determine whether a particular strain is capable of causing meningitis or bacteraemia are largely unknown. We hypothesised that there is a difference in genetic diversity between pneumococci associated with different disease outcomes. We therefore defined the core-genomes of bacteremic and meningitic isolates in Malawi to identify key genes that are essential for invasion.

Methods: 140 randomly sampled genomes (70 meningitis and 70 bacteremia isolates) were submitted to high-throughput Illumina sequencing. We clustered encoded genes using OrthoMCL to identify orthologs and define core-genes and then identified differences in the distribution of core-genes. To understand the strain structure, the phylogeny of the strains was re-constructed using a maximum likelihood approach.

Results: The core-genome of the entire pneumococcal dataset consisted of 1228 genes. Using a two-exponential model we found that meningitis-associated isolates had a larger core-genome than bacteremia-associated isolates (1338 vs. 1284; R² 99.7 and 99.8 respectively; p< 0.001). These highly conserved meningitis genes consisted largely of virulence factors and metabolic genes. This core genome difference is neither clonally driven nor the result of a dominant clonal-complex. HIV status did not influence genetic diversity.

Conclusions: Meningitis-causing pneumococcal isolates have a highly conserved complement of virulence factors and metabolic genes not present in all pneumococci isolated from blood. This genome analysis approach can be used to better understand pneumococcal biology and identify novel vaccine targets.
DISTINCTIVE EFFECTS ON DIVERSIFYING SELECTION BY TWO MECHANISMS OF IMMUNITY AGAINST STREPTOCOCCUS PNEUMONIAE

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Background and aims: Antigenic variation to evade host immunity has long been assumed to be a driving force of diversifying selection in pathogens. In pneumococcus, proteins that are the target of either antibody mediated immunity (B cell antigens) or CD4+ Th17 cell mediated immunity (T cell antigens) have been documented. The effector activity of the T cell immunity has been shown to act in trans, clearing co-colonizing pneumococci that do not bear the relevant antigen. It is thus unclear whether the T cell immunity allows benefit of antigenic variation and contributes to diversifying selection.

Methods: Immunized or control mice were challenged by a mixture of antigen-negative and antigen-positive pneumococcus. The competitive advantage of the antigen-negative strain in the presence of T cell immunity was evaluated. Pneumococcal genes showing signs of diversifying selection were systematically identified and the association of such genes with antigenicity for either B cells or CD4+ Th17 cells was examined.

Results: We found that T cell immunity almost equally reduced nasopharyngeal colonization by both the antigen-positive strain and the co-colonized, antigen-negative strain. B cell antigen genes were significantly more likely to be under diversifying selection than the T cell antigen genes, which were indistinguishable from nonantigens. Within the B cell antigens, epitopes recognized by human antibody showed stronger evidence of diversifying selection.

Conclusions: Th17 cell-mediated immunity against pneumococcal colonization creates only a small degree of competitive benefit from antigenic variation. Th17 cell-mediated immunity does not measurably contribute to diversifying selection of the targeted antigen, unlike antibody-mediated immunity.
THE DEVELOPMENT OF A GLOBAL PNEUMOCOCCAL STRAIN BANK

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Background and aims: Current pneumococcal conjugate vaccines approved for use in children are effective against strains included in the vaccines, but they do not cover all pneumococcal serotypes. The development of a protein vaccine effective against all strains representing the 90+ pneumococcal serotypes could provide broad protection to children worldwide. Access to diverse pneumococcal strains prevalent in the developing world is needed to ensure that new vaccines can protect populations in these regions. The aim of this project is to accumulate an extensive, diverse, well characterized, collection of pneumococcal strains causing invasive disease and pneumonia, recovered primarily from children in developing countries, but also including representative strains of established clonal lineages from industrialized regions.

Methods: Pneumococcal isolates included in the strain bank were serotyped by Quellung, screened for antimicrobial susceptibility by disk diffusion and genotyped by multilocus sequencing typing (MLST).

Results: We have currently characterized 4847 isolates, both from pneumococcal disease and carriage from 60 different countries, including 30 developing countries. Genotyping by MLST has identified 269 new alleles and 939 new sequence types within isolates included in the bank, primarily identified from developing countries and unique to those regions.

Conclusions: The isolate bank has significantly expanded the known pneumococcal population structure, particularly from developing world countries where little information has previously been available. As the isolate bank is maintained it will continue to provide a valuable source of well-annotated isolates for commercial and academic vaccine researchers as well as for other areas of research.
MOLECULAR DETERMINANTS OF RESISTANCE AMONG MACROLIDE NON-SUSCEPTIBLE S. PNEUMONIAE ISOLATES OTHER THAN ERM\textsubscript{B} OR MEF\textsubscript{A/E}

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Pneumococcal macrolide resistance is usually expressed as one of two phenotypes: the M phenotype, mediated by \textit{mefA/E} gene, or the MLS\textsubscript{A} phenotype, mediated by methylases encoded by \textit{erm} genes. Other mechanisms such as changes in ribosomal proteins L4 and L22, and mutations in 23S rRNA, have also been reported. We previously screened 4535 erythromycin-resistant (MIC≥1µg/ml) isolates submitted to CDC’s Active Bacterial Core surveillance program pre (1999) and post (2002-2007) PCV7 introduction, for the presence of \textit{ermB} and \textit{mefA/E} genes. Of these isolates, 41(0.88%) were negative for both \textit{ermB} and \textit{mefA/E}, or expressed a phenotype discordant with their genotype. The aim of this study was to identify molecular determinants associated with macrolide resistance in these isolates. PCR was performed on all 41 isolates for \textit{ermTR} and PCR products verified by sequencing. Mutations in the genes coding for L4 and L22 ribosomal proteins and for domain II and V of 23S rRNA were identified by PCR and DNA sequencing. Of these 41 isolates, 22(54%) had substitutions in the L4, 4(9.8%) had substitutions or insertions in the L22, 1(2%) had mutations in domain II of 23S, 18(44%) had mutations in domain V of 23S, and 1(2%) tested positive for the presence of \textit{ermTR}; 10 isolates had more than one mechanism, and 5 isolates remained unresolved. We identified some of the known mutations in the 23S rRNA, L4 and L22 proteins and although alternative mechanisms of macrolide resistance remain rare among \textit{S. pneumoniae}, we also identified new mutations and mutation combinations within these ribosomal genes.
ANALYSIS OF BETWEEN-STRAIN COMPETITION ACCOUNTING FOR IMPERFECT DETECTION OF MULTIPLE COLONIZATION

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Background: Vaccination-induced replacement in colonisation by pneumococcal non-vaccine-serotypes has occurred in several epidemiologic settings. It is not clear to what extent such replacement can be explained by competitive interactions between pneumococcal strains (serotypes). Inferences about between-strain competition from data about colonisation may be influenced by less-than perfect detection of multiple simultaneously colonising strains.

Materials and methods: We employed three longitudinal datasets of pneumococcal colonisation in infants (US Navajo, The Gambia) and toddlers (Denmark) to study between-strain competition. In all datasets special efforts were made to detect multiple colonisation. We characterised the strength of competition as the relative reduction in the expected time spent doubly colonised per acquisition, compared to if there was no competition, and the mode of competition as whether such reduction is due to reduced acquisition or enhanced clearance of double colonisation. In addition to the base-case assumption of 100% sensitivity to detect double colonisation, we also analysed the data assuming imperfect (50%) sensitivity.

Results and conclusions: In each dataset, the strength of between-strain competition was found to be strong (>80% with >0.95 probability) when assuming perfect detection sensitivity, and considerable (>50% with >0.95 probability) even when assuming imperfect sensitivity. Regardless of the assumed level of detection sensitivity, competition was always identified in acquisition. Competition in clearance was more difficult to identify and depended on the detection sensitivity. The findings of strong between-strain competition in general and competition in acquisition in particular are in agreement with the prompt and strong replacement in colonisation in a vaccinated population.
PNEUMOCOCCAL HISTIDINE TRIAD (PHT) PROTEINS AFFECT ATTACHMENT TO RESPIRATORY EPITHELIAL CELLS

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1Helsinki, Finland, 2Rixensart, Belgium

Background: PhtA, PhtB, PhtD and PhtE are conserved pneumococcal surface proteins, which have proven promising as vaccine candidates. Bacterial attachment to laminin is essential in binding to damaged epithelium and translocation of bacteria into the bloodstream. Transcriptional co-regulation of phtD with lmb suggests a functional link between PhtD and laminin binding protein. In this study we analyzed the role of Pht proteins in binding of pneumococcus to respiratory epithelial cells.

Methods: Live bacteria were incubated with human nasopharyngeal epithelial cells (Detroit-562) and lung epithelial cells (A549 and NCI-H292). The proportion of adherent bacteria was determined by plate-counting. Pneumococcal strains R36A, 2-D39, 3-43, 4-CDC and 19F-2737 with all Pht proteins deleted (Pht¯) were compared with their wild-type counterparts. Each analysis was repeated five times.

Results: Binding of Pht¯ to A549 was significantly reduced in 3-43 and 19F-2737, and slightly, though not significantly, in the 2-D39 and 4-CDC backgrounds (Student’s paired t-test). Binding of Pht¯ to NCI-H292 was significantly reduced in 2-D39 and 19F-2737 backgrounds. Binding of pneumococci to Detroit-562 did not differ between the Pht¯ and wild-type strains.

Conclusions: Based on the preliminary results it appears that lack of Pht proteins reduces binding of pneumococcus to lung, but not to nasopharyngeal epithelial cells. This is in concordance with mouse studies, where deletion of Pht proteins has been shown to attenuate the virulence of the infecting strain in pneumonia. The impact of Pht proteins on the adhesion process may vary with the genotype and capsular serotype of the strain.
MULTIPLE BACTERIAL SPECIES SIMULTANEOUSLY INFECT MANY CHILDREN WITH ACUTE OTITIS MEDIA WITH SPONTANEOUS OTORRHOEA (AOMSO) IN A PARTIALLY PCV-IMMUNISED POPULATION

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Background and aims: Interplay between respiratory viral infections and upper respiratory colonising bacteria and between bacterial species may be important in the aetiopathogenesis of acute otitis media, especially in the context of ecological changes induced by conjugate pneumococcal vaccines.

Methods: We prospectively studied children with AOMSO presenting to the emergency service at Coimbra Children's Hospital, Portugal during winter 2010-11. After consent, each child had demographic and clinical data recorded and swabs taken from the nose and aural discharge and stored at minus 80°C in STGG broth until batched analysis by semi-quantitative bacterial culture and PCR for respiratory viruses.

Results: 34 of 70 (49%) children (mean age 3.3y range 0.2-13.2y) cultured bacteria from aural discharge(B) of whom 12(35%) had two or more otopathogens (pneumococcus(Sp)-21, H. influenzae(Hi)-9, M. catarrhalis-14, S. pyogenes-4 isolates). Few (9/70;13%) were on antibiotics at the time of study and current antibiotic use or in previous month did not predict culture negative results. 56/68(82%) had received PCV(≥1dose). In 8/8 children with aural Hi and available nose swab, Sp was also present either in ear, nose or both. 36 viruses(V) were identified in 32 children (13FLU, 12ADV, 9RSV, 1HMPV, 1PIF3). Among 70 children there were 18 with V only, 20 B only, 14 V+B, 18 neither.

Conclusions: This study shows a higher frequency of mixed bacterial middle ear infection in children with AOMSO than previously reported. It also suggests Hi AOMSO is usually associated with Sp colonisation or infection. However detectable bacterial-viral co-infection occurred in only 20% of these children.
96-MLST, A NEW STREPTOCOCCUS PNEUMONIAE SEQUENCE TYPING SCHEMA IDENTIFIES GENETICALLY DISTINCT LINEAGES WITHIN MLST CLONAL COMPLEXES

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Background and aims: The accessibility of genome analysis tools has allowed the diffusion of a Streptococcus pneumoniae genotyping method based on the sequence of seven housekeeping gene fragments (Multi-Locus Sequence Typing). MLST related strains can be grouped into clonal complexes (CCs), ideally including genotypes with a common pattern of descent from a predicted founder. The growing number of identified Sequence Types (STs) has caused the breakdown of distinct clones into one CC (CC156, predicted founder ST156). CC156 includes strains with MLST allelic profiles differing in all seven genes and heterogeneous in terms of capsular types and pilus islet-1 presence and clade. Here, a new multi-locus typing schema (96-MLST) was applied on CC156 strains to identify distinct evolutionary lineages.

Methods: 96-MLST is based on the sequencing of ninety-six genomic loci, selected to be conserved over 39 complete pneumococcal genomes. 96-MLST was applied to representative CC156 strains.

Results: The discriminatory power of 96-MLST was found to be comparable to whole-genome sequencing, when tested on the available complete genomes. Based on 96-MLST, at least 5 lineages corresponding to formerly distinct CCs and homogeneous for capsular types and pilus islet-1 presence can be identified within the CC156. ST4945, whose identification was the primary cause of the CC156 breakdown, can be unequivocally assigned to one of the identified lineages by 96-MLST.

Conclusion: The identification of new STs has reduced the discriminatory power of the classical MLST approach. 96-MLST represents an alternative to whole-genome sequencing to identify genetically distinct evolutionary lineages within the pneumococcal population.
DIFFERENCES OF GENETIC DIVERSITY OF PNEUMOCOCCI ACCORDING TO CLINICAL PRESENTATION IN PATIENTS WITH INVASIVE DISEASE

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Background and aims: The objective of this study was to investigate the clonal composition and genetic diversity of S. pneumoniae isolates according to clinical presentation in children and adults with invasive disease.

Methods: All invasive isolates received in Catalonia Reference Molecular Laboratory for Invasive Pneumococci (CRMLIP) between January 2009-July 2011 were characterized by MLST and serotyping. Genetic diversity of isolates was estimated using the Simpson's index of diversity.

Results: A total of 1272 invasive pneumococci were received in CRMLIP. Clinical manifestation, serotype and clonal type were available for 1186 isolates and those were included in the study. Overall, 50 serotypes and 221 clonal types were detected. Table 1 shows the differences among rank order of serotypes, clonal type and index of genetic diversity according to clinical manifestation.

<table>
<thead>
<tr>
<th>Clinical Manifestation</th>
<th>Rank Order of serotypes (%)</th>
<th>Rank Order of Clonal Type MLST (%)</th>
<th>Simpson’s Index Diversity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empyema (n=108)</td>
<td>1(37%) 19A(16%)</td>
<td>306(32%) 1223(6%)</td>
<td>0.889 (0.835-0.940)</td>
</tr>
<tr>
<td>Pneumonia (n=771)</td>
<td>1(19%) 7F(13%)</td>
<td>306(15%) 191(13%)</td>
<td>0.953 (0.945-0.961)</td>
</tr>
<tr>
<td>Bacteremia (n=175)</td>
<td>19A(12%) 7F(11%)</td>
<td>191(9%) 156(5%)</td>
<td>0.979 (0.972-0.986)</td>
</tr>
<tr>
<td>Meningitis (n=104)</td>
<td>19A(10%) 3(9%)</td>
<td>191(8%) 180(5%)</td>
<td>0.985 (0.979-0.992)</td>
</tr>
<tr>
<td>Others (n=28)</td>
<td>1(11%) 19A(11%)</td>
<td>180(7%) 230(7%)</td>
<td>0.989 (0.977-1)</td>
</tr>
<tr>
<td>All Types (n=1186)</td>
<td>1(17%) 7F(11%)</td>
<td>306(13%) 191(11%)</td>
<td>0.961 (0.955-0.966)</td>
</tr>
</tbody>
</table>

Conclusion: Genetic diversity was different depending on clinical presentation. Strains causing pneumonia and especially empyema had lower genetic diversity than isolates causing other infections which suggests a selection process to cause lung-damage.
A NOVEL MECHANISM OF PNEUMOCOCCAL TOLERANCE TO NUTRIENT STARVATION BY CLPL UTILIZING BOTH ATP AND ADP AS ENERGY SOURCES

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ATP hydrolysis is a rate-limiting step in folding/refolding by heat shock proteins (HSPs). However, the function of HSPs in ATP depletion/starvation remains unknown. Here we show that ClpL induced by starvation in Streptococcus pneumoniae increased viability utilizing diverse nucleotides. During starvation, the ΔclpL mutant showed higher cell death and ATP accumulation than the wild type. When ClpL was under the control of a fucose promoter, ClpL levels were proportional to cell viability, but inversely proportional to ATP levels. Recombinant ClpL hydrolyzed not only ATP, but also other nucleotides including ADP and ATPγS. ClpL specifically bound to denatured rhodanese and green fluorescent protein, prevented aggregation of native rhodanese at high temperature, and disaggregated denatured rhodanese in the presence of either ATP or ADP. Thus, ClpL appears to confer pneumococci tolerance to nutrient starvation by utilizing stand-alone chaperone and broad-spectrum ATPase activities.
NON-TYPEABLE PNEUMOCOCCI WITH UNCONVENTIONAL CAPSULE GENE LOCI

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**Background:** Carriage of non-typeable (NT) pneumococci has increased since the use of PCV7 began. Although NT pneumococci from Europe were shown to have unconventional capsule gene loci (cps) with aliB-like ORF1 and ORF2 (named as aliC and aliD respectively), little is known about NT pneumococci from other geographic locations.

**Methods:** Fifty-two NT pneumococcal isolates were obtained in Brazil, Korea, and United States. The NT isolates were tested for cpsA, aliC, and aliD genes by PCR and were speciated by MLST. The DNA sequence of unconventional cps was determined.

**Results:** The NT pneumococci could be divided into three null capsule clades (NCC), 11 NCC1 (aliC/-aliD-), 32 NCC2 (aliC+/aliD+), and 9 NCC3 (aliC/-aliD+). NCC1 isolates have a novel gene [named pneumococcal surface protein K (pspK)] in their cps. pspK encodes a protein (~500aa) with a signal peptide, a long alpha-helical region containing a “YPT” motif, and an LPxTG motif. The cps (7.3 kb in the center) of one Brazilian NCC2 isolate was 100% identical to that of a European isolate (AY653211). Some NCC3 isolates have a large part of cps that is 92% similar to S. mitis strain B6 cps (FN568063). MLST analysis grouped all NCC1 strains and most of NCC2 strains as pneumococci but all NCC3 isolates as non-pneumococci.

**Conclusions:** NT pneumococci with unconventional cps can be divided into three clades based on genes aliC and aliD, and a new gene, pspK. Discovery of NT pneumococci with identical cps loci in different locations suggests their infectiousness. NCC3 isolates are often non-pneumococci.
Pneumococcus is a leading cause of invasive bacterial disease. In this study, the role of a sucrose phosphorylase (GtfA) in Streptococcus pneumoniae was investigated. The gtfA mutant was constructed in S. pneumoniae using serotype 2 strain (D39) by inserting ermB cassette, and effect of purified GtfA enzyme on virulence was determined. The gtfA mutant showed significantly reduced cytotoxicity and increased antibiotic resistance than those of the wild type in vitro. Since bacterial adhesion is primarily mediated by interaction between bacterial ligand and host cells, effect of gtfA on adherence was examined in vivo. The gtfA mutant showed significantly decreased adherence than the wild type. And, gtfA overexpressing revertant showed significantly higher cytotoxicity and increased adherence than the wild type. Moreover, when the promoter of gtfA gene is replaced by fucose-driven promoter (F-gtfA) and F-gtfA strain was exposed to L-fucose, adherence was dose-dependently increased to A549 cells. Interestingly, gtfA mRNA expression was significantly induced when D39 was exposed to FBS. These results indicate that the GtfA is required for S. pneumoniae virulence as well as attachment to lung cells, and might have an important role in the pathogenicity of S. pneumoniae by regulating adherence.
A CHEMICAL APPROACH TO TARGET VALIDATION OF S. PNEUMONIAE ENDA FOR CONTROL OF PNEUMOCOCCAL INFECTION

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Background and aims: From initial colonization to establishment of disease, Streptococcus pneumoniae utilizes numerous strategies to evade and overcome components of the human immune response. Understanding the mechanisms by which S. pneumoniae evade host defenses and cause invasive infection exposes new avenues for therapeutic options. One unexplored target is the pneumococcal surface endonuclease, EndA, which is involved in at least two activities that enable the pathogen to evade immune defenses. EndA’s ability to degrade the DNA backbone of neutrophil extracellular traps (NETs) allows pneumococci to physically escape an immune response, whereas EndA’s role in transformation plays a central part in the adaptation of the pathogen to host defenses. Given that both of these activities promote invasive infection, we hypothesize that EndA facilitates S. pneumoniae infection and pathogenesis by its cardinal roles in NET escape, transformation, or a combination of both.

Methods: Our work utilizes small-molecule inhibitors of EndA as a means to elucidate the roles of EndA as a mediator of pneumococcal pathogenesis. We have developed novel high-throughput screening assays to identify and characterize in vitro inhibitors of EndA.

Results: We have confirmed a set of small-molecules and tested their ability to inhibit NET degradation or transformation in microbiological assays.

Conclusions: Functionally selective inhibitors of EndA are being exploited to tease apart the relative importance of EndA’s activities in S. pneumoniae infection. Moreover, the identification of small-molecule inhibitors of EndA offers great potential for validating EndA as a druggable target and developing adjunctive therapies for the control of pneumococcal infection.
WHOLE-GENOME SEQUENCE OF STREPTOCOCCUS PSEUDOPNEUMONIAE ISOLATE IS7493

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\textit{Streptococcus pseudopneumoniae} is a recently designated species belonging to the viridans group streptococci (VGS). Despite having >99\% 16S rRNA gene identity with \textit{Streptococcus pneumoniae} and \textit{Streptococcus mitis}, it exhibits DNA-DNA hybridization values of < 70\% and is phenotypically distinct. Unlike \textit{S. pneumoniae}, \textit{S. pseudopneumoniae} is optochin resistant in the presence of 5\% CO\textsubscript{2}, is bile insoluble, and lacks the pneumococcal capsule. Its pathogenic potential and the underlying genetic identity are not well characterized in relation to those of \textit{S. pneumoniae} or the commensal \textit{S. mitis}.

To define genomic differences and establish genetic targets for clear identification of these closely related organisms, whole-genome shotgun sequencing (331,392 raw reads) of a representative \textit{S. pseudopneumoniae} patient isolate, IS7493, and initial assembly were performed with the Genome Sequencer FLX (Roche, Basel, Switzerland). Isolate IS7493 was obtained from the sputum of a patient with human immunodeficiency virus (HIV) who had documented pneumonia. Details of the comparative genomic characterization will be presented.

We conclude that \textit{S. pseudopneumoniae} is a hybrid strain between \textit{S. pneumoniae} and \textit{S. mitis} lacking key virulence factors and therefore more akin to a commensal. However, due to the propensity for horizontal gene transfer, \textit{S. pseudopneumoniae} may be able to acquire virulence genes and become pathogenic in humans.

This study is published in part in J Bacteriol November 2011, p. 6102-6103, Vol. 193, No. 21
THIOL PEROXIDASE IS AN ESSENTIAL COMPONENT IN OXYGENATED ENVIRONMENTS OF STREPTOCOCCUS PNEUMONIAE

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Background and aims: Streptococcus pneumoniae produces exceptionally high levels of H₂O₂. Since S. pneumoniae lacks catalase, the question of how it defends itself against hazardous H₂O₂ levels is of critical importance. The psa locus encodes an ABC Mn²⁺-permease complex (psaBCA) immediately upstream of psaD coding for a putative thiol peroxidase (tpx). This study explored the role of Tpx in pneumococcal survival and the mechanism controlling Tpx expression.

Methods: In-vitro Tpx activity was assayed using a thioredoxin-thioredoxin reductase system. Tpx-cysteine redox state was determined by the thiol trapping method and mass spectrometry. In-vivo studies used a mouse model of pneumococcal disease that develops after intranasal infection.

Results: The in-vitro assay showed that Tpx reduced H₂O₂ efficiently. Thiol trapping and mass spectrometry established that Tpx-cysteine undergoes selective oxidation under conditions where H₂O₂ is formed. Challenging pneumococci with H₂O₂ resulted in tpx upregulation, while psaBCA expression was oppositely affected, implying that psa expression is regulated by H₂O₂. Tpx was found to be localized in both the cell wall and the cytoplasm, suggesting its dual role in H₂O₂ sensing and scavenging. In-vivo experiments revealed higher tpx levels in tissues isolated from aerated niches of infected mice (nasopharynx and lungs), compared to the blood. In addition, a Δtpx-mutant showed a significant decrease in its survival following intranasal challenge.

Conclusions: psaD encodes a functional Tpx, involved in H₂O₂ scavenging. Tpx expression is modulated, both in-vivo and in-vitro, in accordance with H₂O₂ levels, reinforcing the conclusion that it has a role in maintaining homeostatic peroxide levels.
RECENT CHANGES IN THE PROPORTION OF PNEUMOCOCCI CARRYING PILI-ISLANDS: CONSEQUENCES OF VACCINE USE AND FUTURE PROSPECTS

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Background and aims: Two pilus-like structures (PI-1 and PI-2) have been recognized in pneumococci and suggested as potential vaccine targets. We demonstrated previously that PI-1 was a clonal property of *S. pneumoniae* and that only 27% of the invasive strains carried the *rlrA* islet. The introduction of the 7-valent conjugate vaccine (PCV7) resulted in changes in serotype frequency that can also affect pili prevalence. The aim of this study was to evaluate the distribution of the pilus islets after PCV7 availability.

Methods: A collection of 623 invasive isolates recovered from children and adolescents (< 18 years) after PCV7 introduction (2003-2009) was characterized in terms of PI-1 and PI-2 presence. The results were analyzed in terms of pilus islet association with antibiotic resistance, serotype, PFGE and MLST.

Results: Overall, 49% of the invasive pneumococci presented one of the pilus islets. A high correspondence between serotype, PFGE and presence and type of pilus was observed (Wallace coefficient >0.800). The *rlrA* islet was identified in 15.6% of the strains, most of them expressing serotypes 6B, 9V, 14, 19A and 19F. PI-2 islet was found in 37.6% of the invasive strains and was mainly associated with serotypes 1 and 7F.

Conclusion: A decrease in the presence of the *rlrA* islet was observed after PCV7 availability. This change is associated with the decrease of vaccine serotypes since the majority of the strains carrying PI-1 expressed vaccine serotypes. In contrast, PI-2 islet became more prevalent due to the predominance of serotypes 1 and 7F.
IMPACT OF COMPETENCE STIMULATING PEPTIDE VARIANTS ON GENETIC EXCHANGE AND BIOFILM FORMATION

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Background and aims: In Streptococcus pneumoniae the competence-stimulating peptide, encoded by the comC gene, controls competence development and influences biofilm growth. In the present study, we explored the influence of pherotype, defined by the two major comC allelic variants (comC1 and comC2), on biofilm development and recombination efficiency.

Methods: The biofilm forming capacity and the intra and inter-pherotype genetic exchange of isogenic strains differing in the comC allele were evaluated. The biofilm forming capacity of a genetically diverse set of invasive isolates was determined.

Results: Isolates recovered from infections and presenting comC1 show a higher capacity to form biofilms. Biofilm architecture evaluated by confocal laser scanning microscopy showed that isogenic strains carrying comC1 form biofilms that are denser and 35% thicker than those carrying the comC2 allele. Evaluation of competence in liquid culture and in biofilms of isogenic strains of different pherotypes showed that strains carrying the comC1 allele yielded more transformants than those carrying the comC2 allele. In contrast to mixed planktonic growth, within mixed biofilms inter-pherotype genetic exchange is less frequent than that occurring between bacteria of the same pherotype.

Conclusion: Since biofilms are a major bacterial lifestyle, these observations may explain the genetic differentiation between populations with different pherotypes reported previously. Considering that biofilms have been associated with colonization our results suggest that strains carrying the comC1 allele may be more transmissible and more efficient at persisting in carriage. Both effects may help explain the higher prevalence of the comC1 allele in the pneumococcal population.
INTERACTION BETWEEN BACTERIAL RESPIRATORY PATHOGENS IN THE NASOPHARYNX OF AMERINDIANS PANARE CHILDREN AND ADULTS FROM BOLIVAR STATE, VENEZUELA


Background and aims: Nasopharyngeal colonization is the first step for invasive disease due to *Streptococcus pneumoniae*. Studies have described a negative association between colonization with *S. pneumoniae* and *S. aureus*. Data concerning interactions between *S. pneumoniae* and the other pathogens in the nasopharynx are limited.

Methods: Surveys of nasopharyngeal carriage of 556 Panare Amerindians; 390 children (0 to 15 years) and 163 adults (mean age 28 years) for *S. pneumoniae*, *H. influenzae*, *M. catarrhalis* and *S. aureus* were conducted in ten rural Amerindians Panare communities between 2004 and 2011. Standard microbiological methods were used.

Results: Pneumococcal carriage prevalence was 59% among children and 13.5% among adults. Carriage of *H. influenzae*, *M. catarrhalis* and *S. aureus* among children and adults was 7.4% and 2.5%, 19% and 1.8% and 20% and 30%, respectively. Multivariate analysis showed positive associations between *S. pneumoniae* and *M. catarrhalis* in children (Odd Ratio [OR] 2.13; 95% CI 1.19, 3.82) while adult pneumococcal carriage was associated with co-colonization of *H. influenzae* (OR 7.10; 95% CI 0.95, 13.22). No significant association between *S. aureus* and *S. pneumoniae* was found.

Conclusions: Colonization and hence the possible risk of invasive disease by *S. pneumoniae* in Amerindians Panare is increased in children colonized with *M. catarrhalis* and adults with *H. influenzae*. Understanding of upper respiratory tract microbial ecology will contribute to improvement and development of treatment and preventions strategies in high risk populations.
TN6078: A NOVEL MOBILE GENETIC ELEMENT DISCOVERED IN PNEUMOCOCCAL OUTBREAK STRAINS HAS GEOGRAPHY-SPECIFIC INVASIVE DISEASE ASSOCIATIONS

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**Background and aims:** Genomic accessory regions (ARs) of the pneumococcal chromosome encode strain-specific genes. Nearly 50 ARs are known, some of which may contribute to virulence, but little is known about AR functions in general and mechanisms underlying their polymorphism. Our aim was to characterize ARs from a serotype 12F, clonal complex 1527 outbreak strain from Alaska.

**Methods:** Suppressive subtractive hybridization was used to identify sequences unique to the outbreak strain in comparison to two diverse reference strains. Multiplex PCR was used to screen for the presence/absence of ARs in cross-sectional samples of 281 pneumococci (148 invasive, 133 carriage) from Alaska and 265 pneumococci (132 invasive, 133 carriage) from Israel.

**Results:** One AR was statistically over-represented from invasive isolates in Alaska; it was positive in 43 invasive and 15 carriage isolates (\(P=0.0002\)). Comparisons of full-length sequences from this AR revealed flanking BOX element components and 146bp direct repeats with intervening open reading frames. The direct repeats suggested that the AR might encode a novel mobile genetic element. This hypothesis was confirmed by the detection of extrachromosomal circular forms and hybrid coupling sequences from excised molecules. The element was named Tn6078. Interestingly, the element was not over-represented from invasive isolates in Israel; it was positive in 12 invasive and 15 carriage isolates (\(P=0.685\)).

**Conclusions:** These results provide new insight into the nature and polymorphism of one AR and reveal geographic differences in the association of this AR with disease, possibly due to geographic differences in clone distribution and gene flow.
CAPSULAR SWITCHING AS A STRATEGY TO INCREASE VIRULENCE AND ERODE VACCINE EFFECTIVENESS

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Background and aims: Capsular switch events, in which Streptococcus pneumoniae acquires a new capsule operon, have been reported with increasing frequency following implementation of conjugate polysaccharide vaccines. We hypothesized that compared to donor strains, the newly emerged recombinants may exhibit enhanced virulence thus increasing the likelihood of disease due to serotype replacement. We compared the virulence of wild type (wt) serotype 6A (vaccine serotype, new capsule recipient), and 6C strains (non-vaccine serotype, capsule donor) with that of 6A-C mutants (6A chassis with 6C capsule) in a chinchilla model of experimental otitis media (EOM).

Methods: Invasive serotype 6A strain was transformed into two independently constructed mutants using DNA of 6C strains recovered from asymptomatic nasopharyngeal colonization. Proportion of animals developing culture-positive EOM in a chinchilla model was assessed to evaluate strain virulence. Deposition of Complement (C3) on bacteria surface was also evaluated in vitro.

Results: Both 6A-C mutants were more virulent than 6Cwt donor strains, but less than the 6Awt in EOM. EOM developed in 13/22 (59%) and in 15/22 (68%) ears challenged with first and second 6A-C mutants, compared to 5/40 (12.5%) challenged with 6Cwt [p< 0.001 for both], EOM developed in 20/20 (100%) ears challenged with 6Awt. C3 deposition on either mutant was lower compared to 6Cwt, but higher than on 6Awt strain consistent with the observed virulence in EOM.

Conclusions: Capsular transformants 6A-C demonstrate reduced C3 binding and increased virulence for EOM compared to 6C wt demonstrating the potential of capsular switch events to result in new phenotypes.
Poster No 126

PRELIMINARY RESULTS FROM A LONGITUDINAL STUDY OF THE NASOPHARYNGEAL MICROBIOTA OF INFANTS ON THE THAILAND-BURMA BORDER

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Background: *Streptococcus pneumoniae* does not live in isolation in the nasopharynx, but little is known about the early establishment of the nasopharyngeal microbiota and its changes over time and through periods of illness and antimicrobial treatment. Between 2007-2010, 511 nasopharyngeal swabs were collected from 20 infants in Maela, Thailand. Samples were taken monthly, from birth to 24 months of age, with additional swabs if the infant was diagnosed with pneumonia according to WHO clinical criteria.

Methods: DNA was extracted from the swabs using a modification of the FastDNA Spin Kit For Soil protocol and barcoded for 16S rRNA gene sequencing on the 454 platform. The dataset was analysed using Pyrotagger and Mothur. Here we describe preliminary observations from the first 182 swabs and 8 controls.

Results: Controls allowed the identification of kit contaminants which were screened out of downstream analysis. The available data cover the first 5-12 months of life for 20 infants. We found differences in the nasopharyngeal microbiota with age, for example in the relative abundance of *Staphylococcus aureus* and *Streptococcus pyogenes*. Swabs before, during and after episodes of respiratory disease show changes in the proportions and presence/absence of different OTUs. One 10-month-old infant diagnosed with pneumonia was treated with amoxicillin; a swab 8 days later shows a 90-fold increase in relative abundance of the streptococcal OTU compared to illness and pre-illness samples. The following month colonisation dropped below detectable levels, presumably as a result of further antimicrobial use for urinary tract and skin infections.
THE ROLE OF CAPSULAR POLYSACCHARIDE IN BIOFILM FORMATION AND COLONIZATION BY STREPTOCOCCUS PNEUMONIAE

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Background: The capsular polysaccharide (capsule) of S. pneumoniae is a critical virulence factor required for passage through mucus in the nasopharynx, colonization, and preventing opsonophagocytosis. However, acapsular strains exhibit an enhanced capacity to form biofilms in vitro, suggesting that capsule impedes this process by masking underlying surface-associated molecules involved in adherence. Additionally, biofilm-defective mutants are attenuated in colonizing the nasopharynx, implicating that these two processes are linked. These data suggest that capsule expression is tightly regulated with a high level to evade host surveillance and lower levels to promote adherence to the epithelium and colonization. We therefore hypothesize that after passing through the mucus, S. pneumoniae downregulates capsule to expose critical surface molecules, facilitating the formation of biofilms, and host colonization.

Methods: We constructed a TIGR4 strain with a constitutively active promoter (P_cat) and determined gene expression and capsule levels by qPCR and SDS-PAGE analysis. Competition experiments in the nasopharynx colonization, lung and blood infection models determined fitness of the P_cat mutant relative to wild-type. Additionally, adherence to A549 epithelial cells was examined.

Results: The P_cat mutant expresses significantly higher levels of total amounts of capsule, adherence to A549 cells was strongly reduced and P_cat demonstrated a severe fitness defect in all models of infection.

Conclusions: To establish a successful infection and cause disease, the expression of capsule is tightly regulated. The inability to modulate capsule levels leads to a severe defect in pneumococcal adherence to epithelial cells, colonization of the host nasopharynx and infection of the lung and blood.
INTERRELATIONSHIP OF STREPTOCOCCUS PNEUMONIAE WITH HAEMOPHILUS INFLUENZAE AND STAPHYLOCOCCUS AUREUS

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Background and aims: High prevalence of bacterial nasopharyngeal (NP) co-infections has been reported in children. We examined the interaction of Haemophilus influenzae, Staphylococcus aureus and Streptococcus pneumoniae NP colonization on the risk of acquisition of a non-colonizing bacterium.

Methods: Pneumococcal-vaccine naive children had NP swabs done at 1.6, 2.5, 3.5, 4.5, 7.4, 9.5, 12.5, 16.2 and 24.2 months of age. Swabs were cultured for S.pneumoniae, H.influenzae and S.aureus using standard microbiologic methods. Multivariate Generalized Estimating Equation models were used to quantify the subsequent risk of acquiring a particular bacterium if previously colonized by another.

Results: The observed probability of co-occurrence was higher than expected and not random. The interrelationships of the three bacteria, estimated by odds ratios (OR) and 95% confidence intervals (CI), are presented (KEY: Vaccine-serotypes (VT)=4,6B,9V,14,18C,19F and 23F). The interrelationships were bidirectional but differed quantitatively on occasion. Carriage of VT and S.aureus increased the risk of H.influenzae colonization (OR 2.92, 95% CI 1.68-5.08).

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Any pneumococcus</th>
<th>7-valent vaccine serotypes(VT)</th>
<th>Non-vaccine serotypes</th>
<th>H.influenzae</th>
<th>S.aureus</th>
<th>H.influenzae and S.aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any pneumococcus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.57-2.72</td>
<td>0.51-0.90</td>
<td>1.01-2.99 1.73</td>
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<tr>
<td>7-valent vaccine serotypes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.23-2.34</td>
<td>0.29-0.54</td>
<td>1.19-3.86 2.14</td>
</tr>
<tr>
<td>Non-vaccine serotypes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.29-2.43</td>
<td>0.34-0.61</td>
<td>1.67-5.59 3.06</td>
</tr>
<tr>
<td>H.influenzae</td>
<td>(1.65-2.79) 2.15</td>
<td>(1.12-2.01) 1.50</td>
<td>(1.02-1.84) 1.37</td>
<td>-</td>
<td>(0.38-0.97) 0.61</td>
<td>-</td>
</tr>
<tr>
<td>S.aureus</td>
<td>(0.50-0.91) 0.68</td>
<td>(0.47-0.99) 0.68</td>
<td>(0.54-1.08) 0.77</td>
<td>(0.36-0.99) 0.60</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Conclusions: These data underscore the synergy between S.pneumoniae and H.influenzae and the antagonistic effect between S.aureus and S.pneumoniae colonization. Monitoring the impact of pneumococcal immunization on the ecology of NP colonization is warranted.
A PHYLOCHIP APPROACH TO STUDY EAR DISCHARGE MICROBIOMES IN ACUTE AND CHRONIC SUPPURATIVE OTITIS MEDIA IN AUSTRALIAN ABORIGINAL CHILDREN

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Background and aims: Chronic suppurative otitis media (CSOM) is diagnosed in ~20% of young Aboriginal children in remote communities in Australia. Culture data from ear discharge are limited, but suggest a respiratory bacterial aetiology at initial perforation (eg. pneumococcus and non-typeable Haemophilus influenzae) and an increased proportion of other pathogens during progression to CSOM (eg. Pseudomonas aeruginosa). Our primary aim is to use PhyloChip to examine and compare the microbiomes of ear discharge specimens from children at initial perforation (acute otitis media with perforation, AOMwP) and after progression to CSOM.

Methods: Using PhyloChip, a 16S rDNA microarray platform, the primary outcome is the comparison of microbiomes of ear discharge specimens from children with AOMwP (n=10) and CSOM (n=10). The secondary outcome is the analysis of paired nasopharyngeal and unrelated auditory canal swabs.

Results: Initial pilot PhyloChip data on 5 ear discharge swabs from AOMwP identified 67 phyla. The core microbiome common to all swabs contained 12 phyla and 25 families. These were typical of aerobic and anaerobic otitis media pathogens, other human flora, and environmental bacteria. PhyloChip analysis detected numerous phyla and families not previously considered in otitis media, including Archaea.

Conclusions: We will present an approach to studying the microbiomic signatures in ear discharge to identify critical constituent bacteria in polymicrobial communities associated with disease progression from AOMwP to CSOM. The diversity of ear discharge microbiomes may help to explain CSOM treatment failure in high-risk populations. It could support therapies aimed at full pathogen eradication from the middle ear.
LACK OF CORRELATION BETWEEN PNEUMOCOCCAL AND STAPHYLOCOCCUS AUREUS COLONIZATION IN A POPULATION WITH WIDESPREAD USE OF PCV7

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Background and aims: An antagonism between colonization by pneumococcal PCV7 serotypes (VT) and Staphylococcus aureus has been documented in unvaccinated populations. We aimed to evaluate if there had been changes in this antagonism in the era of PCV7.

Methods: Nasopharyngeal samples were collected from pre-school children in two periods, before PCV7 was available (1996-1998, n=2,111) and when around 70% of the target population had received at least one vaccine dose (2006-2007, and 2009, n=2,100). Pneumococcal and S. aureus isolation and pneumococcal serotyping were done by standard procedures. Statistical analysis was used to compare data from the two periods.

Results: Pneumococcal carriage ranged from 60% to 70% and was not affected by introduction of PCV7. Carriage of S. aureus increased from 14% to 17% (p< 0.001) and simultaneous carriage of both species increased from 6% to 10% (p< 0.001). A negative association between the two species was observed in the pre-PCV7 period (p=0.046), particularly among VT and S. aureus (p=0.044; OR 0.69, 95% CI 0.50-0.94). No correlation was found in the PCV7 period between the two species (p=0.052) or between S. aureus and VT or non-VT (p=0.077).

Conclusion: The antagonism between pneumococcus and S. aureus seems to have disappeared in the pneumococcal population as a whole or when divided into VT and non-VT. Further investigation is needed to clarify which specific serotypes or genetic properties contribute to the observed scenario.

Emergence of Multidrug Resistant and Vaccine Replacement Serotypes of Streptococcus Pneumoniae: Comparative Genomic Analysis of 150 Isolates

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Background and aims: Multidrug resistant and vaccine replacement pneumococcal serotypes are emerging. Comparative genomic analysis of vaccine and non-vaccine serotypes from an 18-year longitudinal and geographically defined pneumococcal population enables mapping of the evolutionary history of emerging serotypes and multidrug resistant vaccine escape clones.

Methods: 150 isolates, collected in Atlanta, GA as part of ABCs surveillance in the Georgia Emerging Infections Program, and encompassing 23 serotypes, 82 MLST types, resistance to 13 antibiotics, and 11 disease outcomes, were sequenced using 454 (>20x coverage). Genome comparisons were conducted with Sybil (http://sybil.sourceforge.net/).

Results: We are characterizing the population’s genomic variability under antibiotic pressure and/or vaccine pressure. We are also defining the armamentarium of pneumococcal resistance elements and mobile elements involved in resistance dissemination. We discovered that strain GA17545, displaying high-level efflux-mediated resistance to macrolides, harbors the MEGA resistance element and a Clostridium difficile-like transposon that together are replacing pneumococcal pathogenicity island 1 (PPI-1). PPI-1 was also replaced by MEGA in strain GA16242. PPI-1 was reported as required for pathogenicity and is typically conserved among pneumococcal isolates, yet GA17545 and GA16242 were isolated from cases of meningitis and bacteremia, respectively.

Conclusion: Genome comparisons among this collection of isolates reveal new insights into determinants of antibiotic resistance and virulence.

This project was funded with federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services under contract number HHSN272200900007C and grant number 5R01A1070829. Pneumococcal surveillance and isolate collection was funded by CDC.
LIMITED IMPACT OF PHEROTYPE ON PNEUMOCOCCI CO-COLONIZING THE NASOPHARYNX

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Background and aim: A recent study by Vestrheim et. al showed that pneumococcal pherotypes often co-exist in the nasopharynx and suggested that the impact of pherotype-mediated fratricide on competition is limited. Here we determined the pherotype of pneumococcal strains from Portuguese co-colonized samples.

Methods: Nasopharyngeal samples (n=158) containing two strains selected according to distinct colony morphology and posterior confirmation of distinct capsular types were selected from cross-sectional studies conducted between 2001 and 2010. Pherotypes were assigned by multiplex PCR detection of CSP-1 or CSP-2. Co-colonization of pneumococcal pherotypes was assumed to occur independently and the multiplicative rule for independent events [Prob(A and B)=Prob(A)xProb(B)] was used to estimate the distribution of concordant or discordant pherotypes, based on the overall distribution of pherotypes in the co-colonized samples.

Results: Of the 366 co-colonizing isolates 63.7% were of pherotype CSP-1 (n=233) and 36.3% were of pherotype CSP-2 (n=133). The estimated and observed proportions of concordant (53.8% and 52.5%, respectively) and discordant (46.2% and 47.5%, respectively) pherotypes in the co-colonized samples were compared and the differences were not significant (p=0.9, $\chi^2$ test).

Conclusions: Our results show that pherotypes CSP-1 and CSP-2 are dominant among colonizing isolates. The estimated and observed pherotype distributions of the co-colonizing strains were not significantly different, corroborating the hypothesis that pherotype-mediated fratricide does not seem to be an important mechanism of within-host competition. Further studies are needed to clarify this issue.

Background and aim: Potential pathogenic bacteria as well as a wide variety of viruses can be detected at high rates in the nasopharynx of asymptomatic young children. Interactions between microbial species are assumed to be important in the pathogenesis of respiratory infections. We aimed to investigate co-occurrence patterns between viruses and bacteria at the nasopharyngeal niche of healthy children.

Methods: We randomly selected 986 nasopharyngeal samples of 6- to 24-month-old children that had participated in a previous study. We tested for the presence of 20 common respiratory viruses by real-time PCR. S. pneumoniae, H. influenzae, M. catarrhalis and S. aureus were isolated by conventional culture methods. Information regarding risk factors were obtained by questionnaires. We performed multivariate logistic regression analyses followed by partial correlation analysis to identify the overall pattern of potential interactions.

Findings: We observed significant associations between S. pneumoniae and the other bacteria, several viruses and risk factors (Table 1). In addition, we observed several other significant bacterial-bacterial, viral-bacterial and viral-viral associations.

Interpretation: We showed that both bacteria and respiratory viruses are abundantly present in the nasopharynx of otherwise healthy young children. Furthermore, we demonstrated distinct associations between bacteria, viruses and environmental factors. This sheds more light on the complexity and dynamics of the microbial composition of this specific niche.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>S. pneumoniae in presence of covariate N (%)</th>
<th>S. pneumoniae in absence of covariate N (%)</th>
<th>aOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. influenzae</td>
<td>357 (72)</td>
<td>250 (51)</td>
<td>1.62 (1.20–2.18)</td>
</tr>
<tr>
<td>M. catarrhalis</td>
<td>485 (68)</td>
<td>122 (44)</td>
<td>1.81 (1.31–2.50)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>34 (40)</td>
<td>573 (64)</td>
<td>0.59 (0.36–0.98)</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>272 (74)</td>
<td>335 (54)</td>
<td>1.77 (1.20–2.18)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>46 (79)</td>
<td>561 (61)</td>
<td>2.01 (1.00–4.03)</td>
</tr>
<tr>
<td>Enterovirus b</td>
<td>101 (79)</td>
<td>408 (58)</td>
<td>1.89 (1.13–3.16)</td>
</tr>
<tr>
<td>Day care attendance</td>
<td>424 (68)</td>
<td>183 (50)</td>
<td>1.77 (1.28–2.45)</td>
</tr>
<tr>
<td>Presence of siblings</td>
<td>371 (69)</td>
<td>236 (52)</td>
<td>2.28 (1.67–3.11)</td>
</tr>
<tr>
<td>Recent use of antibiotics</td>
<td>33 (36)</td>
<td>574 (64)</td>
<td>0.26 (0.16–0.42)</td>
</tr>
<tr>
<td>PCV7 vaccination</td>
<td>281 (58)</td>
<td>326 (65)</td>
<td>0.70 (0.52–0.93)</td>
</tr>
</tbody>
</table>

a Adjusted for the additional variables age, presence of WU polyomavirus and human coronavirus.

b Enterovirus was analyzed in a subset of 831 samples. Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; N, number; PCV7, 7-valent pneumococcal conjugate vaccine.
A HIGH-THROUGHPUT MUTANT ANALYSIS APPROACH COMBINED WITH NETWORK ANALYSIS YIELDS NEW INSIGHTS AND INTERVENTION STRATEGIES FOR FIGHTING BACTERIAL INFECTIONS

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Background: The unknown function of many pathogen genes is hampering our ability to design new approaches and strategies to battle infectious diseases. This makes developing high-throughput, cost-effective approaches to matching genes with phenotypes imperative.

Methods: By combining simultaneous competition growth profiles of tens-of-thousands of mutants using our recently developed massively parallel sequencing method Tn-seq with network analysis, we derive a high quality dataset rich in discovery.

Results: Probing fitness of complex mutant libraries in 18 different in vitro conditions and in three different in vivo disease states yielded thousands of phenotypes that allowed us to study conditional gene essentiality, discover leads for gene-function and drug-action and match in vitro stress conditions with in vivo disease states. Here we highlight the discovery of new genes required for the growth on different carbon sources, transformation and DNA repair, and coping with iron or oxidative stress. We describe the discovery of a dual role ABC-transporter (withstand physiological stress and provide antibiotic resistance), and we show how an antibiotic triggers a secondary response in DNA repair that, when interfered with, leads to a more efficacious antibiotic. Lastly we show how regulatory modules can be uncovered through mapping genome-wide genetic interaction profiles and how this led us to identify a pathway that can be targeted to prevent infection.

Conclusions: We show that Tn-seq combined with genetic interaction profiling and network analysis can lead from uncovering basic biology to direct application by the identification of molecular pathways as direct targets, and novel intervention strategies.
EARLY BIOFILMS PRODUCED BY STREPTOCOCCUS PNEUMONIAE STRAIN D39 ARE REGULATED BY THE LUXS/AI-2 QUORUM SENSING SYSTEM

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Streptococcus pneumoniae is the leading cause of mortality in children worldwide and forms highly organized biofilms in the nasopharynx, lungs and middle ear mucosa. The LuxS/AI-2-controlled quorum sensing (QS) system has recently been implicated in virulence and persistence in the nasopharynx, but its role in biofilms had not been studied. This study shows that this QS system plays a major role in controlling S. pneumoniae biofilm formation. Our results demonstrate that the luxS gene is encoded by invasive isolates and normal flora strains in a region that contains genes involved in division and cell wall biosynthesis. The luxS gene was maximally transcribed, as a monocistronic message, in the early-mid log phase of growth and this coincides with the appearance of early biofilms. Demonstrating the role of the LuxS system in regulating S. pneumoniae biofilms, at 24 h post-inoculation two different D39ΔluxS mutants produced ~80% less biofilm biomass than the wt strain D39. The complementing strains encoding either the luxS in a plasmid or integrated as a single copy into the genome restored biofilm levels to that of the wt. Moreover, a soluble factor secreted by wt strain D39 or purified Al-2 restored the biofilm phenotype of D39ΔluxS. Our results also demonstrate that this system regulates, during the early-mid log phase of growth, levels of lytA transcript, an autolysin previously implicated in biofilm production, and also the transcript of ply, pneumococcal pneumolysin. In conclusion, the luxS-controlled QS system is a key regulator of early biofilm formation by S. pneumoniae strain D39.
DIVERSITY OF CAPSULAL GENES OF PNEUMOCOCCAL SEROTYPE 6A, 6B, 6C, AND 6D ISOLATES FROM KOREAN CHILDREN


Background and aims: Evolution of capsular genes of the newly-identified serotype 6D Streptococcus pneumoniae strains have not been fully studied yet in contrast to other serotypes of serogroup 6. We have investigated the diversity and genetic relatedness of serogroup 6 isolates focusing on 6D.

Methods: Multilocus sequence typing (MLST) was performed to investigate 160 isolates of serogroup 6 from clinical specimens in children during 1991-2010. Three of capsular polysaccharide genes (cps), wciP, wzy, and wzx were analyzed for 74 isolates which include 6A (n=20), 6B (n=20), 6C (n=10) and 6D (n=24). The cps profiles of these genes were subjected to neighbor-joining analysis in MEGA5.

Results: Three STs, ST3171 (n=4), ST189 (n=4) and ST282 (n=16) were found in 6D isolates. The latter two STs were closely related to clonal complex (CC) 81, which clustered with serotype 6A isolates. Serotypes 6B and 6C frequently shared the same ST or CC with 6A. Analysis of cps genes of each serotype showed 2-4 profiles based on allele designation. In the phylogenetic analysis of cps genes, all profiles of serotype 6D were found in 6B clade when previously published cps profiles were taken together. Serotype 6C isolates showed most distinct clade because of wzy allele which had a constant 6 base pair deletion within, but the profiles were close to 6A clade.

Conclusions: MLST analysis showed that serotype 6D isolates displayed close relatedness with 6A. The cps profiles of 6D suggested capsular genes of 6D were originated from 6B.
NASOPHARYNGEAL PNEUMOCOCCAL COLONIZATION DENSITY AS A MARKER FOR DISEASE SEVERITY IN ADULT PNEUMONIA

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Background/aims: High genomic load of pneumococcus from blood or cerebrospinal fluid has been associated with increased mortality. We aimed to analyze whether nasopharyngeal (NP) colonization density in pneumonia patients is associated with markers of disease severity or poor outcome.

Methods: Quantitative lytA rtPCR was performed on NP swabs in adults hospitalized for pneumonia at Chris Hani Baragwanath Hospital, Soweto, South Africa. Pneumonia etiology was considered pneumococcal if any of sputum culture or Gram stain, urinary pneumococcal C-polysaccharide-based antigen, blood culture or whole blood lytA real-time (rt) PCR revealed pneumococcus. Infection- and prognosis-related biomarkers were measured using commercially available assays (KRYPTOR) for midregional pro-adrenomedullin (MR-proADM), midregional pro atrial natriuretic peptide (MR-proANP) and copeptin and for procalcitonin (PCT Liasensitive).

Results: Among patients with X-ray-confirmed pneumonia, mean NP colonization densities increased with increasing CURB65 scores (p=0.02). In HIV-infected patients with pneumococcal pneumonia, NP colonization density was higher among non-survivors (n=8) than survivors (n=76; 7.7 vs. 6.1 log cfu/ml, respectively; p=0.02) and among those who had pneumococcus identified from blood cultures and/or by whole blood lytA rtPCR than those with non-bacteremic pneumococcal pneumonia (6.6 vs. 5.6 log cfu/ml, p=0.04). NP colonization density correlated positively with PCT (Spearman correlation coefficient r=0.37, p<0.0001), ProADM (r=0.40, p=0.008), copeptin (r=0.30, p=0.01), but not with ProANP or CRP.

Conclusions: NP colonization density serves not only as a diagnostic but also as a severity marker for pneumococcal pneumonia in adults. It correlates with mortality, clinical severity scores, prognostic biomarkers and biomarkers which indicate the likelihood of a bacterial infection.
Poster No 138

REDUCTION IN INFANT PNEUMOCOCCAL CARRIAGE AT 7 MONTHS OF AGE FOLLOWING PNEUMOCOCCAL POLYSACCHARIDE VACCINATION IN PREGNANCY: "PNEUMUM" RANDOMISED CONTROLLED TRIAL

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Background and aims: The ear infection burden and early onset pneumococcal colonisation among Indigenous Australian children is well documented. We conducted a maternal pneumococcal vaccine trial and report vaccine efficacy (VE) against nasopharyngeal pneumococcal carriage of 23vPPV types at seven months (a priori co-primary outcome) and post-hoc analyses of VE against other carriage types.

Methods: Pregnant women were randomised to receive pneumococcal polysaccharide vaccine (23vPPV) during the third trimester, at delivery, or seven months post delivery. Four colonies were serotyped for each positive swab using standard methods whilst blinded to the allocation status.

Results: The consent rate was 50% (313/632), enrolment rate 73% (227/313) and retention rate through to seven months post delivery for the carriage endpoint was 87% (197/227). Infant carriage of 23vPPV types at seven months was 23% (15/66) among infants of unvaccinated mothers (controls) versus 12% (8/67) for infants of women vaccinated during pregnancy (VE 47%; 95%CI 15-76%, p=0.11). By including 6A as a "vaccine-related" serotype in a post-hoc analysis, VE was 59% (95%CI 12-80%, p=0.02), whilst VE against carriage of any serotype was 35% (95%CI 3-57%, p=0.04). There was no discernible difference in carriage between infants of mothers vaccinated at delivery to those of the control group (data not shown).

Conclusions: Although failing to reach statistical significance in the a priori analysis, reductions in carriage of 23vPPV related types offer encouragement for further investigation of pneumococcal vaccination in pregnancy as does the strong community support for the trial. Results of the ear assessments are pending.
Poster No 139

INCREASED RISK OF INVASIVE PNEUMOCOCCAL DISEASE (IPD) IN SELECTED RISK GROUPS

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Background and aims: It is well known that patients with many immunological, malignant and pulmonary diseases are overrepresented in studies of IPD but few studies have estimated how much the risk is increased in different diseases.

Methods: Retrospective review of case records from 2977 patients living in Västra Götaland region (mean population 1,512,233) with isolates of pneumococci from blood, CSF, synovial, pleural, pericardial, peritoneal fluid 1996-2008. Information on incidence of selected diagnoses in the general population was obtained from recent Swedish publications and official statistics.

Results: The risk was significantly increased in myeloma (154-fold), in chronic lymphatic leukemia (29-fold), lung cancer (22-fold), HIV (16-fold) SLE (14-fold), asplenia (14-fold), rheumatoid arthritis (4.9-fold), COPD (3.5-fold) and diabetes mellitus (3.2-fold).

Conclusions: There is a considerable risk for patients with certain hematological malignancies, solid tumors and other chronic immunocompromising diseases to acquire IPD.
Poster No 140

BRONCHOALVEOLAR INTERLEUKIN-1 BETA: A MARKER OF BACTERIAL BURDEN IN CRITICALLY ILL CHILDREN WITH PNEUMOCOCCAL PNEUMONIA

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Objective: To assess the relationship between concentrations of bronchoalveolar cytokines and bacterial burden in children with a presumptive diagnosis of Pneumococcal pneumonia.

Interventions: According to the time course of Pneumococcal pneumonia at the time of study with bronchoalveolar lavage, 121 children were divided into two subgroups: referral (n=61), and treated (n=60) Pneumococcal pneumonia. Bronchoalveolar lavage was performed in the most abnormal area on chest radiograph by fiberoptic bronchoscope. The concentrations of bronchoalveolar lavage interleukin-1 beta were measured.

Measurements and main results: 84 patients had a positive bacterial culture (bronchoalveolar lavage > or = 10 colony-forming units/mL), and made up 82% of pathogens recovered at high concentrations. The concentrations of bronchoalveolar lavage interleukin-1 beta were 204.2 +/- 15.7 and 38.2 +/- 12.6 pg/mL (mean +/- se) in the children with positive and negative bacterial culture, respectively (p < .001). Bronchoalveolar lavage interleukin-1 beta was significantly higher in the children with Pneumococcal pneumonia (p < 0.001), compared with values in patients without these features. The relationship between bacterial load and concentrations of bronchoalveolar lavage interleukin-1 beta was very strong in the children with referral.

Conclusions: Since the concentration of bronchoalveolar lavage interleukin-1 beta was correlated with bacterial burden in the alveoli, it may be a marker for progressive and ongoing inflammation in critically ill children who have not responded to Pneumococcal pneumonia therapy and who have persistence of bacteria in the lung.
Poster No 141

PNEUMOCOCCI CAUSING MULTIPLE EPISODES OF ACUTE EXACERBATIONS IN PATIENTS WITH CHRONIC RESPIRATORY DISEASE (AECRD): GENETIC CHARACTERIZATION OF PERSISTENT STRAINS

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\textsuperscript{1}Hospitalet de Llobregat, \textsuperscript{2}Madrid, \textsuperscript{3}Majadahonda, Spain, \textsuperscript{4}Oxford, UK

Objectives: To analyze the MLST genotype and sequences of PBP and QRDRs of persistent pneumococcal isolates causing AECRD over an extended period of time.

Methods: Fifty pneumococci recovered from sputum samples (1995-2010) from 13 adult patients with \textgreek{\geq}3 AECRD caused by isolates with the same serotype and PFGE pattern were studied. MLST loci, PBP genes (\textit{pbp2X, pbp1A, pbp2B}) and the QRDRs of \textit{parC, parE} and \textit{gyrA} were PCR-amplified and sequenced.

Results: The average time between the first and last episode was 582 days (SD = 362). Before PCV7 introduction in 2001, serotype 9V was the most frequently recovered (4 of 5 patients) whereas seven serotypes were detected among isolates from the remaining 8 patients (2002-2010). All but two patients received multiple courses of \textit{\beta}-lactam treatment and all 50 strains were resistant to penicillin and other antimicrobial groups; however, the PBP sequences were stable over time, apart from one variable nucleotide in \textit{pbp2x} observed among isolates from three patients. No changes in the MLST genotype or the penicillin MIC were observed. In contrast, 7 of 11 patients treated with FQs had FQ-resistant isolates. In three patients, the initially FQ-susceptible persistent strain developed resistance (due to mutations in \textit{parC} and \textit{gyrA}) after FQ therapy, and in the remaining four patients, the persistent strain was FQ-resistant since the first episode.

Conclusions: QRDR changes involved in FQ resistance were frequently observed in persistent strains after FQ treatment; however, the PBP sequences and MLST genotypes of these strains were stable over a long period of time.
ACTIVE SURVEILLANCE OF VIRAL AND BACTERIAL RESPIRATORY TRACT INFECTIONS IN SUBJECTS ≥ 60 YEARS: IMPLICATIONS FOR THE IMMUNIZATION STRATEGIES


Background and aims: The epidemiology and etiological diagnosis of the upper and lower respiratory tract infections are partially unclear, particularly in the outpatient setting. To fill this gap, an epidemiological survey was performed in a cohort of subjects aged ≥60 years presenting a clinical picture of Influenza Like Illness (ILI) and Community-Acquired Pneumonia (CAP).

Methods: 2,551 subjects were monitored with active surveillance from November 2010 to April 2011. Biological samples (oropharyngeal swab and blood) were collected from patients presenting ILI and CAP. A "recapture" of the clinical cases was performed through monthly phone calls. The detection of the major etiologic agents (12 viruses and 6 bacteria, including Streptococcus pneumoniae) was performed by multiplex gene amplification. Serological and molecular techniques were used for characterization of influenza and Streptococcus pneumoniae.

Results and conclusion: Influenza and pneumococcal vaccination coverage rates were 71% and 6%, respectively. Cumulative incidence of ILI was 1.76% (95%CI: 1.24-2.29) and increased to 4.23% (95%CI: 3.44-5.03) after "recapture". The same was observed for the incidence of CAP that resulted 0.12% (95%CI: 0-0.25) and 0.27% (95%CI: 0.07-0.48) after "recapture". A swab was obtained from 47 (1.8%, 95%CI: 1.3-2.3) patients with ILI: 38.3% of the cases were attributable to influenza, mainly the A/H1N1 strain. No cases of bacteremic CAP were detected, while 25% of swabed patients with ILI resulted carriers of Streptococcus pneumoniae (12.5%) and Haemophilus influenzae (12.5%). Effectiveness of influenza vaccine in preventing laboratory-confirmed ILI was 65.3% (95%CI: 13.1-86.2) in a scenario of good matching between circulating and vaccinal strains.
Understanding of host genetic factors that contribute to susceptibility/resistance to pneumococcal disease may pave the way for targeting therapy or prophylaxis to high-risk individuals as well as helping to unravel the complexities of the immune response to *S. pneumoniae*-infection.

Inbred mouse strains allow exploration of the influence of genetic elements on disease resistance in an otherwise uniform background. Previous work identified two mouse strains with differing responses to type 2 pneumococcal pneumonia and bacteraemia: the highly resistant BALB/c and the susceptible CBA/Ca mouse strain. Genomic analysis of F₂ BALB/c x CBA mice revealed a single major linkage with survival and bacteraemia on proximal chromosome 7, within a region of approximately 8cM near to *D7Mit77*. This quantitative trait locus (QTL) is associated with susceptibility to IPD and was named *Spir1* (*Streptococcus pneumoniae* infection resistance 1).

Congenic mice with a 99.9% BALB/c genome into which the CBA/Ca *Spir1* locus was inserted showed susceptibility to IPD, confirming that genetic elements within *Spir1* influence disease-resistance. The presentation will show genetic and susceptibility data collected to narrow the QTL region to less than 0.5 cM (fewer than 5 genes) in order to identify the causative gene(s) of susceptibility and subsequently to investigate how the identified gene(s) influences the pathology of pneumococcal disease.
PNEUMOCOCCAL CARRIAGE IS UNAFFECTED BY HIV EXPOSURE IN MALAWIAN INFANTS UP TO SIX MONTHS OLD

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Background and aims: HIV exposed and uninfected infants are believed to be at high risk of pneumonia and S.pneumoniae is the major contributor to this. Carriage prevalence of S.pneumoniae is higher in HIV-infected African adults, thus we hypothesised that HIV exposed children are colonised earlier and more frequently than their HIV-unexposed counterparts.

Methods: Using a matched birth cohort study we measured naso-pharyngeal carriage of S.pneumoniae in infants born to HIV infected and uninfected mothers in a rural setting in Malawi. For each HIV-exposed uninfected baby up to three HIV unexposed babies are recruited matched on family size and birth date. Swabs were collected from the mother and infant every 4 weeks starting at 6 weeks of age. Pneumococci were looked for using conventional methods and serotyped using latex agglutination and the Quellung method.

Results: 54 HIV-exposed and 132 non-exposed mother/infants were recruited. Overall HIV+ mothers had detectable carriage nearly twice as often as the HIV- mothers, OR 1.9 [95%CI1.0-3.5] throughout the year but with seasonal variation. Despite this there was no difference in time to first colonisation (median time to colonisation 10 weeks for both groups of infants) or incidence of pneumococcal colonisation during the first 6 months by HIV exposure status, (44.1 and 40.4 incident carriage events per 100 child-months in the HIV non exposed and exposed infants respectively).

Conclusion: Despite higher pneumococcal carriage in HIV+ mothers, there is no evidence of increased carriage in HIV-exposed uninfected offspring, although carriage incidence is high in all infants in this region.
HIV EXPOSURE AND RISK OF PNEUMONIA IN MALAWIAN CHILDREN UNDER 5 YEARS OLD
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**Background and aims:** HIV-exposed children are believed to be at high risk of pneumonia and may be a target group for enhanced pneumococcal protection. We have studied under 5 children (U5C) attending a rural health centre nested within an extensive demographic and HIV serosurvey (~35,000 population) to measure the impact of HIV exposure on respiratory disease.

**Methods:** An observational cohort study. U5C presenting to a rural health clinic with fever are diagnosed and managed following set protocols. They are linked to their mother’s HIV status through the demographic information system. Child years of observation are calculated for all children within the demographic survey and disaggregated by HIV exposure status.

**Results:** Between May 2009 and April 2011 there were 4497 attendances of which 2048 were diagnosed as pneumonia according to Integrated Management of Childhood Illness (IMCI) criteria, 6 (0.3%) had a positive blood culture for *S.pneumoniae*. In a random subset 17 (9.5%) of 179 had evidence of radiographic pneumonia. Using the first year study data (HIV exposure data complete for this period), rates of IMCI pneumonia were similar in infants, 167 [95% CI 148-187] and 150 [87-259] per 1000 child years in unexposed and exposed respectively. During the 2nd and 3rd and the 4th and 5th years of life there was a non-significant increase in rates in the HIV exposed group, 117 [106-129] vs 178 [112-283] and 30 [24-36] vs 42 [13-131] respectively.

**Conclusion:** To date there is evidence of a modest increase in rates of respiratory disease in the HIV-exposed group in the post-infant period.
THE ROLE OF ACUTE VIRAL INFECTIONS ON PNEUMOCOCCAL ACQUISITION IN YOUNG ANDEAN CHILDREN

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Background/aims: Animal-models suggest viral infections favor acquisition of specific pneumococcal serotypes. We assessed whether viral infections increased pneumococcal acquisition in young children.

Methods: We enrolled a prospective cohort of young Andean children. Through weekly visits, we identified acute respiratory illness (ARI) and obtained nasal swabs for viral detection using molecular methods. Monthly nasopharyngeal (NP) samples were obtained to detect pneumococcal colonization. We assessed whether an ARI occurring between NP samples (termed episodes) increased the risk of pneumococcal acquisition.

Results: From May-November 2009, 527 children were evaluated. Less than 3% and 5% of them had received influenza and pneumococcal conjugate vaccine, respectively. There were 286 pneumococcal acquisition episodes (new serotype compared to prior sample) and 492 non-acquisition episodes (no colonization or colonization with the same serotype as the prior sample). Although acquisition of any new pneumococcal serotype did not increase following any ARI compared with no ARI (odds ratio [OR]: 1.26, 95% CI: 0.93-1.71), exposure to any ARI increased the risk of acquisition of the most common colonizing serogroups: 6, 14, 19, 23 and nontypables, significantly (OR: 1.50 [1.01-2.24]). The odds of acquisition of any pneumococcal serotype tended to be higher following an influenza-ARI (1.52 [0.55-4.19]) or a multiviral-ARI (3.82 [0.70-20.93]), whereas exposure to rhinovirus, human metapneumovirus and respiratory syncytial virus were not associated with acquisition (1.13 [0.74-1.71], 0.94 [0.18-4.99] and 0.84[0.18-3.90], respectively).

Conclusion: This ongoing study lends support to the hypothesis that ARIs selectively facilitate acquisition of common-colonizing pneumococcal serotypes. Influenza and multi-viral infections appear to facilitate pneumococcal acquisition in this population.
PRELIMINARY ANALYSIS OF NASOPHARYNGEAL CARRIAGE AND LOWER AIRWAY INFECTION IN PCV7 AND PHID-CV10 VACCINATED AUSTRALIAN INDIGENOUS CHILDREN WITH BRONCHIECTASIS

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**Background and aims:** Indigenous children in Australia’s Northern Territory have the world’s highest recorded rate of bronchiectasis. *Streptococcus pneumoniae* and nontypeable *Haemophilus influenzae* (NTHi) dominate the upper and lower airway bacteriology in these children. PCV7 was introduced for Indigenous children in 2001 and replaced by PHID-CV10 in 2009 and PCV13 in 2011. We investigated the impact of vaccination on nasopharyngeal (NP) carriage and lower airway infection (LAI) in Indigenous children with bronchiectasis.

**Methods:** Children presenting for high-resolution computed tomography (HRCT) and bronchoscopy were eligible for enrolment. NP swabs and bronchoalveolar lavage (BAL) fluid were collected under anaesthetic, stored at -80°C and processed as previously described. LAI was defined as >10⁴ cfu/mL BAL fluid. Data were recorded on vaccines administered since birth.

**Results:** From 2007 to 2011, 104 Indigenous children aged 5.2 to 154.6 (median 28.5) months with HRCT-confirmed bronchiectasis were enrolled; 64 (62%) were male. While 88 children had received ≥2 doses of PCV7 (82 had ≥3 doses), only 13 had ≥2 doses of PHID-CV10 as primary course. *S. pneumoniae* NP carriage in PCV7 children was 42%; PHID-CV10 was 23% (overall 37%). NTHi carriage was 53% and 38% respectively (overall 50%). *S. pneumoniae* LAI in PCV7 children was 17%; PHID-CV10 was 31% (overall 16%). NTHi LAI was 33% and 23% respectively (overall 31%). Low numbers precluded any significance.

**Conclusions:** There is currently insufficient data to draw conclusions regarding vaccine impact. Studies are ongoing to assess the impact of pneumococcal conjugate vaccines on respiratory microbiology and clinical outcomes in this population.
IMPACT OF RECENT ANTIBIOTICS ON NASOPHARYNGEAL CARRIAGE AND LOWER AIRWAY INFECTION IN AUSTRALIAN INDIGENOUS CHILDREN WITH NON-CYSTIC FIBROSIS BRONCHIECTASIS

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Background and aims: Australia’s Indigenous children have the world’s highest bronchiectasis rates. Many clinicians prescribe long-term azithromycin to reduce exacerbations despite a lack of effectiveness and safety (antibiotic resistance) data. We report the impact of macrolides (primarily azithromycin) and beta-lactam antibiotics on nasopharyngeal (NP) bacterial colonization, lower airway infection (LAI) and resistance.

Methods: Children presenting for high-resolution computed tomography (HRCT) and bronchoscopy were eligible for enrolment. NP swabs and bronchoalveolar lavage (BAL) fluid were collected under anaesthetic, stored at -80°C and processed as described previously. LAI was defined as >10^4 cfu/mL BAL fluid. Data were recorded on antibiotics administered in the 2 weeks before bronchoscopy.

Results: From 2007 to 2011, 104 Indigenous children aged 5.2 to 154.6 (median 28.5) months with HRCT-confirmed bronchiectasis were enrolled; 39 had received macrolide and 26 beta-lactam antibiotics (4 received both). Recent macrolide use was associated with reduced NP carriage of Streptococcus pneumoniae (Risk Ratio [RR] 0.44, 95% Confidence Interval [CI] 0.25, 0.76), nontypeable Haemophilus influenzae (RR 0.70, 95%CI 0.44, 1.10) and Moraxella catarrhalis (RR 0.47, 95%CI 0.23, 0.96). Recent beta-lactam use was associated with reduced carriage of S. pneumoniae (RR 0.18, 95%CI 0.06, 0.54). No significant reduction in LAI was recorded for any pathogen in children who received macrolides or beta-lactams. Children given macrolides were significantly more likely to carry (RR 3.75, 95%CI 1.24, 11.4) and be infected by (RR 6.67, 95%CI 1.49, 29.8) azithromycin-resistant S. pneumoniae.

Conclusions: Clinical benefits of azithromycin therapy need to be assessed alongside potential harms of antibiotic resistance.
PNUEMOCOCCAL PROSTHETIC JOINT INFECTIONS - A DISEASE OF UNKNOWNS

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Background and aims: Pneumococcal prosthetic joint infections (PPJI) are uncommon but cause substantial morbidity requiring prolonged antimicrobial therapy, may require surgical removal and can be fatal. We describe an elderly patient with a prosthetic knee infection due to serotype 4 (ST 206) *Streptococcus pneumoniae* treated successfully with antimicrobial therapy without surgical removal of the prosthesis and reviewed the management and laboratory results published for other PPJIs.

Methods: A review of literature regarding PPJIs over the last 40 years identified only 23 other cases.

Results: None of the reviewed cases included serotyping or molecular typing data for the pneumococci causing infection. Only 2 cases included the minimum inhibitory concentrations (MICs) to antibiotics used for treatment. Where a preceding pneumococcal illness was described, a pneumonic manifestation always featured and occurred within a month of the manifestation of prosthesis infection. There is no consensus regarding the route or duration of antibiotic treatment for PPJI and no consensus regarding whether to retain or remove the infected prosthesis (removed in 9 cases, retained in 6 and not documented in the others).

Conclusions: Most published cases of PPJI are written from the perspective of its orthopaedic surgical management and little is known regarding the molecular epidemiology of the causative pneumococcal isolates. Further reports focused on the nature of the causative organism may help to elucidate if there is a tissue tropism of serotypes involved in the pathogenesis of PPJI or why a preceding chest infection appears to be a common feature of these infections.
MANAGING A PERFECT STORM - HOST PREDISPOSITION, RECURRENT INVASIVE PNEUMOCOCCAL DISEASE (IPD), UNRESPONSIVENESS TO VACCINE AND A UNIQUE PNEUMOCOCCUS

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Background and aims: A non-neutropenic patient with low grade non-Hodgkin’s lymphoma and hypogammaglobulinaemia recovered from pneumococcal pneumonia and septicaemia (serotype 7F; ST191) subsequent to influenza A H1N1 (2009). Both infections were potentially vaccine preventable. The patient then developed pneumococcal meningitis due to a serotype 35F with a unique sequence type (ST7004) which was not vaccine preventable. Patient management was influenced by host predisposition to IPD, antibiotic intolerance and poor response to polysaccharide pneumococcal vaccine.

Methods: Case report, MLST database and literature review.

Results: Indirect immunofluorescence with anti-human immunoglobulin confirmed a poor or intermediate response to serotypes in Pneumovax II. Prophylactic clarithromycin was initiated. Immunoglobulin transfusions were also commenced.

ST7004 is a single locus variant of ST1635 which has been seen associated with the serotype 35F capsule in England. The spi gene in ST7004, which differentiates it from ST1635, is the same spi gene present in ST191 which could have arisen from the first disease episode. On review of the serotype 35F isolates within the MLST database (n=66), 16 (24%) were from carriage, 11 (17%) from cerebrospinal fluid and 10 (15%) from blood with no diagnosis stated.

Conclusions: We explore the difficulties managing recurrent IPD when host factors predispose to IPD and unresponsiveness to vaccine. Potentially an environment was created where horizontal gene transfer could occur, creating a new sequence type and facilitating IPD with a non-vaccine serotype. Reports in the MLST database suggest an association with carriage by serotype 35F, but when IPD occurs, it may predispose to meningitis.
IMPAIRED INFLUENZA-SPECIFIC CD4+ T-CELL IMMUNITY IS ASSOCIATED WITH AN INCREASE IN PNEUMOCOCCAL CARRIAGE IN HIV-INFECTED AFRICAN ADULTS

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Background: HIV-infected adults are at an increased risk of invasive pneumococcal disease (IPD). Impaired influenza-specific immunity might also predispose HIV-infected patients to pneumococcal carriage. We have therefore measured the impact of HIV on naturally-acquired influenza-specific T-cell immunity and pneumococcal carriage in Malawian adults.

Methods: 46 HIV-uninfected and 48 HIV-infected adults commencing Highly-Active Anti-Retroviral Therapy (HAART) (sampled pre-HAART, 6months and 12months) were recruited at the Queen Elizabeth Central Hospital, Malawi. We measured pneumococcal carriage using standard microbiological techniques. We also measured influenza-specific CD4+ T-cell immune responses in PBMCs by CFSE proliferation assay. Results are given as medians with IQR.

Results: The pneumococcal carriage rates were 16% in HIV-uninfected adults, 27% in HIV-infected patients pre-HAART, 40% at 6months and 41% at 12months on HAART. The proliferative influenza-specific responses in HIV-infected patients at 6months following HAART were similar with those detected at baseline (3.3%[0.6-8.3] vs 2.5%[0.0-10.2]; p>0.05), and did not differ with those detected at 12months (3.3%[0.6-8.3] vs 1.9%[1.1-7.4]; p>0.05). The proliferative influenza-specific responses at 12months following HAART were lower than HIV-uninfected individuals (1.9%[1.1-7.4] vs 14.7%[7.2-23.2]; p<0.04).

Conclusion: The study demonstrates that the high level of pneumococcal carriage and the impaired influenza-specific CD4 T-cell immunity in HIV-infected adults did not reconstitute even after 12months on HAART. Together these might contribute to the increased pneumococcal invasive disease seen in HIV-infected patients even after HAART.
THE IMPACT OF HIV ON NASOPHARYNGEAL CARRIAGE OF STREPTOCOCCUS PNEUMONIAE IN CHILDREN FROM MALAWI

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1Blantyre, Malawi, 2Liverpool, 3London, UK, 4Karonga, Malawi, 5Cambridge, UK

High HIV sero-prevalence has been observed previously among Malawian children with invasive pneumococcal disease (IPD). However, the association between HIV infection and pneumococcal carriage in our context remains unknown. Our study investigated the impact of HIV on both carriage serotype distribution and prevalence of multiple serotype carriage. From a wider cohort of 200 samples, a subset of nasopharyngeal samples were collected from children who were either HIV negative, HIV positive or with unknown HIV status, including both longitudinal and cross-sectional data sets. To determine serotypes present, purified DNA was analysed using a molecular serotyping microarray. Initial results defined 43 distinct serotypes, 11 of which were covered by the 13-valent pneumococcal conjugate vaccine (PCV13). Based on this initial data, PCV13 coverage was 49% and multiple carriage rates were high at 47%, with co-colonised samples expressing two (34%), three (12%) or four capsular types (1%). Multiple carriage was slightly higher in HIV negative than HIV positive children but this did not reach significance. The impact of anti-retroviral therapy (ART) on pneumococcal carriage was determined by analysing longitudinal samples collected pre and up to 12 months post ART. The data indicates multiple carriage increases post ART following immune reconstitution. High rate of multiple carriage promotes horizontal gene transfer between strains, consequently affecting vaccine efficacy and also the spread of antimicrobial resistance. In Malawi, there is significant heterogeneity of non-vaccine serotypes in circulation and so in our setting such population diversity may provide fertile ground for serotype replacement disease post vaccination.
Poster No 153

CLINICAL IMPACT OF PRECEDING RESPIRATORY VIRAL INFECTION IN PATIENTS WITH PNEUMOCOCCAL PNEUMONIA


Background: Aim of this study is to investigate clinical significance of preceding or simultaneous respiratory viral infection (RVI) on the patients with pneumococcal pneumonia.

Methods: A case-control study was conducted at a teaching hospital from January 2008 through October 2011. Study subjects included adult patients with pneumococcal pneumonia who had S. pneumoniae isolation from clinical specimens and received virus culture or (real-time) reverse transcriptase PCR tests for RVI (n=178). A 1:4 matched cases with positive RVI test (n=24) and controls (n=96) were randomly selected. A multivariate logistic regression analysis was performed to identify clinical severity.

Results: During the study period, a total of 649 episodes of S. pneumoniae isolation were recorded. Of 178 patients with pneumococcal pneumonia who received tests for RVI, 24 cases (13.5%) showed preceding or simultaneous RVI; influenza A virus (n=10), rhinoviruses (4), respiratory syncytial virus (4), parainfluenza viruses (3), influenza B virus (2), coronavirus (1) and metapneumovirus (1). Four patients showed pneumococcal bacteremia. There was no significant difference in demographic and laboratory characteristics, comorbid illness, mortality and antibiotic therapy between case and controls. However, clinical severity based on the pneumonia severity index (PSI) and APACHE II score were significantly higher in the case patients. In the multivariate logistic regression analysis, higher APACHE II score remained significantly associated with preceding RVI (odds ratio 1.22, 95% confidence interval 1.11-1.34).

Conclusions: This study suggests that preceding or simultaneous respiratory viral infection in the patients with pneumococcal pneumonia might be a factor influencing on the clinical severity of pneumococcal pneumonia.
Poster No 154

A CASE OF EPIDURAL ABSCESSES AS A COMPLICATION OF ACUTE MASTOIDITIS CAUSED BY STREPTOCOCCUS PNEUMONIAE IN A 35 MONTH-OLD CHILD

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Background and aims: Neurological complications of acute mastoiditis are rare but can be life threatening. Their presentation may be masked by the use of antibiotics. We report a case of acute otitis media progressing to acute mastoiditis, epidural abscess formation and lateral sinus thrombosis in a child.

Methods: A 35 month-old female child who was unimmunized with pneumococcal conjugate vaccine presented with intermittent fever for 2 weeks and otalgia for 3 days. On the 4th day of hospitalization, she had left postauricular swelling with redness. Both otomastoiditis, an abscess formation in overlying soft tissue and an epidural abscess in left posterior fossa, lateral sinus thrombosis were revealed by means of a computed tomography and magnetic resonance imaging. However, she had not any symptoms or signs of increased intracranial pressure.

Results: Mastoidectomy, surgical drainage of epidural abscess were performed. Sixteen milliliters volume’s white to yellow colored pus was drained. Cultures of pus later grew streptococcus pneumoniae. Antibiotic therapy was carried on for 8 weeks postoperatively. The patient recovered without any sequelae including hearing.

Conclusions: The rarity of neurological complications, in addition to the insidious onset and subtle symptoms of mastoiditis associated with antibiotic therapy, can make diagnosis extremely difficult. However, early, aggressive diagnosis and treatment can make a successful outcome. Also, extended use of pneumococcal conjugate vaccine can make reducing the occurrence of invasive pneumococcal diseases.
Background and aims: Viruses can play a pivotal role in host cell mediation of bacterial superinfection. However, the mechanisms underlying this synergism are unclear. The objective of this study was to describe a comprehensive picture of the cellular interaction between the colonizing bacteria and the host in the presence or absence of a virus infection.

Methods: Gene expression profiles of Detroit-562 cells infected with respiratory syncytial virus (RSV), parainfluenza virus 3 (HPIV3) or mock infected were analyzed using microarrays. The reciprocal response of Streptococcus pneumoniae strain TIGR4 (serotype 4) in the presence of either mock- or viral-infected cells was also analyzed. Significantly expressed genes were identified using Significance analysis of Microarray (SAM) as well as a ≥ 2-fold change ratio cut-off.

Results: The results showed that the adherence of S. pneumoniae to human nasopharyngeal cells was significantly augmented in the presence of RSV or HPIV3 infection. Global gene expression profiling of the host cells revealed the enhanced transcription of carcinoembryonic antigen-related cell adhesion molecules (CEACAM1), CD47, fibronectin, interferon stimulated genes as well as other host cell adhesion molecules that have not hitherto been described. In the presence of virus-infected human epithelial cells, pneumococci increased transcription of genes (e.g. fibronectin-binding gene, glnQ) that may code for new ligands possibly to match the newly expressed receptors on the host cell.

Conclusions: Our findings revealed bacterial genes and cell adhesion molecules that can potentially be used to control pneumococcal adherence occurring secondary to a viral infection.
GENERAL MORBIDITY AS RISK FACTOR FOR INVASIVE PNEUMOCOCCAL DISEASE IN INUITS FROM GREENLAND - A HIGH INCIDENCE AREA

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Background and aims: Invasive pneumococcal disease (IPD) is frequent in native Arctic populations, but little is known about factors accounting for this. The study aim was to determine whether general morbidity measured by all-cause hospitalisations prior to IPD increases the risk of IPD in native Greenlanders (Inuits).

Methods: All microbiological testing in Greenland takes place at the national microbiology laboratory in the capital Nuuk. All IPD cases in the years 1994-2008 were registered from laboratory files. The Greenland Inpatient Register contains information of all hospitalisations in Greenlandic hospitals. Using these nation-wide registers a cohort of all native Greenlanders was formed and the relative risks (RR) of IPD for hospitalisations in the period 5-½ years prior to IPD by ICD8 and ICD10 diagnosis groups were estimated using Poisson regression analysis.

Results: 121 IPD patients were identified, hereof 100 being Inuits with information of background factors. 53 percent of IPD patients had been hospitalised (range 1-12 times) in the study period. Hospitalisation for any reason was associated with increased IPD risk (p=0.02), but mainly for those hospitalised 10+ times (RR 4.97, 95% CI 1.14-15.5, compared with persons hospitalised 2 times). Main diagnosis groups associated with increased IPD risk were cancers (RR 2.95), haematological (RR 3.92), eye (RR 2.6), endocrinological (RR 2.21), respiratory (RR 2.01), digestive (RR 1.62), pregnancy related (RR 1.94), and infectious diseases (RR 1.46), although only significant for cancers.

Conclusions: General morbidity increases the risk of IPD but may only explain a smaller fraction of IPD cases in Greenland.
Poster No 157

PRESENTATION AND OUTCOME OF INVASIVE PNEUMOCOCCAL DISEASE IN CHILDREN WITH UNDERLYING DISEASE

J. Marco del Pont, A. De Cristófano, L. Verdier, C. Faragó, Capital Federal, Argentina

Objective: To describe the way of presentation and the outcome of pediatric patients with invasive pneumococcal disease (IPD) and a previous underlying disease admitted at the Hospital Italiano de Buenos Aires.

Material and methods: Descriptive and observational study. We included all the patients with an underlying disease and a confirmed IPD admitted at the Hospital Italiano de Buenos Aires from 2000 to 2011. The data were processed in Epi Info 3.5.3.

Results: We evaluated 56 patients, 60.7% male, with a median age of 48 months (8-102), 30.4% had received antibiotics previously. The underlying diseases were: oncological disease 18%, liver disease 14%, heart disease 12.5%, respiratory disease 11%, S. Down 7%, other 37.5%. The Clinical presentations were: respiratory disease 57.2% (in this group 6.6% had pleural drainage), sepsis 19.6%, peritonitis 8.9%, CNS 7.2%, other 7.2%. A 12.5% had a nosocomial infection. The 67.9% of the isolates were sensitive to penicillin. Five patients died (8.9%) with a median age of 84 months (24-144). The clinical presentations were: sepsis in 2 patients, meningitis, respiratory disease and an abscess, one patient in each one. There was no statistical significance in the outcome between the ones who received antibiotics previously and the ones who not and between the ones who

Conclusion: The invasive pneumococcal disease remains a problem in patients with underlying disease. The data increase the importance of the incorporation of specific vaccines for this population of patients.
COMPARISON IN THE INVASIVE PNEUMOCOCCAL DISEASE IN PEDIATRIC PATIENTS WITH AND WITHOUT UNDERLYING DISEASE

J. Marco del Pont, A. De Cristófano, C. Faragó, L. Verdier, Capital Federal, Argentina

Objective: Find differences in the way of presentation and the outcome of the invasive pneumococcal disease (IPD) between kids with and without underlying disease.

Material and methods: Observational analytic study. We included all patients with confirmed diagnosis of IPD admitted to the pediatric areas of the Hospital Italiano de Buenos Aires from 2000 to 2011. The data were processed with Epi Info 3.5.3

Results: We included 117 patients, 56 with any underlying disease and 61 with no history.

<table>
<thead>
<tr>
<th></th>
<th>Without underlying disease</th>
<th>Without underlying disease</th>
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</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>61 (52.1%)</td>
<td>56 (47.8%)</td>
</tr>
<tr>
<td>Male</td>
<td>34 (55.7%)</td>
<td>35 (62.5%)</td>
</tr>
<tr>
<td>Median age</td>
<td>24 meses (12-48)</td>
<td>48 meses (8-102)</td>
</tr>
<tr>
<td>Previous treatment with antibiotics</td>
<td>5 (8.1%)</td>
<td>17 (30.4%)</td>
</tr>
<tr>
<td>Nosocomial cases</td>
<td>1 (1.6%)</td>
<td>7 (12.5%)</td>
</tr>
<tr>
<td>Sensitivity to penicillin</td>
<td>72.4%</td>
<td>67.9%</td>
</tr>
<tr>
<td>Clinical Presentation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>33 (54%)</td>
<td>30 (53.5%)</td>
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<tr>
<td>Suppurative pleuropulmonary</td>
<td>7 (11.4%)</td>
<td>2 (3.5%)</td>
</tr>
<tr>
<td>Sepsis</td>
<td>8 (13.1%)</td>
<td>12 (21.4%)</td>
</tr>
<tr>
<td>CNS</td>
<td>8 (13.1%)</td>
<td>4 (7.1%)</td>
</tr>
<tr>
<td>Peritoneal</td>
<td>1 (1.6%)</td>
<td>5 (8.9%)</td>
</tr>
<tr>
<td>Others</td>
<td>4 (6.5%)</td>
<td>3 (5.3%)</td>
</tr>
<tr>
<td>Deceased</td>
<td>1 (1.6%)</td>
<td>5 (8.9%)</td>
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</tbody>
</table>

Conclusion: In patients with underlying disease the median age was twice compared with the other group, mortality was higher in the group with underlying disease although not statistically significant because a small number of patients died. The most common infection was pneumonia in both groups.
EFFECT OF UPPER RESPIRATORY TRACT VIRAL INFECTION ON PNEUMOCOCCAL LOAD IN THE NASOPHARYNGES OF CHILDREN LIVING IN RURAL KENYA

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Background: Respiratory viral infection predisposes to bacterial pneumonia and viruses facilitate transmission of nasopharyngeal bacteria. We aim to see how acquisition of a viral upper respiratory tract infection (URTI) affects nasopharyngeal pneumococcal concentration, by starting with a pilot study to refine the analysis plan for the main dataset.

Methods: Nasopharyngeal flocked swabs were collected twice per week from 50 households in rural Kenya, November 2009 - May 2010. A respiratory multiplex PCR was performed. Swabs before, during and after acquisition of viral infection in 9 children < 5 years with URTI symptoms underwent real-time quantitative lytA PCR (qPCR) to detect pneumococcal concentration. A Kruskal-Wallis rank test was used to compare log pneumococcal concentration before, during and after viral infection.

Results: All participants carried pneumococcus and presence of multiple respiratory viruses was common. The temporal pattern was suggestive of a rise in pneumococcal concentration once viral infection occurred but there was considerable heterogeneity. Pneumococcal concentration was greater during or after viral infection than before viral infection, p = 0.062.

Conclusions: This pilot study suggests that there are likely differences in nasopharyngeal pneumococcal load with the onset of viral URTI. The pattern of responses in individual subjects informs the definition of episodes of infection and co-infection to apply to the formal study of 50 households.
INCIDENCE OF PNEUMONIA IN A CASE OF SICKLE CELL DISEASE BEFORE AND AFTER PNEUMOCOCCAL VACCINATION: A CASE REPORT

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Background: Children with Sickle Cell Disease (SCD) are vulnerable to early mortality from pneumococcal infections including invasive pneumonia. Current interventions employ antibiotics and pneumococcal vaccines. This paper reports a case of invasive pneumonia in a Ugandan child with homozygous (ss) SCD; discussed is the role of pneumococcal vaccination in reducing incidence of hospitalizations due to pneumonia in SCD in Uganda.

Case description: A 15-year old Ugandan male was first admitted at age 3 weeks to Mulago National Referral Hospital for cough and dyspnoea; he was diagnosed as ss Sickle Cell sickler with community-acquired invasive pneumonia, treated with Cotrimoxazole and discharged 72 hours later on Amoxicillin. He was re-admitted for severe pneumonia at 38 months, and given IM Ampicillin, IV Ceftriaxone and his first dose of polysaccharide pneumococcal vaccine. Within 6 years post-vaccination, only 2 admissions for pneumonia were reported, managed with Benzyl-penicillin injection, Erythromycin and Cloxacillin. A second dose of pneumococcal vaccine was given at 88 months; no hospitalizations due to pneumonia have been reported to-date since.

Results: Number of hospitalizations due to pneumonia dropped significantly from 1 episode in 1.5 years prior to pneumococcal vaccination to 1 in 3 years after the first dose and no episodes after the second dose.

Conclusion: Decreased incidence of invasive pneumonia in SCD, represented by decreased number of hospitalizations could strongly be attributed to pneumococcal vaccination. Polysaccharide pneumococcal vaccine is suitable for prophylaxis of the large SCD population in Uganda, and it is therefore recommended that it be made more affordable/ available locally.
PNEUMOCOCCAL INFECTION ASSOCIATED WITH ATOPIC DERMATITIS IN CHILDREN

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Atopic dermatitis in children is an immune pathological condition that can create the premises for infections frequent with Staphylococcus aureus, Streptococcus pneumoniae or fungi.

Method: From 221 children diagnosed with atopic dermatitis have been selected 78 cases (0-18 years) with moderate and severe forms who presented pneumococcal infection varied located. Were compared 2 groups: group 1 (41 cases)- atopic dermatitis associated with comorbidities, group 2 (37 cases)- atopic dermatitis without comorbidities.


Conclusions: We have objectified an increased incidence of pneumococcal infection in children with atopic dermatitis associated with comorbidity and severity SCORAD index > 40. Also be noted a high prevalence of penicillin-resistant strains of the Streptococcus pneumoniae in atopic dermatitis in children with or without comorbidities.
CLINICAL COURSE OF CHILDREN WITH INVASIVE PNEUMOCOCCAL DISEASE (IPD) ACCORDING TO PNEUMOCOCCAL RESISTANCE TO PENICILLIN

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Objective: Because of the controversies that penicillin resistance influences the evolution of the disease caused by pneumococcus, we aim to evaluate the effect of penicillin resistance on the clinical picture of IPD in children < 60 months of age treated with penicillins.

Methods: We retrospectively analyzed data of 164 children with confirmed pneumococcal infection. 108 children treated with penicillin were selected and divided into two groups according to the penicillin sensitivity of the bacterial isolate. The outcomes treatment success or failure during early (EP) (72hs) and late progression (LP) (7-21 days) periods, and duration of hospitalization were compared between the two groups. The influence of covariates was controlled by a logistic regression model. We use CLSI 2004 criteria for penicillin resistance.

Results: Early improvement frequencies were similar among children with sensitive isolates (42/65, 64.6%) and those with resistant isolates (19/43, 44.2%), as well as the occurrence of LP treatment failure [12/65 (18.5%), vs. 5/43 (11.6%), respectively]. In contrast, two factors were determinants of poor outcomes: the clinical severity (EP: OR=8.7; 95%CI 1.9, 39.9); (LP: OR=9.1; 95%CI 2.9, 28.9) and occurrence of meningitis at admission (LP OR=9.1, 95%CI 2.9, 28.9). Of the 4 patients with meningitis two had MIC equal to 2mg/ml and others equal to 0.12 mg/ml, 3 were cured and one died.

Conclusions: The "in vitro" penicillin sensitivity does not determine the outcome of children with pneumococcal disease. However, the severity of the clinical presentation and the diagnosis of meningitis are indicative of adverse outcomes.
PNEUMOCOCCAL CARRIAGE IN HIV-INFECTED AND HIV-UNINFECTED MOTHERS IN SOUTH AFRICA

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1Johannesburg, 2Sandringham, South Africa, 3Atlanta, GA, USA

Background and aim: HIV infection increases the risk of IPD. Nasopharyngeal (NP) and/or oropharyngeal (OP) colonization with Streptococcus pneumoniae is a pre-requisite for IPD. We investigated the risk of pneumococcal colonization and colonization risk-factors in HIV-infected and -uninfected mothers.

Methods: 251 mother-baby pairs (126 HIV-infected mothers) had 9 schedule visits when the baby was 1.9-24.2 months old, at which demographic data, OP and NP swabs were collected for pneumococcus culture and serotyping. HIV-infected and -uninfected mothers were compared by Chi-square test and risk-factors for colonization were identified by regression analysis.

Results: 1845 swabs were collected (986 from HIV-infected). The incidence of acquisition of new serotypes was 14.8% in HIV-infected and 16.0% in HIV-uninfected mothers (p=0.5). Serotypes included in the 7-valent pneumococcal conjugate vaccine (PCV7) and PCV13 accounted for 48.6% and 57.5% of the recovered serotypes in HIV-infected and 25.3% and 40.4% in HIV-uninfected mothers, respectively (RR=1.9, p< 0.001 [PCV7] and RR=1.4, p=0.001 [PCV13]). In multivariate analysis, factors negatively associated with mothers' colonization were: increasing age (OR=0.96, p=0.004) and increasing number of people in the household (OR=0.93, p=0.02). Pneumococcal colonization in the baby, over time, was positive associated with carriage in the mother for all serotypes (OR=1.91, p< 0.001) and similarly for PCV7 and PCV13 serotypes (OR=2.2, p< 0.001 and OR=2.6, p< 0.001, respectively).

Conclusions: The increase in carriage in mothers of colonized babies and the higher prevalence of PCV-serotypes in HIV-infected mothers suggests that infant PCV vaccination might have an important indirect impact in settings with high HIV burden.
IN THE ERA OF PNEUMOCOCCAL CONJUGATE VACCINES, ANTIBIOTIC CONSUMPTION REMAINS A MAIN DRIVING FORCE OF ANTIMICROBIAL RESISTANCE

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Background and aims: To improve knowledge on pneumococcal colonization in Portugal, we compared colonization patterns between two regions: Montemor-o-Novo, a rural area, and Oeiras, an urban area.

Methods: Nasopharyngeal samples from day care center (DCC) attendees in Montemor-o-Novo (n=756) and Oeiras (n=1088) were collected between January-March of 2009-2010. Socio-demographic and clinical data were obtained. Pneumococci were isolated, serotyped and antibiotyped. A multivariable logistic regression analysis was used to evaluate association between carriage, and socio-demographic and clinical variables, such as region, number of children in DCC, age, antibiotic use and use of pneumococcal conjugate vaccines.

Results: Carriage (c.a. 60%) and PCV7 use (complete schedule, c.a. 70%) were comparable in both regions. In Oeiras there were higher rates of antimicrobial resistance (32.4% vs 21.6%, p< 0.001) and higher rates of antimicrobial consumption one month before sampling (16.7% vs 11.6%, p=0.004). In the multivariate model, antimicrobial consumption one month before sampling was the variable that better explained the differences in antibiotic resistance between the two regions (OR=2.58, 95%CI: 1.61-4.17). Independently of the region, older age (OR=0.92, 95% CI: 0.86-0.99) and antibiotic consumption one month before sampling (OR=0.27, 95% CI: 0.13-0.59) were protective factors for pneumococcal colonization.

Conclusions: Antibiotic use is a protective factor for pneumococcal colonization but is a risk factor for colonization by resistant strains. In the era of widespread use of PCVs in Portugal, antibiotic consumption remains a driving force for maintenance of antimicrobial resistant pneumococci in the community.

THE PNEUMOEL PROJECT: PNEUMOCOCCAL COLONIZATION AMONG THE ELDERLY IN PORTUGAL

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Background and aims: This study, pioneer in Portugal, aimed to evaluate pneumococcal carriage in adults aged over 60 years.

Methods: Between April and November of 2010, the nasopharynx and oropharynx of adults (>60 years), living in Oeiras (n=651), an urban area, and Montemor-o-Novo (n=647), a rural area, were swabbed. Pneumococci were identified by standard procedures and by PCR detection of genes cpsA and lytA. Isolates were antibiotyped and serotyped. Association between pneumococcal carriage, socio-demographic and clinical factors were evaluated using a logistic regression.

Results: A total of 1,298 adults were enrolled. Only 4.1% were smokers. Fifty-nine percent of the participants were vaccinated with the seasonal influenza vaccine and only 3.5% with the 23-valent pneumococcal polysaccharide vaccine. Twenty-nine (2.3%) adults carried bona fide capsulated pneumococci. One adult carried two different pneumococcal strains in the nasopharynx. The 30 strains expressed 18 capsular types. Sixteen isolates showed antimicrobial resistance. There were more adults carrying pneumococci in the rural area (3.4%) than in the urban area (1.1%) (p=0.004). Smoking increased seven-fold the odds for carriage (OR=7.4, CI 95%: 2.56-21.27).

Conclusion: The prevalence of pneumococcal carriage in the elderly is low and serotype diversity is high. Smoking is an important risk factor for colonization whereas living in Oeiras is a potential protective factor.

CHARACTERISTICS OF RECURRENT VERSUS NON-RECURRENT PNEUMOCOCCAL MENINGITIS


Objective: Compare characteristics of patients with recurrent versus non-recurrent pneumococcal meningitis.

Materials and methods: We performed a retrospective study of patients hospitalized between 2005-2011 for pneumococcal meningitis. The diagnosis was established by characteristic appearance on Gram stain, positive culture for S. pneumoniae and/or a positive latex agglutination reaction of cerebral spinal fluid (CSF) samples. The meningitis was considered to be recurrent if the patient experienced at least two episodes at least 4 weeks apart. We excluded relapsing disease.

Results: We identified 124 pneumococcal meningitis episodes. Contiguous dissemination from a local site of infection was suspected in 71 (57%), while 7 (6%) patients had hematogenous spread. Thirty-three percent of patients had an underlying condition associated with impaired immune response. Fifteen (13%) patients with recurrent meningitis were identified with a total of 77 recorded prior meningitis episodes. Although most patients had only one previous episode, two had recurrences of 11 and 40 episodes. In the latter, phenoxymethylpenicillin prophylaxis reduced the number of recurrences. The combination of pneumococcal vaccine and penicillin provided longer recurrence-free periods. Median age of patients with recurrent vs. non-recurrent meningitis was 29.5 vs. 54 years (p<0.001). Recurrent meningitis patients had survival of 94% and CSF leakage of 55%, while non-recurrent cases had survival of 70% (95%CI, p=0.04) and CSF leakage of 13% (95%CI, p<0.001).

Conclusions: Patients with recurrent meningitis were younger, more likely to have a cranial bone discontinuity and better survival. The combination of pneumococcal vaccine and penicillin prophylaxis can provide an additional benefit in certain cases.
PERSISTENCE OF S. PNEUMONIAE (SPN) IN MIDDLE EAR FLUID (MEF) IN PEDIATRIC PATIENTS WITH RECURRENT ACUTE OTITIS MEDIA (AOM)


Background and aims: AOM is the most common disease caused by Spn. Our aim was to evaluate the persistence of pneumococci in patients (pts) with relapses of AOM.

Methods: We included 324 pts from 05/2009 to 08/2010 (with follow-up to 02/2011); with first episode (ep.) of AOM diagnosed by otomicroscopy with purulent effusion retained in the middle ear. Tympanocentesis and culture of MEF was performed. Pts were treated with amoxicillin 80mg/k/d during 10 days and evaluated at days: 1, 2, 7 and 30. Resolution of AOM was considered when patients were free of signs and symptoms of infectious disease. Spn strains were serotyped by Quellung reaction. The genetic relatedness was evaluated by SmaI PFGE in Spn sharing the same serotype.

Results: A total of 55/324 pts (17%) had more than 1 ep. of AOM during the follow-up period. Spn was responsible for the relapses in 12/55 pts (22%) and the same serotype persisted in 8/55 pts (14,5%) (total:20 ep). In these 8 pts, Spn serotypes were: 14 (N=3 pts; 9 ep.), 19A (N=2 pts; 5 ep.) and serotypes 6B, 9V and 23B (1 pt each; 6 ep). Different clonal types were found between pts. The same Spn PFGE type was observed in all episodes of the same patient. All pts resolved AOM between episodes. The median time between ep. was 35 days. After 7 days, purulent exudate in middle ear was not observed in 17/20 ep. (85%).

Conclusions: The resolution of AOM between episodes did not discard relapses caused by Spn strains including those carrying the same clonal type. Persistence was not associated to any particular clon.
Poster No 168

EARLY AND LATE MORTALITY IN ADULTS WITH INVASIVE PNEUMOCOCCAL DISEASE (IPD) IN CALGARY, CANADA

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Background/aims: IPD continues to cause significant mortality and 60% of deaths occur within 5 days of presenting to hospital. Our aim was to analyze differences in early and late mortality compared to survival in patients with IPD from the Calgary *S. pneumoniae* Epidemiology Research (CASPER) Study.

Methods: In 1,001 cases of IPD in adults in the CASPER study from 2000-2009, the case-fatality rate was 11.9%. Multinomial regression was performed to analyze 3 outcomes: early mortality (< 5 days post-presentation), late mortality (5-30 days post-presentation), and survival. The model tested for modification and confounding by age, gender, primary diagnosis, Charlson comorbidity index, smoking status, and time to appropriate antibiotic treatment.

Results: Patients with severe IPD had increased risk of early and late death. In multinomial regression with survivors as baseline, the risk of early death in those with a Charlson index score ≥2 was 5.5X (1.8-16.4); the risk of late death was 5.8X (1.2-26.8) (if less severe disease) and 19.8X (0.95-415.7) (if severe disease). Patients who never received appropriate antibiotics had 3.6X (1.6-8.1) the risk of early death. Patients receiving appropriate antibiotics >48 hours after presentation had 5.5X (1.8-16.4) the risk of late death. Being female was nearly significant as a risk for early death, while age was not associated with risk of early or late death.

Conclusions: Severe IPD and multiple comorbidities increase the risk of early and late death, while age does not. Delay in receiving appropriate antibiotics increases the risk of death and may be a modifiable factor.
Poster No 169

INVESTIGATING THE ROLE OF PNEUMOCOCCAL NEURAMINIDASE ACTIVITY IN ISOLATES FROM PNEUMOCOCCAL HAEMOLYTIC URAEMIC SYNDROME

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Background and aims: Streptococcus pneumoniae is a rare but increasing cause of atypical haemolytic uraemic syndrome associated with a higher mortality rate than diarrhoea-associated HUS. This study aimed to determine the importance of neuraminidase A (NanA) and genomic diversity in pathogenesis of pneumococcal HUS (p-HUS).

Methods: Gene sequencing, phylogenetic analysis, activity assays and comparative genomic hybridization were carried out on NanA alleles from p-HUS-causing isolates and control strains. The isolates selected for this study were p-HUS isolates and control isolates matched by serotype and ST and isolated from patients with invasive pneumococcal disease (IPD), but not causing p-HUS.

Results: The NanA sequence of 33 isolates was determined and mutations at 142 amino acid (AA) positions were identified, representing 14% of positions in the wild-type protein. 18 protein alleles were recognised. When comparing NanA allelic diversity with ST and disease profile in the isolates tested, NanA alleles clustered mostly by ST. No particular NanA allele was associated with p-HUS. There was no significant difference in overall neuraminidase activity between p-HUS isolates and controls when induced/uninduced with Neu5ac. Comparative genomic hybridization showed little difference in genetic content between the p-HUS isolates and the controls. Results of gene expression studies identified 12 genes differentially regulated in all p-HUS isolates compared to controls.

Conclusions: Although bacterial genetics may be important in p-HUS progression, and NanA enzyme activity may contribute to pathogenesis, other factors such as defects in regulators of the complement system or auto-antibodies against factor H probably play a more significant role.
PNEUMOCOCCAL OTITIS MEDIA IN RELATION TO ACQUISITION OF CARRIAGE AND RESPIRATORY INFECTION

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Background: For considering prevention of pneumococcal acute otitis media (PncAOM) with vaccines, it is important to understand the relationship between nasopharyngeal carriage, respiratory infection and PncAOM.

Methods: 329 children were followed prospectively in FinOM Cohort Study by nasopharyngeal samples collected at scheduled visits and at sick visits arranged for diagnosing PncAOM during respiratory infection. Using samples obtained < 2 months apart, episodes of serotype-specific carriage and non-carriage were defined. Acquisition was defined to occur at half-way between two samplings with different carriage status. Episodes of respiratory infection (sick episodes) were defined based on consecutive sick visits, a new episode starting only if ≥ 30 days had elapsed since the previous sick visit. We estimated the hazard of PncAOM during risk episodes, defined as overlapping periods of carriage and a sick episode, stratified according to timing of carriage acquisition.

Results: There were 321 carriage episodes with known acquisition times, producing 457 risk episodes with 913 person-weeks and 114 PncAOMs. If carriage had started within 4 weeks before or within 1 week after the sick episode onset, the PncAOM hazard was 0.051 per day (90%CI 0.042-0.063) for the first month of carriage. By contrast, if carriage had started earlier than 1 month before or more than 1 week after the sick episode onset, the PncAOM hazard was markedly lower (0.012 per day, 90%CI 0.009-0.015).

Conclusion: A new carriage is more prone to proceed to PncAOM during respiratory infection than established carriage or carriage acquired after the acute phase of the infection.
ASSOCIATION BETWEEN PNEUMOCOCCAL COLONISATION AND VIRUS DETECTION IN THE NASOPHARYNX IN WHO-DEFINED CLINICAL PNEUMONIA


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Background: Viral respiratory tract infections may predispose to pneumococcal disease, although the relationship to colonisation and precise mechanisms are not fully established.

Methods: During a longitudinal pneumonia epidemiology study in 965 Burmese-Karen infants, we collected paired nasopharyngeal swabs and aspirates from 474 infants presenting with their “first ever” WHO-defined clinical pneumonia episode. In addition to pneumonia specimens, monthly surveillance swabs for detection of pneumococcal colonisation were collected between 1-24 months of age. Pneumococcal colonisation was confirmed by swab culture and viral infection was identified by rRT-PCR (adenovirus, hMPV, influenza viruses, and RSV) of the aspirates.

Results: Overall, cohort infants first became colonised by pneumococcus early (median 45.5 days) and carriage was common (pneumococcus detected in 76.3% of surveillance swabs). The median age at first pneumonia diagnosis was 207 days (range 10 - 726). At least one virus was detected in 66.2% of aspirates on presentation (rank order: RSV, adenoviruses, hMPV, influenza viruses). Adjusting for age, antimicrobial consumption in the previous 30 days, isolation of pneumococcus from the two preceding surveillance swabs, and detection of multiple viruses, we found a trend towards a positive correlation between influenza virus detection and pneumococcal colonisation (OR 2.79; 95% CI 0.87 - 9.0; p=0.085). Interestingly, RSV detection was negatively correlated with pneumococcal colonisation (78.2% vs 67.7% colonised for RSV negative vs positive; OR 0.56 (95% CI 0.35 - 0.90; p=0.016)).

Conclusion: This carriage data suggests that not all viral infections may necessarily have the same effect on pneumococcal colonisation of the nasopharynx in vivo.
Introduction and objective: Children born to kidney transplanted mothers are exposed to immunosuppressive drugs during gestation. Many of them are also born prematurely. Both conditions can reduce maternal antibody transfer to the fetus. This study evaluated transplacental antibody transfer of Streptococcus pneumoniae antibodies in pairs of kidney transplanted women and their offspring. Methods: Twenty mother-infant pairs from kidney transplanted group (TX) and 24 healthy mother-infant pairs of full-term children (CTL) were evaluated. Placental antibody transfer against the seven Streptococcus pneumoniae serotypes (pn:4,6B,9V,14,18C,19F,23F) present in the 7-valent conjugate vaccine were evaluated by ELISA in maternal and cord blood. Total IgG plasma levels were measured by nephelometry.

Results: TX neonates were born at a mean earlier gestational age than CTL neonates (TX:37.4 weeks x CTL:39.3 weeks, p=0.003). Maternal S. pneumoniae (mcg/mL) and total IgG plasma levels (g/L) were similar in solid organ transplanted and control women (IgG:8.9x9.6; pn4:2.7x3.1; pn6B:6.1x7.1; pn9V:6.1x4.7; pn14:19.4x14.1; pn18C:3.5x3.6; pn19F:7.6x5.8; pn23F:8.1x5.4; p>0.05 for all analyses). Placental antibody transfer was similar between TX and CTL groups: while mean total IgG mother-infant ratio was 1.3 and 1.2, respectively, mean pneumococcal antibody ratio was always lower than 1 (pn4:0.8x0.8; pn6B:0.9x0.7; pn9V:0.7x0.7; pn14:0.9x0.8; pn18C:0.6x0.7; pn19F:0.6x0.7; pn23F:0.7x0.8), but similar between groups (t test, p>0.05 for all comparisons). Neonates from TX and CTL groups had comparable levels of total IgG and pneumococcal antibody levels at birth (IgG:10.3x11.7; pn4:2.1x2.5; pn6B:4.0x5.1; pn9V:4.1x3.3; pn14:13.6x10.7; pn18C:2.3x2.7; pn19F:5.0x4.2; pn23F:5.2x3.9).

Conclusion: Women on immunosuppressors due to kidney transplantation are able to provide their neonates with adequate amounts of pneumococcal antibodies.
UNDERSTANDING INVASIVE PNEUMOCOCCAL DISEASE INDUCED DURING INFLUENZA VIRUS CO-INFECTION: A ROLE FOR VIRAL HEMAGGLUTININ IN FACILITATING PNEUMOCOCCAL PNEUMONIA


Background: Viral infections of the upper respiratory tract, such as influenza A virus (IAV) infection, are a major susceptibility factor to pneumococcal disease. While a number of viral mechanisms have been identified that contribute to induction of invasive pneumococcal disease, the role of viral surface proteins in facilitating disease is less well understood.

Methods: We used a novel infant mouse model in which mice are colonized with serotype 6 S. pneumoniae followed by infection with IAV. A series of reverse genetically engineered IAV was used to identify a role for viral surface proteins in induction of pneumococcal pneumonia and sepsis.

Results: Co-infection of S. pneumoniae colonized mice with IAV resulted in 10-50-fold increased bacterial load in the nose while only infection with H3 viruses and not H1 viruses resulted in significantly increased (>1000 fold) bacterial load in the lungs and blood. The ability of IAV to facilitate secondary pneumococcal pneumonia and sepsis was independent of viral replication in the lungs and histological analysis suggested that there was no correlation with the ability of the virus itself to induce inflammation in the lungs. Preliminary analysis of co-infected mice demonstrated the presence of a neutrophil infiltrate and IL-1b, IL-6, IL17A, IFNg and TNF in lung homogenates from mice co-infected with H3 IAV strains but not in mice co-infected with H1 IAV strains.

Conclusions: these data demonstrate the importance of the viral hemagglutinin in facilitating bacterial disease and provide an exciting new insight into the complex interactions between S. pneumoniae and IAV.
CEFDITOREN INCREASES COMPLEMENT MEDIATED IMMUNITY AND ENHANCES BACTERIAL CLEARANCE OF PNEUMOCOCCAL ISOLATES WITH HIGH LEVELS OF ANTIBIOTIC RESISTANCE

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Background: Prophylactic measures against S. pneumoniae are an effective strategy to prevent Invasive Pneumococcal Disease (IPD). The successful outcome against IPD, largely depends on the host immune system and the effectiveness of the antimicrobial chemotherapy. The aims of this study were to explore the ability of cefditoren to increase C3b, C1q and CRP deposition on the surface of different pneumococcal resistant strains opsonized with serum containing pneumococcal antibodies and investigate whether cefditoren might accelerate bacterial clearance from blood in the presence of specific antibodies.

Methods: Three strains of serotypes 6B, 19F and 23F (MICs to cefditoren: 1µg/ml, 2µg/ml and 4µg/ml respectively) were used for this study. Sera containing pneumococcal antibodies were obtained from mice immunized against the different heat killed strains. C3b, C1q and CRP deposition was analyzed by flow cytometry and bacterial clearance was investigated using a mice sepsis model.

Results: Deposition of C3b, C1q and CRP was significantly increased when the different isolates were incubated in the presence of 0.5MIC and 0.25MIC of cefditoren and serum containing specific antibodies. Administration of sub-therapeutic doses of cefditoren in immunized mice decreased significantly bacterial counts from blood during the first 24 h for all the strains investigated in comparison to the individual groups (lethal, antibiotic and passive immunization).

Conclusions: Antimicrobial chemotherapy with beta-lactams antibiotics such as cefditoren enhances bacterial killing in the presence of specific antibodies by activating complement mediated immunity against S. pneumoniae. Prophylactic strategies in combination with antibiotic treatment may overcome clinical failure by pneumococcal isolates with increased resistance.
PREVALENCE OF ANTIBODIES TO PNEUMOCOCCAL SURFACE PROTEIN ANTIGENS (CSPAS) AND ASSOCIATION WITH NASOPHARYNGEAL Colonization AND HIV STATUS IN AFRICAN WOMEN

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Background: CSPAs of S. pneumoniae are being investigated as vaccine candidates, with the potential of protecting against pneumococcal disease irrespective of capsular serotype.

Objectives: To characterize natural-induced antibody seroprevalence to 15 CSPAs; and explore the association between antibody titers and nasopharyngeal colonization in HIV-infected and uninfected women.


Results: The study enrolled 565 women, including 197 HIV-uninfected and 368 HIV-infected, at a median age of 27 years. HIV-uninfected women had significantly higher antibody titers than HIV-infected women against antigens associated with the cell wall, either through choline-binding domains or LPXTG motifs, and toxins. These included higher geometric mean antibody titers against PspA, PspC, PdB, SP 0082, LytB, IgA1-proteinase, and PcsB. Lower antibody titres against PspC, SP 2027, SP 0082, LytB and PcsB were evident in HIV-uninfected women colonized with pneumococci compared to un-colonized women. There was, however, no association between antibody titres and presence of pneumococcal colonization in HIV infected women.

Conclusion: The inverse association between antibody titres against PspC, SP 2027, SP 0082, LytB and PcsB, and prevalence of pneumococcal colonization in HIV-uninfected women, indicate that these antigens may have potential as vaccine-candidates against pneumococcal disease. Lower antibody titres to select CSPAs in HIV-infected women may explain in part the heightened susceptibility to pneumococcal disease in these individuals.
IMMUNOGENICITY OF PNEUMOCOCCAL CONJUGATE VACCINE (PCV7) AT 6 AND 14 WEEKS AND BOOSTER DOSE AT 9 MONTHS


Background: PCV7 (Prevenar™) vaccination was introduced as a two dose primary series at 6 and 14 weeks with a booster dose at 9 months of age in South Africa. The aim of the study was to determine quantitative and qualitative antibody responses following the primary and booster doses of PCV.

Methods: Children were immunized with PCV7 concurrently with other routine childhood vaccines. Serum serotype-specific IgG antibody concentrations was measured by standard ELISA one month after the 1st and 2nd dose of PCV; and prior to and two weeks following the booster dose. Opsonophagocytic killing assay (OPA) were undertaken for serotypes 9V, 19F and 23F following the 2nd and booster dose.

Results: 250 children were enrolled and received PCV at mean ages (in weeks) of 6.3 (SD=0.04), 15.8 (SD=0.1) and 39.9 (SD=0.05). Quantitative antibody responses are tabulated.

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1Geometric mean antibody concentration and 95% Confidence interval.
2Proportion with antibody concentration ≥0.35 μg/ml shown in square parenthesis.

Immunogenicity following PCV at 6 and 14 weeks and booster dose at 9 months of age.

[Immunogenicity of PCV as a 2+1 schedule.]

The proportion of children with OPA ≥8 to 9V, 19F and 23F were 80%, 70% and 78%, following PCV dose-2 and increased significantly to 95% (p< 0.001), 83% (p=0.002) and 91% (p< 0.001), respectively, after the booster dose.

Conclusions: Children demonstrated good antibody responses following a two-dose primary series and robust anamnestic responses after a booster dose of PCV. The proportion of children with OPA killing activity was modest following the initial two doses and improved significantly following the booster dose of PCV.
Poster No 177

DOES NATURAL ACQUIRED SEROTYPE-SPECIFIC ANTIBODIES PROTECT AGAINST SEVERE PNEUMOCOCCAL PNEUMONIA?

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Background: We aimed to evaluate if natural acquired serotype-specific capsular polysaccharide (CPS) antibodies were protective against severe pneumococcal community-acquired pneumonia (CAP).

Methods: From a prospective study of 235 adult patients, hospitalised for radiologically confirmed CAP, 20 bacteraemic patients with Streptococcus pneumoniae isolated from blood culture, and 25 non-bacteraemic patients with S. pneumoniae isolated from sputum samples of good quality (>5 leukocytes per epithelial cell) with negative blood cultures, were included. At admission, serum samples were collected and analysed with ELISA for serotype-specific CPS antibodies against the causative pneumococcal isolates. The total immunoglobulin antibody titres were measured by optical density and calculated as a percentage of a standard serum, and presented in arbitrary units. The vaccination frequency in the population was very low.

Results: Non-bacteraemic patients had significantly higher titres of antibodies against the causing serotype, compared to bacteraemic patients (mean value 64.3 ± 46.4 vs. 33.3 ± 33.4; P = 0.016; t-test). Time duration from onset of illness to serum sampling was short but slightly longer (P = 0.32) for non-bacteraemic patients (4.9 ± 6.0 days) than for bacteraemic patients (3.5 ± 2.3 days). Fifty percent of bacteraemic patients belonged to Pneumonia Severity Index (PSI) classes IV-V, compared to 40% of non-bacteraemic patients.

Conclusions: Natural acquired antibodies against CPS may be protective against invasive pneumococcal pneumonia. Time duration from onset of illness to sampling was short for both groups of patients and, therefore, the measured antibodies were likely not induced by the immunologic response to the actual pneumococcal disease.
AUGMENTED PASSIVE IMMUNOTHERAPY TO TREAT ACUTE PNEUMOCOCCAL INFECTIONS

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Even when optimal antibiotic therapy is administered, mortality from acute pneumococcal infections remain high in the first 48 hours of admission. Augmented passive immunotherapy (API) is a combined treatment of immunoglobulin and the immunomodulating pneumococcal peptide P4. In murine models of invasive pneumococcal disease, API significantly reduced mortality by upregulating the phagocytic function of neutrophils and macrophages. We tested whether ex vivo API affects the phagocytic ability of human alveolar macrophages (AM).

Bronchoalveolar lavage (BAL) was collected from healthy adults (n=25) and AMs purified by adherence to culture plates. For opsonophagocytosis assays (OPA), opsonised ST 2 or 6B pneumococci, complement and 10µg P4 or PBS were incubated for two hours. Uptake was determined by enumeration of pneumococcal colonies. Intracellular oxidation during OPA was determined using a fluorescent reporter probe. Following OPA, AMs were assessed for activation markers (HLA-DR, CD206, CD11b, CCR7, CD163, CD86). Intracellular oxidation levels of AMs were also determined in the presence of PBS, LPS or P4 alone.

Opsonophagocytosis was up-regulated in healthy (n=12, p< 0.0001, paired t-test) and carbon loaded (n=13, p=0.0002, paired t-test) volunteers. Activation marker MFI following OPA were similar between treated and non-treated cells. In the presence of opsonised pneumococci and complement, P4 treated AMs significantly increased their intracellular oxidation compared to PBS treated AMs. P4 alone did not increase intracellular oxidation levels.

In the presence of opsonised pathogen, API treatment of human AM resulted in increased phagocytic function of healthy and carbon loaded volunteers.
THE EICOSANOID HEPOXILIN A₃ PLAYS CRITICAL ROLE IN PMN TRANSEPITHELIAL MIGRATION AND INVASIVE DISEASE IN STREPTOCOCCUS PNEUMONIAE LUNG INFECTION

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Background and aims: Pulmonary infection by Streptococcus pneumoniae triggers neutrophil influx into alveoli, leading to significant tissue damage. Pulmonary inflammation may also promote translocation of S. pneumoniae across lung epithelium leading to life-threatening septicemia. We investigated the role of hepoxilin A₃ (HXA₃), a 12-lipoxygenase- (12-LOX) derived eicosanoid PMN chemoattractant during S. pneumoniae lung infection.

Methods: We measured basolateral-to-apical PMN migration across polarized monolayers of lung epithelial cells infected with S. pneumoniae, as well as retrograde (i.e. apical-to-basolateral) bacterial transmigration. We quantified lipid mediators of inflammation by mass spectrometry and assessed the role of 12-LOX on PMN and bacterial transmigration in vitro and on inflammation, disease and survival during murine infection.

Results: Apical infection by pneumococci elicited epithelial secretion of HXA₃ and a robust 12-LOX-dependent basolateral-to-apical PMN migration across monolayers of respiratory epithelium. PMN migration across monolayers also increased S. pneumoniae apical-to-basolateral epithelial transmigration. Mice pretreated with a 12-LOX inhibitor or genetically lacking 12-LOX activity, when challenged with a lethal pulmonary dose of S. pneumoniae, exhibited dramatically reduced lung inflammation, suffered little or no bacteremia and survived uniformly.

Conclusions: Results suggest that S. pneumoniae-elicited PMN migration is largely mediated by HXA₃, and that acute pulmonary inflammation facilitates high-level bacteremia, at least in part by epithelial barrier disruption during PMN transmigration across lung epithelia.
NONSPECIFIC INNATE IMMUNITY GENERATED IN THE LUNGS OF MICE FOLLOWING FLAGELLIN INSTILLATION IS PROTECTIVE AGAINST STREPTOCOCCUS PNEUMONIAE CHALLENGE

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Background and aims: Pneumococcal septicemia is a major cause of infant mortality in developing countries. Amongst treatment strategies, penicillin was the antibiotic of choice, but increasing incidence of antibiotic resistance among pneumococci has forced the scientists to look for unconventional means of controlling this hardy pathogen. With steadily increasing knowledge on molecular mechanisms of innate immunity, recent swing is to target this arm of immune system. In the present study flagellin was evaluated for boosting the innate immune response against Streptococcus pneumoniae.

Methods: In the present study 5ug of purified flagellin, prepared from a clinical isolate of Stenotrophomonas maltophilia, was administered intranasally in mice. The animals in the test group were subsequently challenged, 4 hours after flagellin instillation with Streptococcus pneumoniae D39 type 2. Various inflammatory mediators were estimated in the lung homogenates.

Results: Treatment with flagellin proved beneficial in controlling lung infection in the test group as a decrease in bacterial count was observed. This was accompanied with decrease in inflammatory mediator like malondialdehyde, lactate dehydrogenase and increase in nitric oxide levels at all time points. A decrease in the level of antioxidants like catalase and superoxide dismutase was also observed. An early infiltration of neutrophils in the lungs was observed in the flagellin treated group on histopathogical examination of tissue. In addition an increase in phagocytic function of alveolar macrophages was also observed.

Conclusions: These results suggest that flagellin can be used to mount nonspecific resistance against S. pneumoniae in mouse lungs.
SHORT-TERM SEROTYPE-SPECIFIC IMMUNITY; THE KEY TO COMPETITION AND DIVERSITY OF STREPTOCOCCUS PNEUMONIAE?

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Observed serotype replacement following pneumococcal conjugate vaccination is thought to be the result of competition between the pneumococcal serotypes. This essential dynamic is commonly modelled through an artificial parameter. We explore if serotype specific and serotype non-specific immunity could constitute the underlying mechanism governing competition and coexistence and study possible implications.

An individual based model is employed where each individual could carry any combination of 20 imaginary pneumococcal serotypes. These differ by their duration of carriage (4 to 8 weeks in < 2y). Following acquisition an individual gains both serotype specific immunity and typically shorter lasting non-specific immunity. Longer term immunity is reflected through carriage duration and susceptibility declining with age. Vaccination is assumed to be 70% efficacious and serotype specific.

Serotypes in the model compete through non-specific immunity, which leads to a reduced pool of susceptibles to be infected by other serotypes. The difference in duration of carriage and non-specific immunity determines levels of multiple carriage. Serotype specific immunity limits a type's growth and allows stable coexistence. Serotypes carried at low prevalence show high variance in carriage levels, which, if their invasive potential was high, would result in apparent outbreaks. Vaccination against some serotypes induces replacement by others and a subsequent extension of the vaccine formulation can cause re-emergence of previously controlled serotypes due to a reduction in competition.

Immunity patterns may determine patterns of competition and coexistence observed for the pneumococcus. The use of higher valency vaccines could, paradoxically, result in the return of previously controlled serotypes.
CONSISTENT ANTI-PROTEIN ANTIBODY RESPONSES TO STREPTOCOCCUS PNEUMONIAE IN DIVERSE POPULATIONS

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Background: Mechanisms of naturally-acquired adaptive immunity to Streptococcus pneumoniae remain poorly defined, but may include antibody against surface protein antigens. The large genetic variability between S. pneumoniae strains and between human populations perhaps suggests that the dominant protein antigens may vary between geographical areas. We have therefore investigated the strength of naturally acquired antibody responses to a range of pneumococcal protein antigens between individuals and in three diverse populations and whether IgG to protein antigens could be protective.

Methods: Antibody responses in pooled and individual sera were investigated using immunoblots and a multiplex assay of 18 recombinant pneumococcal antigens. Protective efficacy was assessed using in vitro assays of neutrophil phagocytosis and killing of unencapsulated S. pneumoniae opsonised with IgG-replete or depleted sera.

Results: Immunoblots demonstrated that individuals respond to a large number of protein antigens. Multiplex assays indicated that there was some variation between individuals in responses to specific pneumococcal antigens. However, anti-protein antibody responses in pooled sera from Malawi and intravenous immunoglobulin products from both Europe and the USA had remarkably similar patterns of antigen dominance. There were consistently high levels of antibody to the antigens PhtD, PspC, PspA and PsaA and weak responses to Eno, NanA and SltA. Neutrophil phagocytosis and killing of unencapsulated S. pneumoniae was reduced in IgG depleted serum.

Conclusions: These data demonstrate that naturally-acquired antibody responses to a range of conserved S. pneumoniae protein antigens are conserved amongst diverse populations and are functionally relevant. These antigens could be useful components for a polyvalent protein vaccine.
DETERMINANTS OF ANTIBODY RESPONSES TO PROTEIN ANTIGENS FOLLOWING NASAL COLONISATION WITH STREPTOCOCCUS PNEUMONIAE


Streptococcus pneumoniae colonisation of mice induces serum antibody to protein antigens protective against invasive pneumococcal disease (IPD). A large number of bacterial proteins are expressed during colonisation, but little is known about which antigens dominate the antibody response and which anti-protein antibodies mediate protection.

We investigated which S. pneumoniae proteins are immunodominant following murine colonisation. Sera from colonised (D39 or TIGR4) inbred (CBA/Ca and C57BL/6) and outbred (CD1) mice were studied using Western blotting of bacterial lysates, and responses quantified by ELISA and Luminex assay. Protein abundance was measured by nHPLC-QTOF mass spectrometry.

Functional importance of antibody in protection was studied by bloodstream infection of mice with mixed inocula of WT and mutant bacterial strains deficient in target antigens.

A limited number of proteins dominated the response to colonisation irrespective of bacterial strain or mouse genetic background. Overall, bacterial strain was more important that mouse background. There was a bias towards surface proteins, in particular PspA, Phd and lipoproteins which did not reflect protein abundance. The functional importance of colonisation-induced antibody to PspA was demonstrated by showing a competitive advantage to PspA-deficient bacteria over the parent WT strain during bloodstream infection in previously colonised mice compared to uncolonised controls.

We are currently utilising imaging approaches in WT and genetically-modified mice to investigate how B-cell interaction with bacterial proteins leads to immunodominance of specific bacterial surface proteins. Deeper understanding of such mechanisms of natural immunity will help explain predisposition to IPD and may lead to improved vaccine design.
FUNCTIONAL RESPONSES TO PNEUMOCOCCAL VACCINE DELIVERED AT BIRTH TO KENYAN INFANTS

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Background: In Kenya, invasive pneumococcal disease (IPD) occurs early in life. We recently showed that PCV7 is safe and immunogenic when immunization begins at birth (Scott et. al., CID 2011). Here we describe functional responses to the vaccine.

Methods: Infants born to HIV-ve mothers in Kilifi were randomised to receive PCV7 either at birth or 6w and then at 10w and 14w with EPI vaccines. Functional activity of sera to serotypes 4, 6B, 14, and 23F was assessed by OPA and compared to the serum IgG and antibody avidity measured by ELISA in a subset of 18 and 36w sera.

Results: 6B OPA GMT was significantly higher at 18w in the newborn group (6753 vs 3539, p=0.023) while 23F OPA GMT was higher in the EPI group at 36w (592 vs 1121, p=0.048). OPA correlated positively with IgG concentration and there were moderate but significant correlations for all the four serotypes at each time point. Some differences between the vaccine groups were only significant with either ELISA or OPA although the general trend was similar for both assays. There was a poor correlation between OPA and avidity.

Conclusions: PCV7 starting at birth induces functional antibody similar to the EPI schedule and ELISA and OPA generally correlated well.
IMMUNOGENICITY AND SAFETY OF A 10-VALENT PNEUMOCOCCAL CONJUGATE VACCINE IN CHILDREN AND ADOLESCENTS WITH LEUKAEMIA

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Background: Australian immunisation guidelines recommend a pneumococcal conjugate vaccine (PCV) booster following a haematological malignancy diagnosis. This study measured the serum immune responses to a 10-valent vaccine (PCV10) during chemotherapy for paediatric leukaemia.

Methods: Between May 2010 - January 2011, thirty-nine participants with leukaemia were recruited to an open-label study in Melbourne, Australia. Group 1 (n=27) had received a primary infant PCV7 course and had a single PCV10 dose. Group 2 (n=12) were PCV naïve and had three doses at two-monthly intervals. Serum was taken at baseline and 1-month post each dose. Anti-pneumococcal serotype specific IgG levels were measured by ELISA (10 serotypes) and opsonophagocytic assay (OPA) in 4 serotypes (1, 6F, 19F, 23F).

Results: Median age at enrolment was 6.2 years (1.7 -17.2 years) with 62% male. The median time from diagnosis to baseline serology was 7.4 months (1.6-36.8 months). At baseline, protective GMC above the threshold (> 0.35 µg/ml) ranged from 5.3% (serotype 4) to 71% (serotype 19F). More than 60% of participants in both groups were above threshold post immunisation for 7 of the 10 PCV serotypes. OPA analysis correlated with ELISA for all serotypes [(r) range =0.51-0.83]. The main adverse event following immunisation was tenderness at the injection site 73% (27/37), with one reported fever > 38°C.

Conclusion: It is safe and immunogenic to administer PCV10 immunisation during therapy for paediatric leukaemia. Long-term follow-up is required to determine duration of protection and response to a PCV 'booster' recommended at 6 months post completion of chemotherapy.
NASOPHARYNGEAL BACTERIAL COLONISATION EARLY IN LIFE MODULATES MUCOSAL IMMUNITY IN CHILDREN

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Background and aims: Mucosal immunity can be modulated by many factors in the postnatal infant. This study examined the effect of bacterial colonisation within the first 30 days of life on salivary IgA and specific salivary IgA antibody levels to Streptococcus pneumoniae (Spn), nontypeable Haemophilus influenzae (NTHi) and Moraxella catarrhalis (Mcat) in Aboriginal and non-Aboriginal children living in a semi-arid rural community in Western Australia.

Methods: Saliva samples and nasopharyngeal aspirates/swabs were collected at regular intervals from children aged 0-2 years as part of prospective birth cohort study. Bacterial load was estimated by culture quadrant score. Immune parameters were measured by ELISA. Early colonisation was defined as presence of a positive culture with Spn, NTHi or Mcat in the first 30 days of life.

Results: Compared with non-Aboriginal infants, in Aboriginal children, the bacterial load in the upper airways (P< .001) and the rate of increase in bacterial load (P< .002) were greater. Total salivary IgA was higher (P< 0.02) and the rate of increase of IgA levels was greater (P< .01). The combined bacterial load of Spn, NTHi and Mcat was positively correlated with the total salivary IgA level (P< .02). In Aboriginal children early colonisation was significantly associated with a lower initial specific antibody response for Mcat and NTHi (P< 0.008). In non-Aboriginal infants early colonisation was associated with a more rapid increase in Spn antibody (P< 0.02).

Conclusion: Nasopharyngeal colonisation in the first 30 days of life modulates mucosal immune responses in the upper airways. Specific antibody modulation is antigen dependant.
HUMAN PNEUMOCOCCAL CHALLENGE BOOSTS MUCOSAL ANTIBODIES BUT CARRIAGE IS NECESSARY TO INCREASE SYSTEMIC RESPONSES

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**Background and aims:** Experimental human pneumococcal challenge can be used as a tool to mimic antigen exposure and establish carriage. We compared challenge and carriage in healthy volunteers in order to assess whether challenge alone or carriage are immunizing events in the mucosal and systemic compartments.

**Method:** Healthy volunteers (n=30) were inoculated with 23F or 6B in 2 different protocols. Carriage was determined by serial nasal washing and plating. Capsular and protein specific IgG and IgA were measured by ELISA in nasal wash, serum and bronchoalveolar lavage (BAL) before and after pneumococcal inoculation. A subset of colonized volunteers (n=3) were re-challenged with the same strain 7 - 11 months after clearance of colonization.

**Results:** Pneumococcal challenge with undetectable carriage resulted in increased mucosal (nasal wash and BAL) levels of pneumococcus-specific IgG and IgA but this change was not seen in serum. Anti-PspA IgG levels increased in lung and decreased in serum after challenge. The overall experimental carriage rate was 54% (7 out of 13). Carriage lasted between 3 and 8 weeks. Subjects in whom pneumococcal carriage was established showed increased immunoglobulin responses in both serum and mucosal samples. Serum anti-capsular IgG levels were increased in volunteers with carriage and this correlated with an increase in opsonophagocytic activity (OPA) to the inoculated strain.

**Conclusion:** Carriage is an immunizing event which elicits increased immunoglobulin response and OPA and protects against carriage re-acquisition. Exposure without carriage also boosts levels of existing mucosal immunity in healthy adults.

**Acknowledgements:** The BMGF GCE and the NIHR BRC
CATCH-UP VACCINATION WITH 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE IN PNEUMOCOCCAL VACCINE-NAÏVE CHILDREN AND CHILDREN PREVIOUSLY VACCINATED WITH 7-VALENT PNEUMOCOCCAL CONJUGATE VACCINE

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Background/aims: Many children aged < 5 years have been previously vaccinated with 7-valent pneumococcal conjugate vaccine (PCV7) or are pneumococcal vaccine-naïve, and are eligible for catch-up vaccination with 13-valent pneumococcal conjugate vaccine (PCV13). These four studies evaluated PCV13 in children aged ≤5 years fully or partially immunized with PCV7, or not previously immunized with pneumococcal vaccine.

Methods: Study designs are shown in the Table.

<table>
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<tr>
<th>Study designs for catch-up vaccination with PCV13.</th>
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<td><strong>Age in months (mo) or years (y)</strong></td>
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<td>5 mo (≥140 to ≤196 days)</td>
<td>12 mo (≥336 to ≤392 days)</td>
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<td>Previous pneumococcal vaccination</td>
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<td>PCV7 at age 3 mo</td>
<td>PCV7 at ages 3, 5 mo</td>
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<td>PCV13 dosing schedule</td>
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<td>PCV13 at ages 3, 5 and 12 mo to complete a 2+1 schedule (ages 3, 5, 12 mo)</td>
<td>PCV13 at age 12 mo to complete a 2+1 schedule (ages 3, 5, 12 mo)</td>
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<td>Concomitant vaccination</td>
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<td>Recommended pediatric vaccines permitted</td>
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<td>Blood drawn</td>
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<td>1 mo after infant series; prior and 1 mo posttoddler dose</td>
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Results: IgG GMCs for the 7 serotypes contained in PCV7 were generally similar (1.55-35.51 µg/mL) in all groups following immunization with PCV13. For the 6 additional serotypes, IgG GMCs were 1.34-14.65 µg/mL (Sweden) and 1.1-5.3 µg/mL (France) posttoddler dose, and 1.11-14.18 µg/mL (USA) and 1.42-6.03 (Poland) after the catch-up dose. IgG GMCs for all 13 serotypes increased from pre- to postimmunization in all groups; where measured, fold rises ranged from 1.95- to 177.31. PCV13 was generally safe and well tolerated in a catch-up setting.

Conclusions: Catch-up vaccination schedules induced antibody responses to PCV13 serotypes in children either naïve to or previously fully or partially vaccinated with pneumococcal conjugate vaccine.
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PNEUMOCOCCAL SPECIFIC IL-17 POSITIVE CD4 T CELLS ARE PRESENT IN BAL AND INCREASE THE ANTI-PNEUMOCOCCAL FUNCTION OF ALVEOLAR MACROPHAGES

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**Background and aims:** Murine models show that pneumococcal-specific IL-17+ CD4 T cells are essential for protection against colonisation. Our aim was to show that 1) pneumococcal specific IL-17+ T cells are present in the human adult airway, 2) human alveolar macrophages (AMs) express IL-17 receptors and 3) IL-17 stimulation of AMs increases pneumococcal uptake

**Methods:** Human BAL cells (n=9) were stimulated with pneumococci (6B) in the presence of Brefeldin A. CD4 T cells were stained for intra-cellular cytokines (TNF, IFNγ and IL-17) and positive cells measured as a percentage of CD4 T cells by flow cytometry. IL-17RA and RC receptors were measured on AMs using flow cytometry. AMs for an opsono-phagocytosis assay were adhered to a 96-well plate. Washed AMs were co-cultured with opsonised serotype 2 pneumococci with or without 10ng (125ng/ml) or 50ng (625ng/ml) of rhIL-17, in triplicate, for 2 hours in the presence of complement. Colonies were enumerated on agar to determine % uptake.

**Results:** Pneumococci stimulated CD4 T cells produce more IL-17 (mean %±SD 0.12±0.09 vs control 0.039±0.02, p=0.011), TNF (mean %±SD 0.24±0.19 vs control 0.09±0.06, p=0.016) but not IFNγ compared to non-stimulated cells. The mean (±SD) fluorescence intensity for IL-17RA was 187±113 (n=7) and for RC 111±71 (n=8) units on AMs. Finally, 10 or 50ng rhIL-17stimulated AMs exhibit 20(±25.8%SD) and 33(±22%SD) % greater pneumococcal uptake, respectively, compared to vehicle treated AMs (n=3).

**Conclusion:** 1) BAL pneumococcal-specific CD4 T cells produce IL-17. 2) AMs express IL-17 receptors and 3) following stimulation exhibit enhanced anti-microbial activity.
Influenza virus predisposes individuals to secondary infections with the bacterium *Streptococcus pneumoniae*. Despite the prevalence of OM, our understanding of disease pathogenesis is limited. Using a novel disease model for pneumococcal OM, we show that infant mice co-infected with *Streptococcus pneumoniae* and influenza virus had significantly higher bacterial load in the middle ear, middle ear inflammation and hearing loss compared to mice colonised with *S. pneumoniae* alone. Here, we show that this phenotype is dependent on the viral hemagglutinin (HA). Of the two HA subtypes currently circulating in the human population, only H3 viruses induced disease. The ability of H3 viruses to induce pneumococcal OM reflected their ability to induce inflammation in the middle ear, and was dependent on the active replication of the virus. These findings were confirmed using human middle ear epithelial cells, emphasising the clinical relevance of these data. We then used the observation that influenza virus induces pneumococcal OM to study the role of different adjuvants in vaccination. Here, we show that mucosal vaccination with pneumococcal surface protein A in combination with cholera toxin subunit B (CTB) protected mice against influenza virus-induced replication of *S. pneumoniae* in the middle ear cavity. Mice vaccinated with CTB and PspA showed significantly higher antibody responses compared to other adjuvants. Our findings strongly emphasize the value of using well-defined, clinically relevant animal models of disease for vaccination purposes, in particular for complex multi-factorial diseases such as pneumococcal OM.
IMPAIRED IMMUNOLOGICAL MEMORY TO STREPTOCOCCUS PNEUMONIAE PROTEIN ANTIGENS IN AFRICAN POPULATIONS WITH HIGH RATES OF COLONISATION AND HIV SEROPREVALENCE

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¹Blantyre, Malawi, ²Liverpool, ³Bristol, UK

Background and aims: Immune memory is a key to the development of natural protection against Streptococcus pneumoniae (Spn). We have previously demonstrated age-related mucosal Th1 and Th17 CD4+ T cells reactive to Spn, controlled by antigen-specific Tregs; and shown that African adults are immunologically primed, presumably through colonisation. To investigate the role of immunological memory in control of pneumococcal colonisation/ invasion in immunocompromised and immunocompetent populations, we have characterised Spn immunity in HIV-infected adults and mucosal immunity in children undergoing routine tonsillectomy.

Methods: T cell proliferation and intracellular expression of IFN-gamma/IL17, and B cell antibody response to Spn antigens were measured in blood from HIV-infected and uninfected Malawian adults. Mucosal regulation of immune memory in otherwise healthy Malawian children was assessed by CD25⁺(Treg) depletion.

Results: T cell and B cell Spn-specific memory was dysregulated even in adults with asymptomatic HIV-infection compared to healthy controls. Further immune deterioration was associated with increased nasopharyngeal carriage but not impairment in CD4 T cell IL17 production. Immune reconstitution with antiretroviral therapy (ART) was not associated with decreased carriage. Palatine tonsillar T cell immunity to Spn occurred early in Malawian children with rapid emergence of Treg activity.

Conclusions: These data highlight the complex relationship between pneumococcal colonisation, cellular immunity, and its regulation in immunocompromised and immunocompetent populations. They raise the possibility that where pneumococcal carriage rates are high, development of immune regulation to Spn in children and poor reconstitution of Spn immunity in HIV infected adults leave individuals vulnerable to invasive disease.
COMPARISON OF ANTIBODY RESPONSE TO POLYSACCHARIDE AND CONJUGATE PNEUMOCOCCAL VACCINES IN HIV-INFECTED ADULTS IN SÃO PAULO, BRAZIL

Y.-L. Ho, M.C.C. Brandileone, C.F. Rizek, A.P. Brandão, M.H. Lopes, São Paulo, Brazil

Background and aims: The risk of invasive pneumococcal disease (IPD) and mortality are higher in HIV-infected patients than in uninfected individuals. Strategy to reduce the burden of IPD is crucial. Pneumococcal polysaccharide vaccine 23-valent (PPV) is recommended for HIV-adults, but its immunogenicity is still controversial. Few trials with 7-valent pneumococcal conjugate vaccine (PCV7) in HIV-adults revealed disparate results. This study aims to compare antibody response to PPV and PCV7 in HIV-infected adults.

Methods: A randomized, blinded clinical trial with HIV-adults aged 18-60 years, CD4 count ≥200 cells/mm³, was conducted in the city of São Paulo, Brazil. To compare the immunogenicity of PPV and PCV7 to serotypes 6B, 9V and 14, total IgG antibody concentrations to serotypes 6B, 9V and 14 were measured by ELISA, according to the Training Manual for Elisa for the Quantitation of Streptococcus pneumoniae Serotype Specific IgG of WHO Pneumococcal Serology Reference Laboratories.

Results: Of the 331 volunteers recruited, 210 received PCV7, and 111, PPV. No statistically significant differences were observed between both groups in demographic characteristics, information related to HIV-infection and pre-vaccine antibody concentration.

Sixty days after vaccination, the proportion of individuals with antibody concentration ≥0.35µg/mL was similar to both groups. However, for serotype 9V, geometric means concentration (GMC) was statistically higher in the PCV7 group. Proportion of individuals with >4-fold increase in IgG GMCs was higher to serotypes 6B and 9V in the PCV7 group.

Conclusion: These results suggest conjugate vaccine may be more immunogenic than PPV for serotypes 6B and 9V in HIV-adults.
CORRELATION OF SERUM AND SALIVARY ANTIBODY IN HIV-INFECTED (HIV+) AND HIV-UNINFECTED (HIV-) CHILDREN VACCINATED WITH PNEUMOCOCCAL CONJUGATE VACCINE (PCV)

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Background: There is no data on the association of serum and salivary anti-capsular antibody responses to PCV in HIV+ children. We analysed the correlation between serum and salivary IgG antibody concentration following a three dose primary series and post-booster of PCV in HIV+ and HIV- children.

Methods: Infants received PCV at 6, 10 and 14 weeks, half of whom also received a booster at 15-18 months. Vaccine-serotype salivary IgG was measured by luminex assay and serum antibody by standard ELISA one month post-primary series, 9 months of age, prior to the booster dose and two weeks later. The study included HIV+ infants with CD4+ ≥25% randomised to immediate antiretroviral treatment (HIV+/ART+) or deferred ART until clinically or immunologically indicated (HIV+/ART-). Furthermore, HIV- infants born to HIV- mothers (M-/I-) and HIV+ mothers (M+/I-) were enrolled.

Results: Correlations for each serotype were significantly different from zero at all timepoints and increased over time in all groups. Similar correlations were observed between HIV+/ART+, HIV+/ART- and M-/I- children. The effect of serum on saliva antibody was larger in these HIV-exposed infants compared to M-/I- infants until receipt of the booster dose for most serotypes, however, this was no longer evident post-booster.

Conclusions: We hypothesise that differences in the strength of correlation in HIV-exposed children compared to M-/I- infants may be due to impaired integrity of oral mucosa, resulting in greater transudation of serum IgG onto mucosal surfaces, in HIV-exposed children. The effect of our observation on pneumococcal colonization is being investigated.
DO MATURATION OF INFANT IMMUNITY AND MICROBIOME PREDICT RESPONSES TO PNEUMOCOCCAL CONJUGATE VACCINE IN INFANTS?

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Background/aims: We are determining the relationship between maturation of the infant immune system and intestinal microbiome and antibody responses following 7-valent pneumococcal conjugate vaccine (PCV7) in infants.

Methods: We immunized 17 healthy breastfed U.S. infants with PCV7 at 2, 4 and 6 months, sampled blood and stool at birth, 1, 2, 6, 12 months. We characterized CD4+ T and B cell subsets by flow cytometry, microbiome by high-throughput 454 sequencing of 16S ribosomal DNA (>2,000 transcripts/sample) and IgG and IgM to 5 serotypes (ST4, 6B, 9V, 14, 19F) by ELISA.

Results: Among 10 infants to date, the proportion of memory CD4+ T cells (not CD45RA+62L+11dim) remained stable (15.8-18.8%) over time but with substantial variance (SD 5.0-8.7%), and activation (CD3+HLA-DR+) remained low (< 2%). At birth, >95% of B cells were naive (IgD+IgM+CD27-). Intestinal meconium was sterile at birth. The phylum Bacteroides predominated at 1-2 months, but phylum Firmicutes comprised 50% of bacterial transcripts by 6 months, with significant inter-infant variance. Levels of IgM to pneumococcal ST were undetectable at birth-1 month, present in 2/10 infants pre-PCV7 at 2 mos, and 9/10 (500-700 ELISA units) at 6 and 12 mos post-doses 2 and 3. ST-specific IgG fell from 1100 EU at birth to 260 EU at 2 mos, increasing to >2,400 and >1,000 EU at 6 and 12 mos, respectively.

Conclusions: Waning maternal ST-specific IgG had no impact on vaccine responses, and IgM persisted through 1 year. We are correlating the diversity of microbial species and the variance in infant immune maturation over time with the magnitude of responses to PCV7.
Poster No 195

PERSISTENCE AND FUNCTIONAL ACTIVITY OF RESPONSES TO 7-VALENT PNEUMOCOCCAL CONJUGATE COMPARRED TO 23 VALENT PNEUMOCOCCAL POLYSACCHARIDE VACCINE IN THE ELDERLY


Background: The potential benefits of conjugate vaccine use in the elderly remain unclear.

Methods: We performed an open-label, randomized study that compared PCV7 with PPV23 in 50-80yr olds. Vaccinees received either one dose of PCV7 or PPV, or two doses; PCV7+PCV7 or PCV7+PPV separated by 6 months. Primary responses (IgG) and persistence to one year have previously been reported (Goldblatt et al. CID 2009). Here we report antibody persistence to 2 years (n=269) and functional activity (OPA) to serotypes 4/6B/14/23F on a subset (n=114).

Results: IgG for most serotypes 2y post initial vaccination were similar for all groups irrespective of age and for all except 19F remained above pre-vaccination levels. Type 4 IgG fell slowest between 12-24 months for those given 1xPCV7 while 9V and 18C IgG at 24 months was lowest for the 1xPPV group. OPA titers for 4, 6B, 14 and 23F pre, 4-6 weeks post the first and/or second vaccine and at 2y were similar for all groups and correlated well with ELISA. Serotype 4 titers at 2 years were lower for those immunized with a single dose of PPV.

Conclusions: Responses to vaccine remain above baseline at 2 years for all except 19F irrespective of vaccine schedule. Functional antibody agrees well with binding IgG in the elderly. http://www.clinicaltrials.gov/NCT00197821.
CROSS REACTIVE FUNCTIONAL ANTIBODY TO 19A AND 19F FOLLOWING PCV7 OR PCV13 AMONG NAVAJO AND WHITE MOUNTAIN APACHE INFANTS

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Background and aims: In March 2010, 13-valent pneumococcal conjugate vaccine (PCV13) replaced PCV7 for routine vaccination among Navajo and White Mountain Apache communities. We compared PCV13 and PCV7 immunogenicity to inform our understanding of the clinical impact of the change.

Methods: We aimed to collect 100 blood specimens from children receiving only PCV7 or only PCV13 at 3 time points: post-dose3, pre- and post-boost (600 specimens). Sera were separated, stored at -70°C and tested by ELISA (CPS and 22F adsorption). Of the post-dose3 sera, a preliminary subset (n=30) were tested by multiplexed opsonophagocytic assay (OPA) for functional antibody to serotypes 19A and 19F (titer >1:8 considered positive) and then retested after adsorbing sera with 19F or 19A capsular polysaccharide to assess type specific contribution to killing.

Results: Post-dose3 sera in 15 PCV7 and 15 PCV13 recipients matched for 19F IgG concentrations were assessed by 19F and 19A-OPA. PCV13 recipients had higher functional anti-19F responses than PCV7 recipients (483 vs 208, p=0.04). 19F-IgG correlated with 19F-OPA. 15/15 PCV13 compared to 1/15 PCV7 recipients had 19A-OPA activity. 19A polysaccharide adsorption resulted in a 25% reduction in 19F-OPA activity in PCV7 recipients despite absent 19A-OPA activity. In PCV13 recipients, heterologous adsorption of sera resulted in a 40% reduction in activity for both OPA’s suggesting that some 19F killing in PCV13 recipients is mediated by 19A antibodies.

Conclusions: This preliminary data suggests that PCV13 may have enhanced activity against 19F due to cross reactive antibodies induced by 19A.
MOLECULAR CONSEQUENCES OF THE INTERACTION OF PNEUMOCOCCAL CAPSULAR POLYSACCHARIDE TYPE 1 WITH IMMUNE CELLS

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Pneumococcal capsular polysaccharide (PCP) is its major virulence factor. Pneumococcal strains devoid of the capsule are avirulent. Protection against pneumococci is primarily mediated by opsonic anticapsular antibodies. Serotype 1 is a significant contributor to the incidences of invasive pneumococcal disease worldwide. PCP is present in free soluble form and on the surface of pneumococci in infected individuals, and can potentially interact with the immune cells. We analyzed the molecular consequences of the interaction of PCP from serotype 1 (PCP1) with immune cells. Using flowcytometry we demonstrated that PCP1 binds to the surface of B, macrophage and dendritic cell lines but not to T cell line. PCP1 bound to the surface of the RAW264.7 cells in a dose dependent manner. Treatment of various immune cells with PCP1 resulted in the production of proinflammatory cytokines. PCP1 induced cytokine production was not cell line or cytokine specific. Immunoblotting and inhibitor experiments with RAW264.7 cells revealed that PCP1 exerted its proinflammatory activity by inducing MAP kinase pathway. Using HEK293T transfectants expressing TLR1 and TLR2, we demonstrated that PCP1 induced IL-8 production involved TLR2. TLR2 blocking antibody inhibited PCP1 induced TNF-α from RAW264.7 cells. Acetyl groups present in PCP1 made a small but significant contribution towards PCP1 induced proinflammatory responses. Analysis of anti-PCP1 antibody responses in mice immunized mice with soluble PCP1 or whole heat killed pneumococcal strain ATCC 6301 (serotype 1) showed similar antibody isotype profiles. Our data suggests that PCP1 is an important modulator of innate and adaptive immune response against pneumococci.
**IMMUNOSENESCENCE CONTRIBUTES TO INCREASED PNEUMOCOCCAL COLONIZATION IN AGED MICE**

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**Introduction:** Nasopharyngeal colonization with *Streptococcus pneumoniae* is usually asymptomatic, though an important prerequisite for disease. Elderly are especially at increased risk for severe infections.

**Aim:** To study potential mechanisms of increased risk of pneumococcal infection in an elderly mouse model.

**Methods:** Female C57Bl/6 mice aged 18-23 months (elderly) and 3-4 months (young controls) were intranasally inoculated with serotype 6B pneumococci. We determined density and duration of colonization 1-2 times a week for 4 weeks. In addition, we studied dynamics of immune cells infiltrating into the nasopharynx by flow cytometry. Cell signalling in the NALT was studied using real-time semi-quantitative PCR.

**Results:** Although there was no significant difference in colonization density between elderly and young mice at day 3, elderly were colonized significantly longer. We observed greater immune cell numbers in the nasopharynx of elderly before colonization plus a larger increase in cell influx in the days post-inoculation compared to young controls. In young mice we observed an influx of monocytes/macrophages at day 7, strongly correlating with clearance of bacteria. These dynamics were less apparent in elderly mice. Also, young mice significantly increased expression of IL-1β and NLRP3 after colonization, while elderly did not significantly up-regulate these genes.

**Conclusion:** Elderly mice were not able to clear pneumococcal colonization as quickly as controls. Whether this process is causally related to the observed dynamics in monocytes/macrophages needs further study.
SURGICAL AND PATHOLOGICAL FEATURES OF 123 CASES OF PNEUMOCOCCAL PERITONITIS IN YOUNG FEMALES (F-PnP) OF THE METROPOLITAN REGION (MR), CHILE

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Background: F-PnP is a relatively uncommon, poorly understood manifestation of invasive pneumococcal disease (IPD). In the MR, a prospective, 14-year systematic surveillance detected 123 cases of F-PnP (the largest clinical heretofore reported), representing ~9% of all IPD cases recorded in females < 15 years of age. Most cases were due to S.pneumoniae serotype 1 in previously healthy school- age girls (Table 1).

Aim: To characterize the MR's case-series of F-PnP, we re-inspected the patients' medical records for information not captured during the IPD surveillance-study.

Methods: Past medical history, surgical procedures, intraoperative findings and histopathology reports were reviewed in relation to S.pneumoniae serotypes: St-1 (N=63 cases); non-1 (N=19) and untyped (N=41).

Results: (Table 2) Most chronically ill patients (7/8) were associated with non-St-1; 50% of those with non-St-1 isolates who underwent laparostomy exhibited overt signs of appendicular peritonitis. Conversely, all 63 St-1 S.pneumoniae isolates came from previously healthy girls; 6 patients presented with pneumonia or pleuropneumomia concomitant with peritonitis, and >75% were reported without meaningful visceral abnormalities upon abdominal surgery. Histopathological findings were consistent with surgeons' intraoperative assessments.

Comment: Whereas YF-PnP has traditionally been attributed to ascending genital-tract infection, our study suggests that haematogenous spread, rather than ascending infection, was the leading pathogenic mechanism in the MR's case-series. Isolation of S. pneumoniae from blood and/or peritoneal fluid of patients with overt appendicular peritonitis is intriguing, since pneumococci are not part of the intestinal flora.
PCV-ELICITED ANTIBODY PERSISTENCE AND RESPONSES TO PCV13 IN CHILDREN PREVIOUSLY VACCINATED WITH 4 DOSES OF EITHER PCV7 OR PCV13

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Background/aims: Assessed antibody persistence ≥24 months after toddler dose of PCV13 or PCV7 and immunogenicity and safety of follow-on dose of PCV13 in children previously vaccinated with four doses of PCV.

Methods: Children vaccinated in a previous study at ages 2, 3, 4 and 12 months with PCV7 (PCV7/PCV7) or PCV13 (PCV13/PCV13), or 3 doses of PCV7 and one toddler dose of PCV13 (PCV7/PCV13) received PCV13 at age 3 years. Pneumococcal IgG responses were measured prevaccination, 4-7 days, and 1 month postvaccination. Safety events were collected.

Results: 262 subjects enrolled. All groups had comparable antibody levels prevaccination for the 7 common serotypes; for the 6 additional serotypes, groups previously administered PCV13 had higher levels than the PCV7/PCV7 group. The follow-on dose of PCV13 was immunogenic regardless of previous regimen. One month postvaccination, all groups had similar responses to the common serotypes (IgG GMCs: 4.56-51.07 µg/mL). Groups previously vaccinated with PCV13 had higher responses to the additional serotypes (1.81-23.09 µg/mL) than the PCV7/PCV7 group (1.60-14.38 µg/mL); lower bounds of 95% CIs of serotype response ratios of groups previously administered PCV13 compared to the PCV7/PCV7 group were >1 for most additional serotypes. Groups previously vaccinated with PCV13 demonstrated an early response to the additional serotypes at 4-7 days postvaccination. Safety was comparable across groups.

Conclusions: Antibody persisted 2 years after a toddler dose of PCV13. An additional dose of PCV13 was safe and immunogenic regardless of previous vaccination with PCV7 or PCV13. Responses reflected a memory response.
IMMUNOGENICITY AND SAFETY OF 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE: POPULATION DIFFERENCES OBSERVED IN ASIAN, EUROPEAN, AND AMERICAN PAEDIATRIC STUDIES

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¹Seoul, Republic of Korea, ²Taipei, Taiwan R.O.C., ³Sapporo, Japan, ⁴Berlin, Germany, ⁵Mumbai, India, ⁶Mainz, Germany, ⁷Collegeville, PA, ⁸Pearl River, NY, USA

Background/aims: Paediatric studies with PCV13 in Korea, Japan, Taiwan, India, USA, and Germany were reviewed.

Methods: Infants received PCV13 and other vaccines according to national immunization programs.

Results: For most serotypes, ≥95% of subjects had IgG concentrations ≥0.35 µg/mL postinfant series. IgG levels were generally higher in Korea, Japan, and Taiwan than India, USA, and Germany (Table 1, Table 2); posttoddler results were similar. PCV13 was safe; redness and swelling were more frequent after subcutaneous dosing (Japan).

Conclusions: Asian populations generally showed higher immune responses. Clinical significance of population differences in immune responses is currently unknown.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Korea (n=83)</th>
<th>Japan (n=175-176)</th>
<th>Taiwan (n=80)</th>
<th>India (n=185-204)</th>
<th>USA (n=251-252)</th>
<th>Germany (n=284-285)</th>
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<tr>
<td>23F</td>
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<td>1.93</td>
<td>1.38</td>
<td>1.33</td>
<td>1.26</td>
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</table>

(Table 1. 7 PCV7/PCV13 serotypes: GMCs [µg/mL])

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Korea (n=83)</th>
<th>Japan (n=176)</th>
<th>Taiwan (n=80)</th>
<th>India (n=200-206)</th>
<th>USA (n=249-252)</th>
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<td>1.44</td>
<td>2.19</td>
<td>1.33</td>
</tr>
<tr>
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<td>2.27</td>
<td>2.57</td>
<td>2.59</td>
</tr>
<tr>
<td>19A</td>
<td>5.94</td>
<td>6.97</td>
<td>3.69</td>
<td>2.76</td>
<td>2.07</td>
<td>3.26</td>
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</table>

(Table 2. 6 PCV13-unique serotypes: GMCs [µg/mL])
LONG-TERM FOLLOW UP STUDY TO THE FIJI PNEUMOCOCCAL PROJECT: INVESTIGATION OF IMMUNE HYPORESPONSIVENESS FOLLOWING A 12-MONTH PNEUMOCOCCAL POLYSACCHARIDE BOOSTER

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Background & Aim: The Fiji Pneumococcal Project (FiPP) examined the immunogenicity of reduced dose schedules comprising pneumococcal conjugate (PCV) and polysaccharide (PPS) vaccines. Following a micro-PPS (20%) challenge dose at 17 months, children who received the PPS at 12 months of age exhibited immune hyporesponsiveness in terms of serotype-specific IgG and opsonophagocytic (OPA) levels. The 12-month PPS dose also had no impact on vaccine-type carriage. We set out to examine anti-pneumococcal immunity in the same children at 4-6 years of age.

Methods: Blood samples were taken pre- and 28-days post-PCV13 immunisation from the first 101 children (now 4-6 years old) re-consented into this study. To date, analysis includes specific IgG measurement to all 23 serotypes in PPS as well enumeration of the memory B cell response to serotypes 1, 3, 4, 6B, 14, 19F and 23F.

Results: Significantly lower pre-PCV13 IgG levels for 6B (p=0.003), 22F (p=0.024) and 23F (borderline p=0.053) were detected in children who received the 12-month PPS; after PCV13, only serotype 22F IgG was lower in PPS-vaccinated children (p=0.009). Memory B cell levels were not different before PCV13. However, significantly lower number of 23F-specific B cells (p=0.021) post-PCV13 were found in children given PPS at 12 months of age.

Conclusion: Altered pneumococcal immunity to a limited number of serotypes was observed in children 3-4 years after they received PPS at 12 months of age. The clinical implications of this are unknown. Analysis is ongoing to evaluate the impact of the PPS booster in this study.
CHARACTERIZATION OF HUMAN TH17 RESPONSES TO STREPTOCOCCUS PNEUMONIAE: COMPARISONS BETWEEN ADULTS AND CHILDREN IN A DEVELOPED AND A DEVELOPING COUNTRY

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**Background:** Intranasal exposure to *Streptococcus pneumoniae* and either mucosal or parenteral immunization with a recently developed killed pneumococcal whole cell vaccine, confer Th17-mediated protection against *S. pneumoniae* nasopharyngeal colonization in mice. Given the ongoing efforts to develop this vaccine for infants and children in developing countries, we analyzed Th17 responses to the whole cell antigen (WCA) and individual pneumococcal antigens in Swedish and Bangladeshi children and adults.

**Methods:** Cytokine production from peripheral blood mononuclear cells (PBMCs) stimulated with pneumococcal antigens was analyzed by ELISA and flow cytometry.

**Results:** PBMCs from Swedish adults produced IL-17A after stimulation with WCA, the pneumolysoid PdT and the protein required for cell separation in group B streptococci (PcsB). IL-22 and IFN-γ responses were also detected, but originated from separate CD4+ T-cell subsets. PBMCs from Swedish children produced lower levels of IL-17A in response to WCA compared to adults, whereas no such difference was noted from Bangladeshi samples, where responses by children and adults were both significantly higher than those in Sweden. High IL-17A responses to stimulation with WCA were also observed in children with proven or probable pneumococcal pneumonia.

**Conclusions:** Our results demonstrate the presence of Th17-type T cells that are specific for pneumococcus in both children and adults. The different levels of Th17 responses to pneumococci in children and adults in developing and developed countries, which may at least partly be due to differences in pneumococcal exposure, are important factors to consider in clinical evaluation of candidate pneumococcal protein-based vaccines. Supported by PATH.
KINETICS OF SALIVARY IGA AND IGG VACCINE-SEROTYPE ANTIBODY IN HIV-INFECTED (HIV+) AND -UNINFECTED (HIV-) CHILDREN FOLLOWING PNEUMOCOCCAL CONJUGATE VACCINE

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Background: Salivary IgG and IgA may play a role in protecting against pneumococcal nasopharyngeal colonization. We compared vaccine-serotype salivary IgA and IgG antibody concentrations following the primary series and after booster dose of PCV in HIV+ and HIV- children.

Methods: Infants received PCV at 6, 10 and 14 weeks and half of them received a booster at 15-18 months. Vaccine-serotype salivary IgG and IgA antibody was measured by luminex assay one month post-primary series, at 9 months, just prior to the booster dose and two weeks thereafter. The study included HIV+ infants with CD4+ ≥25% randomised to immediate antiretroviral treatment (HIV+/ART+) or deferred ART until clinically or immunologically indicated (HIV+/ART-). Furthermore, HIV-infants born to HIV- mothers (M-/I-) and HIV+ mothers (M+/I-) were enrolled.

Results: Salivary IgA and IgG concentrations were similar one-month following the primary series and at 9 months of age between all four groups, except that HIV+/ART+ children had higher IgA (to serotypes 4,14, 23F) and IgG (to serotypes 9V and 19F) concentrations than M-/I- one-month post primary series. IgA antibody concentrations were however higher in M-/I- compared to any other groups of HIV-exposed children for all serotypes prior to the booster dose. The booster dose was associated with robust salivary IgA and IgG antibody responses in all four groups and antibody concentrations did not differ between groups thereafter.

Conclusions: HIV exposure was associated with lower durability of salivary IgG and IgA antibody compared to M-/I- children. A PCV booster dose induced anamnestic mucosal antibody responses.
DEVELOPMENT OF CROSS-REACTIVE ANTIBODIES TO THE PROLINE-RICH REGION OF PNEUMOCOCCAL SURFACE PROTEIN A (PSPA) IN CHILDREN

M. Melin1, S. Hollingshead2, J. Palomäki1, P. Coan2, 1Helsinki, Finland, 2Birmingham, AL, USA

Background: The highly variable alpha-helical part of PspA is immunogenic and elicits protective antibodies. Serologically cross-reactive PspAs have been grouped into two major families. Our previous studies indicated that children develop antibodies preferentially to the same PspA family they have been exposed to. The proline-rich part of PspA, which is also present in most PspCs, often contains a highly conserved epitope called the non-proline-block (NPB). In this study we assessed the ability of antibodies induced by infection to recognize this part of PspA.

Methods: Sera of children (n=47) with history of pneumococcal carriage or otitis media, collected in the FinOM Cohort Study, were analyzed for presence of IgG antibodies to CD-2 (an antigen containing proline-rich/NPB epitopes) as well as to PspA family 1 and 2 antigens by ELISA. The PspA families expressed by the pneumococcal isolates of the children were characterized in a previous study.

Results: The antibody titers to the CD-2 antigen were significantly higher in the sera of children who had been exposed to pneumococci, compared to the pre-exposure sera of the same children. Moreover, the antibody titers to CD-2, in contrast to PspA family 1 and 2, were similar in children exposed to pneumococci expressing either family 1 or family 2 PspA.

Conclusions: The results indicate that the proline-rich part of PspA elicits antibodies during infection. The fact that a rise in antibody titer was generally seen in response to pneumococcal exposure underscores the potential of the “common” epitopes of this region to elicit broad cross-protection.
Background: Discovering new aspects of pneumococcal pathogenesis may identify new therapeutic approaches. We have previously identified four cell wall proteins with known enzymatic activity (PPP, GtS, NOX, and FBA). We now show that these proteins moonlight in the cell wall as adhesins. Their putative target molecules have been identified, and peptides derived from the target molecules have been tested for ability to interfere with bacterial adhesion in vitro and severe disease development in vivo.

Methods: The ability of the proteins to interfere with bacterial adhesion to A549 cells and the adhesion of null mutant bacteria were tested. To identify putative target molecules, a phage display library was screened with the four recombinant proteins. The insert peptides in the phages that bound the protein were sequenced and aligned against the human genome. Peptides derived from putative target molecules were tested for ability to inhibit bacterial adhesion in vitro and prevent disease development in infected mice.

Results: The recombinant proteins inhibited bacterial adhesion, and the viable null mutant bacteria exhibited reduced adhesion in vitro, as well as in vivo virulence in mice. Peptides derived from target molecules were capable of inhibiting bacterial adhesion in vitro and reducing colonization and mortality in infected mice.

Conclusion: Some of the proteins found to reside in the pneumococcal cell wall have an adhesin function. Identification of their target molecules enabled the synthesis of peptides capable of interfering with bacterial adhesion in vitro and reducing disease development in infected mice.
DEVELOPMENT OF MULTIPLEX BEAD-BASED IMMUNOASSAY FOR DETECTING ANTIBODIES TO 16 PNEUMOCOCCAL PROTEINS IN PEDIATRIC SERA

M. Tal1, O. Liron1, S. Samira1, N. Rachamim1, D. Warino2, M. Paton2, P. Pierre Cambron3, Y. Mizrahi Nebenzahl4, R. Dagan4, R. Ellis1, 1Ness Ziona, Israel, 2Lee’s Summit, MO, USA, 3Rixensart, Belgium, 4Beer Sheva, Israel

Background: We developed and qualified a multiplex bead-based immunoassay (Luminex) for simultaneous detection of natural antibodies to 16 pneumococcal proteins. The assay was engineered to evaluate the serum antibody responses during childhood and to naturally occurring pneumococcal infections for eventual use in clinical studies of pneumococcal protein vaccines.

Methods: Each of the 16 candidate vaccine antigens was expressed in E. coli and purified, then chemically bound to Luminex beads. We identified a secondary antibody that can detect both human and rabbit IgGs, enabling the use of rabbit hyper-immune antisera as assay calibrators in the absence of human protein-specific sera.

Results: The standard curve was an optimized pool of 16 monospecific anti-protein rabbit antisera, which were diluted for assigning Relative Unit values as assay standards for each anti-protein antibody. We selected a specific human serum as an inter-assay standard for detecting all 16 antibody signals with fluorescence values within specified control limits. Specificity of ≥92% was established by the ability of each of the 16 proteins to block its specific signal (except one, which inhibited 54%). We also determined intra- and inter-assay precision, linearity with dilution, lower limits of detection and quantification, and sample stability for completing assay qualification.

Conclusion: The lowest serum control could be diluted ten additional 2-fold serial dilutions and still detect all protein signals as indicated by a recovery range of 84-116%. Therefore, the multiplex-immunoassay can be employed for pediatric sera to detect anti-pneumococcal protein antibodies, which are at variable and often very low titer.
THE IMPACT OF T-CELL PERTUBATIONS ON PNEUMOCOCCAL CONJUGATE VACCINE RESPONSES IN HIV-INFECTED ADULTS

L. Munk-Petersen, T. Gravesen Johannesson, O.S. Søgaard, M. Tolstrup, C. Erikstrup, L. Østergaard, Aarhus, Denmark

Background: HIV-infected persons have excess risk of infections caused by Streptococcus pneumoniae. Furthermore, these individuals are often hyporesponsive to pneumococcal immunization. In this study, we assessed the impact of specific CD4+ T subpopulations on antibody responses to 7-valent pneumococcal conjugate vaccine in persons with HIV.

Methods: Ninety-five HIV-infected adults were immunized twice with double-dose 7-valent pneumococcal conjugate vaccine with or without 1 mg CPG 7909 (toll-like receptor 9 agonist) at baseline and 3 months. Serum IgG concentrations for vaccine serotypes were quantified by ELISA at baseline and 3, 4, and 9 months post-vaccination. Pre-vaccination CD4+ T-cell populations were assessed by flow cytometry and classified as naïve (CD45RA+CCR7+CD27-), effector memory (EM) CD45RA-CCR7-CD27+, central memory (CM) CD45RA-CCR7+CD27+, terminal differentiated (TD) CD45RA-CCR7-CD27-, TH17 (IL17a+), and regulatory T cells (T-reg) CD25+CD125+FoxP3+.

Results: As expected, total CD4+ cell counts predicted IgG responses at all time points. Neither total counts or fractions of naive and TD CD4+ T cell counts were associated with antibody responses whereas increasing counts and fractions of CM, EM, TH17, and T-reg cells were all positively associated with high post-vaccination IgG titers. Increased expression of CD38, a marker of activation on CD4+ T cells strongly correlated with poor antibody responses.

Conclusion: High pre-vaccination levels of CD4+ T cell subsets associated with immunological memory and inflammatory regulation (TH17 and T-reg) predicted good antibody responses following immunization with pneumococcal conjugate vaccine. In turn, the negative effect of immune exhaustion (high CD38 expression) highlights the detrimental effects of HIV-associated destruction of immune competence.
THE LECTIN-LIKE OXIDIZED LOW-DENSITY LIPOPROTEIN RECEPTOR (LOX-1) SYNERGIZES WITH TLR2 AND 9 TO INDUCE IL-8 IN PNEUMOCOCCI INFECTED BRONCHIAL EPITHELIUM


Background and aims: Streptococcus pneumoniae (S. pneumoniae) is a major cause of pneumonia, one of the most common causes of death due to infectious diseases worldwide. LOX-1 is a type II membrane receptor and a member of the scavenger receptor family which has been characterized as the receptor for oxidized low-density lipoprotein (OxLDL). Although S. pneumoniae and OxLDL have the protein phosphorylcholine in common, which share the ability to bind to LOX-1, little is known about the role and regulation of this receptor in Pneumonia. The goal of this study was to investigate the role of LOX-1 in S. pneumoniae caused infection of bronchial epithelium.

Methods: S. pneumoniae R6x; Bronchial epithelial cell line: BEAS-2B; siRNA for: LOX-1, TLR (Toll Like Receptor) 2 and 9; LOX-1 overexpression plasmids; Pneumococcal adhesion assay; confocal microscopy; IL-8 ELISA.

Results: We observed that bronchial epithelial cells express LOX-1. Furthermore, infection of the bronchial epithelial cells with pneumococci leads to a time dependent increase of the expression of LOX-1. Inhibition of LOX-1 expression with specific siRNAs reduced the adhesion of S. pneumoniae to bronchial epithelium as well as the expression of IL-8, a key cytokine for leukocyte migration and inflammation in pneumonia. Accordingly overexpression of LOX-1 in bronchial epithelial cells increased the expression of IL-8. Moreover the induction of IL-8 so far attributed to TLR2 and 9 could be modulated by LOX-1 in pneumococci-infected bronchial epithelial cells.

Results: LOX-1 might be important for immunomodulation in the course of S. pneumonia induced pneumonia.
PCV13 IMMUNOGENICITY AND CROSS REACTIVE ANTIBODY ASSESSMENT AMONG NAVAJO AND WHITE MOUNTAIN APACHE INFANTS

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Background and aims: In March 2010, 13-valent pneumococcal conjugate vaccine (PCV13) replaced routine PCV7 among Navajo and White Mountain Apache communities. We compared PCV13 and PCV7 immunogenicity to inform changes in nasopharyngeal carriage and disease following PCV13 use.

Methods: We aimed to collect blood from children receiving only PCV7 or PCV13 at 3 time points: post-dose3, pre- and post-boost (n=100 per vaccine-time-group; n=600 total specimens). Sera were separated, stored at -70°C and tested by ELISA (CPS and 22F adsorption, WHO reference assay) for PCV13 serotypes plus 6C and by OPA; only ELISA data are reported here. We compared ELISA GMC (mcg/mL) between the groups.

Result: Between April 2010-October 2011, 534 sera were collected. Preliminary ELISA results (PCV7 serotypes plus 6A and 19A), for 137 post-dose3 sera (43 PCV7 , 94 PCV13) show a significantly higher GMC for PCV13 compared with PCV7 recipients for 4 of 9 serotypes (6A, 14, 19A, 19F; p< 0.001) and no differences for the remaining 5 serotypes tested. The two groups differed significantly (p< 0.05) in average age at dosing for doses 1 and 3 (PCV13 vs. PCV7: dose1, 58 vs 70 days; dose3, 208 vs 226) and interval between dose3 and post-dose3 blood (PCV13, 41 days; PCV7, 70 days, p< 0.01). The interval between doses differed only for dose2-dose3 (PCV13, 67 days; PCV7, 79 days, p< 0.05).

Conclusions: PCV13 recipients had higher antibody concentrations for some serotypes than PCV7 recipients not explained by differences in dosing ages. Additional ELISA and OPA results are in progress.
SEROTYPE SPECIFIC HYPORESPONSIVENESS TO A BOOSTER DOSE OF PNEUMOCOCCAL VACCINE IN PNEUMOCOCCAL VACCINE CARRIERS


Background: A reduced response to pneumococcal vaccine has been demonstrated in children carrying some pneumococcal serotypes at the time of primary immunisation. We sought to determine whether carriage also influenced responses to a booster dose.

Methods: We evaluated booster responses to PPV23/PCV7 delivered at 9 months of age to Kenyan infants in a trial of pneumococcal vaccine delivered soon after birth.

Results: The serotype specific geometric mean fold rise in antibody concentration between 36 and 37 weeks were higher among non-carriers compared to carriers of a homologous serotype (Table 1).

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Non carriers at 36 weeks</th>
<th>Carriers at 36 weeks</th>
<th>Ratio*</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>GM</td>
<td>95%CI</td>
<td>No.</td>
</tr>
<tr>
<td>4</td>
<td>234</td>
<td>4.95</td>
<td>(4.38, 5.59)</td>
<td>0</td>
</tr>
<tr>
<td>6B</td>
<td>228</td>
<td>13.61</td>
<td>(11.59, 15.98)</td>
<td>6</td>
</tr>
<tr>
<td>9V</td>
<td>230</td>
<td>5.36</td>
<td>(4.76, 6.05)</td>
<td>4</td>
</tr>
<tr>
<td>14</td>
<td>224</td>
<td>2.80</td>
<td>(2.48, 3.16)</td>
<td>10</td>
</tr>
<tr>
<td>18C</td>
<td>230</td>
<td>7.83</td>
<td>(6.95, 8.81)</td>
<td>3</td>
</tr>
<tr>
<td>19F</td>
<td>204</td>
<td>7.19</td>
<td>(6.11, 8.45)</td>
<td>30</td>
</tr>
<tr>
<td>23F</td>
<td>230</td>
<td>10.44</td>
<td>(8.89, 12.25)</td>
<td>2</td>
</tr>
</tbody>
</table>

*GM ratio: carrier to non-carrier

Conclusions: Responses to PCV or PPV are influenced by carriage at the time of boosting although careful analysis of prior responses to PCV needs to be undertaken to establish if the association is causal or if carriage in itself is associated with a propensity to lower responses.
THE EFFECT OF PREVIOUS PPV23S ON SEROTYPE-SPECIFIC MEMORY B CELL RESPONSE TO PCV13 IN ASPLENIC B-TALASSEMICS


Background and aims: Immunological hyporesponsiveness associated with repeated doses of pneumococcal polysaccharide vaccine (PPV) in individuals at increased risk for pneumococcal disease has been attributed to the depletion of serotype-specific memory B-cell pool and has been demonstrated by reduced antibody responses. We investigated the effect of repeated PPVs on memory B cell (MBC) responses to the 13-valent pneumococcal conjugate vaccine (PCV13) in asplenic β-thalassemics.

Methods: Thirty seven adults (20 male, mean age 35.7 years) were vaccinated with PCV13. All patients had received 0-4 PPVs in the past and 1 PCV7 seven years earlier. Blood samples for cell and sera isolation were taken at baseline, 7 and 28 days post-PCV13. Serotype-specific MBCs stimulated with SAC, CpG and PKW were quantified by ELISpot and serum PS-specific antibodies to 3, 19A, 19F, 9V and 23F by a modified, double absorption ELISA.

Results: Patients with >2 PPVs and a short interval (<5 years) since their last PPV had consistently lower MBC increment at 1 month post-PCV13, compared to subjects with ≤2 PPVs and >5 years interval for all serotypes studied (for PS 9V and PS19F, p=0.029 and p=0.045, respectively). Evaluation of both factors by regression analysis revealed that the effect of repeated PPVs was reduced with time (for PS9V: p=0.078). ELISpot findings were correlated with serotype-specific antibody responses.

Conclusions: The extensive use of PPV attenuates serotype-specific B cell memory response and impairs PCV13 immunogenicity. This negative impact is reduced with time suggesting that PPVs should be used cautiously in asplenic β-thalassemics.
SERA FROM HEALTHY ADULTS CONTAIN ANTI-PNEUMOCOCCAL SURFACE PROTEIN A ANTIBODIES THAT SHOW CROSS-REACTIVITY WITH CARDIAC MYOSIN AND ANTI-MYOSIN ANTIBODIES

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Background and aims: Pneumococcal Surface Protein A (PspA) is one of the most protective pneumococcal vaccine antigens. However, its cross-reactivity with myosin has raised certain concerns of the induction of autoimmune cardiac disorders. This seems unlikely, since pneumococcal infections have no clinical history which relates with cardiac injuries. Here we analyzed healthy subject sera regarding the presence of antibodies to PspA and their cross-reactivity with cardiac myosin in order to evaluate the risks of using PspA as a vaccine antigen.

Methods: Human sera of healthy adult laboratory employees at Instituto Butantan (n = 12) were purified by affinity chromatography using Cyanogen bromide-activated-Sepharose®4B (Sigma) coupled to PspA or to cardiac myosin. The purified antibodies were tested against both antigens by Western blot and enzyme immunoassay.

Results: The study showed that healthy adults have specific IgG antibodies against PspA (20.4 µg/mL ± 1.4) and against myosin (7.7 ± 0.6). The antibodies to PspA were able to cross-react with cardiac myosin in western blot analysis. However, the affinity purified anti-myosin antibodies did not recognize PspA in the enzyme immunoassay nor in western blot.

Conclusions: The presence of anti-PspA antibodies in the sera of healthy adults and their cross-reactivity to cardiac myosin suggests that the presence of cross-reactive antibodies does not represent a risk to the development of an autoimmune response. Further studies will be performed in order to identify the regions of myosin molecule recognized by anti-PspA antibodies and their relation with the rheumatic heart disease pathogenesis.

Financial Support: FAPESP, Butantan Foundation
NO EVIDENCE OF HYPO-RESPONSIVENESS FOLLOWING RE-CHALLENGE AT 3-5 YEARS IN PNG CHILDREN VACCINATED WITH PNEUMOVAX AT 9 MONTHS

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Background: Concerns have been raised regarding hyporesponsiveness following pneumococcal polysaccharide vaccine (PPV) in young children even if primed with pneumococcal conjugate vaccine (PCV). This may have implications in high-risk populations such as PNG, where a high proportion of invasive pneumococcal disease may not be covered by 10- or 13-valent PCV. We investigated antibody responses in children at 3-5 years who had received PPV at 9 months with or without priming with 7vPCV and compared their responses to unvaccinated controls.

Methods: Children received 7vPCV in a 1-2-3 or 0-1-2-month schedule or no PCV. 259 received PPV at age 9 months, 240 followed to 18 months. 150 who were located at age 3-5 years were challenged with a 1/5th normal dose of PPV as were 130 unvaccinated 3-5-year-olds. Blood was collected pre- and post-challenge PPV to measure antibodies to serotypes 2, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F.

Results: Among children who received PPV, serotype-specific GMCs were maintained or increased between 18 months and 3-5 years (18 months 0.45-5.5µg/mL, pre-challenge 1.00-13.01µg/mL) Generally GMCs were lower in unvaccinated children (n=86) than in PPV-vaccinated children (n=80). 63%-99% of unvaccinated children and 72%-99% of PPV-vaccinated children had serotype-specific antibody titres ≥1.0µg/mL pre-challenge; equivalent proportions post-challenge were respectively 82%-97% and 78%-99%.

Conclusions: Pneumococcal serotype-specific GMCs at 18 months were maintained to 3-5 years suggesting natural stimulation of antibody responses through carriage. To date we found no evidence of hyporesponsiveness in 3-5-years-old children given a PPV-challenge dose after PPV dose at 9 months.
IGG AGAINST PNEUMOCOCCAL PROTEINS IS PRESENT IN MIDDLE EAR EFFUSION OF CHILDREN WITH A HISTORY OF RECURRENT ACUTE OTITIS MEDIA


**Background:** Vaccines including conserved antigens from *Streptococcus pneumoniae* may reduce the burden otitis media (OM, middle ear infection). Little is known about the immunogenicity of these protein antigens in young children with recurrent acute otitis media (rAOM).

**Methods:** Using a multiplex bead-based assay, we measured IgG levels in arbitrary units (AU/mL) against pneumococcal proteins PspA1, PspA2, CbpA and Ply in serum of 172 children under 3 years of age with a history of at least 3 episodes of AOM. IgG against pneumococcal proteins were also measured in 139 MEE samples collected from 87 children. Anti-protein IgG levels in MEE were standardized according to total IgG in MEE as determined by nephelometry and expressed in AU.

**Results:** IgG against all pneumococcal proteins was detected in serum of children with rAOM, with a geometric mean of 132 AU/mL (PspA1), 692 AU/mL (PspA2), 269 AU/mL (CbpA) and 214 AU/mL (Ply). IgG against all protein antigens was also detected in MEE, being 19 AU (PspA1), 589 AU (PspA2), 3419 AU (CbpA) and 3138 AU (Ply). There was a strong correlation between IgG levels in serum and MEE.

**Conclusion:** These data suggest that pneumococcal proteins are immunogenic in children with a history of rAOM. The strong correlation between IgG levels in serum and MEE may imply that antibodies are produced in serum and transported to the middle ear. Vaccines including these conserved pneumococcal protein antigens may have the potential to reduce the burden of pneumococcal AOM.
IMMUNOGENICITY OF A PNEUMOCOCCAL CONJUGATE 7-VALENT VACCINE AND IMPACT ON CARRIAGE IN VENEZUELAN CHILDREN AT RISK OF INVASIVE PNEUMOCOCCAL INFECTION

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Background and aims: We evaluate the immunogenicity of the PCV7 vaccine and impact on pneumococcal carriage in children at high risk for invasive pneumococcal disease.

Methods: 82 children (2-59 months) with sickle cell anemia (n=22), chronic heart disease (n=19), HIV infection (n=12), immune-suppressive therapy (n=11) and others (n=18) were enrolled and vaccinated according to their age with PCV7 followed by a booster dose of PS-23. Blood samples and nasopharyngeal swabs for determination of IgG antibody concentration and isolation of S. pneumoniae respectively were obtained before the first vaccine dose and 1 month after PS-23 booster.

Results: Pneumococcal carriage prior the first immunization was found in 27% (n=22) of children. One month after completion of the vaccination scheme pneumococcal carriage was found in 22% (n=17). Table 1 shows the distribution of the serotypes pre- and post-vaccination

<table>
<thead>
<tr>
<th>Serotypes</th>
<th>Pre- Vaccination N(%)</th>
<th>Post Vaccination N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV7</td>
<td>12 (14,6)</td>
<td>3 (3,8)</td>
</tr>
<tr>
<td>PCV13 (additional serotypes)</td>
<td>4 (4,9)</td>
<td>7 (9)</td>
</tr>
<tr>
<td>Non vaccine types (NVT)</td>
<td>6 (7,3)</td>
<td>7 (9)</td>
</tr>
</tbody>
</table>

Table 1

Previous primary immunization,55% of the subjects had antibody titers $\geq 0.35 \, \mu g/mL$ for the 7 serotypes. After boosting,100% of the subjects showed high geometric mean concentrations for all serotypes, ranging from 1.75 $\mu g/mL$ (serotype 23F) to 17.16 $\mu g/mL$ (serotype 14)

Conclusions: The introduction of PCV7 appears to be associated with a shift to carriage non-PCV7 serotypes. PCV7 is highly immunogenic and safe however, immunization with PCV-13 may offer better protection in this high risk population.
VACCINATION OF HEALTHY ADULTS WITH 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE AND 23-VALENT PNEUMOCOCCAL POLYSACCHARIDE VACCINE AT DIFFERENT INTERVALS

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Background/aims: 13-valent pneumococcal conjugate vaccine (PCV13) elicits robust immune responses in adults. The effect of dosing intervals on immune responses to sequential vaccination with PCV13 or 23-valent pneumococcal polysaccharide vaccine (PPSV23) in healthy adults aged 60-64 years not previously vaccinated with PPSV23 was assessed.

Methods: In one study subjects received PCV13/PCV13 (n=180), PCV13/PPSV23 (n=302) or PPSV23/PCV13 (n=238) at a 1-year interval. In another study, subjects received PCV13/PCV13 (n=108), PCV13/PPSV23 (n=108) or PPSV23/PPSV23 (n=189) at a 3-4 year interval. Functional antibody titers 1 month post-vaccination were determined by opsonophagocytic activity (OPA) assays; geometric mean titer (GMT) ratios (vaccination 2/vaccination 1) assessed.

Results: At a 1-year interval OPA GMTs after PCV13/PCV13 and PCV13/PPSV23 were significantly lower than after PCV13 for 7/13, and 8/13 serotypes respectively (upper limit of GMT ratio 95% CI < 1). For the 3-4 year interval, GMTs after PCV13/PCV13 and PCV13/PPSV23 were significantly greater than after PCV13 for 7/13 serotypes for both comparisons (lower limit of GMT ratio 95% CI >1); GMTs after PPSV23/PPSV23 relative to PPSV23 were significantly lower for 8/12 shared serotypes. GMTs after PCV13/PPSV23 were significantly greater than PPSV23 at both 1 (6/12) and 3-4 (9/12) year. GMTs after PCV13/PCV13 relative to PPSV23/PPSV23 were significantly higher for 12/12 serotypes.

Conclusions: PCV13 enhances responses to subsequent PPSV23 at 1 year and PPSV23 or PCV13 3-4 years later for most serotypes, reflecting PCV13 inducing immunological memory. In contrast, responses to a second PPSV23 dose administered 3-4 years after a first PPSV23 are diminished, indicating lack of memory effect.
NUMBER OF INFANT PCV DOSES AND TYPE OF BOOSTER MAY AFFECT IMMUNE RESPONSES TO PREVENAR13 IN CHILDHOOD

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Background: Two or three infant 9-valent-pneumococcal-conjugate-vaccine (PCV9) doses primed for memory responses to pneumococcal polysaccharide vaccine (PPSV23) at 12 months, but responses to PCV13 at 7 years were inferior among those who received PPSV23 dose at 12 months vs. PCV9. Here we analyse the effect of the number of priming doses on responses to PCV13 at 7 years.

Methods: 7-year-old children, vaccinated with 2 or 3 doses of PCV9 in infancy and PPSV23 or PCV9 at 12 months, were vaccinated with PCV13 (N=89). IgG antibodies, avidity and opsonophagocytosis (OPA) were measured before, 1 and 4 weeks after vaccination.

Results: Children who received PPSV23 at 12 months exhibited similar OPA responses following PCV13 administration irrespective of whether they received 2 or 3 infant doses of PCV9. In contrast, children who received PCV9 at 12 months exhibited higher OPA responses after PCV13 if they had also been primed with 3 infant doses of PCV 9.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>1</th>
<th>4</th>
<th>5</th>
<th>6B</th>
<th>9V</th>
<th>14</th>
<th>18C</th>
<th>19F</th>
<th>23F</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 PCV9+PPSV23</td>
<td>213</td>
<td>2804</td>
<td>282</td>
<td>10263</td>
<td>1542</td>
<td>2818</td>
<td>3195</td>
<td>1731</td>
<td>1869</td>
</tr>
<tr>
<td>3 PCV9+PPSV23</td>
<td>234</td>
<td>1922</td>
<td>260</td>
<td>12467</td>
<td>2650</td>
<td>3366</td>
<td>3275</td>
<td>863</td>
<td>1462</td>
</tr>
<tr>
<td>2 PCV9+PCV9</td>
<td>898</td>
<td>2049</td>
<td>466</td>
<td>9958</td>
<td>1604</td>
<td>3575</td>
<td>3207</td>
<td>891</td>
<td>2201</td>
</tr>
<tr>
<td>3 PCV9+PCV9</td>
<td>1230</td>
<td>5510</td>
<td>912</td>
<td>12211</td>
<td>2041</td>
<td>2794</td>
<td>7650</td>
<td>1404</td>
<td>3016</td>
</tr>
</tbody>
</table>

[Table. OPA titers 4 weeks after PCV13 vaccination]

Conclusion: Both the number of PCV9 infant doses and type of vaccine used as the booster dose may influence the memory responses to PCV13 at 7 years of age.
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IMMUNOGENICITY/REACTOGENICITY OF 2-DOSE CATCH-UP VACCINATION WITH 10-VALENT PNEUMOCOCCAL NON-TYPEABLE HAEMOPHILUS INFLUENZAE PROTEIN-D CONJUGATE VACCINE (PHID-CV) DURING FOURTH YEAR OF LIFE

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Background/aims: To assess the immunogenicity, safety and reactogenicity of 2-dose PHID-CV catch-up vaccination in children in their fourth year of life.

Methods: 2-dose PHID-CV catch-up vaccination was evaluated in children constituting the age-matched unprimed control groups in two phase III, open studies assessing immunological memory\(^3,4\) (age at dose-1: Study-A: 36-50 months; Study-B: 36-46 months). Immune responses were measured (pre-vaccination and 1 month post-dose-2) using 22F-ELISA and OPA assays. Solicited local/general (≤4 days post-vaccination) and unsolicited symptoms (≤31 days post-vaccination) were recorded. SAEs were recorded until study end.

Results: In both studies, robust immune responses were observed for each vaccine pneumococcal serotype (VT); PHID-CV was also immunogenic for cross-reactive serotypes 6A and 19A (Table). Post-dose-2 anti-protein-D antibody GMC was 785.9 and 960.4 EL.U/mL in Study-A and Study-B, respectively. In both studies, strong increases in antibody GMCs/OPA GMTs were observed pre- to post-vaccination. Pain was the most frequent solicited local symptom and irritability and drowsiness the most common solicited general symptoms (both studies). One SAE was reported (Study-B); it was not considered causally vaccine-related.

Conclusions: 2-dose PHID-CV catch-up vaccination administered to children during their fourth year of life was well-tolerated, and elicited immune responses against VTs that were within the ranges or higher than those observed following 3-dose priming\(^3\).

\(^1\)Prymula ISPPD 2012(abstract-116)
\(^2\)Silfverdal PIDJ 2011;30:e155-63
\(^3\)Prymula Lancet 2009;374:1339-50
\(^4\)Silfverdal PIDJ 2009;28:e276-82

Table. Percentages of children with anti-pneumococcal antibody concentrations ≥2.0 µg/mL (GSK’s 22F-ELISA) and OPA titres ≥8 after 2-dose catch-up vaccination with PHID-CV (ATP cohort for immunogenicity). Doses administered 2 months apart; immune responses measured 1 month post-dose-2.

<table>
<thead>
<tr>
<th>Serotypes</th>
<th>Study A</th>
<th>Study B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotype 1</td>
<td>% of children with antibody concentrations (≥2.0 µg/mL, 95%CI) N=200</td>
<td>% of children with OPA titres ≥8 (95%CI) N=201</td>
</tr>
<tr>
<td>Vaccine serotypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>100 (98.3, 100)</td>
<td>93.0 (88.6, 96.1)</td>
</tr>
<tr>
<td>4</td>
<td>100 (98.3, 100)</td>
<td>100 (98.3, 100)</td>
</tr>
<tr>
<td>5</td>
<td>100 (98.3, 100)</td>
<td>90.4 (85.4, 94.1)</td>
</tr>
<tr>
<td>6B</td>
<td>92.3 (87.9, 95.0)</td>
<td>95.4 (91.5, 97.9)</td>
</tr>
<tr>
<td>7F</td>
<td>100 (98.3, 100)</td>
<td>100 (98.3, 100)</td>
</tr>
<tr>
<td>19F</td>
<td>99.5 (97.4, 100)</td>
<td>100 (98.1, 100)</td>
</tr>
<tr>
<td>14</td>
<td>100 (98.3, 100)</td>
<td>100 (98.3, 100)</td>
</tr>
<tr>
<td>18C</td>
<td>100 (98.3, 100)</td>
<td>100 (98.3, 100)</td>
</tr>
<tr>
<td>23F</td>
<td>94.3 (90.2, 97.0)</td>
<td>95.3 (90.4, 97.7)</td>
</tr>
<tr>
<td>Cross-reactive serotypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6A</td>
<td>77.5 (71.2, 83.8)</td>
<td>95.3 (91.3, 97.8)</td>
</tr>
<tr>
<td>23A</td>
<td>96.2 (92.6, 98.3)</td>
<td>94.4 (90.2, 97.2)</td>
</tr>
</tbody>
</table>

N = maximum number of children with available results. N may vary between serotypes.

[Table. Immune response after 2-dose catch-up]
EFFECT OF AGEING ON CONCENTRATION, AVIDITY AND FUNCTIONAL ACTIVITY OF NATURALLY-ACQUIRED ANTI-PNEUMOLYSIN ANTIBODIES

B. Simell¹, E. Holmlund¹, T. Jaakkola¹, A. Reunanen¹, H. Käyhty¹, M. Väkeväinen², ¹Helsinki, ²Espoo, Finland

Background: Compromised immune function as a consequence of normal human ageing is widely accepted to play a role in increased susceptibility of the elderly to bacterial infections. We evaluated the effect of ageing on concentration, avidity and functional activity of naturally-acquired IgG antibodies to pneumolysin - a pneumococcal intracellular toxin and a protein vaccine candidate.

Methods: Concentration and avidity (AI) of anti-pneumolysin IgG antibodies in the sera of younger (aged 30 to 64 years) and elderly (aged 65 to 97 years) unvaccinated adults were measured by enzyme immunoassay. Haemolytic inhibition microassay (HIMA) was used to measure the functional activity of anti-pneumolysin antibodies.

Results: The geometric mean concentration (gmc) of anti-pneumolysin antibodies was significantly lower in the elderly (≥65 years) compared to the younger adults (< 65 years; p< 0.001) (Table1). In the avidity and functionality of anti-pneumolysin antibodies, a decreasing trend with increasing age was detected, while the difference between the younger and elderly adults was not statistically significant (Table 1). Stratification of data to six age groups (30-44, 45-54, 55-64, 65-74, 75-84 and ≥85 years) showed a clear decrease in anti-pneumolysin antibodies with increasing age.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>&lt;65 years</th>
<th>N</th>
<th>≥65 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMC (95%CI)</td>
<td>300</td>
<td>25.48 (23.05-28.17)</td>
<td>300</td>
<td>18.66 (16.64-20.97)</td>
</tr>
<tr>
<td>AI (95%CI)</td>
<td>59</td>
<td>1.49 (1.34-1.65)</td>
<td>59</td>
<td>1.38 (1.22-1.55)</td>
</tr>
<tr>
<td>HIMA (95%CI)</td>
<td>60</td>
<td>301 (236-384)</td>
<td>60</td>
<td>204 (150-277)</td>
</tr>
</tbody>
</table>

Conclusions: The concentration of naturally-acquired anti-pneumolysin antibodies decreases with increasing age. The significance of finding in protection against pneumococcal disease needs to be determined.
**Poster No 221**

**EFFECT OF PNEUMOCOCCAL CARRIAGE AT THE TIME OF PCV9 ADMINISTRATION ON THE SEROTYPE-SPECIFIC POST-PRIMARY ANTIBODY CONCENTRATIONS**

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**Background:** Pneumococcal carriage at the time of pneumococcal conjugate vaccine (PCV) administration has been shown to interfere with the subsequent immune response in infants. We evaluated the effect of pneumococcal carriage at the time of immunization with a 9-valent PCV (PCV9) on post-primary series antibody concentrations in South African infants.

**Material and methods:** Children in a double-blind randomized trial received PCV9 at 6, 10 and 14 weeks of age (N=350). Nasopharyngeal swabs to detect pneumococcal carriage were taken at ages 6, 10 and 14 weeks. The concentrations of IgG antibodies against serotypes 4, 6B, 14, 19F and 23F were measured by enzyme immunoassay in serum samples taken at 18 weeks of age.

**Results:** The concentrations of anti-6B, anti-19F and anti-23F IgG antibodies were significantly lower in the PCV9-vaccinated children who had carried the specific type at 6, 10 and/or 14 weeks as compared to the non-carriers.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Carrier of the serotype at 6, 10 and/or 14 weeks</th>
<th>Non-carriers</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>N=6, GMC (95% CI): 5.39 (1.64-17.79)</td>
<td>N=47, GMC (95% CI): 3.05 (1.88-4.94)</td>
<td>0.41</td>
</tr>
<tr>
<td>6B</td>
<td>N=14, GMC (95% CI): 2.19 (0.79-6.11)</td>
<td>N=44, GMC (95% CI): 6.47 (3.85-10.89)</td>
<td>0.046</td>
</tr>
<tr>
<td>14</td>
<td>N=9, GMC (95% CI): 3.52 (1.16-10.71)</td>
<td>N=43, GMC (95% CI): 2.36 (1.31-4.28)</td>
<td>0.56</td>
</tr>
<tr>
<td>19F</td>
<td>N=43, GMC (95% CI): 1.64 (1.04-2.56)</td>
<td>N=35, GMC (95% CI): 3.27 (2.16-4.96)</td>
<td>0.027</td>
</tr>
<tr>
<td>23F</td>
<td>N=26, GMC (95% CI): 0.91 (0.49-1.70)</td>
<td>N=38, GMC (95% CI): 3.45 (2.60-4.56)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Conclusions:** Pneumococcal carriage interferes with serotype-specific immune response to primary series of PCV having potential implications for immunization programs. The immunogenicity may improve by diminution of vaccine-type strains in vaccinated populations over time.
PERSISTENCE OF OPSONOPHAGOCYTIC ACTIVITY OF CIRCULATING ANTIBODIES AT 19 MONTHS OF AGE AFTER ALTERNATIVE PCV7 REGIMENS IN ISRAELI INFANTS

N. Ekström¹, B. Simell¹, N. Givon-Lavi², H. Käyhty¹, R. Dagan², ¹Helsinki, Finland, ²Beer-Sheva, Israel

Aim: To compare the persistence of functional circulating antibodies induced by three alternative regimens of PCV7 by measuring opsonophagocytic activity (OPA).

Methods: Of 543 Israeli infants originally enrolled sets of samples taken at 19 months of age were randomly selected to represent each regimen (N=30/group). Concentration and functionality of serum IgG antibodies were determined by enzyme immunoassay and multiplex OPA assay, respectively.

Results: Majority of samples showed detectable OPA titer. GMOPAs were higher in the 3+1 vs. 2+1 group (P≤0.05 for serotypes 6B and 9V). In the 3+0 group, the GMOPAs were significantly lower than in the boosted groups (P≤0.02), excluding serotypes 4 and 6B in the 3+0 compared to the 2+1 group. Interestingly, the GMOPAs in the 3+0 group had not declined from the 13 month level.

<table>
<thead>
<tr>
<th>Immunization schedule</th>
<th>Geometric mean IgG concentration (EIA;μg/ml) at 19 months of age</th>
<th>Geometric mean OPA titer; GMOPA (% of infants with detectable OPA) at 19 months of age</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4,6,12 mo (3+1)</td>
<td>0.45  1.66  0.58  0.76</td>
<td>1083 (100)  2649 (100)  4905 (100)  3309 (97)</td>
</tr>
<tr>
<td>2,4,6 mo (3+0)</td>
<td>0.15  0.82  0.35  0.30</td>
<td>243 (97)  476 (80)  1057 (93)  493 (93)</td>
</tr>
<tr>
<td>4,6,12 mo (2+1)</td>
<td>0.46  1.76  0.52  0.67</td>
<td>597 (100)  730 (93)  2810 (100)  1617 (100)</td>
</tr>
</tbody>
</table>

[Table 1]

Conclusions: Significant differences in circulating antibody functionality were observed between alternative PCV7 regimens. Clinical significance of this finding requires further research.
Poster No 223

RISK FACTORS FOR PNEUMOCOCCAL NASOPHARYNGEAL COLONIZATION BEFORE AND AFTER PNEUMOCOCCAL CONJUGATE VACCINATION IN PERSONS WITH HIV

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Background and aims: HIV-infected individuals have excess rates of invasive pneumococcal disease. Further, HIV infection increases the risk of pneumococcal colonization and repeated colonization, and reduces time to new colonization. We assessed nasopharyngeal pneumococcal colonization before and after pneumococcal immunization and identified risk factors for pneumococcal carriage.

Methods: 96 adult HIV patients were randomized to receive a double dose 7vPnC (Prevnar, Pfizer) at 0 and 3 months, with either the adjuvant (1 mg CPG 7909) or a placebo (phosphate-buffered saline) added to it; 48 patients received CPG 7909 and 48 placebo. Nasopharyngeal swabs were obtained prior to immunization at 0 and 9 months.

Results: In total, 22 patients (23%) were colonized, 11 at baseline only, four at both baseline and 9 months, and seven at 9 months only. Compared to non-colonized patients, more colonized patients were smokers, had lower CD4+ nadir and had an AIDS-diagnosis. Immunization, antiretroviral treatment and the CPG adjuvant had no impact on colonization.

Conclusions: These findings suggest that certain leukocyte subsets involved in the immunity against pneumococcal colonization and infection are irreversibly lost during profound immune depletion in the course of HIV infection. In addition to immunization, preventive strategies aimed at limiting immune deterioration are needed to pneumococcal disease in HIV patients.
CHEMOENZIMATICALLY MODIFIED S. PNEUMONIAE SEROTYPE 14 CAPSULAR POLYSACCHARIDE AND ITS HUMORAL RESPONSE IN A MICE MODEL.


Charged bacterial polysaccharides antigens can modulate the immune response. In this work we present the preparation of charged derivatives of the neutral capsular polysaccharide from S. pneumoniae serotype 14 (CPS14) using chemical and enzymatic methods, and their characterisation. The antigenic properties of the products were evaluated in a mice model.

Cationic and anionic derivatives were prepared by partial or total N-deacetylation of the D-GlcNAc residue or by enzymatic and chemical partial oxidation of the D-Gal residues, respectively. The products were analysed by chemical and spectroscopical methods. The immunochemical similarity with native CPS-14 polysaccharide was analysed using a polyclonal serotype-specific latex reagent.

To analyze the humoral response, mice were inoculated with either saline or one of the following antigens: CPS14, CPS14 conjugated to BSA, N-deacetylated CPS14, zwitterionic CPS14 derivative or serotype 1 capsular polysaccharide. Quil A was included in all the formulations as adjuvant. Animals were inoculated on days 0 and 14, and blood was collected on days 0, 14, 28 and 49.

Anti-CPS14 and anti zwitterionic CPS14 total IgG and IgG isotypes were determined by ELISA assays. The results showed that the zwitterionic CPS14 derivative induced the secretion of anti-CPS14 antibodies, but cationised CPS14 derivatives do not.
Evasion of complement mediated opsonophagocytosis of opaque S. pneumoniae confers resistance to acute pneumococcal otitis media in mice

H.H. Tong, Q. Li, Y.X. Li, G.L. Stahl, J.M. Thurman, Columbus, OH, Boston, MA, Aurora, CO, USA

Background and aims: Considerable evidence has implicated that phase variation between a transparent and an opaque colony phenotype of Streptococcus pneumoniae (Spn) plays a significant role for its survival in host. The aim of this study was to define the role of Spn opacity variants in complement mediated host defense against pneumococcal acute OM (AOM).

Methods: The susceptibility to AOM of mice deficient in complement factor B and C2 (Bfl/C2−/−), C1qa (C1qa−/−), and factor B (Bf−/−) was investigated. The bacterial burden in the middle ear and blood, histology, in vivo complement C3 activation, and opsonophagocytosis were compared.

Results: The bacterial titers of opacity variants in the middle ear lavage fluid samples of Bfl/C2−/−, Bf−/− and C1qa−/− were significantly higher than those of the transparent variant at 48 and 72 h post infection. There were more opaque Spn bacteremia occurred in complement deficient mice compared to transparent Spn bacteremia. The middle ear mucosa inoculated with opaque variants was severely inflamed and significantly thickened with more extensive inflammatory cell infiltration than those infected with transparent Spn. Although Spn opacity variants induced increased C3 activation equivalently in the middle ear, decreased C3b deposition on opaque Spn impedes opsonophagocytosis of Spn by mouse neutrophils middle ears was evident compared the transparent phenotype.

Conclusions: The opacity phenotypes effect the complement system mediated otoimmune defense against pneumococcal OM.
ANTIBODY RESPONSES TO TWENTY SEVEN PNEUMOCOCcal SURFACE PROTEIN ANTIGENS IN THE FIRST TWO YEARS OF LIFE

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Background and aims: We sought to determine the kinetics of natural development of serum antibodies to pneumococcal surface protein vaccine-candidates in a cohort of Burmese/Karen refugee infants in whom we have documented a high prevalence of pneumococcal nasopharyngeal carriage.

Methods: 234 mother-infant pairs were followed from birth for twenty four months. Nasopharyngeal swabs (NPS) were taken from mother and infant at monthly follow-up visits to detect pneumococcal colonisation. Blood was collected at delivery (mother and cord) and from the infants at each monthly review. Serum IgG antibodies to twenty seven pneumococcal surface proteins were quantified by a multiplexed electrochemiluminescence assay.

Results: 2,624 serum specimens were analysed. Trans-placental transfer of antibody was almost 100% for all proteins. Concurrent maternal pneumococcal colonisation was associated with significantly higher titres to four surface exposed proteins in mother sera. Cord blood IgG titres to two of the 27 proteins were associated with delayed infant nasopharyngeal acquisition in a univariate model. Antibody profiles in the infants varied by protein and colonisation status, with the median age at nadir of 4 months (range: 2m-8m). By 24 months, the GMC of nine proteins were greater than or equal to the cord blood GMC. The concentrations at 24 months of age of 7/27 proteins were positively correlated with overall colonisation duration.

Conclusions: Infant antibody responses to vaccine-candidate pneumococcal surface proteins were varied and, although we demonstrated higher titres in colonised mothers, the overall protective effects of these on early infant colonisation were minimal.
FUNCTIONAL ANTIBODY RESPONSES AFTER REDUCED DOSE SCHEDULE WITH A PNEUMOCOCCAL CONJUGATE VACCINE INTO THE SECOND YEAR OF LIFE

E. van Westen\textsuperscript{1}, H. van Dijken\textsuperscript{1}, T. Zborowski\textsuperscript{2}, G. Rodenburg\textsuperscript{2}, D. Goldblatt\textsuperscript{3}, N. Rots\textsuperscript{1}, L. Sanders\textsuperscript{2}, G. van den Dobbelsteen\textsuperscript{1}, Bilthoven, \textsuperscript{2}Utrecht, The Netherlands, \textsuperscript{3}London, UK

Background and aims: The 7 valent pneumococcal conjugate vaccine (PCV7) has been introduced in most developed countries although with differences in the number and age of primary doses and timing of the booster dose. The aim of this study was to determine the immune responses after a 2 primary dose schedule and the impact of PCV7 booster doses given at 11 or at 24 months of age.

Methods: In a randomized controlled setting, children received PCV7 at 2 and 4 months (2-dose group); at 2, 4 and 11 months (2+1 dose group); or no PCV7 (control group). All children received a PCV7 dose at 24 months of age. Antibody opsonophagocytic activity was evaluated, using a multiplex opsonophagocytosis assay (MOPA), in blood samples collected at 12, 24 and 25 months of age.

Results: At 12 months, the 2-dose group showed higher OPA titers compared with unvaccinated controls for several serotypes. Higher OPA responses were observed after a booster dose at 11 months of age compared with 2 primary doses or no vaccine doses for all vaccine serotypes. After an additional PCV7 vaccination at 24 months robust increases in OPA responses were observed in both vaccinated groups for all serotypes.

Conclusions: A PCV7 booster dose after 2 primary doses adds to immune responses, however antibody titers wane rapidly. PCV7 vaccination early in life primes for robust quantitative and functional antibody responses at 24 months. A booster dose later in the second year of life may have a positive effect on sustained immunity.
**NOVEL INSIGHTS INTO IMMUNITY TO COLONIZATION FROM AN INFANT MOUSE MODEL**

S. Kuil, N. Patterson, J. Ortega, A. Walduck, K. Short, O. Wijburg, Melbourne, VIC, Australia

**Background:** Colonization of the nasopharynx with *S. pneumoniae* in infants occurs at increased incidence and duration compared with adults. This study investigated the role of immune mediators in clearance of primary colonization with *S. pneumoniae* in infant mice in comparison with adult mice.

**Methods:** Five-day old C57BL/6 mice and adult (6 week old) mice were colonized with *S. pneumoniae* EF3030 (type 19F) and clearance of bacteria from the nasopharynx was monitored by viable count.

**Results:** Following inoculation with a single dose of EF3030, bacterial load in neonatal mice was approximately 10^e5 CFU for at least 40 days and complete clearance took 65 days. In contrast, colonization levels in adult mice were ~100-fold reduced and highly variable, with clearance occurring within 21-28 days after colonization. Differences in colonization of neonatal and adult mice were not due to age-dependent differences in nasal flora or nasopharyngeal anatomy. Neonatal colonization induced antigen-specific antibody responses in wild-type mice, which were first detectable at 21 days after colonization. Clearance from neonatal mice lacking antibodies (uMT-/-) was significantly delayed compared with neonatal CD4+ T cell deficient mice, while CD4+ T cell deficient mice did not develop *S. pneumoniae* specific IgG responses and IgM responses were significantly reduced compared with C57BL/6 mice.

**Conclusions:** This study demonstrates that neonatal mice provide a useful model to study pneumococcal colonization in children. The results to-date suggest that clearance of *S. pneumoniae* from neonatal mice might involve different immune mechanisms than in adult mice and warrant further investigation into colonization-induced immunity and the role of antibodies.
DEVELOPMENT AND VALIDATION OF A LUMINEX ASSAY FOR QUANTIFICATION OF IgG SERUM ANTIBODIES AGAINST PNEUMOCOCCAL PROTEINS

Z. Ditse, P.V. Adrian, L. Kuwanda, M.R. Madimabe, S.A. Madhi, Johannesburg, South Africa

Background: Enzyme-linked immunosorbent assay (ELISA) is widely used for antibody quantification, however, separate assays are required for each antigen. The Luminex platform is a sensitive, flow cytometric, high-throughput screening system that allows for a single small volume sample to be tested simultaneously for different antigens.

Objectives: We established a multiplex Luminex assay for the simultaneous quantification of naturally acquired serum antibodies against pneumococcal surface protein A (PspA), pneumococcal surface protein C (PspC), pneumococcal surface adhesion A (PsaA) and pneumolysoid (PdB), and compared its performance characteristics to single-plex ELISAs.

Methods: Archived serum samples were analysed from 100 HIV-uninfected individuals, including 20 samples each from children aged 10, 18, 28 and 48-72 months of age and adults of median age of 27 years.

Results: The ELISA assays correlated well with the Luminex assay, with correlation coefficients (R²) of 0.85, 0.90, 0.86 and 0.96 for PspA, PspC, PdB and PsaA respectively (Fig 1.). The two assays displayed similar sensitivity and specificity characteristics.

Conclusion: The broader dynamic range of the Luminex assay and the capacity to analyze multiple antigens make it an ideal high throughput platform with which to evaluate antibodies to multiple pneumococcal protein antigens.
A FRAMEWORK FOR ESTIMATION OF SEROTYPE-SPECIFIC AND OVERALL VACCINE EFFICACY AGAINST PNEUMOCOCCAL COLONISATION

K. Auranen¹, M.E. Halloran², H. Rinta-Kokko¹, ¹Helsinki, Finland, ²Seattle, WA, USA

Background: Evaluating vaccine efficacy against pneumococcal colonisation is an area of growing interest. Previous estimates of vaccine efficacy against specific serotypes and against all vaccine-serotypes may not be comparable because of unadjusted effects of baseline prevalence of vaccine-type colonisation, between-serotype competition, multiple colonisation, and time-lag between vaccination and the measurement of colonisation. There is need to standardise the statistical methodology for estimation of vaccine efficacy against pneumococcal colonisation.

Methods and results: We developed a general framework for defining and estimating serotype-specific and overall (all vaccine-type) vaccine efficacy for susceptibility to acquisition of colonisation. In this framework, efficacy estimates can be obtained from a cross-sectional study, i.e., from one sample per study subject in a phase III trial setting. Serotype-specific estimates are adjusted for the confounding effects of between-strain competition in colonising hosts and can be generalised to estimation of efficacy against the transmission potential of colonisation. We review several existing data sets, demonstrating that the published estimates of serotype-specific vaccine efficacy against colonisation are generally negatively biased.

Conclusions: The methodology to obtain coherent estimates of serotype-specific and overall vaccine efficacy against pneumococcal colonisation facilitates the use of colonisation endpoints in vaccine trials and enables the comparison of vaccine efficacy estimates obtained in different epidemiological settings.
Poster No 231

SAFETY/REACTOGENICITY RESULTS OF A PHASE I CLINICAL TRIAL OF AN INVESTIGATIONAL PNEUMOCOCCAL PROTEIN-BASED VACCINE IN ADULTS

G. Leroux-Roels¹, C. Maes¹, F. De Boever¹, M. Traskine², J.U. Rüggeberg², D. Borys², ¹Ghent, ²Wavre, Belgium

Background/aims: Highly conserved pneumococcal proteins such as pneumolysin toxoid (dPly) and histidine-triad protein D (PhtD) are being studied to develop new vaccines. Different formulations of an investigational vaccine (GlaxoSmithKline Biologicals) were evaluated for safety/reactogenicity when administered to adults in 2-dose primary series followed by a booster dose.

Methods: In a phase I double-blind study (NCT00707798), healthy adults (aged 18-40 years) were randomised (1:2:2:2:2:2:2) to receive 2-dose priming with 1 of 6 formulations (dPly or dPly-PhtD at low and high dose combined/not combined with 10-valent pneumococcal non-typeable Haemophilus influenzae protein D conjugate vaccine [PHID-CV]) at months 0 and 2 or 23-valent pneumococcal polysaccharide vaccine (month 0) followed by placebo (month 2). Two groups continued to the follow-up phase II study (NCT00896064) which investigated the safety/reactogenicity of a third dose of 1 of 2 formulations 5-9 months after primary vaccination. Solicited local/general (within 7 days post-vaccination), unsolicited symptoms (within 31 days post-vaccination) and SAEs (throughout each study) were recorded.

Results: Of 156 enrolled and vaccinated adults, 146 completed the primary immunisation study. 43 adults continued to receive a booster dose; all completed this study. During both studies, for any formulation, ≤8.9% of doses were followed by grade 3 solicited local or general AEs; no fever >39.5°C (oral temperature) was reported. Unsolicited AEs considered causally related to vaccination were reported following ≤33.3% of investigational vaccine doses. No SAEs were reported for adults receiving investigational vaccine formulations.

Conclusions: All the investigational formulations of the pneumococcal protein-containing vaccine were well tolerated when administered to adults.
A NOVEL BACTERIAL HEAT-SHOCK PROTEIN-BASED VACCINE TO PROTECT AGAINST PNEUMOCOCCAL DISEASE

L.S. Brackenbury¹, C. Bignell², P. Cecchini², C. Entwisle², S. Clarke², S. Hill², K. Dalton², C.A. Colaco², R. Garland¹, C.R. Bailey², N.A. Williams¹,
¹Bristol, ²Cambridge, UK

Background and aims: Despite the introduction of pneumococcal polysaccharide-based vaccination programs, there remains a pressing need to develop a cost-effective vaccine capable of inducing pan-serotype protection. Heat shock proteins (hsps) are molecular chaperones which aid protein folding and prevent aggregation. Their expression is increased during cellular stress where they associate with numerous proteins in heat shock proteins complexes (HspCs). Bacterial HspCs have been shown to activate both innate and adaptive immune responses and to induce protective immunity against tuberculosis. Using patented ImmBioVax™ technology (ImmunoBiology Ltd), we have developed and tested a pneumococcal-derived multi-component vaccine enriched in HspCs.

Methods: HspC vaccines were produced following the induction of heat shock (+/- acid stress) in a ply deletion mutant of the Rx1 strain. Immunogenicity was tested in mice and both T and B cell responses analysed. T cell cytokine production was determined using a Milliplex cytokine immunoassay kit and antibody responses assessed by ELISA. In addition human T cell assays were performed and cytokine production analysed as above.

Results: The vaccines were shown to consist of multiple Hsps and bacterial antigens. Vaccine-specific antibodies were detected in mice and antibody functionality using an opsonophagocytic assay will be assessed. Memory T cell responses to the vaccines were readily detectable in both humans and mice but were neither Th1 nor Th2 biased.

Conclusions: We believe that this approach offers an exciting opportunity to produce a novel, cost-effective protein-based vaccine capable of inducing multi-serotype immunity and present evidence to support this.
Pneumococcal Surface Protein A (PspA) Inhibits Complement Deposition on Pneumococci Surface by Competing with the Binding of C-Reactive Protein (CRP)

D.E. Briles, R. Mukerji, A.J. Szalai, Birmingham, AL, USA

PspA is a major virulence factor whose surface presence inhibits classical pathway complement (C3). Its C-terminal choline-binding domain attaches PspA to the phosphorylcholine moieties on the pneumococcal surface. C-reactive protein (CRP) also binds phosphorylcholine and its attachment to pneumococci enhances C3 deposition through the classical pathway. All human and mouse sera contain at least low levels of CRP. Here we investigated the possibility that PspA inhibits classical complement pathway by competing with the binding of CRP to pneumococci. We demonstrated, using flow cytometry and PspA+ and PspA− strains, that the absence of PspA leads to both enhanced CRP deposition and C3 deposition on pneumococci. Furthermore, when a pneumococcal eluate containing PspA was added to the PspA− mutant, there was lowered deposition of CRP on the pneumococcal surface compared to a pneumococcal eluate that contained no PspA. This blocking effect on CRP was not observed when a recombinant PspA, which lacks the choline-binding region, was added to the PspA− mutant. When C3 deposition was measured, a similar blocking effect was observed by the PspA+ eluate. Furthermore, there was a significantly higher amount of C3 deposition, on the PspA− strain, using normal mouse serum from wild-type mice compared to that from CRP knockout (CRPKO) mice. When CRP was added back to the CRPKO serum there was a dose dependent increase in C3 deposition. Therefore, this study describes a novel mechanism for complement inhibition by a bacterial protein: inhibition of both CRP surface binding and subsequent CRP-mediated complement deposition.
Are Efficacy Trials of Pneumococcal Protein Vaccines Feasible? A Strategic Approach to the Regulatory Pathway

C.V. Broome¹, E.R. Zell², C.G. Whitney², ¹Berkeley, CA, ²Atlanta, GA, USA

Pneumococcal Protein Vaccines (PPV) are expected to provide broader coverage of pathogenic pneumococcal serotypes and thus might prevent a higher proportion of severe childhood pneumonias compared to currently licensed pneumococcal conjugate vaccines (PCV). However, since PCV are efficacious, may provide protection outside of the target age range, and anti-capsular immunity, a surrogate for PCV effectiveness, will not predict PPV effectiveness, the licensure of PPV should be based on clinical protection in humans.

We have calculated the sample size needed to compare PPV+PCV to PCV alone, estimating the expected incidence in the PCV comparison group from the population receiving PCV9 in the Gambia efficacy trial (Cutts 2005). To detect PPV efficacy against invasive pneumococcal disease due to serotypes not in PCV, sample size per group varied between 7000 and 17,000. To detect a clinical endpoint of percent decrease in radiologic pneumonia, sample size per group varied between 4000 and 35,000. While feasible, such trials will be challenging; a strategic approach should begin now to support needed studies such as population based age specific incidence of serotypes not in PCV in countries with widespread PCV use. Studies of candidate PPV's, including Phase 2 studies, impact on nasopharyngeal carriage, and key animal model studies should include a PCV control group. The urgency and complexity of assessing PPV effectiveness warrant a global collaborative approach to design clinical studies. Such a transparent global process would provide a forum for discussing when remaining questions about effectiveness could only be resolved with Phase 4 (post-licensure) effectiveness studies.
EVALUATION OF THE PRODUCTION PROCESS FOR A WHOLE CELL PNEUMOCOCCAL VACCINE


Background: The conjugate pneumococcal vaccines are very expensive, making them unaffordable for developing countries. Furthermore, they do not provide coverage for prevalent serotypes in many regions. A whole cell pneumococcal vaccine (WCPV) would represent an alternative low cost and serotype-independent vaccine.

Aims: To evaluate different production processes for a WCPV using a non-encapsulated strain of Streptococcus pneumoniae.

Methods: Fermentation procedures at bench (10 L) and pilot scale under cGMP conditions (60 L) were performed using animal-free medium. Samples were taken at different cell concentrations and used for vaccine preparation at 10 L scale. cGMP lots were recovered and purified by tangential flow microfiltration in 0.1 µm hollow-fibers. Cells were harvested at the end of exponential phase, concentrated to 1/6 of the initial volume and washed 6 times with Ringer's lactate plus 0.2% glucose. The cell suspension was inactivated with β-propiolactone (1:4000 v/v).

Results: Analyzes of 13 different WCPV lots presented comparable growth curves and production of acetic and lactic acids. cGMP production lots contained ~10^9 CFU/mL at the beginning of concentration and the average maximum specific growth rate during exponential phase was 1.0 h^{-1}. The product recovery in cGMP lots was over 80% after concentration.

Conclusions: The scale-up was successfully achieved and our results showed the consistency of the production process of pneumococcal whole cell vaccine, which enables the large scale production for human immunization. Other fermentation strategies are being analyzed to increase cellular mass per batch.

Supported: By PATH, Fundação Butantan, Instituto Butantan.
A MULTIVALENT PNEUMOCOCCAL PROTEIN VACCINE COMPRISING FUSIONS OF PNEUMOLYSOID WITH EPITOPE/FRAGMENTS FROM CBPA AND/OR PSPA

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Background and aims: Pneumolysin, CbpA and PspA are proven protective immunogens against invasive pneumococcal disease in animal models. Here we test the efficacy of pneumolysin toxoid (L460D), fused with protective epitopes from CbpA (YPT and NEEK), or the proline-rich region of PspA (PRN), ± larger PspA fragments.

Methods: Mice were immunized IP thrice with antigen combinations in alum adjuvant at 2 week intervals, and challenged in diverse mouse infection models with multiple S. pneumoniae strains.

Results: Intratracheal challenge with TIGR4 models pneumonia, sepsis and meningitis with baseline survival < 30%. Several constructs elicited significantly improved survival of >60%. Combinations of antigens improved survival over L460D alone. Only YPT-L460D-NEEK elicited significant protection against meningitis. Protection from nasopharyngeal colonization was also strongest using a combination of YPT-L460D-NEEK and the PspA a-helical region. In an intraperitoneal sepsis model, YPT-L460D-NEEK yielded dose dependent protection against strain D39 with >66% survival, compared with 40% for toxoid alone and 20% for alum alone. Other IP challenge studies demonstrated improved protection against type 1, 2 and 6A strains when YPT-L460D-NEEK was supplemented with the PspA a-helical region. In an intravenous challenge model, all fusions that contained PRN were highly protective, as was the PspA a-helical region. With a focal lung infection model, the best protection was obtained with a mixture of a-helical PspA and either L460D or YPT-L460D-NEEK, or a mixture of YPT-L460D-NEEK and PRN-L460D.

Conclusion: The strongest and broadest protection in diverse pneumococcal challenge models is elicited by the fusion protein YPT-L460D-NEEK combined with a-helical PspA.
PREPARATION OF PNEUMOCOCCAL TYPE 5 AND 23 GLYCOCONJUGATES WITH CARRIER CRM197 USING ROBUST CONJUGATION METHODS

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Streptococcus pneumoniae (S. pneumoniae), a gram-positive bacterium, is the most common causative agent of severe bacterial pneumonia in children and adults and also causes meningitis, sepsis and otitis media. The introduction of polysaccharide conjugate vaccines (PCV) has resulted in improved immunity, especially in children, and a drop in disease caused by covered serotypes. Although the developing world experiences the highest morbidity and mortality rates from S. pneumoniae infection, the relatively high cost of PCV makes them difficult for low-resource countries to afford without substantial assistance. PATH, the US Food and Drug Administration (USFDA), and Chengdu Institute of Biological Products (CDIBP) are collaborating to advance the development of a safe, affordable and effective pneumococcal conjugate vaccine. The partners are currently developing methods for preparing and testing conjugate vaccines against pneumococcal serotype 5 and 23 polysaccharides. Pneumococcal serotype 5 polysaccharide was size-reduced by microfluidization prior to coupling. A water soluble carbodimide method was used to couple a mutant of diphtheria toxin CRM197 via an adipic acid dihydrazide linker to pneumococcal serotype 5 polysaccharide. The type 23 polysaccharide was conjugated to CRM197 by a modified reductive amination method of Lee and Frasch. Both methods are robust and were easily transferred to CDIBP for further vaccine development.
Streptococcus pneumoniae is responsible for significant morbidity and mortality. After introduction of current pneumococcal vaccines, serotype shifts in carriage and disease, including capsular switch and presence of antimicrobial resistance, have been found. Here we report live attenuated vaccine strain which is avirulent and can protect from systemic and mucosal pneumococcal diseases. Pep27, an autolysis-inducing factor of S. pneumoniae is known to mediate LytA-dependent and -independent lysis and it was thus expected to effect virulence. The loss of Pep27 had a much larger than expected decrease in virulence and has made the pep27 mutant strain sufficiently avirulent to be used as a live vaccine. The pep27 mutation unexpectedly had lower level of capsular polysaccharide than the wild type (type 2, D39) strain. Moreover, the pep27 mutant showed rapid clearance by 24 h post intranasal infection, and was not detected in lung and blood suggesting that mutant could not invade into the tissue. Even when $2 \times 10^8$ CFU were injected intravenously the mutant was not detected in the blood or brain after 4 h. Whereas 4 h after injection of $6 \times 10^6$ CFU of the wild type parent D39 strain, bacteremia was readily detected. Two dose intranasal immunizations with the live pep27 mutant in the absence of adjuvant elicited IgG antibody and serotype-independent protection against lethal intranasal challenge. Moreover, intranasal immunization of mice with an inactivated pep27 mutant provided protection from lethal challenge. Thus Pep27 was essential for virulence, and intranasal immunization with the pep27 mutant could provide protective immunity.
PSPA-PNEUMOLYSIN FUSION PROTEINS INDUCE PROTECTION AGAINST STREPTOCOCCUS PNEUMONIAE INFECTION IN MICE

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PspA and detoxified mutants of pneumolysin (Pds) have been shown to be immunogenic and protective in different animal studies. Furthermore, the combination of these proteins was able to increase protection against pneumococcal sepsis in mice, possibly by impairing the ability of the bacteria to evade complement deposition.

Objective: the aim of this study was to evaluate the antibody response and protection induced by PspA-Ply fusion proteins.

Methods: 2 pneumolysoids were generated by site-directed mutagenesis, and inserted in a previous construction of pAE-6xHis expression vector containing the PspA gene fragment. The recombinant proteins were purified and used to immunize BALB/c mice. The antibodies were produced and tested for their ability to increase C3 deposition onto pneumococcal surface, by FACS. Protection was evaluated by intravenous challenge with strain A66.1

Results: PspA-Pd fusion proteins induced antibodies that strongly recognized both proteins included in the formulation. These antibodies were also capable of binding to intact pneumococci, and promote an increase in the levels of C3 deposited onto the bacterial surface. Moreover, in some cases, the complement activating effect of such antibodies was stronger than that of sera made against each protein alone. The hybrid proteins were also able to induce protection against fatal challenge in mice.

Conclusion: the results indicate that the use of PspA-Pd fusions is a promising vaccination strategy in mice, leading to the production of antibodies that can activate key factors involved in the control of pneumococcal infection.

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BLP-BASED PNEUMOCOCCAL PROTEIN VACCINES ARE PROTECTIVE AGAINST COLONIZATION AND PNEUMONIA

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Here we describe protection against pneumococcal disease induced by pneumococcal protein vaccines adjuvanted with non-living bacterium-like particles (BLPs). BLPs consist of processed cells of Lactococcus lactis, a food-grade bacterium, that are loaded or mixed with (combinations of) proteinaceous antigens. BLPs are heat stable particles that activate, through TLR-2 signaling, the innate as well as the adaptive immune system.

To identify the most optimal protection against pneumococcal disease, the protective potential of various single or combinations of pneumococcal antigens was evaluated in mouse models of pneumococcal pneumonia and colonization. The antigens included IgA1p, PpmA, SlrA, PspA and pneumolysin mutants, which were formulated with BLPs and tested for their effectiveness in inducing protection after intranasal or parenteral administration. Comparisons were made with the benchmark adjuvants CpG1826, Alum and Cholera Toxin.

The results show that several of the antigens tested, induced significant protection against pneumonia and colonization. The highest protection against pneumonia was observed for vaccines that contained the IgA1 protease, pneumolysin mutant, or a combination of these. In colonization models, IgA1p protease and SlrA provided significant protection against colonization by multiple pneumococcal serotypes. BLPs enhanced immune responses against all antigens, both in terms of serum IgG levels and IL17A induction by spleen cells and/or in whole blood stimulation.

In conclusion, combinations of pneumococcal antigens with BLPs were shown to be protective against pneumococcal disease, indicating that BLPs offer a promising platform for future pneumococcal vaccine development.
PREVALENCE OF ANTIBODIES TO PNEUMOCOCCAL COMMON PROTEIN ANTIGENS (CPAs) AND THEIR ASSOCIATION WITH PNEUMOCOCCAL COLONIZATION IN HIV-INFECTED AND HIV-UNINFECTED CHILDREN

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Background: CPAs are being investigated as potential pneumococcal vaccine candidates. CPAs are likely to be immunogenic in infancy and may provide protection irrespective of the pneumococcal serotype. There are limited data on natural antibody kinetics against CPAs in African populations.

Objectives: To characterize the naturally-induced antibody titres to 15 CPAs; and explore the association between antibody titres and pneumococcal nasopharyngeal colonization in HIV-infected and -uninfected children between 4 and 7 years of age.

Methods: Cross-sectional cohort of 286 children (HIV-uninfected=212, HIV-infected=74), median age 5.61 (range: 3.89 to 7.09 years). Titres to CPAs were measured using a 15-plex Luminex assay for the following proteins: PspA, PspC, LytB, IgA1-proteinase, SP 0082, PdB, PcsB, PsaA, SP 0609, SP 0749, PpmA, StrA, StrP, SP 2027 and SP 2194. Nasopharyngeal swabs were evaluated for pneumococcal colonization with standard microbiologic methods.

Results: HIV-uninfected children had significantly higher antibody titres against cell wall associated proteins and toxins (PspA, PspC, PdB, SP 0082, LytB, IgA1 proteinase and PcsB). In contrast, membrane associated proteins (PsaA, SP 2027, PpmA and StrA) were associated with significantly lower antibody titres in HIV-infected compared to HIV-uninfected children. Higher titres against PdB and PcsB were associated with lower prevalence of pneumococcal colonization.

Conclusion: Dysfunctional cell mediated immune responses in HIV infected individuals may explain the dichotomous immune response to cell wall and membrane associated proteins in relation to HIV infection status, and suggests that HIV status can be used as a probe to measure the degree of cell surface exposure of an antigen.
COMPARISON OF PROTECTIVE ACTIVITY AGAINST PNEUMOCOCCAL RESPIRATORY INFECTIONS BY NASAL IMMUNIZATION WITH LACTOCOCCUS LACTIS-PSpA AND LACTOCOCCUS LACTIS-PpPA

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Background: Streptococcus pneumoniae is a serious public health problem, especially in developing countries, where available vaccines are not part of the vaccinationschedule. The rapid emergence of multidrug-resistant S. pneumonia strains throughout the world has led to an increased attention on the prevention of pneumococcal infections by vaccination.

Aim: To compare the Protective activity against pneumococcal respiratory infections by nasal immunization with Lactococcus lactis-PspA and Lactococcus lactis-PppA.

Methods: A recombinant strains of Lactococcus lactis that can express the pneumococcal surface protein (PspA) and pneumococcal protective protein A (PppA) on its surfaces were developed separately. The presence of PspA and PppA on the cell surface of the recombinant strains such as Lactococcus lactis PspA and PppA was confirmed by Immunodetection assays. The induction of systemic and mucosal specific antibodies in mice was studied after intranasal immunization of Lactococcus lactis PspA and Lactococcus lactis PppA.

Results: The presence of Immunoglobulins such as IgG, IgM, IgA, anti-PspA and anti-PppA antibodies the serum and bronchoalveolar lavage fluid of mice were detected, which showed that PspA expressed in L. lactis and PppA expressed in L. lactis was able to induce a strong mucosal and systemic immune response.

Conclusion: The results presented in this study demonstrate the effectiveness of nasal immunization with PppA and/or PspA expressed as a protein with L. lactis: it elicited cross-protective immunity against different pneumococcal serotypes, it induce protection against both systemic and respiratory challenges.

Acknowledgement: We sincerely thank to Shantha Biotechnics, Hyderabad for providing the recombinant PspA and PppA proteins.
ADJUVANT PROPERTIES OF BORDETELLA PERTUSSIS IN COMBINATION WITH THE PNEUMOCOCCAL SURFACE PROTEIN A

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Pneumococcal Surface Protein A (PspA) is a surface protein that elicits protection against models of pneumococcal infection in animals. The immunomodulatory properties of the whole cell Bordetella pertussis vaccines wP and wP_low (wP with low levels of LPS), as adjuvants to PspA, were reported by our group. The characterization of this adjuvant activity is the aim of our present studies. Here, BALB/c mice were immunized by the nasal route with PspA5 in combination with purified components such as pertussis-derived MPLA and pertussis toxin (PT), the wP and wP_low vaccines or the BPLOW strain (a B. pertussis mutant which expresses low amounts of PT and the FHA adhesin). Immunization with 3 doses of PspA5-wP, PspA5-BPLOW or PspA5-PT induced high concentrations of anti-PspA5 IgG in sera and conferred protection against a pneumococcal invasive challenge. Conversely, no significant induction of anti-PspA5 IgG or improvement in protection was observed in mice vaccinated with PspA5-MPLA. Improvements in the immunization protocol showed that a single dose of PspA5-wP or PspA5-wP_low, containing 5µg of the antigen, was sufficient to confer protection. Moreover, low amounts of the antigen (0.5µg of PspA5) combined with wP or wP_low in three doses, produced the same effect. These conditions will be used to better evaluate the other formulations. Our data suggests that the adjuvant properties of B. pertussis may be based on the interaction of different bacterial components with the immune system. The induction of high levels of anti-PspA IgG seems to be an important factor to confer protection against this model.
IS IT FEASIBLE TO PRODUCE CAPSULAR POLYSACCHARIDE IN CONTINUOUS CULTIVATION?


Background and aims: *Streptococcus pneumoniae* is a major cause of meningitis, pneumonia, and bacteremia. The capsular polysaccharides of pneumococci have been shown to be essential for their virulence. The maintenance of cells under steady state makes continuous culture system an excellent tool for metabolic studies. In this process, the growth rate can be manipulated as a function of the dilution rate (D). The objective of this work was to evaluate the influence of D on polysaccharide serotype 14 (PS14) production.

Methods: The culture medium was chemically defined (Van der Rijn, 1980) and different growth rates (equivalent to D) were tested: 0.1h\(^{-1}\), 0.3h\(^{-1}\), 0.4h\(^{-1}\), 0.5h\(^{-1}\) and 0.6h\(^{-1}\). The biomass was measured by the optical density at 600nm and the production of PS14 by capture ELISA. Sugar consumption and production of organic acids was determined by HPLC.

Results: The highest PS14 productivity (72.3mg/L.h) occurred in D=0.5h\(^{-1}\). The PS production was well below the others in 0.6 h\(^{-1}\), probably because this value is close to \(\mu_{max}\) (0.7h\(^{-1}\)). The highest yield factor (21 mg PS14/ g consumed glucose) were obtained at 0.4h\(^{-1}\), showing greater efficiency in glucose conversion to the product than other D.

Conclusion: The PS14 production in continuous cultivation with chemically defined medium was higher (160mg/L) than that previously obtained in our laboratory using complex medium (100mg/L). The results showed the feasibility of PS production in continuous cultivation.

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O-ACETYLATION IN PNEUMOCOCCAL SEROTYPE 15B CAPSULAR POLYSACCHARIDE (PS) COMPARED TO 15C RESULTS IN TYPE-SPECIFIC PROTECTION FOLLOWING IMMUNIZATION WITH 15C-CRM VACCINE

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Introduction: Pneumococcal capsular polysaccharide [Ps] from serotypes 15B and 15C have similar composition with the exception that 15B-Ps is the o-acetylated variant. The genetic basis for this difference has been reported as due to phase variation resulting in premature stop in translation in the o-acetyltransferase gene in 15C isolates. Previous studies suggest that functional antibodies against 15B and 15C are type specific.

Methods: We evaluated a 15C-crm conjugate [prepared by GSK Bio] for protection against experimental otitis media [EOM] due to S. pneumoniae 15C. Following immunization with 15C-crm conjugate, chinchillas were challenged intranasally with inoculum enriched for SP15C. Four days later barotrauma was performed and animals examined daily for EOM. Middle ear (ME) cultures were obtained and proportion and density of ME infection compared in immunized and controls. Immune responses against 15C and 15B Ps were evaluated by ELISA and flow cytometry.

Results: Response to both 15C and 15B Ps was confirmed by ELISA and flow cytometry. None of 8 immunized animals developed EOM due to serotype 15C compared to 3 of 4 controls [p= 0.01]. However, EOM due to SP15B developed in 4 of 8 animals immunized with 15C-crm.

Conclusion: We observed protection against EOM due to SP15C following immunization with 15C-crm conjugate but breakthrough due to SP 15B. As previously reported following immunization with 23V Ps vaccine, protection was type-specific despite use of a conjugate vaccine and eliciting antibody to both 15C and 15B. Further understanding of cross protection within SP serogroups is critical for future vaccine development.
REGULATION OF PNEUMOCOCCAL PILUS-1 EXPRESSION PERMITS IMMUNE EVASION IN EXPERIMENTAL OTITIS MEDIA (EOM)

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Background and aims: The pneumococcal pilus-1 consists of the RrgB protein which forms the backbone and two ancillary proteins, RrgA and RrgC. Pilus-1 expression is reported as biphasic; permitting heterogeneity within the bacterial population. To delineate the role of pilus-1 in EOM, we evaluated colonization and disease due to wt Streptococcus pneumoniae (SP) 19F Taiwan-14 and otherwise isogenic pilus-1 deficient mutant as well as potential for a chimeric protein candidate (RrgB321) [containing three RrgB variants] for prevention of middle ear (ME) disease.

Methods: Chinchillas were challenged intranasally with either wt 19F Taiwan-14 or a 19F Taiwan-14 pilus-1 deficient mutant. Four days following inoculation, animals underwent barotraumas, ME status was assessed and direct culture performed. Subsequently, new cohorts were immunized with RrgB321 and challenged with 19F Taiwan-14 as above. Pilus-1 expression was analyzed in SP isolated from nasopharynx and ME fluids in RrgB321 immunized and controls animals.

Results: Culture positive EOM developed following challenge with either 19F Taiwan-14 wt or its pilus-1 deficient mutant. Upon challenge with wt 19F Taiwan-14 no differences in proportion or density of colonization or culture positive EOM was observed following immunization with RrgB321 despite high titer, specific immune response. Reduced pilus-1 expression was observed in SP recovered from nasopharyngeal washes of all the animals and from ME fluids of immunized chinchillas.

Conclusion: In the chinchilla model, pneumococcal pilus-1 was not essential for EOM. Immune evasion following RrgB321 immunization was likely due to the biphasic regulation of pilus-1 expression.
NOVEL PNEUMOCOCCAL ANTIGENS IDENTIFIED THROUGH PROTEOMIC SCREENS USING T\(_{h}17\) CELLS FROM HUMANS PROTECT MICE AGAINST NASOPHARYNGEAL CARRIAGE

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**Background and aims:** Studies have shown that T\(_{h}17\) cells are important in the control of nasopharyngeal (NP) carriage of *S. pneumoniae*. Carriage reduction can potentially decrease the incidence of pneumococcal disease. We previously identified antigens recognized by human T\(_{h}17\) cells through pneumococcal proteomic screens. In this study, we evaluated the immunogenic and protective activity of a subset of these antigens.

**Methods:** Pneumococcal antigens were identified by proteomic screens with T\(_{h}17\) cells from human donors or whole cell antigen (WCA)-immunized mice. A subset of antigens was evaluated as vaccines in C57BL/6 mice. IL-17A secretion in response to WCA or protein of immunization was measured *in vitro* and then animals were intranasally challenged with type 6B pneumococcus. The density of pneumococcal carriage was measured in nasal washes.

**Results:** The antigen most frequently associated with human IL-17A responses was not protective against NP carriage in mice, despite inducing antigen-specific IL-17A after immunization. A second antigen identified in humans but not mice was more protective when administered i.n. than s.c. Protection correlated with IL-17A secretion in response to WCA. Two antigens identified in both mouse and human screens administered s.c. on alum were protective.

**Conclusions:** Pneumococcal antigens that elicit IL-17A responses from human T cells are immunogenic in mice. However, not all protein-specific responses translated to WCA responses or effectiveness against carriage, suggesting immunodominance does not predict protection. A better predictor may be T\(_{h}17\) responses to WCA *in vitro*. This is the first evidence that parenterally-administered T cell-directed subunit vaccines reduce NP carriage.
HEPTAVALENT CONJUGATE VACCINE AGAINST THE LATINOAMERICA MOST PREVALENT SEROTYPES OF STREPTOCOCCUS PNEUMONIAE. PRECLINICAL EVALUATION


Considering serotypes of S. pneumonia more frequently associated with infection in Latinaomerica we designed a vaccine containing 7 serotypes as initial approach to conjugate vaccines with the aim of their introduction in Cuba and in other countries of the region. Several batches were produced of this vaccine candidate as a result of several years of development. The vaccine contain 2µg of each capsular polysaccharide 1, 5, 6B, 14, 18C, 19F and 23F and 4µg for 6B conjugated to tetanus toxoid, with aluminum phosphate as adjuvant. It was used to demonstrate their physicochemical properties as well as for their preclinical evaluation. The studies discussed in the present report include immunogenicity in New Zealand rabbits, prove of memory by a booster with the unconjugated CPS after 4 months and Opsonophagocytic activity of serum. As a result the vaccine elicited high titers of total IgG with high avidity and specificity to the CPS after the second dose. Antibodies were functional as shown by their opsonophagocytic titer over 1:8 for each serotype. On the other hand safety was assessed by several toxicological studies in rats demonstrating the vaccine candidate didn’t induce any sign of unexpected toxicity. In conclusion, the conjugated vaccine candidate is immunogenic and safe in laboratory animals.
THE STRATEGIC PARADIGM FOR INCLUSION OF VACCINE EFFICACY AGAINST CARRIAGE IN THE PNEUMOCOCCAL VACCINE LICENSURE PROCESS

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Background and aims: The current licensure pathways for pneumococcal conjugate vaccines (PCVs) are based on comparative immunogenicity to 7-valent PCV and do not include vaccine efficacy against pneumococcal nasopharyngeal colonization (VEcol). This may impede the speed and breadth of pneumococcal vaccine implementation in two ways: (1) efficacious pneumococcal vaccines may fail licensure because of non-inferiority requirements for multiple serotypes, and (2) public health impact measures (including indirect effects of PCV) are not explicitly considered in the decision making process. Further, the current pathway does not allow for evaluation of protein and other novel-mechanism vaccines, several of which are under development. The PneumoCarr (Pneumococcal Carriage Consortium), funded by the Gates Foundation via the Grand Challenges scheme, has aimed to collect, present and further develop the methodology to include VEcol in the licensure process, believing this to be an important adjunct to the current approach.

Methods and results: The PneumoCarr consortium has developed and published new statistical, modeling and analytic methods to assess existing and newly conducted clinical studies on VEcol. The consortium has also developed a package of evidence for inclusion of VEcol in the licensure (including key evidence reviews as appendixes). This document will be presented to regulators at a convened conference aimed at having licensing bodies formally consider the inclusion of VEcol as part of the licensure criteria for novel pneumococcal vaccines.

Conclusions: The additional information on carriage would help to improve the effectiveness of the pneumococcal vaccine licensure process.
NEW SOY PEPTONE BASED MEDIUM FOR CAPSULAR POLYSACCHARIDE PRODUCTION BY STREPTOCOCCUS PNEUMONIAE

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Background and aims: Capsular polysaccharides are the current antigens in pneumococcal vaccines, but there is a lack of information on polysaccharide production in the literature, probably because this know-how is an industrial secret of pharmaceutical companies. Therefore, producers of developing countries have to establish their own technology to produce pneumococcal vaccines. The aim of this work was to evaluate the influences of the soy peptone Phytone, yeast extract (YE), asparagine and glutamine on pneumococcal polysaccharide serotype 1 (PS1) production using a design of experiments and to elaborate a new culture medium based on the results.

Methods: 12 experiments (2⁴−1 fractional factorial and 4 center points) were carried out in pH-controlled 5L-fermentor. The biomass was measured by the optical density at 600nm and the production of PS1 by meta-hydroxydiphenyl method. Sugar consumption and production of organic acids was determined by HPLC. A value of p < 0.05 was considered for statistical analyses.

Results: Phytone and YE presented a positive effect on biomass production and YE and glutamine on specific growth rate. The production of acetic and lactic acids was negatively influenced by YE and positively by Phytone. Asparagine presented no influence on variables analyzed. Only Phytone influenced significantly the PS1 production with a positive effect.

Conclusion: A new culture medium, containing YE, Phytone, glucose, asparagine, glutamine, salts, choline and phosphate was elaborated. This medium is considerably less expensive than the previously described hydrolyzed casein based medium and yielded higher PS1 production (275mg/L against 163mg/L).

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CONTINUOUS CULTIVATION: A NOVEL TOOL FOR UNDERSTANDING STREPTOCOCCUS PNEUMONIAE METABOLISM

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Background and aims: The capsular polysaccharide (PS) is currently used as antigen in pneumococcal vaccines. However, very little attention has been devoted to understand pneumococcal metabolism and improve PS production. This study aimed to evaluate the effect of glucose (energy source), choline (required for cellular division and infection process) and glutamine (role not completely understood) on metabolism of S. pneumoniae serotype 14 using continuous cultivation in bioreactor, a novel approach to this microorganism.

Methods: Three experiments were carried out at 0.5h⁻¹ dilution rate with 1-L of chemically defined medium, increasing the concentration of each substrate in the feed medium: (i) glucose (2.5-30 g/L), (ii) choline (10-500 mg/L) and (iii) glutamine (0.05-1.0 g/L).

Results: The uncoupling of growth and acid production was verified in condition of glucose excess. In choline excess, the glucose consumption increased twice, probably due to an enhancement of biosynthesis of teichoic and lipoteichoic acids. The excess of glutamine did not have impact on organic acid production and glucose consumption. In all conditions of substrate excess, no significant impact was observed on PS production. In contrast, the conditions of limitation of glucose/choline/glutamine increased yield factors of PS and biomass per glucose consumed as well as the yield factors of PS per biomass produced in values greater than 300% in some cases.

Conclusion: Substrate limitation was the most efficient condition to improve the conversion of substrates in biomass and PS and to avoid the uncoupling of growth and by-product formation in pneumococcal metabolism.

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INCREASED BINDING AND COMPLEMENT DEPOSITION INDUCED BY A PSPA-PDT HYBRID PROTEIN

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Background and aims: Several pneumococcal proteins have been studied as vaccine candidates with promising results. Among these are the surface protein PspA and the pneumolysin toxin (Ply). PspA and Pds (detoxified forms of Ply) have been shown to be immunogenic and protective in different animal studies. Here we constructed a hybrid protein containing PspA fused to PdT (genetically detoxified Ply) and evaluated the humoral immune response induced in mice and the functional properties of the antibodies.

Methods: PspA-PdT was obtained by gene fragment fusion and expression in \textit{E. coli}. BALB/c mice were immunized with PspA, PdT, PspA+PdT (co-administered) or the hybrid PspA-PdT protein and the antibody production was evaluated by ELISA. The anti-sera were tested for their ability to bind to pneumococci and promote complement deposition by FACS.

Results: The hybrid PspA-PdT protein induced similar levels of PspA and PdT IgG when compared to the other groups. The antibodies induced by PspA-PdT bound more effectively to pneumococci than antibodies induced by the co-administered proteins; affinity was similar to antibodies induced by PspA individually. Furthermore, anti-PspA-PdT antibodies showed an increased ability to promote complement deposition than antibodies from mice that received co-administered PspA and PdT.

Conclusion: These results suggest that immunization with the PspA-PdT fusion protein induces antibodies able to promote pneumococcal opsonization more efficiently than the co-administered proteins.

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SAFETY AND IMMUNOGENICITY OF A PNEUMOCOCCAL VACCINE BASED ON THE GENETICALLY MODIFIED PNEUMOLYSIN PROTEIN PLYD1

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Background: Pneumococcal vaccines based on conserved protein antigens have the potential to offer expanded protection against \textit{Streptococcus pneumoniae}. The highly detoxified mutant pneumolysin (PLY) protein, PlyD1 is a candidate for pneumococcal vaccine development.

Aim: Evaluate the safety/tolerability and immunogenicity of PlyD1.

Methods: A phase I, randomized, placebo-controlled, observer-blind, dose ascension study was conducted in healthy adults. Participants received 2 injections approximately 30 days apart of 1 of 3 dose levels of PlyD1 or placebo, after successful completion of an inpatient pilot safety study. Safety endpoints included rates of solicited injection site and systemic reactions, unsolicited adverse events (AEs, SAEs). Immunogenicity endpoints included antigen-specific IgG levels and functional assessment by toxin neutralization.

Results: 10 participants received 1 dose of 10µg PlyD1 and were observed for 24 hours, with no SAE or any other safety concern. 90 participants received 2 doses of vaccine at 10, 25, 50µg or placebo. There were no vaccine-related SAE, no study discontinuations due to adverse events and no decreased tolerability after repeat administration. Most solicited reactions were mild and transient with pain at injection site. Antigen-specific antibody levels increased in a dose related manner and after repeat vaccination. PlyD1 antisera neutralized PLY toxicity to Vero cells.

Conclusions: All dose levels were well tolerated and immunogenic; reactogenicity and immunogenicity showed a dose relationship with a tendency to plateau at the middle dose. Repeat vaccination significantly increased antibody levels, without untoward effects on safety/tolerability.
SAFETY AND IMMUNOGENICITY OF TWO CANDIDATE PROTEIN VACCINE ANTIGENS: PNEUMOCOCCAL HISTIDINE TRIAD D (PHTD) AND CHOLINE-BINDING PROTEIN A (PCPA)

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Background: Pneumococcal vaccines based on conserved protein antigens have the potential to offer expanded protection against Streptococcus pneumoniae. PcpA and PhtD are leading pneumococcal vaccine candidates.

Aims: Phase I study to describe the safety and immunogenicity of 2 candidate pneumococcal protein vaccines.

Methods: Phase I, randomized, observer-blind, open-label followed by placebo-controlled, dose ascension studies were conducted in healthy adults. Participants received 2 injections approximately 30 days apart of 1 of 3 dose levels of PhtD, 1 dose level of PcpA or 1 of 3 dose levels of the bivalent vaccine. Safety endpoints included rates of solicited injection site and systemic reactions, unsolicited adverse events (AEs, SAEs). Immunogenicity endpoints included antigen-specific IgG levels.

Results: 63 participants received PhtD vaccine at 6, 25 or 100µg, 23 received PcpA at 25µg and 83 received bivalent vaccine at 10, 25 or 50µg each protein in 2:1 randomization against placebo (n=25). No vaccine-related SAE was reported. There were no study discontinuations due to adverse events and no decreased tolerability after repeat administration. Most solicited reactions were mild and transient with pain at injection site. Antigen-specific antibody levels increased in a dose related manner and at each dose level after repeat vaccination, with no observed interference due to co-administration of the two protein antigens.

Conclusions: All dose levels were well tolerated and immunogenic. Reactogenicity and immunogenicity showed a dose relationship with a tendency towards a plateau effect at the middle dose. Repeat vaccination significantly increased antibody levels, without untoward effects on safety/tolerability.
THE NEXT GENERATION PNEUMOCOCCAL VACCINE: INTRANASAL IMMUNIZATION WITH TRIMETHYL CHITOSAN ENCAPSULATED -PSAA INDUCES MUCOSAL AS WELL AS SYSTEMIC IMMUNITY

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Background: The licensed polysaccharide-based pneumococcal vaccines only elicit protective antibodies against the infection of serotypes that are included in the vaccine. Mucosal immunization is an effective way of inducing immune responses at mucosal surfaces as well as systemic. PsaA is a highly conserved across all pneumococcal serotypes. Previous research shows that PsaA is a promising component for the next generation of pneumococcal vaccines. Even though a number of mucosal adjuvants have been proposed, trimethyl chitosan (TMC) has been shown to be an effective mucosal adjuvant for various antigens.

Method: In this research we evaluate PsaA encapsulated in TMC-Nanoparticle (NP), Chitosan -NP for pneumococcal mucosal vaccine delivery system. The immunogenicity of nanoparticles delivery system was assessed by measuring anti-PsaA IgG antibody in serum and anti-sIgA antibody titer levels in BALB/c mice serum and secretions after intranasal administration.

Results: Mice immunized with TMC-NP shows high levels of IgG and IgA antibodies compared to the group that received the CS-NP, PsaA alone. High levels of subclasses of IgG1, IgG2 and IgG3 antibodies were also observed in sera of mice immunized with TMC-NP. In addition, high IgG and IgA antibody responses were observed in sera of young mice immunized with PsaA encapsulated in TMC-NP or CS-NP compared with the group received the PsaA alone.

Conclusions: These results reveal that mucosal immunization with pneumococcal PsaA using TMC-NP as adjuvant as well carrier can confer protective immunity against pneumococcal infection.

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THE PROTEIN L460D AND THE CONSTRUCT PRN-L460D PROTECT MICE AGAINST BACTEREMIA AND LUNG INFECTION CAUSED BY THREE DIFFERENT PNEUMOCOCCAL STRAINS

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Conserved widely expressed pneumococcal proteins are candidates for universal serotype independent pneumococcal vaccines.

This study was a blinded evaluation of the protective capacity of pneumococcal protein constructs based on pneumolysoid (L460D), PspA (PRN) and CbpA (NEEK): L460D, L460D-NEEK, YPT-L460D-NEEK, PRN-L460D, PRN-L460D-NEEK, alone or combined (YPT-L460D-NEEK + PRN-L460D) in a mouse model of lung infection and bacteremia, caused by high dose intranasal challenge with aspiration.

Adult NMRI mice were immunized subcutaneously 3 times with 10 µg protein with 0.04% Al(OH)₃+100µg CpG1826. Two weeks after the third dose they were challenged with pneumococci of serotypes 1 (ATCC6301), 4 (PATH10) and 6B (PATH18), and sacrificed 24 hours later to evaluate bacteremia and lung infection.

All protein constructs mixed with CpG1826+Alum significantly reduced bacteremia and lung infection caused by one or more of the three pneumococcal strains. The fusion protein PRN-L460D was the only protein that significantly reduced both bacteremia and lung infection caused by all pneumococcal strains, and 5/10, 2/10 and 6/9 mice had undetectable bacteremia for serotypes 1, 4 and 6B, respectively. Although fewer mice immunized with L460D were completely protected the level of bacteremia (CFU/mL) did not differ between mice immunized with PRN-L460D and L460D, whereas significantly less protection against bacteremia was obtained with L460D-NEEK (all strains), YPT-L460D-NEEK (4 and 6B) and PRN-L460D-NEEK (4), respectively. Serotype 1 and 6B lung infection was reduced similarly by PRN-L460D and L460D.

These protein constructs, particularly PRN-L460D and L460D, are promising vaccine candidates that should be further evaluated combined with other pneumococcal proteins.
ASSAY VALIDATION OF A FOURFOLD MULTIPLEXED OPSONIZATIONASSAY FOR PNEUMOCOCCAL ANTIBODIES AT EWHA CENTER FOR VACCINE EVALUATION AND STUDY, KOREA


Background and aims: Opsonophagocytic killing assay (OPA) is essential reference method for evaluation of pneumococcal vaccines. A fourfold multiplexed OPA (MOPA4) has developed against 13 serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F) of pneumococci by Dr. Nahm in UAB. They showed that it is a high-throughput, reliable, standardized and fully characterized OPA for pneumococcal antibodies.

Methods: To establish the pneumococcal vaccine evaluation assay in Korea, MOPA4 was performed and validated in Ewha Center for Vaccine Evaluation and Study (ECVES). Thirteen target bacteria were provided from UAB, which are resistant to only one of the following antibiotics: optochin, streptomycin, spectinomycin, and trimethoprim.

Results: The essential components for MOPA4, such as bacteria, HL-60 cells, complement, and fetal bovine sera were evaluated and validated. Following optimization of assay conditions, accuracy of MOPA4 was determined by testing 3 QC pool sera in the MOPA4 and the single-serotype assays. The opsonization titers obtained with both assays agreed well. The assay was specific: preabsorbing test sera with homologous polysaccharide (PS) completely abrogated opsonic activity, but an unrelated PS (5 mcg/ml of each) had no effect. Intra- and interassay coefficients of variation were acceptable.

Conclusions: MOPA4 was validated and can be performed for the evaluation of pneumococcal vaccines in ECVES, Korea. MOPA4 should allow evaluation of multivalent pneumococcal vaccines with the limited volume of serum typically available from young children.
MEMORY OF PSPA SPECIFIC IMMUNE RESPONSES IN OFFSPRING DELIVERED FROM IMMUNIZED MOTHER MICE

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Background: Children younger than 2 years old usually have lower levels of pneumococci specific IgG antibody in sera due to the age-related immaturity of immune responses and are vulnerable to pneumococcal infections. It is important to maintain protective immune responses against pneumococci during early childhood. In this study, we further evaluated immunological memory of specific responses in maternal-immunized offspring with pneumococcal surface protein A (PspA).

Method: BALB/c byj mice, 4 wks old female, were intranasally immunized with PspA mixed with cholera toxin B subunit (CTB) for 2 to 3 weeks. After the final immunization, they were mated with male mice for two weeks. Approximately 3 weeks after mating, offspring were obtained and fed by mother for 6 weeks. Offspring at 6 weeks old were once stimulated with PspA alone. After the PspA stimulation, we evaluated changes of PspA specific IgG in sera by ELISA.

Results: Anti-PspA specific IgG antibody in offspring's sera was maintained during the nursing periods and then gradually decreased in the maternal immunized offspring with PspA. After a stimulation with PspA alone, the offspring derived from PspA immunized mother enhanced anti-PspA specific IgG in sera again.

Discussion: Maternal intranasal immunization would be a noble procedure against pneumococcal infections among early childhood. The current results suggested that PspA specific immune response was memorized in offspring maternal immunized with PspA for a considerable period of time. The maternal immunization would be one of the most suitable approaches to induce effective immune protections against pneumococcal infections.
DEVELOPMENT AND EVALUATION OF A RAPID IMMUNOASSAY TO DETECT CELL WALL POLYSACCHARIDE CONTAMINATION IN PNEUMOCOCCAL POLYSACCHARIDES

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Background and aims: Cell wall polysaccharide (CWPS) is a common pneumococcal cell component. Lowest levels of CWPS are expected hence estimation of its contamination in purified pneumococcal capsular polysaccharides (PnPS) is critical in developing pneumococcal conjugate vaccines (PCV). Such evaluations by NMR and dionex methods are laborious, time consuming and cumbersome or may not be used for few serotypes, hence, we evaluated a monoclonal antibody based immunoassay as a fast and simple alternative.

Methods: CWPS specific monoclonal antibodies (MAbs) were developed by hybridoma technology. We report development and partial validation of a rapid bead-assay based on x-MAP technology using Bioplex platform for CWPS estimation in purified PnPS of 12 serotypes. Specific MAbs against CWPS were used in a competitive assay. The assay was validated for various parameters viz., sensitivity, linearity, intermediate precision, accuracy and comparability with conventional ELISA, NMR and dionex results.

Results: Bead-based immunoassay was found to be reproducible, linear, with a high accuracy (80-120% spiking recovery) for all 12 pneumococcal serotypes tested. The 3.5 hour assay was highly sensitive with an LOQ of 40ng/ml. Highly satisfactory correlation was observed in results from ELISA for all serotypes and results corroborated well with NMR and dionex based assay for various serotypes tested.

Conclusions: The benefits of immunoassay assay over conventional NMR and dionex based analyses include ease, sensitivity and speed. Additionally, the immunoassay can be applied to all the pneumococcal serotypes having common CWPS. The method can be highly useful tool in in-process quality control of PCV development.
UTILIZATION OF SUSPENSION ARRAY TECHNOLOGY IN NEW PNEUMOCOCCAL VACCINE DEVELOPMENT FROM CELL BANKING TO FILL FINISH

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Background and aims: Complex methodologies and assays are required for development of multivalent pneumococcal vaccines. We evaluated use of bead based suspension array technology (SAT) in development of various immunoassays for in-process quality control, pre-clinical evaluation and product release.

Methods: Methods for coupling of carrier protein, various pneumococcal polysaccharides (PnPS) and monoclonal antibodies (MAbs) on polystyrene microspheres were standardized. PnPS and CRM coupled beads were used in development of SAT based multiplexed assays for antibody titration in animal sera and hybridoma culture supernatants for up to 24 serotypes. Further, beads were used in development of antigen estimation by competitive multiplexed assays using serotype specific in-house developed MAbs or polyclonal sera. Assays have been qualified for various validation parameters.

Results: Beads coupled with various antigens and antibodies were successfully utilized in developing various immunoassays during the whole process of pneumococcal multivalent vaccine development. A 24-plex assay was very useful in development of highly specific MAbs against Type 1, 4, 5, 6B, 7F, 9V, 14, 23F, CWPS and CRM197. Anti-CWPS MAbs were successfully used in estimation of CWPS contamination in the purified PnPS samples. PnPS-MAbs were successfully used in development of rate nephelometry, ELISA and competitive bead based assays for antigen quantitation in fermenter cultures, purification samples, conjugate bulks and final vaccine formulation. Assay was also utilized in determining extent of adsorption of conjugates on alum adjuvant. Multiplexed IgG estimation assays could help rapid analyses of sera from rabbit and mouse immunogenicity studies.

Conclusions: The Luminex based SAT can be a highly useful tool in development of all new pneumococcal or other multivalent vaccines.
CHALLENGES IN EFFECTIVENESS EVALUATIONS OF NEW PNEUMOCOCCAL VACCINES BASED ON SURROGATE ENDPOINTS* 

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Background: New pneumococcal vaccines comprised of protein antigens or whole killed cells are currently in development. A licensure approach for these vaccines, using surrogate endpoints, presents several challenges. We sought to identify characteristics important for supporting use of a selected biomarker. 

Methods: Product approval summaries of vaccines licensed by the FDA within the last five years, in which effectiveness was inferred from a surrogate endpoint, were reviewed for regulatory examples of considerations for use and choice of biomarker, and relevant licensure pathways. 

Results: Use of a surrogate was supported when protective mechanisms were known, efficacy data was available from trials in the intended population, and in vitro methods could accurately measure vaccine response in immunized individuals. Bridging to a different population or vaccine, using an established surrogate marker, for the same disease manifestation was feasible when the protective mechanism was common and measured responses reliably predicted protection in the new population (e.g. meningococcal conjugate, pertussis vaccines). When an active control vaccine was not available for randomized study participants, inferred effectiveness using a surrogate relied mainly on a quantitative measurement considered to be protective. Regulations exist for accelerated approval of new vaccines to prevent serious/life-threatening diseases, which provide meaningful benefit over available therapies, a reasonably predictive surrogate exists, and confirmatory trials are underway at the time of approval. 

Conclusions: Use of a single surrogate marker to predict manifestations of pneumococcal disease is limited by available clinical efficacy data and assay methods. 

*Reflects views by the authors
A FUSION CONJUGATE VACCINE OF PNEUMOCOCCAL CONSERVED PROTEINS AND VI POLYSACCHARIDE PROTECTS MICE AGAINST PNEUMOCOCCAL COLONIZATION AND DISEASE

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Background: We have shown previously that a “fusion conjugate” vaccine, consisting of a pneumococcal protein genetically fused to pneumolysoid (PdT) and then conjugated to a polysaccharide (PS) results in enhanced antibody responses to the PS and proteins and induces protective CD4+ Th17 cell responses to the proteins. We applied this approach using a Vi PS from Salmonella typhi by conjugating two pneumococcal conserved proteins fusion to PdT to develop a bivalent vaccine against pneumococcal and typhoid diseases.

Methods: Two highly conserved pneumococcal antigens (SP1572 and SP2070) were fused to PdT. SP1572-PdT was then conjugated to Vi PS and SP2070-PdT was conjugated to the conserved pneumococcal cell wall PS. Mice were immunized with a combination of 2 conjugates or equimolar mixtures subcutaneously adsorbed onto aluminum hydroxide. Mice were challenged with pneumococcal strains in a colonization model (Pn type 6B) or in a sepsis model (Pn type 3). Vi antibody was measured by ELISA and opsonophagocytic assay (OPA) using S. typhimurium bearing the Vi capsule.

Results: Mice in the bivalent conjugate group made significantly higher functional Vi antibody, and also higher antibody and T cell responses to the pneumococcal antigens when compared to mice that received equal amount of mixture. Mice that received the bivalent conjugate were protected from type 6B colonization and sepsis challenge with type 3, whereas mice that received mixture were not.

Conclusion: A bivalent conjugate vaccine induced Vi antibodies and OPA and protected mice against pneumococcal colonization and sepsis. Further studies of this vaccine are ongoing.
BROADLY PROTECTIVE PROTEIN BASED PNEUMOCOCCAL VACCINE COMPRISED OF PNEUMOLYSIN TOXOID-CBPA PEPTIDE FUSION

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Background and aims: Infection by Streptococcus pneumoniae remains a health threat worldwide. New pneumococcal vaccines are needed that can provide serotype-independent coverage, avoid serotype replacement, induce protection against pneumonia, and be affordable and accessible for developing countries.

This study aims to test the immunogenicity and protective capabilities of a fusion protein combining a toxoid of pneumolysin (L460D) with receptor binding regions of choline binding protein A (CbpA). These proteins are immunogenic in humans and their importance in disease pathogenesis is well documented. CbpA also generates antibodies cross-reactive with meningeal pathogens N. meningitidis and H. influenzae.

Methods and results: We compared the protective immunogenicity of CbpA domains, linear peptides, or peptides with hairpin loops to mimic the native CbpA structure in mouse pneumonia, sepsis and meningitis models. Immunization with looped peptides of 30-75 a containing the conserved sequences RNYPT and EPRNEEK was highly protective as was passive administration of the cognate monoclonal antibody. Cross protection occurred across serotypes and for other meningeal pathogens as well. Covalent modification of pneumolysin with looped peptides induced high neutralizing IgG titers against pneumolysin, CbpA and whole pneumococci and conferred survival in a pneumonia/sepsis model.

Conclusions: The fusion of modified CbpA YPT and NEEK peptides to L460D generates a pneumococcal vaccine that provides protection against pneumonia, sepsis and meningitis, including additional meningeal pathogens.
IDENTIFICATION OF NOVEL S. PNEUMONIAE CANDIDATE VACCINE ANTIGENS

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Background: Mortality due to pneumococcal infections remains high worldwide, augmented by widespread antibiotic resistance in many pneumococcal strains. To identify protein antigens that may be involved in the development of natural immunity to S. pneumoniae, a pneumococcal cell wall protein-enriched extract was screened using 2-D gel electrophoresis and immunoblotting with sera obtained longitudinally from children attending day-care centers, frequently exposed to S. pneumoniae. We concentrated on proteins for which antigenicity increases with age, coinciding with decreased morbidity. The proteins from this group that do not share homology to human proteins and that are conserved among different S. pneumoniae strains were tested for their ability to elicit protection against S. pneumoniae challenge in animal models.

Methods: S. pneumoniae proteins for which antigenicity increases with age (PPP, GtS, Nox, PsipB, FBA, TF and FtsZ) were amplified from TIGR4 strain, cloned, expressed in E. coli, and purified. Mice were immunized three times intranasally or subcutaneously with these proteins in the presence of adjuvant and challenged two weeks later. Nasopharyngeal and lung colonization levels were quantified 48hrs following bacterial challenge, and survival was monitored daily for seven days.

Results: All seven proteins elicited protective immune responses in mice as determined by reduced nasopharyngeal and lung colonization, prolonged survival, and the ability of antibodies obtained from immunized mice to ex-vivo neutralize bacterial virulence for the intraperitoneal challenge model.

Conclusion: Immunization with proteins that demonstrate age-dependent antigenicity recapitulates the development of natural immunity in children and elicits protective immune responses in mouse challenge systems.
POST-TRANSATIONAL LIPIDATION OF 2 SUBSTRATE BINDING PROTEINS IS ASSOCIATED WITH TLR2 ACTIVITY, ENHANCED IMMUNOGENICITY, AND PROTECTIVE POTENTIAL IN MICE

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**Background and aims:** A protein-subunit pneumococcal vaccine represents an important strategy towards the goal of achieving serotype-independent immunity. Additionally, a vaccine that prevents mucosal colonization regardless of serotype might enhance herd immunity. We recently identified several antigens from proteomic screens that protected mice from pneumococcal colonization in a CD4+ T-cell and IL-17A-dependent manner. Several of these proteins are lipidated substrate binding proteins. We hypothesized that their immunogenicity and mechanism of reducing colonization is in part due to their lipid moieties and activation of Toll-like receptor (TLR) 2.

**Methods:** We cloned, expressed and purified two lipoproteins and their respective mutants that could not be lipidated. These constructs were used to intranasally immunize C57BL/6 mice and their immunogenicity and protective efficacy were compared in a colonization model. The lipidated and non-lipidated proteins were also used to stimulate Human Embryonic Kidney (HEK)-TLR2 cells as well as wild-type and TLR2−/− macrophages from C57BL/6 mice and activation and pro-inflammatory cytokine secretion was evaluated.

**Results:** Immunization with lipidated antigens resulted in 1-2 log higher IL-17A secretion compared with non-lipidated mutants after stimulation of whole blood with pneumococcal whole cell antigen, correlating with more significant protection. Lipidated proteins elicited robust activation of HEK-TLR2 cells while non-lipidated mutants did not. Murine TLR2-deficient macrophages demonstrated ~100x lower TNFa when stimulated with lipidated proteins compared with WT macrophages.

**Conclusion:** These experiments suggest the lipid moieties enhance the immunogenicity and protective efficacy of pneumococcal antigens through activation of TLR2. Active immunization studies in TLR2−/− mice are underway. Supported by PATH.
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PNEUMOCOCCAL WHOLE-CELL VACCINE INDUCES BROAD HUMORAL AND T-CELL IMMUNITY ACROSS MULTIPLE SEROTYPES

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Background and aims: Injecting mice with killed cells of non-capsulated strain RM200 adsorbed on alum (pneumococcal whole-cell vaccine; WCV) reduces nasopharyngeal colonization by capsular serotype 6B and prevents fatal aspiration pneumonia by serotype 3 or serotype 5 strains. To further examine the potential for omni-strain immunity, we examined a panel of clinical isolates and a library of capsule-switch variants in the TIGR4 background.

Methods: IgG binding to these bacteria in sera of rabbits injected with WCV or alum alone was assessed by ELISA without and with adsorption with cell-wall polysaccharide (CWPS). The examined strains were 24 primary pneumococcal isolates including at least 12 different MLS types and 15 serotypes. To investigate the effect of capsulation, isogenic TIGR4 strain constructs with the capsulation genes of 20 different serotypes were evaluated by measuring WCV-immunized sera binding by flow cytometry and IL-17A stimulatory effect in vitro with WCV-immunized T cells.

Results: All strains showed a large IgG rise due to WCV immunization (10-140 fold-rise over alum-immunized control serum), of which most of the antibody (42-94%) was not adsorbed by CWPS. Increased binding of IgG in WCV-immunized sera to the 20 isogenic capsule-switch strains was shown by flow cytometry (MFI range of WCV-immune sera vs. pre-immune sera; 171-702 vs. 3-5). Further, all 20 capsule-switch strains elicited IL-17A in T cells of WCV-vaccinated mice (median IL-17A range for capsule-switch strains vs. media alone; 900-4800 pg/ml vs. 30 pg/ml).

Conclusion: WCV induced both humoral and T₃₁₇ cell-mediated immunity against all tested strains. Supported by PATH.
ANALYSIS OF INHIBITION OF BINDING OF FACTOR H (FH) AND SECRETORY IGA (SIGA) TO PNEUMOCOCCI THROUGH ANTI-PSPC ANTIBODIES

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Background: Pneumococcal surface protein C (PspC) is an important candidate for a cost effective vaccine with broad coverage against pneumococcal diseases. Previous studies have shown that Streptococcus pneumoniae is able to bind both human Factor H (FH), an inhibitor of complement alternative pathway, and human secretory IgA (sIgA) via PspC. PspC was classified in 11 groups based in variations of the protein.

Methods: BALB/c mice were immunized with three different PspC molecules (PspC3, PspC5 and PspC8) for the production of antibodies that were used for Western blot and FACS analysis.

Results and conclusions: Immunization with PspC3 induced antibodies that were able to recognize the majority of the pneumococcal isolates as analysed by Western Blot of whole-cell extract and FACS of intact bacteria. We have also evaluated the interaction of FH and sIgA with pneumococcal extracts and the majority of the isolates tested showed a strong binding to FH and weaker interaction with sIgA through Western Blot. Inhibition of binding assays with whole-cell extract of pneumococci using anti-PspC3 IgG and anti-PspC5 IgG showed a reduction in binding with FH and sIgA. Inhibition in intact bacteria was not observed for the majority of the isolates. We could observe only a small decrease in FH binding through incubation of anti-PspC3 IgG with D39 and D39-DpspA and one clinical isolate showed sIgA binding inhibition through anti-PspC3 and anti-PspC5 antibodies. Inhibition of binding of FH and sIgA to pneumococci through anti-PspC antibodies thus varies considerably with the different isolates.
IMMUNIZATION WITH THE RRGB321 FUSION PROTEIN PROTECTS MICE AGAINST BOTH HIGH AND LOW PILUS-EXPRESSING STREPTOCOCCUS PNEUMONIAE POPULATIONS

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Background and aims: RrgB321, a fusion protein of the three *Streptococcus pneumoniae* pilus backbone RrgB variants, is protective in vivo against pilus islet-1 (PI-1) positive pneumococci. RrgB321 antibodies mediate a complement-dependent opsonophagocytic killing (OPK) of piliated strains. Pilus-1 displays a biphasic expression pattern: two phenotypes, one expressing and one not-expressing the pilus-1 are present in PI-1 positive strains. Here, we evaluated the impact of pilus expression on the OPK, and on the ability of RrgB321 immunization to confer protection in vivo.

Methods: In PI-1 positive strains, the two pilus-expressing phenotypes were separated obtaining the enriched High and Low pilus-expressing populations. Both populations were tested in the OPK assay performed with RrgB321 antisera and pilus-1 expression was evaluated in surviving bacteria. Bacteraemia and survival were analyzed in sepsis and pneumonia models in mice immunized with RrgB321 and challenged with either the H or L population of different pneumococcal strains.

Results: The OPK mediated in-vitro by RrgB321 antisera was dependent on pilus expression of the strain used. During the opsonophagocytosis assay pilus-expressing pneumococci were selectively killed, and no switch towards the pilus non-expressing phenotype was observed. RrgB321 protected mice against challenge with either the H or the L pilus-expressing population in active and passive immunization experiments. This suggests that pilus-1 expression could be up-regulated in vivo.

Conclusions: RrgB321 is protective against PI-1 positive strains regardless of their pilus expression level, thus supporting the inclusion of RrgB321 into a pneumococcal multi-component protein-based vaccine.
VACCINATION OF HUMANS WITH PNEUMOCOCCAL HISTIDINE TRIAD D (PHTD) ELICITS FUNCTIONAL ANTIBODIES

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Background: Pneumococcal vaccines based on conserved protein antigens have the potential to offer broad protection against Streptococcus pneumoniae. PhtD is a leading pneumococcal vaccine candidate.

Aims: To demonstrate functionality of anti-PhtD antibodies elicited by immunization of healthy adults in a phase I clinical study.

Methods: Healthy adults participating in a phase I study were immunized twice with PhtD protein in the presence of aluminum phosphate adjuvant. Pre-immune and post-immune sera were collected and compared in murine passive protection studies. Sera were transferred into CBA/CaHN-Btk xid /J (CBA/N) mice that were challenged one hour post transfer with a lethal dose of Streptococcus pneumoniae strain A66.1 by intravenous injection.

Results: Sera from 18 trial participants were selected for analysis. The post-immunization sera from 11 of these participants were protective against lethal pneumococcal challenge as determined from significant delay-of-death or increase in the percentage of surviving mice compared to the paired pre-immune sera. Studies to determine the role of anti-PhtD in this protection used sera from 5 participants in the presence of PhtD protein and demonstrated that the observed survival increases were due to the presence of anti-PhtD in the post-immune sera.

Conclusions: Immunization of healthy adults with PhtD protein elicited a functional antibody response. The transfer of post-immune sera from these adults into mice increased mouse survival after pneumococcal challenge.
DESIGN OF A STREPTOCOCCUS PNEUMONIAE IMMUNISATION AND CARRIAGE (SPICAR) STUDY TO ASSESS A NOVEL PNEUMOCOCCAL PROTEIN-BASED VACCINE IN GAMBIAN CHILDREN


Background/aims: The efficacy of a pneumococcal conjugate vaccine (PCV) against pneumococcal disease in Gambian children has been demonstrated1, but the potential for replacement carriage and disease has also been recognised2. Common protein-based vaccines offer great potential for next generation pneumococcal vaccines to broaden coverage. This study evaluates the safety/reactogenicity, impact on nasopharyngeal carriage (NPC), and immunogenicity of a pneumococcal protein-based vaccine (GlaxoSmithKline Biologicals) in children in The Gambia.

Design: This phase II, randomised, controlled study (NCT01262872) has 2 components: a safety assessment in children 2-4 years of age, followed by the assessment of impact on NPC, safety and immunogenicity in infants, aged 8-10 weeks at first vaccination. Two formulations of the investigational vaccine are being assessed versus 2 licensed controls (PHID-CV and PCV13), administered according to 2-3-4 months-of-age schedule. Two additional groups will receive either the investigational vaccine or PHID-CV according to an alternative schedule. Nasopharyngeal swabs and blood samples will be collected at defined time points to assess colonisation and immune responses, respectively (Table).

Conclusions: The results of this study on the safety, immunogenicity and possible impact on NPC of a pneumococcal protein-based vaccine in infants will help to define potential of pneumococcal proteins in next generation vaccines for broadening pneumococcal prevention.

1Cutts Lancet 2005;365:1139-46
2Cheung PIDJ 2009;28:990-95

Table. Overview of study procedures for Cohort 2

<table>
<thead>
<tr>
<th>Vaccine received and schedule</th>
<th>Study time point (age of child)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M0 (8-10 wks)</td>
</tr>
<tr>
<td>Formulation 1 3+0</td>
<td>Vac</td>
</tr>
<tr>
<td>Formulation 2 3+0</td>
<td>Vac</td>
</tr>
<tr>
<td>PHID-CV 3+0</td>
<td>Vac</td>
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<tr>
<td>PCV13 3+0</td>
<td>Vac</td>
</tr>
<tr>
<td>Formulation 1 2+1</td>
<td>Vac</td>
</tr>
<tr>
<td>PHID-CV 2+1</td>
<td>Vac</td>
</tr>
</tbody>
</table>

wks, weeks; mo, months; BS, blood sample; M, study month; NP, nasopharyngeal swab; PHID-CV, 10-valent pneumococcal non-typeable Haemophilus influenzae protein D conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; Vac, vaccination with a study pneumococcal vaccine co-administered with routine paediatric vaccines; Co-ad, vaccination with routine paediatric vaccines only; 3+0, 3-dose primary series; 2+1, 2-dose primary series followed by booster.

[Table. Overview of study procedures]
THE POTENTIAL OF CATIONIC LIPOSOME AS A USEFUL VACCINE ADJUVANT AGAINST STREPTOCOCCUS PNEUMONIAE

S.J. Park, K.H. Seo, K.N. Park, Hwasun-Eup, Republic of Korea

Streptococcus pneumoniae (S. pneumoniae) causes serious illnesses such as pneumonia, meningitis, and acute otitis media. Polysaccharide (PS) is a main antigenic determinant of S. pneumoniae. However PS shows a poor immunogenicity as a T cell independent antigen. Liposome is entirely enclosed by a membrane composed of lipid molecules and emerging as a good adjuvant for a vaccine. In this study, we investigated the ability of a cationic liposome as an adjuvant for an infant vaccine against S. pneumoniae through converting an antigen into a T cell dependent antigen.

The ability of liposome to proliferate T cell was evaluated through co-culture of T cells and dendritic cells, which was stimulated with liposomal PS or PS only and stained with a BrdU incorporation method. The influence on APCs of liposomal PS was identified with Bone marrow derived macrophages (BMMs) by FACS analysis. Liposomal PS showed significantly higher T cell proliferation compared to PS only or control group in both in vitro and in vivo. In the test of BMMs, liposomal PS induced higher activation of MHC class II than PS only. And we have verified the ability of inducing IgG against pneumococcal PS and producing interleukin-4 in T cells stimulated with liposomal PS.

In conclusion, a cationic liposome is useful as an adjuvant of a vaccine against S. pneumoniae for infants showing that it can convert a weak T cell-independent PS into a T cell-dependent PS and inducing activation of MHC class II in APCs higher compared to PS only.
IN VITRO INDUCTION OF TH17-CYTOKINES IN PAEDIATRIC ADENOIDAL AND BLOOD LYMPHOCYTE CULTURES BY PNEUMOCOCCAL VACCINE CANDIDATES

C. Pope¹, E. Oliver¹, C. Wright¹, E. Clarke¹, A.D. Ogunniyi², T.J. Mitchell³, R. Malley⁴, A. Finn¹, ¹Bristol, UK, ²Adelaide, SA, Australia, ³Glasgow, UK, ⁴Boston, MA, USA

Studies in mice have shown that CD4+IL-17A-secreting(Th17) cells are involved in antibody-independent immunity to many bacterial species and may play an important role in nasopharyngeal clearance of S.pneumoniae. Candidate protein vaccines against nasal bacteria might exert protection by inducing mucosal immune responses and a tool for assessing such responses would assist the evaluation of new vaccines.

We evaluated the induction of IL-17A and IL-22 by pneumococcal whole cell antigen (WCA) and individual recombinant pneumococcal protein antigens in adenoidal mononuclear cells (AMNC) and peripheral blood mononuclear cells (PBMC) from children aged 1-15 years. Nasopharyngeal carriage of pneumococci was also assessed.

Cytokine generation by PBMC in response to CbpA, PsaA and PspA, and WCA was significantly greater than to media alone, whereas stimulation with PhD generated no response. However, in AMNC cultures, only WCA elicited a significant response.

Children with the lowest PBMC-derived IL-17A responses to recombinant proteins were not detectably colonised with pneumococcus. IL-17A secretion by human CD4+Th17 cells in these cultures was shown by intracellular cytokine staining.

IL-17A responses to different pneumococcal antigens vary between antigens and between individuals but are more consistently present in blood than adenoidal mononuclear leukocytes. These responses appear to be stronger and more reliably present in children detectably colonised with pneumococcus. CD4+T cells are a source of IL-17A among these cells. Future longitudinal studies of carriage, of other antigens and in vaccine recipients will clarify the potential value of this approach as a measure of immunity to colonisation.

Support for this project was provided by PATH.
Poster No 273

PAEDIATRIC MUCOSAL IMMUNE RESPONSES TO CANDIDATE PNEUMOCOCCAL FUSION PROTEIN VACCINES

C. Pope1, E. Oliver1, J. Ma2, K.S. Ross2, T.J. Mitchell2, A. Finn1, 1Bristol, 2Glasgow, UK

Alternative or adjunctive non-capsular pneumococcal vaccines are urgently needed. We have shown in mice that pneumolysin (Ply) and a non-toxic variant (Δ6Ply) have enhancing effects on antibody responses when genetically fused to pneumococcal surface adhesin A (PsaA), a potentially valuable effect for future vaccines. We investigated this effect in human mucosal primary immune cell cultures.

Adenoidal mononuclear cells (AMNC) from children aged 2-10y (n=32) were stimulated with individual proteins, mixed proteins or fusion proteins and proliferative responses and cell permeability were assessed using flow cytometry.

CD4+T-cell proliferative responses to PsaAPly were significantly higher than responses to individual or mixed proteins (all p<0.02). Careful evaluation of cell permeability suggested this may have been due to inhibition of Ply-induced cytolysis by fusion. In contrast, enhanced responses to PsaAΔ6Ply compared to individual or mixed proteins occurred (p<0.04), although only at higher concentrations, in the absence of discernable cell permeability changes.

We have previously described naturally-acquired T-cell mucosal immunity to Ply in children. Residual cytotoxic effects of some pneumolysoids may limit their use in vaccines. However membrane- and PAMP-receptor binding properties of such proteins may confer valuable adjuvant properties. Fusion of pneumolysoids with other antigens may reduce or abolish cytolysis without altering these other effects and may even result in efficient delivery of antigen to the antigen presenting cell surface.

Support for this project was provided by PATH.
SAFETY AND IMMUNOGENICITY OF AN INVESTIGATIONAL VACCINE CONTAINING TWO COMMON PNEUMOCOCCAL PROTEINS GIVEN TO CZECH TODDLERS

R. Prymula¹, P. Pazdiora², M. Traskine³, J.U. Rüggeberg³, D. Borys³, ¹Hradec Kralove, ²Pilsen, Czech Republic, ³Wavre, Belgium

Background/aims: GlaxoSmithKline Biologicals is investigating two highly conserved S. pneumoniae proteins, pneumolysin toxoid (dPly) and histidine-triad protein D (PhtD), to develop new vaccines offering increased pneumococcal serotype coverage. Different formulations of such a vaccine were evaluated for safety/reactogenicity and immunogenicity when administered to children in the Czech Republic in 2-dose primary series plus booster.

Methods: In this phase II, observer-blind study (NCT00985751), children (aged 12-23 months) were randomised (1:1:1:1:1) to receive 1 of 4 investigational vaccine formulations (dPly-PhtD at low or high dose, combined or not with pneumococcal non-typeable Haemophilus influenzae protein-D conjugate vaccine [PHiD-CV]), or licensed PHiD-CV at Study Months 0, 2 and 6. Solicited local/general (within 7 days post-vaccination) and unsolicited symptoms (within 31 days post-vaccination) were recorded. SAEs were recorded to study end. Antibody concentrations against specific vaccine pneumococcal proteins were measured pre-dose 1, 1 month post-dose 2, pre- and 1 month post-booster.

Results: 257 children were enrolled and vaccinated. Observed percentages of solicited local/general symptoms following the different investigational formulations were generally within the same ranges as for PHiD-CV. Grade 3 fever (>40.0°C, rectal) occurred following ≤1.9% of doses, with no statistical difference in per-subject incidence detected post-primary vaccination between investigational formulations and PHiD-CV (confirmatory primary objective). 17 SAEs were reported; none were considered by the investigator to be vaccine-related. Increases in specific vaccine pneumococcal protein antibody GMCs were observed post-primary and post-booster versus pre-timepoints in all groups.

Conclusions: All investigational vaccine formulations containing pneumococcal dPly and PhtD were well-tolerated and immunogenic when administered to toddlers.
A NOVEL LIVE VACCINE STRATEGY FOR OTITIS MEDIA AND INVASIVE PNEUMOCOCCAL DISEASE

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*Streptococcus pneumoniae* remains the number one cause of childhood mortality despite widespread vaccination. A major limitation of the currently licensed vaccines is the lack of strong immunity and protection in the mucosa. Hence there is considerable interest in the generation of novel vaccine strategies that confer both mucosal and humoral immunity in a serotype independent manner. We sought to generate a novel class of live vaccines that are fully attenuated and retain all major antigenic virulence proteins. We have developed such a vaccine via deletion of the Signal Recognition Particle pathway, rendering the pneumococcus avirulent despite the presence of all known virulence factors. Application of the live vaccine induced potent, serotype independent protection against the development of otitis media, pneumonia, and bacteremia in a serotype independent manner. Protection mediated by the live vaccine, but not the commercial polysaccharide vaccine, required CD4 T-cells for the production of high affinity antibodies and corresponding protection against invasive disease. Furthermore, distinct isotypes of protective antibodies was strongly induced with the live vaccine that was distinct from the Prevnar vaccine groups. This represents a novel, alternative strategy to induce robust mucosal and systemic protection against pneumococcal infections.
PROTECTIVE ACTIVITY OF A WHOLE CELL PNEUMOCOCCAL VACCINE IN MICE


Introduction: Currently available pneumococcal vaccines, based on the capsular polysaccharide, either offer low protection for the high-risk groups, or are too expensive and may not cover all the dominant serotypes. Instituto Butantan is developing the production process for a killed whole cell pneumococcal vaccine (SPWCV) derived from the unencapsulated mutant Rx1E PdT ΔlytA of Streptococcus pneumoniae, originally a serotype 2 strain, autolysin negative, carrying a kanamycin resistance and a pneumolysin defective gene.

Objectives: In this work we evaluated the protection induced in mice by the SPWCV against challenge with a virulent strain of S. pneumoniae.

Methods: BALB/c female mice were subcutaneously immunized with 200µl of SPWCV (1µg or 10µg/dose + aluminum hydroxide 0.24mg/dose, in Ringer lactate), in two doses with 15 days interval. Two weeks after the last dose the mice were challenged with live encapsulated S. pneumoniae A066 strain (1.2 x 10^4 cells/0.5 ml, i.p) and observed for survival during 10 days. One day before the challenge the animals were bled for IgG evaluation.

Results and conclusion: SPWCV in the dose of 10µg elicited respectively 85.7% and 80% of survival after challenge, in two different lots of vaccine, while the lower dose was not effective. In these groups significant levels of IgG were detected. Our results demonstrate the protection afforded by the new whole cell pneumococcal vaccine in this animal model, also suggesting that this protection assay could be considered as a tool in quality control testing of vaccine lots.

Supported: Fundação Butantan/ FAPESP/ CNPq/ PATH (Program for Appropriate Technology in Health).
IMMUNOGENICITY OF A 15-VALENT PNEUMOCOCCAL POLYSACCHARIDE-PROTEIN CONJUGATE VACCINE COMPARED IN INFANT RHESUS MONKEYS AND NEW ZEALAND WHITE RABBITS

J.M. Skinner1, L. Indrawati1, J. Cannon1, J. Blue1, M. Winters1, J. MacNair2, N. Pujar2, W. Manger1, Y. Zhang1, J. Antonello1, J. Shiver1, M. Caulfield1,2, J.H. Heinrichs1,1 West Point, PA, 2Brooklyn, NY, USA

Preclinical evaluation of pneumococcal conjugate vaccines (PCV) in animal models is essential prior to licensure. We have developed a 15-valent pneumococcal conjugate vaccine (PCV-15) containing polysaccharides (PS) from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F conjugated to CRM197 and formulated on aluminum phosphate adjuvant. Immunogenicity was evaluated in infant rhesus monkeys (IRM) following three (half human) vaccine doses and adult New Zealand white rabbits (NZWR) following two (full human) vaccine doses using a multiarray electrochemiluminescence (ECL) assay to measure serotype-specific IgG antibodies. Our vaccine, PCV-15, was compared to another licensed PCV (Prevnar®, Pfizer), as well as to a 15-valent pneumococcal PS vaccine equivalent in dose to PCV-15. The results indicated that, similar to human infants, neither IRMs nor NZWRs responded well to unconjugated PS. Antibody responses in IRM to PCV-15 and Prevnar® were comparable for the 7 serotypes in common and post-vaccination responses to PCV-15 were > 10-fold higher than baseline for the 8 additional serotypes. PCV-15 was also immunogenic in NZWR for all 15 serotypes. Prevnar®-immunized NZWR showed comparable antibody responses for the 7 serotypes contained in the vaccine as well as cross-reactivity to serotypes 6A and 19A. Both IRM and NZWR are suitable models to evaluate antibody responses to PCVs. The NZWR model offers a lower cost and shorter timeline alternative compared to the IRM model.
Poster No 278

STREPTOCOCCUS PNEUMONIAE SEROTYPE 19A CAPSULAR POLYSACCHARIDE PRODUCTION USING DISPOSABLE BAG TECHNOLOGY AND SINGLE-STEP CHROMATOGRAPHIC PURIFICATION

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Background and aims: Streptococcus pneumoniae is a major disease burden in children, the elderly, and patients with HIV and other immunosuppressive conditions. The S. pneumoniae capsular polysaccharide (CPS) is the main virulence factor and is therefore a main component in pneumococcal vaccines. Disposable bag technology has mostly been used for cell line cultivations and to a lesser extent bacterial fermentation. The use of disposable technology is becoming an attractive option for manufacturing, and the cultivation characteristics of S. pneumoniae, a facultative anaerobe, make it a suitable candidate for this technology. The aim of the study was to test the hypothesis that S. pneumoniae could be cultivated using disposable bag technology and purified using a single step chromatographic procedure to produce high levels of CPS. S. pneumoniae serotype 19A (Pn 19A) was used for these studies.

Methods: Cultivation of S. pneumoniae was performed using disposable bag technology. After fermentation, the culture was inactivated using phenol and clarified using flow through centrifugation. The purification procedure for Pn19A included CTAB precipitation and celite fractionation using 20-60% ethanol followed by sodium chloride extraction and ultrafiltration.

Results: A yield of 5.8 g crude and 1.2 g purified CPS (meeting WHO specifications) was obtained from a 7L cultivation. The purified CPS was conjugated to derivatized BSA and TT.

Conclusion: Production of Pn19A CPS was achieved through the cultivation of S. pneumoniae using disposable bag technology and generated sufficient yields of high quality PS suitable for conjugation.
EVALUATION OF DNA VACCINES EXPRESSING PNEUMOCOCCAL SURFACE PROTEIN A FROM CLADE 4 AGAINST AN INTRANASAL LETHAL CHALLENGE MODEL IN MICE

C.F.M. Vadesilho¹, D.M. Ferreira², A.T. Moreno¹, E.N. Miyaji¹, ¹São Paulo, Brazil, ²Liverpool, UK

Background and aims: PspA (Pneumococcal surface protein A) is a promising candidate antigen for the development of protein vaccines against pneumococcal disease. This work aims at analyzing the immunization with a PspA fragment from clade 4 (PspA4Pro) in an intranasal lethal challenge model.

Methods: BALB/c mice were immunized with the recombinant protein PspA4Pro adjuvanted with alum or with the DNA vaccine pSec-pspA4Pro and serum antibodies were analyzed through ELISA and FACS. Mice were challenged intranasally for the analysis of survival and of the cellular infiltrate in the lungs. Secretion of cytokines by splenocytes after stimulation with PspA in vitro was also analyzed.

Results: High antibody concentrations were elicited by PspA4Pro protein and DNA immunizations and both strategies led to full protection of mice against the intranasal lethal challenge. PspA4Pro induced an IgG response characterized by a high IgG1/IgG2a ratio, leading to a lack of binding of anti-PspA IgG2a antibodies to pneumococci in vitro, which is in contrast to the response elicited by pSec-pspA4Pro. Animals immunized with recombinant PspA4Pro and pSec-pspA4Pro showed a similar infiltration of neutrophils, macrophages, CD4⁺T and CD8⁺T cells (but not B cells) in the lungs after the intranasal challenge. Splenocytes from mice immunized with pSec-pspA4Pro showed increased secretion of IFN-γ, while cells from animals vaccinated with PspA4Pro showed increased secretion of IFN-γ, IL-17 and IL-5.

Conclusion: DNA vaccination with PspA induced protection against an intranasal lethal challenge in mice, with major qualitative differences in the humoral and cellular immune responses when compared with protein plus alum.
USE OF NEPHELOMETRIC METHOD FOR SEROTYPE-SPECIFIC QUANTIFICATION OF THE CUBAN HEPTAVALENT GLYCOCONJUGATE VACCINE AGAINST PNEUMOCOCCUS

Y. Valdes¹, R. Abdul-Nour², A. Michaelides², J. Pedroso¹, M. Montalvo¹, V. Fernandez¹, V. Verez¹, ¹Havana, Cuba, ²Ottawa, ON, Canada

A heptavalent conjugate vaccine against pneumococcus is actually in development stage in Cuba. The first vaccine candidate includes the seven serotypes more frequently reported in Latin-American: 1, 5, 6B, 14, 18C, 19F and 23F bounded to tetanus toxoid as carrier protein and adsorbed to AlPO₄ as adjuvant. A crucial quality controls for this type of products is the quantification of serotype specific carbohydrate in the final vaccine container. The present work aims to establish a nephelometric procedure for specific serotype quantification of the heptavalent Cuban conjugate vaccine active ingredients and compare their results with those obtained by a sandwich ELISA method.

During the study, we evaluated different sizes of polysaccharide to obtain the calibration curve, the influence of thimerosal used as preservative in the vaccine and different treatment conditions of samples. We compared the results obtained by this method and those reported in the quantification by ELISA. As results we defined the factors influencing the nephelometric quantification of the Cuban vaccine candidate. At the same time we have found differences in the behavior for each serotype.
EFFECT OF VACCINATION WITH DIFFERENT DOSES OF PLYD1 ON THE IMMUNE RESPONSE AND PROTECTION IN MICE

H. van Dijken\(^1\), E. van Westen\(^1\), L. Myers\(^2\), D. Salha\(^2\), M. Ochs\(^3\), R. Hopfer\(^4\), G. van den Dobbelsteen\(^1\), \(^1\)Bilthoven, The Netherlands, \(^2\)Toronto, ON, Canada, \(^3\)Marcy l’Etoile, France, \(^4\)Swiftwater, PA, USA

Background and aims: Pneumolysin is a conserved protein of *Streptococcus pneumoniae* and a leading candidate for a protein-based vaccine. However, the native protein is a toxin and therefore is not suitable as a vaccine candidate. PlyD1 is a highly detoxified pneumolysin mutant that contains three amino acid substitutions T65C, G293C and C428A (Oloo et al. 2011). Various concentrations of PlyD1 were used to immunize mice to determine antibody responses and level of protection.

Methods: Balb/c mice were subcutaneously immunized on day 0, 21 and 42 with 1, 2.5, 5 or 10µg of PlyD1; 10µg of PdB or a placebo. Blood samples were taken to measure antibody levels (ELISA) and neutralizing activity (HIMA). At day 63, mice were challenged intranasally with serotype 6B and survival after challenge was determined.

Results: In this study, there was no clear dose-response effect found in the quantity of antibodies directed against Ply as measured by ELISA. Immunization with increasing concentration of PlyD1 did however increase the neutralizing activity of the antibodies gradually. After challenge with Pn6B, the median survival was longer after immunization with 5 and 10µg PlyD1 and 10µg PdB.

Conclusions: In this study, increasing doses of PlyD1 did not induce higher antibody levels, but did increase the functionality of the antibodies. Immunization with PlyD1 and PdB prolonged the survival of mice after challenge with Pn6B.

PlyD1 is capable of inducing functional antibodies that inhibit the toxic activity of pneumolysin which supports its use as a vaccine candidate for a protein-based pneumococcal vaccine.
Poster No 282

DEVELOPMENT OF PROTEIN GLYCAN COUPLING TECHNOLOGY AND THE DESIGN OF NOVEL INEXPENSIVE MULTIVALENT PNEUMOCOCCAL VACCINES


Through basic research on bacterial glycosylation systems we have developed Protein Glycan Coupling Technology (PGCT) whereby novel protein/glycan structures are produced in E. coli. A major advantage of the recombinant approach is that an inexhaustible and purified supply of glycoconjugate vaccine can be produced at low cost.

Funded by a recent phase II Grand Challenge Explorations Gates Foundation grant we have initiated PGCT towards the development of novel combinations of a pneumococcal capsule based conjugate vaccine. The technology requires the cloning of candidate capsule loci, modification of target protein carriers to accept the glycan and a novel bacterial oligosaccharyl transferase enzyme to couple the glycan with the protein in E. coli.

To date we have expressed S. pneumoniae type 4 and 8 capsules in E. coli and have successfully modified the ExoA and S. pneumoniae pneumolysin carrier proteins to accept capsule candidates. Currently, these combinations are being tested with a bank of 30 bacterial oligosaccharyl transferases to produce a recombinant pneumococcal glycoconjugate vaccine candidate.

Although these studies are at an early stage for S. pneumoniae, candidate glycoconjugate vaccines have been constructed using PGCT for other pathogens such as Shigella and Francisella. Our overall aim will be to produce a "best of both worlds" S. pneumoniae vaccine of a conserved protein vaccine candidate coupled to an appropriate immunostimulatory glycan capsule combination.
MULTIPLE ANTIGEN PRESENTING SYSTEM - A NOVEL VACCINATION PLATFORM FOR DESIGNING A PNEUMOCOCCAL VACCINE AGAINST INVASIVE DISEASE AND CARRIAGE

F. Zhang, Y. Lu, C. Thompson, M. Herd, R. Malley, Boston, MA, USA

Background and aims: A combination of B- and T-cell immunity to the organism may represent an optimal vaccine strategy against pneumococcus. We developed a novel platform to promote antibody responses to pneumococcal polysaccharide (PS) and Th1/Th17 responses to multiple pneumococcal proteins included in the same vaccine.

Methods: Several macromolecular complexes (using our multiple antigen presenting system technology, or MAPS) were made, in which protein antigens fused to affinity molecules are combined with PS that is minimally modified with an affinity counterpart molecule. We varied PS and protein characteristics in MAPS complexes to determine the effects on immunogenicity and protection.

Results: We show that MAPS technology allows the creation of affinity-based “conjugates” with high efficiency (>90%), specificity, while preserving antigenicity of PS or proteins. PS size and PS/protein ratio affect the strength of MAPS-induced antibody and T cell responses. Immunization of mice with MAPS made with PS of different serotypes (3 or 7) results in similar anti-PS antibody levels as when a conventional conjugate vaccine is used. Mice immunized with MAPS containing conserved cell wall polysaccharide and 5 pneumococcal proteins developed strong anti-CPS antibody and Th1/Th17 cell responses to all proteins. This pilot 5-valent MAPS successfully protected mice against sepsis and colonization.

Conclusions: We describe here a novel vaccine platform that induces potent antibody responses to proteins and PS, and Th1/Th17 responses to protein antigens. This approach may be useful for the design of inexpensive conjugates or vaccines targeting pathogens for which Th1/17 (in addition to antibody) responses are desirable.
Late Breakers
DEVELOPMENT OF AN IMMUNOASSAY TO QUANTIFY CAPSULAR POLYSACCHARIDE OF STREPTOCOCCUS PNEUMONIAE SEROTYPE 1 IN SAMPLES OF VACCINE PRODUCTION PROCESS

D.B. Figueiredo, B.V. Marthos, A.L.S. Ferri, V.M.R. Gogola, V.M. Gonçalves, São Paulo, Brazil

Background and aims: The capsular polysaccharide (CP) of S.pneumoniae is the antigen of all current vaccines, but there is a lack of information on CP production in the literature, probably because this knowledge is an industrial secret of pharmaceutical companies, which leads producers in developing countries to develop their own technology. An efficient method for quantification of CP in samples of the production process is required to follow the CP during fermentation and to evaluate the purity and yield throughout the purification steps. Therefore, the aim of this work was to develop a methodology to quantify the amount of CP produced by S.pneumoniae serotype 1 (CP1) during its growth in bioreactor and the subsequent purification process.

Methods: IgG against CP1 from rabbit serum (Statens Serum Institute, Denmark) was purified by Protein A-Sepharose chromatography and biotinilated. A sandwich ELISA was established using anti-CP1 rabbit serum as coating, CP1 from ATCC as standard, biotinilated anti-CP1 IgG and Streptavidin-peroxidase for color development. A chess-board titration was applied to determine the ideal proportion among ELISA components.

Results: This methodology showed high sensitivity, detecting CP1 concentrations of 4ng/mL, and good reproducibility, giving comparable results among several quantifications. Compared to other quantification method, the MDHS (m-hydroxydiphenyl with sulfamate addition), this sandwich ELISA showed better sensitivity: ELISA detected 2µg/mL of CP1 in fermentation samples, while MHDS detected concentrations above 30µg/mL.

Conclusion: A robust quantification method was developed to evaluate culture conditions and purification protocols for maximizing the CP1 yield and purity.

Supported by CAPES and FAPESP
Introduction/Aim: An alarming increase in infections due to penicillin non-susceptible pneumococci (PNSP) has been documented in nearly all countries. Increasingly, PNSP are also resistant to other antibiotics, and a growing number of clinical failures following the use of these agents have been reported.

Methodology: We undertook a prospective (longitudinal) study on patient's with microbiologically proven community acquired pneumococcal pneumonia (CAP), meningitis and bacteraemia in the medical wards of Aminu Kano Teaching Hospital Kano, Nigeria, over the period June 2009 to May 2011. Patients with mixed bacterial infection were excluded. Pneumococcus strains were identified by its alpha-haemolysis, Gram stain morphology and susceptibility to optochin. Isolates were tested against a panel of antibiotics using E-test strips, and interpreted according to the CLSI criteria. 0.06µg/ml was used as break point for penicillin. The patients were followed-up 1 month after discharge.

Results: Total number of patients was 132. Twenty two (16.7%) of the isolates were fully sensitive to penicillin while 73 (55.3%) and 37 (28%) were intermediately and fully resistant respectively. One hundred and twenty seven (96.2%) of the isolates were fully resistant to co-trimoxazole. Eleven (8.5%) were fully resistant to amoxicillin and 104 (78.8%) and 17 (12.9%) were intermediately resistant and fully susceptible. Resistance to penicillin was shown to infer resistance to other antibiotics. Infection with multidrug resistant pneumococci was associated with mortality (p value = 0.003).

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
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<tbody>
<tr>
<td>Penicillin</td>
<td>22 (16.7%)</td>
<td>73 (53.3%)</td>
<td>37 (28%)</td>
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<tr>
<td>Amoxicillin</td>
<td>104 (78.8%)</td>
<td>17 (12.9%)</td>
<td>11 (8.5%)</td>
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<tr>
<td>Ceftriaxone</td>
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</tr>
<tr>
<td>Cefuroxime</td>
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<td>35 (26.5%)</td>
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<tr>
<td>Co-trimoxazole</td>
<td>5 (3.8%)</td>
<td></td>
<td>127 (96.2%)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>81 (61.4%)</td>
<td>34 (28.8%)</td>
<td>17 (12.9%)</td>
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<tr>
<td>Chloramphenicol</td>
<td>106 (80.3%)</td>
<td>1 (0.8%)</td>
<td>25 (18.9%)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>84 (63.6%)</td>
<td>14 (10.6%)</td>
<td>34 (25.8%)</td>
</tr>
</tbody>
</table>

Conclusion: Ceftriaxone should be part of empirical antibiotic treatment of Invasive Pneumococcal Infection, in Nigeria were resources are limited amoxicillin can be use as an alternative or chloramphenicol in case of beta-lactam hypersensitivity.
A REAL-TIME U-HEALTH MONITORING SYSTEM FOR THE CONFIRMATION OF INFLUENZA

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The influenza is known worldwide as one of the fearful virus. The general symptoms which are caused by it are shivering, fever, cough and so on. However, what’s worse, is that it could lead up to death, because of the developing of complication such as a pneumonia. In order to resolve that, we developed the system which quantitatively analyze the virus as a first steps at the place where the virus is occurred. Then, it confirms the virus in an hour through a system which could conduct the real-time RT-PCR. Also, we developed the U-Health monitoring system for prompt process which could be achieved by combining all process into one. The data which measured at the scene is send to a server through wireless networks so the monitoring system could analyze it. We could get a higher accuracy at the virus confirmation through two analytic processes and predict the place where virus could be occurred through analysis of pattern of received data. These predictions protect health of the people and minimize the damage from virus by blocking the propagation of virus into local community.
Poster No 303

THE HEALTH ECONOMIC EVALUATION OF NEW PNEUMOCOCCAL VACCINES IN HONG KONG

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Background and aims: To examine the health and economic impact of 10-valent pneumococcal non-typeable haemophilus influenza protein-D conjugate vaccine (PHiD-CV) compared with 13-valent pneumococcal conjugate vaccine (PCV-13) in the public sector of Hong Kong.

Methods: A Markov Cohort model simulates the health and economic outcomes of invasive disease (ID) (meningitis and bacteremia), community acquired pneumonia (CAP), and acute otitis media (AOM) over a life-time caused by S pneumoniae and Non-typeable H influenzae in an annual birth cohort of 82,100 newborns. Healthcare costs include hospitalization rates, medical visits, medications and specific interventions such as myringotomies. The epidemiology and disease management data inputs are Hong Kong specific, vaccine efficacy and utility values were obtained from clinical trials and international literature. The study was performed from a healthcare payer’s perspective using a 5% discount rate for both costs and QALYs.

Results: Model projections indicate that PCV-13 and PHiD-CV have an approximately equivalent impact on prevention of deaths caused by ID and pneumonia. PCV-13 is projected to prevent 6 additional cases of meningitis and bacteremia, while PHiD-CV is projected to prevent a greater number of AOM cases (13,229 events). Given a price parity assumption (HKD275), PHiD-CV vaccination is estimated to save an additional HKD44.6M (or HKD34.1M discounted). These cost savings are linked to the reduction in AOM disease burden) and also generates more QALYs.

Conclusions: PHiD-CV appears to have a better quality of life impact than PCV-13. Under price parity assumptions, PHiD-CV dominates PCV-13 as it also has a larger cost offsets.
EFFECTIVENESS OF PNEUMOCOCCAL CONJUGATE VACCINE (PCV) AGAINST PRESUMED BACTERIAL PNEUMONIA (PBP) IN SOUTH AFRICAN HIV-UNINFECTED CHILDREN: A CASE-CONTROL STUDY

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Background: PCV-7 was introduced in South Africa in April 2009 with doses at 6, 14 and 40 weeks and no catch-up.

Aim: A case-control study to evaluate PCV effectiveness against PBP in South African HIV-uninfected children.

Methods: Study sites included two urban (CHBH and Red Cross) and one rural (Ngwelezane) hospital. PBP cases were children age-eligible for ≥1 dose of PCV hospitalized for clinical pneumonia, with either alveolar consolidation on CXR per WHO criteria (CXR-AC) or other CXR infiltrate with serum CRP≥40mg/l. At least one hospital control matched for age, HIV status and hospital was enrolled for each PBP case. For PBP cases at CHBH, we also enrolled ≥3 community controls matched by age. Effectiveness was calculated as 1 minus matched odds ratio for vaccination. Up-to-date vaccination was receipt of ≥2 doses if 16-40 weeks or ≥3 doses if >40 weeks of age.

Results: To date, 696 cases (551 [79.2%] with CXR-AC) were matched to 952 hospital controls, with mean ages of 38.8 and 39.1 weeks, respectively. 527 cases (85.6% with CXR-AC) were also matched to 1484 community controls. The effectiveness of up-to-date PCV was 27.3% (95%CI: 0.49.2) when using hospital controls and 58.9% (95%CI: 37.5-72.9) using community controls. The effectiveness of two doses in children aged 16-40 weeks was 40.7% (95%CI: 2.0-64.1) and 56.8% (95%CI: 23.1-75.7) using hospital and community controls, respectively.

Conclusions: PCV prevents PBP in HIV-uninfected children. Varied results using different control groups suggest potential biases in control enrollment that need further exploration.
Poster No 305

EPIDEMIOLOGY AND ANTIMICROBIAL RESISTANCE OF COMMUNITY ACQUIRED PNEUMONIA IN CHILDREN

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Community acquired pneumonia (CAP) is a common serious infection in childhood. Bacterial resistance is widespread, with large geographical variations related to behaviors in antibiotics prescription. Identification of etiologic organisms of community-acquired pneumonia must be done to guide the physicians for proper antimicrobial use.

**Aim:** to identify the causative organisms most frequently isolated from children hospitalized for invasive lower respiratory tract infection and analyze their susceptibility to the antimicrobial agents most often used in pediatric practice.

**Methods:** 296 immunocompetent children hospitalized in Jeddah clinic hospital with community acquired pneumonia from January 2010 through September 2011 were enrolled in the study. Their ages ranged between 6 weeks and 15 years old. Chest radiograph, complete blood picture (CBC), C reactive protein and sputum culture and sensitivity were done for all patients.

**Results:** 109 (35.82%) subjects were infants, 43.58 % were >1 year ≤ 5 years and 20.6% were > 5 years. A pathogen was identified in 34.12 % of children, 56.4% were typical respiratory bacteria and 43.56% were reported by the laboratory as normal commensals. Sputum cultures grew Streptococcus pneumonia in 8.77% of respiratory pathogens, coagulase positive Staphylococcus (19.3%), group B β-hemolytic Streptococcus (8.77%), Escherichia coli (33.3%), klebsiella spp (14%), Pseudomonas (14%). High antimicrobial resistance was recorded for penicillin, amoxicillin- clavulanate, cefaclor, cephalexin and cefuroxime in gram+ve organisms. 21% of Escherichia coli and 50% of klebsiella spp were resistant to septrin.

**Conclusions:** Higher incidence of CAP due to E coli was recorded. There is increasing antimicrobial resistance to penicillin and second generation cephalosporins.
Poster No 306

PCR ASSESSMENT OF CARRIAGE AMONG POPULATION WITH HIV PREVALENCE: FREQUENT COLONIZATION, MULTIPLE SEROTYPES, AND INTRA-SEROTYPE VARIABILITY WITHIN WZY PCR-SEROTYPING TARGET

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Background and aims: Broth preculture and multiplex-PCR (MPCR) improve pneumococcal (Pn) detection in carriage studies. We assessed carriage serotype (st) distribution among Kenyan adults and children using these approaches.

Methods: Nasopharyngeal (NP) specimens (also oropharyngeal from adults) were collected during October-December 2009 from 235 healthy children (< 5 years old) and 158 adults (118 [75%] HIV-positive). Specimens were pre-cultured in broth and plated for Pn isolation. DNAs extracted from broths were screened for 40 serotypes using MPCR.

Results: Among children, isolation-based screening revealed 193/237 (81.4%) positive NPs, with st19F (n=30), st23F (n=20), st6A (n=15), st6B (n=13), and st14 (n=11) most prevalent; 8 specimens had 2 stts. MPCR detected 221/237 (93.2%) positive NPs, with st 6A/6B, 19F, 23F, and 14 most prevalent. Single stts were detected in 98/205 (47.8%) typeable specimens, 2-4 stts in 104 (50.7%) and 5-7 stts in 3 (1.5%). Among adults, 53/158 (33.5%) NP/OPs were culture-positive, with st19F (n=8), st11A (n=6), and st3 (n=5) most prevalent; no specimens had >1 serotype. MPCR detected 154/158 (97.5%) carriage, with serogroups 10F/10C/33C, 18, and 33F/33A/37 most prevalent. Single stts were observed in 10/151 (6.6%) typeable specimens, 2-4 in 38 (25.2%), 5-7 in 67 (44.4%), and 8-12 in 36 (23.8%). MPCR-derived wzy amplicons from presumed sg18 (from 5 adults) and st5 (from 2 adults) were each unique, sharing 89-99% (sg18) and 97% (st5) sequence identity.

Conclusions: MPCR demonstrated remarkable carriage frequency and co-carrriage of high numbers of serotypes among this HIV-prevalent population. Intra-serotype wzy sequence variability was observed between adult specimens. Compared to isolation-based screening, MPCR offers a markedly different assessment of carriage strain distribution.
DEVELOPMENT AND VALIDATION OF MULTIPLEX REAL-TIME PCR ASSAY FOR IDENTIFICATION OF RESPIRATORY VIRUSES AND BACTERIA IN PNEUMONIA SAMPLES

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Background and aims: The Emerging Pathogens Laboratory of Fondation Mérieux and Fast Track Diagnostics (FTD) co-developed a real time multiplex PCR assay for the detection of 25 respiratory pathogens including viruses, bacteria and atypical bacteria. This assay was verified on referenced viral and bacterial strains then validated on respiratory clinical samples i.e. nasopharyngeal aspirates, nasal swabs and pleural effusion samples from worldwide hospitalized patients. In parallel we developed a quantitative RT-multiplex PCR for the detection of 3 bacteria directly from the patients’ blood samples.

Methods: The real-time multiplex PCR assay (FTD Respiratory 21 PLUS) has been designed to detect influenza A/A (H1N1)/B, coronaviruses, parainfluenza viruses, rhinoviruses, RSV A/B, adenovirus, enterovirus, parechovirus, bocavirus, M.pneumoniae, C.pneumoniae, S.pneumoniae, H.influenzae B and S.aureus. The assay has the ability to be quantitative for the bacteria. A triplex quantitative real-time multiplex PCR was also developed to detect S.pneumoniae, H.influenzae B and S.aureus directly from blood samples.

Results: The two real-time multiplex PCR assays we developed to detect respiratory pathogens are highly sensitive and specific and can detect both mono and co-infections in a semi-quantitative for viruses and quantitative for bacteria. These assays can be used with multiple respiratory or blood samples using either manual or automatic nucleic extraction systems. Both assays were successfully evaluated in different cohorts of children and adults patients hospitalized for bronchiolitis or pneumonia.

Conclusion: Fondation Mérieux with FTD are evaluating both assays in a pilot multicentric study in hospitalized children under five with pneumonia in ten different developing and emerging countries.
Poster No 308

ALTERNATIVE SAMPLING METHODS FOR DETECTING BACTERIAL PATHOGENS IN CHILDREN WITH AN UPPER RESPIRATORY TRACT INFECTION: REVISITING THE GOLD STANDARD

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Background: Transnasal nasopharyngeal sampling is considered the gold standard for detecting bacterial pathogens involved in upper respiratory tract infections (URTI). An simpler method of collecting a representative sample would however be preferable, especially when samples need to be obtained repeatedly. Therefore, we evaluated the accuracy of alternative sampling methods.

Objective: To evaluate the concordance between different sampling methods of the nose and nasopharynx in children with an URTI.

Methods: Children (n=66) aged 0-4 years with rhinorrhea as symptom of an URTI were sampled by (1) a nasopharyngeal swab, (2) a nasal swab, and (3) blowing the nose in a paper tissue, that was subsequently (a) sampled with a swab and (b) transported as a whole in phosphate-buffered saline to the laboratory. Conventional culture methods were used to isolate S. pneumoniae, H.influenzae, M.catarrhalis and S.aureus.

Results: Figure 1 shows the detection rates of individual bacterial pathogens by the different sampling methods. Concordance between the methods was high for each bacterial pathogen (77% to 97%). S.pneumoniae was recovered more frequently from a paper tissue than from a nasopharyngeal swab (P=0.039) and nasal swab (P=0.039).

Conclusion: Culture of a paper tissue with nasal discharge offers a reliable alternative to detect bacterial pathogens in children with rhinorrhea as a symptom of an URTI and was superior to the gold standard in detecting S.pneumoniae.

Figure 1. Bacterial detection rates in children with an URTI

![Graph showing detection rates of bacterial pathogens for different sampling methods]

* = P<0.05 (McNemar test). URTI, upper respiratory tract infection

[Figure 1]
DYNAMICS OF THEORETICAL COVERAGE FOR 7, 10 AND 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINES ACCORDINGLY TO SIREVA SEROTYPES ISOLATED. LATIN-AMERICAN, 2000-2010

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Objectives: To determine theoretical coverage and changes of 7, 10 and 13-valent pneumococcal conjugate vaccines from isolated serotypes of Streptococcus pneumoniae reported by SIREVA II among children < 5 years, for Latin America region and in every single country between the periods 2000-2005 and 2006-2010.

Methods: Retrospective descriptive study, published data SIREVA 2000-2010. Used Epidat3.0® & Excel® to tabulate, graph and analyze theoretical coverage for pneumococcal conjugate vaccines 7, 10 and 13-valent (V), calculating proportions with 95% confidence intervals (95%CI) and Z test.

Results: During 2000-2010, the 13-valent vaccine (86.6%) showed an 11% higher coverage (95%CI: 10.2 to 11.9) than 10-valent (95%CI: 75.6%) and the latter 16.6% higher (95%CI: 15.6 to 17.6) than 7-valent (59%), both differences p< 0.0000. Between 13v and 10v, only 3 countries, Bolivia, Guatemala and Nicaragua, the difference were not statistically significant in the other 14 are significant, while between 10v and 7v, only 4 countries; Costa Rica, Mexico, Nicaragua and Peru were not significant. Changes in Latin America coverage for 13v, 10v and 7v between 2000-2005 and 2006-2010 were in average: slightly increased of 3% (95%CI:1.9-4), decreased -3.2 (95%CI: -3.2 - -0.5) and stable 0.46 (95%CI: -1.1 - 2), respectively.

Conclusions: 13-valent vaccine shows a much higher theoretical coverage for children under 5 years in the region and all countries, and that coverage is increasing in time, as opposed to the 10-valent showing a reduction while vaccine 7-valent is stable.
**Poster No 310**

**ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF STREPTOCOCCUS PNEUMONIAE STRAINS ISOLATED FROM WARAO AMERINDIANS IN VENEZUELA; PRELIMINARY RESULTS**

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**Background:** 388 pneumococcal isolates were recovered from nasopharyngeal samples of children and adults of 157 Warao families. So far, 84 have been serotyped and submitted to an antimicrobial susceptibility study.

**Methods:** The strains were serotyped with a multiplex PCR. Susceptibility was tested by the disk diffusion method. Macrolide resistance was also tested by a PCR method targeting the ermB, mefA and mefE genes. MLST was performed for genomic comparison.

**Results:** 65.5% (n=55) of the strains were susceptible to penicillin. Respectively 76.2 % (n=67) 35.7% (n=30) 29.8% (n=25) and 25% (n=21) of the isolates were resistant to trimethoprim-sulfamethoxazole, tetracycline, erythromycin and clindamycin. 4 strains presented low level resistance to fluoroquinolones. No resistance was found for linezolid, rifampim and vancomycin. Overall 59.5% (n=50) of the isolates were resistant to 2 or more antibiotics and 32% (n=27) presented a multi-drug resistance pattern. 17 of these MDR strains belonged to serogroup 6, and the other belonged to serotypes 4, 23F, 15A, 19A each. Of 10 macrolide resistant strains 9 strain presented the ermB gene, and 1 the mefE gene. Of the multidrug resistant strains belonging to serogroup 6, two belong to a newly described clone.

**Conclusions:** This ongoing study shows a relatively high level of antibiotic resistance in the Warao communities in Venezuela with a variety of underlying mechanisms. The introduction of a conjugate vaccine most possibly can lower the high resistance rates in this Amerindian population since serotypes associated with resistance are mostly vaccine serotypes.
EFFICACY OF 13-VALENT AND 7-VALENT PNEUMOCOCCAL CONJUGATE VACCINES (PCV13;PCV7) IN PREVENTING NASOPHARYNGEAL COLONIZATION WITH PCV7- SEROTYPES: A RANDOMIZED DOUBLE-BLIND TRIAL

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Background/aims: The immunogenicity of PCV7 and PCV13 and efficacy in preventing nasopharyngeal (NP) colonization of the 7 common serotypes (4, 6B, 9V, 14, 19F, 18C, 23F) were assessed.

Methods: Healthy infants in Israel were randomly assigned to receive PCV13 (n=932) or PCV7 (n=934). Eight NP-swabs were collected between ages 2-24m. New NP-acquisitions (PCV13: PCV7 rate ratio; RR) within ages 7-24m, and prevalence (odds ratio; OR) at 5 age points were evaluated. Serotype-specific IgG anticapsular antibodies were assessed at ages 7 and 13m.

Results: Serotypes 4, 6B, 9V, 14, 18C, 23F elicited similar or lower IgG responses after PCV13 than after PCV7 (GMC ratios range: 0.72-1.07); 14 and 23F were significantly lower; however no differences in new NP-colonization acquisition (RR: 0.89, 95%CI 0.75-1.05 for PCV7-grouped) or prevalence (during 7 to 24m) were observed. Serotype 19F elicited significantly higher IgG responses after PCV13 (PCV13:PCV7 GMC ratio: 1.30, 95% CI 1.19-1.42) and significantly reduced NP-acquisition (RR; 0.62, 95% CI 0.45-0.84) and reduced prevalence at all age points.

Conclusions: Trends towards lower immune responses after PCV13 for PCV7-serotypes resulted in no differences between vaccines in impact on NP-colonization. For 19F, PCV13 was more immunogenic, and efficacious in reducing 19F NP-colonization than PCV7; the impact of 19F may be enhanced by 19A. PCV13 is as effective, and for 19F better than, PCV7 in preventing PCV7-serotype NP-colonization.
THE EFFICACY OF THE 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV13) ADDITIONAL SEROTYPES ON NASOPHARYNGEAL COLONIZATION: A RANDOMIZED DOUBLE-BLIND PEDIATRIC TRIAL

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Background/aims: This study assessed the efficacy of PCV13 relative to 7-valent pneumococcal conjugate vaccine (PCV7) on nasopharyngeal (NP) colonization in infants; additional PCV13-serotypes 1, 3, 5, 7F, 6A, 19A were assessed, as was the cross-reacting serotype 6C.

Methods: Healthy infants in Israel were randomly assigned to receive PCV13 (n=932) or PCV7 (n=934) at ages 2, 4, 6, 12 months. Eight NP swabs were collected between ages 2-24m. New NP-acquisitions (rate ratio; RR) within age 7-24m, and prevalence (odds ratio; OR) at each age point were evaluated. Immune responses were measured by ELISA at 7m and 13m.

Results: PCV13 significantly reduced NP-acquisition compared with PCV7 (per-protocol analysis) for (1) serotypes 6A/C or 19A grouped (RR 0.54; 95%CI 0.46, 0.64); (2) additional PCV13-serotypes grouped (RR 0.56; 95%CI 0.47, 0.65); and (3) single serotypes 1, 6A, 6C, and 19A. For serotype 5 there were insufficient events for carriage assessment; serotype 7F showed a non-statistical trend to reduction; no effect could be shown on serotype 3. Prevalence ORs generally showed similar impact. IgG geometric mean concentrations for the 6 additional serotypes at age 7m were 2.6-95.03 fold higher after PCV13 than PCV7.

Conclusions: PCV13 compared with PCV7 was more immunogenic and significantly reduced NP-colonization of the 6 additional serotypes when grouped; with significant reduction in serotypes 1, 6A, 6C, and 19A carriage. PCV13 should be effective in preventing disease due to the additional serotypes by vaccine-induced immunity and indirect herd effect. This is the only PCV13-study that has both efficacy and immunogenicity outcomes.
Poster No 313

13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE IN OLDER CHILDREN AND TEENS EITHER PREVIOUSLY IMMUNIZED WITH OR NAIVE TO 7-VALENT PNEUMOCOCCAL CONJUGATE VACCINE

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Background/aims: 13-valent pneumococcal conjugate vaccine (PCV13) is immunogenic and safe in children aged ≥15 months to < 5 years previously vaccinated with 7-valent pneumococcal conjugate vaccine (PCV7) (Frenck R, et al. Pediatr Infect Dis J 2011;12:1086-1091). Older children may also benefit from vaccination with PCV13. This open-label study evaluated immunogenicity and safety of PCV13 in children aged ≥5 to < 18 years.

Methods: Healthy children aged ≥5 to < 10 years previously vaccinated with ≥1 dose of PCV7 and pneumococcal vaccine-naïve children aged ≥10 to < 18 years received 1 dose of PCV13. For the 5-10yo group, anti-pneumococcal IgG geometric mean concentrations (GMCs) at 1 month postvaccination for each serotype were compared to GMCs of the PCV13 or PCV7 posttoddler dose from an historical control study. For the 10-18yo group, opsonophagocytic activity (OPA) geometric mean titers (GMTs) 1 month postvaccination were compared with OPA GMTs in the 5-10yo group. Safety data were collected.

Results: 598 children enrolled; 200 in each group were included in the comparative analyses. For the PCV7 serotypes, IgG GMCs in 5-10yo group were 8.23-53.56 µg/mL, ≥2.5-fold greater than the historical values. For the 6 additional serotypes, IgG GMCs in the 5-10yo group were 2.38-21.51 µg/mL, ≥1.23-fold greater than the historical values. OPA GMTs were similar in the 5-10yo and 10-18yo groups, except serotype 3 (10-18yo:5-10yo ratio: 0.6; 95% confidence interval, 0.48, 0.67). Safety was comparable in both groups.

Conclusion: PCV13 was immunogenic and safe when administered to older children and teens, regardless of prior PCV7 vaccination.
POTENTIAL SEROTYPE COVERAGE OF THREE PNEUMOCOCCAL CONJUGATE VACCINES AGAINST INVASIVE PNEUMOCOCCAL INFECTION IN ITALIAN ADULTS


**Background:** *Streptococcus pneumoniae* is a major etiologic agent for invasive diseases both among young children and elderly people all over the world. Different vaccines are available, conjugate formulation are known to be significantly more immunogenic both in children and in elderly people. The aim of the work was to assess the potential serotype coverage of three pneumococcal conjugate vaccines (7-, 10- and 13-valent) against invasive pneumococcal disease in a population of Italian adults.

**Patients and methods:** We determined pneumococcal serotypes in adults admitted to hospital with suspicion of invasive bacterial disease and with confirmed bacteremic pneumococcal disease determined by cultures or molecular detection of *S. pneumoniae* in a normally sterile site. Positive samples were serotyped using Realtime-PCR.

**Results:** Between April 2008 and March 2011, a total of 88 patients (age median 61.6 years; Interquartile range 44.9-74.7, M:F ratio 1.2 ) with invasive pneumococcal disease from 10 Italian Regions were serotyped. The most prevalent serotypes were 7F (14.0%), 19A (9.3%), 3 and 22F (8.1%), 12, 15 and 22F (7.0%), 8 (4.7%). Two patients were not typeable due to the paucity of sample. Overall, serotype coverage for PCV-7-, -10 and -13 were respectively 31.3%, 49.8% and 69.7%. The coverage obtainable with PCV13 was 46.4% in patients < 55 years and 63.8% in patients >55 years.

**Conclusion:** The introduction of PCV13 in the vaccination schedule for adults would provide substantial benefit for protection from pneumococcal invasive infections in Italy. The importance of molecular methods for diagnosis and serotyping of invasive pneumococcal disease is confirmed.
ASSOCIATION OF INFANT PNEUMOCOCCAL VACCINATION WITH PNEUMOCOCCAL PNEUMONIA AMONG MOTHERS: A NESTED CASE-CONTROL STUDY USING THE GPRD

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Background: Since widespread implementation of infant immunization with 7-valent pneumococcal conjugate vaccine (PCV7) in the United Kingdom (UK) in 2006, the rate of invasive pneumococcal disease has decreased in children, but not in adults.

Methods: We conducted a matched case-control study of the association between infant PCV7 immunization and risk of pneumococcal pneumonia in mothers from 2006-2010 in a cohort of mother-infant pairs identified from the General Practice Research Database in the UK. Cases were mothers aged 20-40 years with first-time diagnosis of pneumococcal pneumonia. Each case was matched with up to 10 controls on index date of diagnosis, age, birth date of their baby, chronic lung disease, smoking and antidepressant/benzodiazepine use prior to index date.

Results: From 2006 to 2010, the annual incidence rate of pneumococcal pneumonia among mothers increased from 58/100,000 to 87/100,000. We identified 43 cases of pneumococcal pneumonia among mother-infant pairs. The conditional odds ratio of pneumococcal pneumonia in mothers whose infants received a 3-dose series of PCV7 compared to mothers whose infants received 0, 1, or 2 doses was 4.0 (95% CI: 1.0-15.8), and 11.0 (95% CI 1.2-98.6) when compared with mothers whose infants received no vaccinations.

Conclusions: Our results indicate that the incidence of pneumococcal pneumonia may have increased in mothers following the introduction of PCV7, possibly because mothers whose infants received PCV7 are at increased risk for pneumococcal pneumonia. Additional studies to confirm these findings are warranted.
IMPACT OF THE 10-VALENT PNEUMOCOCCAL NONTYPEABLE HAEMOPHILUS INFLUENZAE PROTEIN D-CONJUGATE VACCINE (PHID-CV) ON H. INFLUENZAE COLONIZATION: A RANDOMIZED CONTROLLED TRIAL

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Background: Pneumococcal conjugate vaccination influences the nasopharyngeal bacterial ecosystem. Data regarding effects of PHiD-CV on NTHi and S.pneumoniae colonization are however limited. Of note, the 7-valent pneumococcal conjugate vaccine (7vCRM) was introduced in the Netherlands for all children born after March 31, 2006.

Objective: To examine the effects of PHiD-CV on nasopharyngeal NTHi colonization compared with 7vCRM in Dutch children up to 2 years of age.

Methods: A randomized trial enrolling 780 healthy infants in the Netherlands, conducted between April 2008 and December 2010. Participants were randomly assigned (2:1) to receive either PHiD-CV or 7vCRM at 2-3-4-11 months of age. Nasopharyngeal samples were taken transnasally at 5-11-14-18 and 24 months of age. Bacteria were isolated by conventional culture methods. H.influenzae was differentiated from H.haemolyticus by PCR. Pneumococci were typed by Quellung method. Bacterial loads of S.pneumoniae and H.influenzae were measured by qPCR. Outcome assessors were blinded to group assignment.

Results: Nasopharyngeal NTHi colonization was similar in both treatment groups at each sampling moment and increased from approximately 33% at 5 months to 66% at 24 months of age with comparable bacterial loads. Colonization by the 7 common vaccine pneumococcal serotypes declined with age from around 8% to 3% in both PHiD-CV and 7vCRM vaccinated children alike. Serotype 19A was the predominant colonizer throughout follow-up, regardless of the administered vaccine.

Conclusion: We found no differences in nasopharyngeal NTHi colonization after PHiD-CV immunization compared with 7vCRM in healthy Dutch children.

Trial registration: Clinicaltrials.gov Identifier: NCT00652951

Funding: GlaxoSmithKline Biologicals.
IMMUNOGENICITY OF A BOOSTER DOSE OF 10-VALENT PNEUMOCOCCAL NONTYPEABLE HAEMOPHILUS INFLUENZAE PROTEIN D-CONJUGATE VACCINE (PHID-CV) IN DUTCH CHILDREN

M.R. van den Bergh1,2, J. Spijkerman1,2, K. Swinnen3, N. François3, D. Borys3, L. Schuerens3, R.H. Veenhoven2, E.A.M. Sanders1, *Utrecht, 2Hoofddorp, The Netherlands, 3Wavre, Belgium

Background and aims: Co-administration of different polysaccharide-protein conjugate vaccines might result in immunologic interference. We evaluated the immunogenicity of a booster dose of PHID-CV co-administered with different DTPa-based combination vaccines.

Methods: In this single-blind study, 780 healthy Dutch children who were randomly assigned (1:1:1) to receive a 3-dose priming at 2-3-4 months of age, continued to receive a booster dose of the same vaccines at 11-12 months of age: [1] PHID-CV and DTPa-HBV-IPV/Hib (GlaxoSmithKline Biologicals), [2] PHID-CV and DTPa-IPV-Hib (Sanofi Pasteur MSD) or [3] 7-valent pneumococcal conjugate vaccine (7vCRM, Pfizer Inc.) and DTPa-IPV-Hib. Blood samples were collected before and one month after booster vaccination. Anti-pneumococcal immune responses were measured using GlaxoSmithKline's 22F-ELISA and opsonophagocytic assay. Immune responses to the co-administered vaccine antigens were also assessed.

Results: Anti-pneumococcal antibody geometric mean concentrations (GMCs) are shown in table 1. For each vaccine pneumococcal serotype, percentages of children reaching antibody concentrations ≥ 0.20 µg/mL or opsonophagocytic titers ≥ 8 were in the same ranges in the PHID-CV groups regardless of the co-administered DTPa-based vaccine. Responses to co-administered vaccine antigens were high with comparable seroprotection and seropositivity rates across groups.

Conclusions: Our findings show robust responses to a PHID-CV booster dose co-administered with either DTPa-IPV-Hib or DTPa-HBV-IPV/Hib in 11-12-month-old Dutch children.

Trial registration. Clinicaltrials.gov Identifier: NCT00652951

Funding. GlaxoSmithKline Biologicals.

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<th>Table 1. Anti-pneumococcal antibody GMCs before and 1 month post-booster in Dutch children.</th>
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<td>Vaccine serotypes</td>
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<tr>
<td>1</td>
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<td>4</td>
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<tr>
<td>5</td>
</tr>
<tr>
<td>6B</td>
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<td>7F</td>
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<td>9V</td>
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<td>14</td>
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<td>18C</td>
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<td>19F</td>
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<td>23F</td>
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<tr>
<td>Cross-reactive serotypes</td>
</tr>
<tr>
<td>6A</td>
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<tr>
<td>19A</td>
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N, maximum number of infants with available results; the actual number of infants included in the analysis varies slightly per serotype, depending on the serum availability for testing.

[Table 1]
RAPID ASSESSMENT OF PNEUMOCOCCAL CONJUGATE VACCINE EFFECTIVENESS IN COLOMBIAN CHILDREN < 2 YEARS OLD

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Background: Most used methods to evaluate vaccine effectiveness (VE), like case-control studies, are costly and can take long time periods. We evaluate the VE of 7-valent pneumococcal conjugate vaccine (PCV7) using a cross-sectional survey as a rapid method of VE assessment in Colombian children < 2 years.

Methods: A population survey was carried out on children < 2 years old living in populations where PCV7 was introduced from 2010. A questionnaire was designed to establish vaccination status and the occurrence of syndrome potentially related with pneumococcus (acute lower respiratory disease). ORs were obtained and interpreted as the risk of have been hospitalized by the syndrome of interest among exposed to such vaccine. VE was estimated as 1-OR.

Results: VE of PCV7 was assessed in a probabilistic sample of 2116 children < 2 years old from 4 Colombian cities. The average age were 13.3 months. 1863 (88%) had vaccination card, of these 72% (CI95% 70-74%) had completed schedules to PCV7. Children adequately vaccinated according to their age against pneumococcal were 32% (CI95% 9-49%) less likely to be hospitalized by low respiratory disease, and also showed a protection against consultation due to same syndrome (VE=25% (CI95% 8-38%)).

Conclusion: CPV7 is a effective protective intervention against consultation and hospitalization due to acute lower respiratory disease in a developing country like Colombia. Cross-sectional studies seems to be a valuable tool to conduct quick assessment of vaccines effectiveness in developing countries at a reasonable cost.
RETROSPECTIVE AND PROSPECTIVE INCIDENCE OF ACUTE OTITIS MEDIA (AOM) IN CHILDREN 0-5 YEARS OF AGE FROM CURITIBA, PARANA, BRAZIL

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Background and aims: Acute otitis media (AOM) is a common cause for frequent paediatric bacterial infection and a major reason for antibiotic prescriptions in children, worldwide. AOM incidence data in Brazil is limited. The study aimed at estimating the AOM incidence in children to understand the burden of disease.

Methods: Children (N=2083) aged 0–6 years were randomly selected from a private medical clinic in Curitiba from Sep-08 and Mar-10. Estimation of AOM incidence (overall, by age-group and by vaccination status [PCV7]) was carried out retrospectively by review of medical records 12 months preceding enrolment, or since birth for children aged < 1 year and prospectively by a bimonthly follow-up for one year.

Results: 1136 children were enrolled. Overall incidence of AOM diagnosed retrospectively and prospectively by a physician was 183.6 cases per 1000 person-years (95% CI: 157.96-212.21) and 96.7 cases per 1000 person-years (95% CI: 78.05-118.46), respectively. Table 1 summarises AOM incidence by age group and vaccination status.

Conclusions: AOM incidence in a partially vaccinated population in a private clinic in Curitiba was lower than in other similar-design studies, suggesting possible differences in diagnostic behaviour and health-care access. Estimates found for retrospective and prospective AOM incidence were different; those differences should be further explored. In 2010, Brazil introduced PCV10 in Universal Massive Vaccination, thus methods for documenting the burden of AOM and potential impact of vaccination nationwide should be explored.

Table 1 Retrospective and prospective AOM incidence by age group and vaccination status (ATP cohort)

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Retrospective (N=1113)</th>
<th>Prospective (N=1074)</th>
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<td>Overall</td>
<td>183</td>
<td>96</td>
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<th>Vaccination status by age group (years)</th>
<th>Retrospective (N=1113)</th>
<th>Prospective (N=1074)</th>
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<tr>
<td>Vaccinated 0–&lt;1</td>
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<tr>
<td>1–&lt;3</td>
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<td>13</td>
</tr>
<tr>
<td>1–&lt;3</td>
<td>155</td>
<td>90</td>
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</table>

AOM-incidence, N-number of children included in each group; n-number of AOM episodes in 1000-person-years in each category; P-total number of person-years; CI-confidence interval; 0–<3 years 0–2 years 11 months; 1–<3 years 2–5 years 11 months; *Vaccinated with pneumococcal conjugate vaccine 7-valent.

[AOM Incidence Curitiba]
EFFECTIVENESS OF ONE OR MORE DOES OF 10-VALENT PNEUMOCOCCAL CONJUGATE VACCINE IN BRAZILIAN CHILDREN, 2010-2011 (UPDATED)

C.M. Domingues, 10-Valent Pneumococcal Vaccine Effectiveness Study Group, Brasilia, Brazil

**Background:** In 2010, Brazil began routine infant immunization with 10-valent pneumococcal conjugate vaccine (PCV10). The recommended schedule included 3 doses of PCV10 at 2, 4 and 6 months plus a booster at 12 months. A single dose was offered for catch-up of unvaccinated children 12-23 months old.

**Methods:** Cases of invasive pneumococcal disease (i.e. isolation of Streptococcus pneumoniae from blood, cerebrospinal fluid or other normally sterile site) were identified through laboratory-based surveillance in 10 Brazilian states. Surveillance officers recorded vaccination data from health cards at home visits. We compared vaccination histories among those eligible to receive ≥1 PCV10 dose during 2010-2011 with those of age-matched, community control children to estimate vaccine effectiveness. We calculated effectiveness (1-Odds Ratio) for all serotypes and for PCV10 serotypes using conditional logistic regression.

**Results:** To date, 135 cases of invasive pneumococcal disease among children eligible to receive PCV10 were enrolled; 66 (49%) presented with meningitis and 82 (61%) were caused by PCV10 serotypes. Serotypes 14 (n=41), 6B (n=22), and 6A (n=12) were most frequent. 58 (44%) case-patients versus 312 (60%) of 520 controls received ≥1 PCV10 dose (36 case-patients and 199 controls received one PCV10 dose, 22 and 113 received ≥2 doses). Overall effectiveness of ≥1 PCV10 dose against all serotype disease was 71% (95% CI, 48 to 83) and 85% (95% CI, 64 to 94) against vaccine-serotype disease.

**Conclusions:** Preliminary analyses suggest effectiveness of PCV10 against vaccine-serotype disease; additional data are needed to evaluate specific serotypes and effectiveness of the complete series.
THE ANALYSIS OF EFFICACY, SAFETY OF VACCINATION AGAINST STREPTOCOCCUS PNEUMONIAE IN CHILDREN WITH VARIOUS DEVIATIONS IN A STATE OF HEALTH

A.G. Gayvoronskaya, T.A. Grechukha, M.G. Galytyskaya, Moscow, Russia

Actuality: The diseases, associated with Streptococcus pneumoniae, are a serious problem for public health services. The most effective way of prevention is vaccination.

Aim: To study the acceptability and influence of vaccination against pneumococcal infection in children with various deviations in a state of health in catamnesis.

Materials and methods: 275 children (3 months - 12 years old) were taking part at the research at the department of vaccinal prevention of Scientific Centre of Children’s Health. Patients were subdivided into two groups: healthy children and children with deviations in a state of health. The acceptability of vaccines of Pneumo23 and Prevenar, a current of the basic and accompanying diseases at vaccinated children, frequency of acute respiratory infections were analyzed.

Results: Statistically significant distinctions on frequency and expressiveness of the general and local postvaccinating reactions between 2 groups was not revealed (p>0.05). General and local postvaccinating reactions were rare, especially expressed reactions have been noted only at 7 (4 %) children, immunized by a vaccine of Prevenar and at 4 (4.5 %) children, immunized by a vaccine Pneumo-23. The current of the basic and accompanying diseases remains stable at all vaccinated children throughout all period of supervision. In group of frequently ill children there was a statistically significant differences in frequency of acute respiratory infections within a year after vaccination in comparison with previous year. Conclusion: Vaccination against pneumococcal infection is safe, leads to decrease of frequency of acute respiratory infections within a year after immunization.
DECLINE IN PNEUMOCOCCAL MENINGITIS AFTER INTRODUCTION OF 10-VALENT PNEUMOCOCCAL CONJUGATE VACCINE IN SÃO PAULO, BRAZIL


Background and aims: The 10-valent pneumococcal conjugate vaccine (PHiD-CV) was introduced into the Brazilian Immunization Program in a 3 + 1 schedule for children < 2 years of age in mid 2010. The aim of this study was to evaluate the early impact of immunization on the incidence of pneumococcal meningitis (PM) in the State of São Paulo.

Methods: We analyzed population-based data from the Notifiable Diseases Surveillance System to evaluate trends in the burden of PM before and after the introduction of a publicly funded vaccination program. Changes in the incidence of PM in 2011 were assessed against baseline values from 2001-2009, considering 2010 as a transition year.

Results: We identified 1,332 cases of PM in children aged < 2 years during the study period. The rates of PM in children aged < 2 years declined from an average of 10.2/100,000 persons in the pre-vaccination baseline period (2001-2009) to 5.1/100,000 in 2011. This represents a 50% (p< 0.001) reduction in the incidence rate after the introduction of the vaccine. The proportion of PM cases in children aged < 2 years declined by 59.3%, from 29% to 11.8% (p< 0.001), after the introduction of the vaccine.

Conclusion: The introduction of PHiD-CV into the routine vaccination program provided a rapid and significant reduction in incidence rates of PM in children aged < 2 years, the age group targeted for vaccination. These encouraging results highlight the need of continued surveillance studies to assess the long term impact of this vaccination program.
ANALYSIS OF INVASIVE PNEUMOCOCCAL DISEASE CASES (IPD) AFTER INTRODUCTION OF PCV-10 (PNEUMOCOCCAL CONJUGATE VACCINE), PARANA - BRASIL

E.C. Maluf\textsuperscript{1}, M.S. Wille\textsuperscript{1}, N.D. Haida\textsuperscript{1}, R.C.P. Maluf\textsuperscript{2}, C.F. Cruz\textsuperscript{1}, \textsuperscript{1}Curitiba, \textsuperscript{2}Ribeirao Preto, Brazil

PCV was introduced in the public health system of Brazil to children < 2 years old from 2010. Active surveillance studies to evaluate cases of IPD are important in assessing the impact of the immunization program.

Objective: evaluate the profile of cases of IPD from the introduction of the vaccine in Parana, Brazil.

Methods: This study included IPD cases in children < 2 years old reported to the epidemiological surveillance system of Public Health's Department, Parana, Brazil. All cases were hospitalized and laboratory confirmed (LACEN\textsuperscript{*} PR and IAL\textsuperscript{**}).

Results: main characteristics of the 26 cases included in this study: 9 (34.6\%) were from the capital (Curitiba); cases were more frequent in winter (46.2\%); lethality rate of 15.4\%; main diagnoses: meningitis (53.8\%), pneumonia (42.3\%) and bacteremia (3.9\%); lethality due to meningitis of 21.4\% and 9.1\% to pneumonia. The coverage of PCV (3 doses) was 30.8 \% (8). The coverage of PCV was higher among cases occurring in the capital (5/9) than cases from other cities (3/17) and among meningitis (6/14) compared with cases of pneumonia (1/11). Serotype was identified in 25 cases. The most prevalent ones were 14 (6/25) and 6B (4/25), corresponding to 40\% of the cases.

Conclusions: These findings suggest that the coverage of PCV is not satisfactory and that more prevalent serotypes are present in the vaccine. These results emphasize the importance of investing in strategies to ensure high vaccination coverage and continuing surveillance to monitor changes in serotypes.
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VACCINE-PREVENTABLE MORBIDITY AND MORTALITY DUE TO INVASIVE PNEUMOCOCCAL DISEASE AMONG CHILDREN IN RURAL MOZAMBIQUE

B. Sigaúque¹, D. Vubil¹, I. Mandomando¹, L. Quintó², L. Morais¹, Q. Bassai², S. Machevo¹, S. Acácio¹, T. Nhamposse¹, J. Sacaral¹, P. Aide¹, M.G. Carvalho³, J. Verrani², A. Roca², E. Macete¹, P.L. Alonso¹,², Maputo, Mozambique, Barcelona, Spain, Atlanta, GA, USA

Background: Invasive pneumococcal disease (IPD) is an important cause of illness and death among children in Mozambique. Pneumococcal conjugate vaccine (PCV) will be introduced in the Mozambican routine immunization program in 2012.

Methods: Data from on-going, active, laboratory-based surveillance for IPD among children presenting to Manhiça District Hospital in rural Mozambique were analyzed to calculate incidence (using cases from a defined catchment area), mortality, and the burden preventable through PCV introduction among children < 5 years old for a 10-year period (1/2001 to 12/2010).

Results: Among children < 5 years old there were 668 cases of IPD, including 44 (7%) bacterial meningitis and 624 (93%) bacteremia. A total of 356 were from the catchment area, yielding IPD minimum incidence rates of 522, 426 and 115 per 100,000 among children < 12months, 12-23months and 24-59months old, respectively. Death occurred in 39 (13.2%), 20 (9.3%), and 17 (10.8%) of all cases aged < 12months, 12-23months and 24-59months old. Overall, 484 (72.5%) IPD isolates were available for serotyping (many isolates from early years were not recovered); of those, 63% were covered by PCV-10 and 82% by PCV-13. Among 76 deaths, 36% and 51% were due to PCV-10 and PCV-13 serotypes respectively.

Conclusions: Most IPD episodes and many deaths among children < 5 years old in rural Mozambique could be prevented through PCV introduction. Children < 1 year old have the highest IPD incidence and case fatality rate; PCV impact will be greatest in this age group, but older age groups will also benefit.
GENOMIC DIVERSITY OF STREPTOCOCCUS PNEUMONIAE SEROTYPE 1 ACROSS SUB-SAHARAN AFRICA

S. Harris, Pneumococcal African Genomics Consortium, Cambridge, UK

Background: Serotype 1 strains are responsible for a significant proportion of invasive disease in sub-Saharan Africa and, in some regions, are associated with lethal epidemics. Paradoxically however, serotype 1 strains are rarely detected in carriage in the nasopharynx suggesting a distinct lifestyle/niche compared to other serotypes. The propensity of serotype 1 for disease causation, particularly in Africa, could make it more likely for these strains to fill the disease niche left following the removal of vaccine serotypes and could further increase the risk of lethal epidemics.

Aims and Methods: To understand how the dominant clonal groups of serotype 1 are geographically distributed and to determine whether genetic variants associate with disease manifestations, we carried out whole genome sequencing and phylogenetic analysis on a collection of 267 pneumococci isolated from disease and carriage over across sub-Saharan Africa between 2002 and 2009.

Results: The resulting phylogenetic tree, built using SNP sites across the genome, shows remarkable geographic clustering with distinct genotype clusters representing The Gambia, Niger, Malawi and South Africa. These clusters allow the detection of isolates which, due to their anomalous position on the tree, are likely to represent recent inter-country transmissions. The data also confirm the replacement of one lineage by another in The Gambia.

Conclusions: Taken together with clinical characteristics, these data show the potential for whole genome analysis to clarify the relationships between serotype 1 isolates. Further analysis is underway to determine whether the propensity to cause epidemics is encoded in the pathogen genome.
EXPANSION AND EVOLUTION OF STREPTOCOCCUS PNEUMONIAE SEROTYPE 19A ST320 CLONE: NOT ONLY CAPSULAR SWITCH OF SEROTYPE 19F ST236

Y.-C. Hsieh, Taoyuan, Taiwan R.O.C.

Background: Streptococcus pneumoniae serotype 19A ST320 clone, genetically related to an international Taiwan 19F ST236 clone, emerge to be prevalent in many countries, including in Taiwan.

Method: 19A disease burden was followed up by using national surveillance database. Virulence of 19A ST320 and 19F ST236 were assessed in murine model of sepsis, pneumonia and colonization. By constructing an isogenic serotype 19F variant of 19A ST320 strain, we analyze the role of capsular type and genetic background on the different virulence between 19A ST320 and 19F ST236.

Results: The incidence of invasive pneumococcal disease increased caused by 19A increased from 2.19/100,000 in 2008 to 10.24/100,000 in 2011 among children aged below 5 years old. There is no difference between 19A ST320 and 19F ST236 in sepsis and pneumonia model. 19A ST320 showed a delay of clearance in nasopharyngeal colonization compared to 19F ST236 did. In murine model of colonization by using competitive experiments, 19A ST320 out-compete 19F ST236, showing 20-fold increase (CI of 20.3; P = 0.001). By constructing an isogenic serotype 19F variant of 19A ST320, 19A ST320 out-competed the variant, showing 2-fold increase (CI of 0.47; P=0.04). The isogenic serotype 19F variant of 19A ST320 out-competed 19F ST236, showing 14-fold increase (CI of 14.7; P< 0.001).

Discussion: Genetic evolution of Streptococcus pneumoniae clone from 19F ST 236 to 19A ST320 promote colonization of the nasopharynx, which increase its capacity of spreading and virulence. The difference between the two clones rely not only on capsular switch but also changes of genetic backgrounds.
INTERACTION BETWEEN THE CFTR GENOTYPE OF MICE AND CAPSULAR PHENOTYPE IN A MOUSE MODEL OF PNEUMOCOCCAL LUNG DISEASE

E. Dennis¹, M. Coats², D. Briles¹, M. Crain¹, Birmingham, Montgomery, AL, USA

Streptococcus pneumoniae (SP) is a respiratory pathogen and a leading cause of morbidity and mortality worldwide, but its role in cystic fibrosis (CF) has not been well studied. The major pathogen in CF lung disease, Pseudomonas aeruginosa, is associated with a worse prognosis when it becomes highly mucoid. SP isolated from sputum of CF patients were collected and highly mucoid isolates were unusually common. We hypothesized that mucoid SP would cause more serious lung infections in mice with the CF mutation CtrtmtUncTg (FABPhCFTR) (CF-mice) than in those without the mutation (WT-mice). Congenic CF-mice and WT-mice were infected intranasally with SP isolated from the sputum of patients with CF. A mucoid serotype 3 isolate (CHB756), a less mucoid type 3 (WU2), and a non-mucoid type 19A isolate (CHB1058) were compared. Tracheal lavage fluid, lung homogenates, and blood were collected from mice 5 days post-infection. Significantly more CFUs were recovered from lungs and tracheal lavages of CF-mice infected with CHB756 compared to those infected with CHB1058 (P<0.0001). Significantly more CFUs were recovered from lungs of CF-mice than WT-mice infected with CHB756 (P=0.0408), however no significant difference was observed between CF-mice and WT-mice infected with CHB1058 (P=0.2203). Strain WU2 was also less virulent than CHB756 in CF-mice. Our results indicate that mucoid SP cause lung infection more effectively than non-mucoid SP, and do so more readily in the lungs of CF-mice than in the lungs of WT-mice. This study supports the association of mucoid bacteria with worse prognosis in individuals with CF.
ADDITIONAL EVIDENCE THAT ASTHMA IS ASSOCIATED WITH PNEUMOCOCCAL DISEASE IN CHILDREN: A LARGE POPULATION-BASED COHORT STUDY IN DENMARK

K.M. Shea¹, H.T. Sørensen², S.I. Pelton¹, ¹Boston, MA, USA, ²Aarhus, Denmark

Background: Although asthma has recently been established as a risk factor for pneumococcal disease (PD), few studies have specifically evaluated this association in children.

Methods: We conducted a nation-wide population-based cohort study of the effect of asthma on childhood PD among all singleton live births in Denmark from 1994-2007, prior to the introduction of PCV7. All data were abstracted from Danish Medical Registries. Because underlying comorbidity substantially increases the risk of PD in children, standard methods were used to assess for evidence of biologic interaction between comorbidity and asthma on the risk of PD.

Results: There were 2,253 cases of childhood PD among 888,655 children born in Denmark from 1994-2007. The adjusted incidence rate ratio (IRR) of the effect of asthma on childhood PD was 2.2 (95% CI 2.0, 2.5). Age-stratified IRRs were 2.1 (1.8, 2.9) in children 6< 24 months, 4.1 (3.3, 5.1) in children 24< 60 months, and 2.3 (1.6, 3.2) in children ≥60 months. Evaluation of biologic interaction between asthma and comorbidity in older children revealed that 55% (24< 60 months) - 73% (>60 months) of cases among asthma-exposed children can be attributed to the presence of both asthma and comorbidity at the same time.

Conclusions: These results confirm that asthma is an important risk factor for PD in children, and suggest that children with underlying comorbidities are more sensitive to the effect of asthma on PD than children without comorbidities.
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