### Symposium I: Australian Indigenous Perspectives on Pneumococcal Disease

**Establishment of a randomised controlled pneumococcal vaccine trial among pregnant Indigenous women in the Northern Territory of Australia**


---

### Symposium II: Other Indigenous and Developing Country Perspectives on Pneumococcal Disease

**Activities of reference laboratory in South Asian pneumococcal alliance network**

Lalitha, MK, Ajoykumar P, Thomas, K, Steinhoff, MC and the SAPNA Networking Centres

**Preliminary data on invasive pneumococcal disease in Nepal by Sapna network**

Shah, AS, Sharma, PR, Tuladhar, NR, Gami, FC, Dahal, M, Thomas, K, Lalitha MK, Steinhoff, MC

**Ethnic disparity in the burden of invasive pneumococcal disease in children aged less than 5 years in Fiji**

Colquhoun, S, Russell, F, Carapetis, J, Tikoduadua, L, Pryor, J, Waqatakirewa, L, Mulholland, K

**Multicentre bronchiectasis study: A collaborative and international study of bronchiectasis in Indigenous children**


**A study of invasive pneumococcal disease at the University College Hospital (UCH), Ibadan, Nigeria**

Falade, A, Adegbola, R, Bakare, R, Odekammi, A

**Dynamics of pneumococcal carriage among Warao children in the Delta Amacuro in Venezuela**

Rivera-Olivero, I, Bogaert, D, Bello, T, del Nogal, B, Sluiter, M, Hermans, P, de Ward, J

**Evolution of highly lethal epidemic pneumococcal meningitis in Burkina Faso**

Yaro, S, Koeck, J-L, Lourd, M, Nacro, B for the Clinical Group, Ouedraogo, M, Idohou, R, Lafourcade, B, Hien, A for the Laboratory Group, Traore, Y, Gessner, BD

**Rates of hospitalization and mortality for pneumonia and respiratory illness in China**

Zhao, GM, Black, S, Shinefield, H, Eskola, J

**Pneumococcal meningitis: Surveillance data for Buenos Aires Province, Argentina**

Verzeri, LN, Rosa, S, Brizuela, L Gonzalez Ayala, SE

**Pneumococcal disease – a major burden in children: Could the introduction of pneumococcal conjugate vaccine (PCV) help to reduce this burden in Papua New Guinea?**

Francis, JP, Phuanukoannon, S, Pomat, WS, Reeder, J, Lehmann, D

**Pan Asian review of pneumococcal disease: Regional patterns of disease burden among children and adults to better understand the value of vaccination**

Kilgore, P, Nyambat, B, Han, SH, Jodar, L, Chang, JH, Clemens, J

**Epidemiology of pneumococcal meningitis in children in the district of Colombo, Sri Lanka: A prospective, population based surveillance study**

Batuwanthudawe, R, Rajapakse, L, Abeyesinghe, N, Somaratne, P, Dassanayaka, M

**Clinical and laboratory characteristics of pneumonia with radiological consolidation in Gambian children**


**A cohort study to assess quality of life in young Fijian children who have a history of bacterial meningitis**

Colquhoun, SM, Russell, FM, Carapetis, JR, Tikoduadua, LV, Pryor, J, Wake, M, Mulholland, EK

**Epidemiological and clinical characteristics of community acquired invasive bacterial infections in children aged 2-29 months in The Gambia**

Epidemiology of invasive pneumococcal disease among children in Kilifi district, Kenya
Ndiritu, M, Nyiro, J, Njenga, S, Lewa, P, Mwarumba, S, Lowe, BS, Bauni, E, Scott, JAG, PO2.16

Nasopharyngeal carriage of pneumococci among children in Greenland
Koch, A, Hjuler, T, Krause, TG, Friberg, J, Melbye, M, Olsen, OR, Kaltoft, MS PO2.17

Invasive S. pneumoniae (Pnc) infections in infants and children less than 5 years old admitted to a tertiary hospital in central Philippines
Ladesma, PE, Lupisan, S, Sombrero, L, Quiambao, B, Lucero, M, Gozum, L, Arcay, J, Simoes, E, Herva, E, Ruutu, P PO2.18

Microbial surveillance for Streptococcus pneumoniae in rural Thailand: Lessons learned, laboratory capacity, and the case for quality control
Fischer, JE, Peruski, LF, Wongjindanon, W, Jornrakate, P, Ngetsa, C, Sangsuk, L, Dejsirilert, S, Olsen, SJ, Dowell, SF PO2.19

Bacteremia and bacterial meningitis among children 2 months to 5 years attending to out patient and observation services of Kanti Children Hospital, Kathmandu
Steinhoff, MC, Lalitha, MK, Thomas, K, Sherchan, J, Gauchan, P, Gami, F, Sharma, PR, Singh Shah, A, Tuladhar, NR, PO2.20

Symposium III (Part 1): The Global Epidemiology of Pneumococcal Disease, Resistance and Serotypes

Invasive pneumococcal disease in children <5 years of age living a rural area of Mozambique

Severity and outcome of invasive pneumococcal diseases in an industrialised country (Finland)
Koskikallio, E, Salo, E, Kaijalainen, T, Peitola, H PO3.03

Two nontypable Streptococcus pneumoniae clones associated with acute conjunctivitis in southern Israel

Disease potential of individual Streptococcus pneumoniae (SP) serotypes in invasive disease (IPD), acute otitis media (AOM) and acute conjunctivitis (AC) in infants and young children
Greenberg, D, Shuval, DS, Givon-Lavi, N, Porat, N, Dagan, R PO3.05

Differences in clinical characteristics of invasive pneumococcal infections related to serotype
Troflors, B, Berg, S, Backhaus, E, Kaltoft, MS, Bossen Konradsen, H PO3.06

Serotype and antimicrobial susceptibility patterns of Streptococcus pneumoniae causing invasive disease in The Gambia

Incidence of clinically significant pneumococcal bacteraemia among children presenting to a hospital outpatient department in Kenya

Invasive pneumococcal disease in Singapore children
Chong, C-Y, Mok, Y-H, Tee, N PO3.09


Streptococcus pneumoniae invasive isolates from Uruguayan children and adults: Epidemiological differences among serotypes

Poor potential coverage of pneumococcal conjugate vaccine for pneumococcal meningitis in Papua New Guinean highland children
Phuanukoonnon, S, Michael, A, Nale, G, Johannes, M, Orami, T, Murphy, D, Alpers, M, Lehmann, D, Siba, P, Reeder, J PO3.13

Monitoring antimicrobial resistance and serotypes (the MARS project): Pneumococcal carriage in child care centres in the Northern Territory of Australia, 2003 to 2005

Serotypes and sequence types of Streptococcus pneumoniae isolated from blood in Poland
Sadowsy, E, Hryniewicz, W PO3.15

5th International Symposium on Pneumococci and Pneumococcal Diseases
Increase in macrolide resistance among invasive isolates of *Streptococcus pneumoniae* in Norway, caused by the England 14-9 clone
Sogstad, MKR, Littauer, P, Aaberger, IS, Caugant, DA, Høiby, EA

Invasive pneumococcal disease in England and Wales 1996-2004

Chronic pulmonary disease and risk of invasive pneumococcal infections
Klemets, P, Lyytikäinen, O, Ruutu, P, Ollgren, J, Nuorti, P

The burden of pneumococcal disease and other invasive bacterial infections in children in urban Nepal

*Streptococcus pneumoniae* serotypes isolated from Argentinian children with invasive disease
Vescina, CM, Regueira, M, Gonzalez Ayala, SE, Gatti, B, Cecchini, D, Agosti, MR

Severity of pneumococcal disease among hospitalized children in Bangladesh
Naheed, A, Saha, SK, Khatun, F, Arifeen, SE, Brooks, WA, Sack, DA, Breiman, RF, Luby, SP

Prospective meningitis burden of disease study and rapid assessment of neurological outcomes in children in Fiji
Kunabuli, VL, Mulholland, EK, Tikoduadua, L, Seduadua, A, Pryor, J, Russell, F

Surveillance of antimicrobial resistance of *Streptococcus pneumoniae* isolates in children with invasive pneumococcal infection from Mexico during 1997/2004
Lopez-Enriquez, C, Espinosa-Monteros, LE, Rojas, LV, Gomez-Barreto, D

A multi-Center retrospective analysis of empyema and pleural effusion in hospitalized Asian children
Kilgore, P, Nyambat, B, Han, SH, McIntyre, PB, Shen, XZ, Soenarto, Y, Yong, DE, Chiu, CH, Tharavichitkul, P, Anh, DD, Ngo, TT, Haandsdorff, WP

Longitudinal carriage study of *Streptococcus pneumoniae* serotype or group in Tanzanian children
Oriyo, N, Sam, N, Gillespie, S, Charalambous, B

*Streptococcus pneumoniae* nasopharyngeal colonization in day-care centers of central Greece
Grivea, IN, Chryssanthopolou, DC, The Hellenic antibiotic-resistant respiratory Pathogens (HARP) Study Group

The invasive disease potential of Icelandic pneumococci

Secular variations in age incidence (Inc) and serotypes (St) causing invasive pneumococcal disease (IPD) in children 0 to 59 months of age (MoA) in the metropolitan region (MR), Chile.

Increase of invasive infections caused by vaccine-related and non-vaccine serotypes of *Streptococcus pneumoniae* among children in Barcelona, Spain

Pneumococcal clones causing invasive disease in Iceland, 1990-2004
Kristinsson, K, Jensdottir, H, Erlendsdottir, H, Gunnarsdottir, T

Clinical characteristics, antibiotic resistance and serotype distribution of invasive pneumococcal disease in metropolitan Atlanta after introduction of pneumococcal conjugate vaccine
Albrich, WC, Baughman, W, Schmotzer, B, Farley, MM
Pneumococcal epidemiology in Sevilla and Malaga, Spain
Brueggemann, AB, Arroyo, L, Hausdorff, W, Sanchez-Tatay, D, Moreno, D, Torronteras, R, Mateos, I, Fenoll, A, Obando, I

Family and perinatal risk factors of invasive pneumococcal disease
Hjuler, T, Koch, A, Kaltoft, MS, Wohlfarth, J, Melbye, M

Pneumococcal conjugate vaccine and pneumococcal community-acquired pneumonia in hospitalized children: A retrospective epidemiologic study from 2000 to 2003
Huang, LM, Lin, HC, Huang, YC, Ho, YH

Serotype distribution and antimicrobial susceptibility of pneumococcal bacteremic infections in adults – An international prospective study
Choi, C, McGee, L, Jackson, D, Yu, V, Klugman, K

Respiratory syncytial virus hospitalisation and invasive pneumococcal disease: True true but unrelated
Stensballe, LG, Hjuler, T, Koch, A, Kaltoft, MS, Simoes, EAF

Nasal S. aureus (SA) is inversely related to nasopharyngeal S. pneumoniae (SP) carriage in children and their parents

Twelve months after beginning universal vaccination with 7vPCV. Have we made a difference?
Murphy, D, Hicks, V, Smith, H, Bates, J, Hanna, J

Risk factors for pneumonia and hospitalisation with pneumonia in Auckland, New Zealand

Monitoring antimicrobial resistance and serotypes (the MARS project): Pneumococcal carriage in remote Aboriginal communities in the Northern Territory of Australia, 2003 & 2005

Pneumococcal serotypes among Filipino children admitted in a tertiary care center for infectious diseases in 2000-2005
Capeding, MR, Sombrero, LT, Esparar, GA, Taclibon, AG

Pneumonia among young infants in Bohol Island, Philippines
Quiambao, BP, Ruutu, PJ, Ladesma, EA, Gozum, L, Lupisan, SP, Sombrero, LT, Romano, V, Simoes, EAF, ARIVAC Consortium

Predictors of death among children with community-acquired pneumonia in a referral hospital in Bohol, Philippines
Lupisan, S, Lucero, MG, Ruutu, P, Quiambao, B, Abucejo-Ladesma, E, Gozum, L, Sombrero, L, Simoes, EAF, Riley, I and the ARIVAC Consortium

The evolution of macrolide resistance in Streptococcus pneumoniae isolates over a 20-year period
Syriopoulou, VP, Tsiodras, S, Koutsolioutsou, A, Braoudaki, M, Charissiadou, A, Pangalis, A, Soulis, K, Daikos, G

A national review of the epidemiology of pneumococcal disease in children in Singapore
Chan, FLF, Low, S, Cutter, J, Ma, S, Goh, KT, Chew, SK

Epidemiology of pneumococcal disease in Singapore - A national study
Low, S, Chan, FLF, Cutter, J, Ma, S, Goh, KT, Chew, SK

Invasive pneumococcal disease burden in Hong Kong children
Ho, PL, Chiu, SS, Cheung, CHY, Lee, R, Tsai, TF, Lau, YL

SAPNA Sri Lanka: Pneumococcal surveillance at the largest tertiary paediatric care facility in South Asia – Lady Ridgeway Hospital, Colombo Sri Lanka
Batuwanthudawe, R, de Silva, S, Karunarathne, K, Lalitha, MK, Thomas, K, Steinhoff, M, Abeysinghe, N

Serotype distribution of Streptococcus pneumoniae among children in Bamako, Mali
Sow, SO, Hormazabal, JC, Tapia, MD, Diallo, S, Campbell, JD, Kotloff, K, Levine, M

Invasive pneumococcal disease among children in Bamako, Mali
Tapia, MD, Sow, SO, Diallo, S, Campbell, JD, Keita, M, Doumbia, MN, KeitaM, M, Kotloff, K, Levine, M
Invasive pneumococcal disease in adults in North-Rhine Westphalia, Germany, 2001-2004
van der Linden, MPG, Al-Lahham, A, Reinert, RR

Serotype distribution and antibiotic susceptibility of invasive and respiratory pneumococcal isolates from children < 5 admitted for pneumonia, suspected sepsis or meningitis in Bohol, Philippines
Sombrero, L, Esparar, G, Girasol, F, Herva, E, Lupisan, S, Ruutu, P, Quiambao, B, Gozum, L, Lucero, M, Nissinen, A

Evolution of pneumococcal serotype distribution over a 5 year period in 1-35 month old children in the city of Cordoba, Argentina
Tregnaghi, M, Ceballos, A, Ruttimann, R, Hausdorff, W, Ussher, J, Yudouski, S

Diggle, M, Edwards, G

Relative invasive disease (ID) potential of Streptococcus pneumoniae (S.pn) of specific serotypes and serogroups, in Chilean infants and children from 0 to 24 months of age (MoA)

Evaluation of co-morbidity on the incidence of pneumococcal infection in the far east of Russia
Martynova, AV, Turkutyukov, VB, Skurkhina, JV, Sheparyov, AA

Incidence of respiratory tract infections (RTI) in children < 2 years of age: Impact of environmental risk factors and effectiveness of a pneumococcal conjugate vaccine (PCV7)
Adam, D, Helmerking, M

Symposium IV: Measuring the Impact of Prevention and Treatment Interventions

Measuring the immunogenicity of the PNEUMOVAX®23 vaccine
Marchese, RD, Esser, M, Schlottman, S, Jain, N, Mallette, L, Raab, J, Butterfield-Gerson, K, Norris, M, Sikkema, D, Chirmule, N

Uptake of pneumococcal conjugate vaccine among children in the United States, 2000-2004
Nuorti, JP, Martin, S, Smith, P, Moran, J, Schwartz, B

Invasive pneumococcal disease and the impact of the 7-valent pneumococcal conjugate vaccine in the greater Sydney region, NSW 1998 - 2005
McIntyre, PB, Gilmour, R, Brown, M, Watson, M, Bartlett, M, Gilbert, GL

An electronic system to simplify the WHO process for the radiological diagnosis of pneumonia In research
O'Grady, K-A, Taylor-Thompson, D, Ruben, A

The WHO guidelines for the radiological diagnosis of pneumonia in children: Outcomes of investigator training in the Northern Territory, Australia

Public health potential of a 13vPnC vaccine for immunization of adults in the US
Hackell, JG, Paradiso, PR, Silber, G

A new method of assessing frailty in older patients recruited into pneumonia vaccination trials
Lindley, RI, MacIntyre, R, Ridda, I, McIntyre, P, Sullivan, J, Gilbert, L, Koovor, P, Manolios, N

Vaccination uptake in hospitalised geriatric patients 4-6 months after commencement of a funded national pneumococcal vaccination program for >65's
Ridda, I, MacIntyre, R, Lindley, R, McIntyre, P, Sullivan, J, Gilbert, L, Koovor, P, Manolios, N

Effect of 2 versus 3 pneumococcal conjugate vaccinations Prevnar® on nasopharyngeal carriage, transmission and herd immunity; A randomized, controlled study
van Gils, EJM, Veenhoven, RH, Hak, E, IJzerman, EPF, Rijkers, GT, Sanders, EAM

Multilocus sequence typing scheme for Streptococcus pneumoniae: Novel strains among Gambian carriage isolates
Sankareh, KK, Hanage, WP, Antonio, M, Obaro, S, Greenwood, BM, Adegbola, RA, Spratt, BG

Impact of co-trimoxazole on carriage and antibiotic resistance of Streptococcus pneumoniae and Haemophilus influenzae in HIV infected children in Zambia
Mwenya, DM, Charalambous, BM, Gibbs, D, Nunn, A, Mwansa, JCL, Gillespie, SH
The impact of the Australian Indigenous adult pneumococcal immunisation program
Menzies, R, McIntyre, P

Yields of different pneumococcal tests in community-acquired pneumonia in elderly

Invasive pneumococcal disease in German children vaccinated with pneumococcal vaccines
Reinert, RR, Al-Lahham, A, Siedler, A, van der Linden, M, Toschke, AM, von Kries, R

Clinical predictors of radiological pneumonia and hypoxia in Gambian children

Replacement invasive pneumococcal disease 9 years after introduction of PCV use among a population at high risk for IPD: The Navajo experience


Invasive Streptococcus pneumoniae (ISP) infections continue to cause high mortality
Vanderkooi, OG, Church, DL, MacDonald, J, Scheifele, D, Tyrrell, GJ, Kellner, JD for Calgary Area S. pneumoniae (SP) Epidemiology Research (CASPER)

Data management in a phase III clinical trial: Pneumococcal conjugate vaccine (PCV)
Sanvictores, DH, Lucero, M, Tallo, V, Adamson, J, Chester, A, Nohynek, H, Williams, G for ARIVAC Consortium

Developing a quasi-dynamic model for evaluating vaccines against Streptococcus pneumoniae in infants
Tilden, D, Cottrell, S, Robinson, P, Aristides, M, Wait, S, Hausdorff, W, Bonfatti, L

Epidemiology of community-acquired pneumonias caused by Streptococcus pneumoniae
Martynova, AV, Turkutjukov, VB, Skurihina, E Ju, Sheparjov, AA

Pneumococcal infections in young children in Hong Kong – the pre-vaccine era and cost-effectiveness of pneumococcal conjugate vaccine
Ng, YM, Yau, YS
**Symposium V: Genetic and Molecular Aspects of the Pneumococcus**

**Effect of oxygen availability on membrane composition of *Streptococcus pneumoniae***

**PO5.01**

**Molecular characterisation of invasive paediatric *Streptococcus pneumoniae* isolates collected during a 9-valent pneumococcal conjugate vaccine trial in The Gambia**

**PO5.02**

**Role of the pneumococcal MerR-like regulator in resistance to oxidative stress**

**PO5.03**
Stroeher, UH, Kidd, SP, McEwan, AG, Jennings, MP, Paton, JC

**The two-component signal transduction system RR/HK06**

**PO5.04**
Standish, AJ, Stroeher, UH, Paton, JC

**Evaluation and selection of tandem repeat loci for *Streptococcus pneumoniae* MLVA strain typing**

**PO5.05**
Koeck, J-L, Lafourcade, B, Gessner, BD, Varon, E, Sangare, L, Valjevac, S, Vergnaud, G, Porecel, C

**Differential expression of pneumococcal virulence genes in vivo**

**PO5.06**
LeMessurier, KS, Ogunniyi, AD, Paton, JC

**Characterization of two highly conserved pneumococcal proteins that are essential for in vivo survival and virulence**

**PO5.07**

**Genetic characteristic of the invasive pneumococci collected in elderly population in England and Wales during winter season 2003/04**

**PO5.08**
Pichon, B, Andrews, N, Gungabissoon, U, Wikins, H, Slack, M, Miller, E, George, R

**MLST characterization of *Streptococcus pneumoniae* clones of serotypes 6B, 14, and 23F associated with invasive disease in the Czech Republic**

**PO5.09**
Zemlickova, H, Urbaskova, P, Motlova, J

**Development of genomic array footprinting for the identification of conditionally essential genes in *Streptococcus pneumoniae***

**PO5.10**
Bootsma, HJ, Kloosterman, TG, Burghout, PJ, Bijlsma, JJE, Hermans, PWM, Kuipers, OP

**MLST scheme for high-throughput analysis of pneumococcal isolates**

**PO5.11**
Wicksins, H, Pichon, B, George, R

**Comparative analysis of the pneumococcal capsular loci**

**PO5.12**
Mavroidi, A, Aanensen, DM, Quail, MA, Godoy, D, Kaltoft, MS, Parkhill, J, Bentley, SD, Reeves, PR, Spratt, BG

**Regulation of glutamine and glutamate metabolism by GlnR and GlnA in *Streptococcus pneumoniae***

**PO5.13**
Kloosterman, TG, Hendriksen, WT, Bijlsma, JJE, Bootsma, HJ, Kok, J, Hermans, PWM, Kuipers, OP

**Regulation of gene expression in *Streptococcus pneumoniae* by two-component system 09 is strain-dependent**

**PO5.14**

**Contribution of glutamine synthetase GlnA and its transcriptional repressor GlnR to pneumococcal virulence**

**PO5.15**
Hendriksen, WT, Kloosterman, TG, Estevão, S, Bootsma, HJ, de Groot, R, Kuipers, OP, Hermans, PWM

**Molecular epidemiology of pneumococci causing invasive disease among Oxfordshire children, 1995 – 2005**

**PO5.16**
Brueggemann, AB, Foster, D, Griffiths, D, Crook, DW on behalf of the Oxford Pneumococcal Surveillance Group

**Alterations in predicted primary structure of the penicillin binding protein 2B of 29 penicillin non-susceptible clinical isolates of *Streptococcus pneumoniae***

**PO5.17**
Bengtsson, D, Laurell, MH

**Y-family polymerases of *S. pneumoniae***

**PO5.18**
Henderson-Begg, SK, Livermore, DM, Hall, LMC
Serotypes and genetic characterization of a large collection of drug-susceptible pneumococci isolated from preschool children and comparison with drug-resistant pneumococcal lineages
Sa-Leao, R, Nunes, S, Frazão, N, Sousa, NG, de Lencastre, H

PO5.19

Congruence of pneumococcal population structure resolved by ribotyping and MLST in a defined community

PO5.20

The pathogen and the commensal: Comparison between the genome of Streptococcus mitis B6 and Streptococcus pneumoniae

PO5.21

A functional dlt operon confers resistance to antimicrobial peptides in Streptococcus pneumoniae

PO5.22

Symposium VI: Immune Status and Susceptibility

Difference response with antibodies to PPV and PCV in Asplenic children
Bernatowska, E, Kayhty, H, Mikoluc, B, Pac, M, Berglof, A

PO6.01

Polysaccharide capsule formation is crucial for Streptococcus pneumoniae to evade neutrophil extracellular traps
Beiter, K, Wartha, F, Albiger, B, Normark, S, Zychlinsky, A, Henriques-Normark, B

PO6.02

Pneumococcal contacts induce the development of serum antibodies to pneumococcal neuraminidase (NanA) in children
Simell, B, Jaakkola, T, Lahdenkari, M, Briles, D, Hollingshead, S, Kilpi, TM, Kayhty, H

PO6.03

Mannan-binding lectin levels and polymorphisms in children with recurrent acute otitis media
Wiertsema, SP, Herpers, BL, Walraven, V, Veenhoven, RH, Schilder, AGM, Ruven, HJT, Rijkers, GT, Sanders, EAM

PO6.05

Pneumococcal (Pnc) conjugate vaccine primes mucosal immune responses in Australian Indigenous children for boosting by pneumococcal polysaccharide vaccine (PPV)

PO6.06

Enhanced adaptive immune maturation in neonates born in a high-risk area for invasive pneumococcal disease in Papua New Guinea
van den Biggelaar, AHJ, Nadal, MA, Pomat, WS, Francis, JP, Prescott, SL, Richmond, P, Lehmann, D, Reeder, J, Holt, PG

PO6.08

MyD88-dependent signalling controls bacterial growth during colonization and systemic pneumococcal disease in mice

PO6.09

Immunity to Streptococcus pneumoniae in a healthy blood donor population

PO6.10

The role of CbpA on complement deposition and opsonophagocytosis of Streptococcus pneumoniae
Yuste, J, Ansari, N, Khandavilli, S, Paton, JC, Botto, M, Brown, JS

PO6.11

The use of pneumococcal conjugate vaccine in children with nephrotic syndrome
Wood, N, Richmond, P, McIntyre, P

PO6.12

Opsonophagocytic responses to pneumococcal conjugate vaccine in the elderly

PO6.13

Complement deposition on pneumococcal capsular types 6B and 19F
Melin, M, Jarva, H, Meri, S, Käyhty, H

PO6.14

The role of surface tethered mucins in pneumococcal nasopharyngeal carriage using a primary cell model
Hendy, E, Erlewyn-Lajeunesse, M, Finn, A

PO6.15

Human immune response to meningitis-causing Streptococcus pneumoniae
Pereira, D, Barroso, D, Brasil, P, Rebelo, MC, Jessouroun, E

PO6.16
Effect of aging and gender on naturally acquired antibodies to pneumococcal polysaccharides
Väkeväinen, M, Simell, B, Grönholm, S, Reunanen, A, Käyhty, H

Production of cytokines after stimulation of peripheral blood mononuclear cells with pneumococcal bacteria, polysaccharides, proteins, and vaccines
Vuorela, A, Käyhty, H, Valtonen, H, Julkunen, I, Väkeväinen, M

Dynamics of the immune response in children to the 23-valent pneumococcal capsular polysaccharide vaccine (Pneumovax)
Mlacha, SZK, Warira, A, Scott, JAG

Children with invasive pneumococcal disease have low levels of antibodies to virulence proteins and develop poor antibody responses compared to age-matched children who carry pneumococci in their nasopharynx
Jonsdottir, I, Ingolfsdottir, G, Paton, JC, Kristinsson, KG, Gudnason, T

Functional antibody responses to PCV-PPV regimen in HIV infected children on HAART categorized by entry and Nadir CD4 percentage (PACT1024)
Pelton, SI, Abzug, MJ, Borkowsk, W, Nachman, SA, Levine, M, Song, LY, Fenton, T for the P1024 Team

Population-based surveillance for suspected (S) and radiologically-confirmed (RxC) community acquired pneumonia (CAP) in children 1-35 months of age (MoA), in 6 municipalities (Mn) of the metropolitan region (MR), Chile.

Correlation of naturally acquired anti-pneumococcal IgG antibody levels with nasopharyngeal carriage in Gambian villagers
Saaka, M, Akisanya, A, Hill, PC, Sankareh, K, Jeffries, D, Nakua, E, Cheung, YB, Lahai, G, Greenwood, BM, Adegbola, RA

Development of antibodies to pneumococcal proteins PhtD, CbpA and LytC in Filipino pregnant women and their offspring in relation to pneumococcal carriage
Holmlund, E, Quimba, B, Ollgren, J, Jakkola, T, Hermand, P, Neyt, C, Poolman, J, Nohynek, H, Käyhty H

Symposium VII: The Nasopharyngeal Ecosystem and Relationship to Disease

Nasopharyngeal carriage of Streptococcus pneumoniae in Gambian villagers
Hill, PC, Akisanya, A, Sankareh, K, Cheung, YB, Saaka, M, Lahai, G, Greenwood, BM, Adegbola, RA

Pneumococcal nasopharyngeal carriage and penicillin resistance patterns in young children in Fiji
Russell, FM, Carapetis, JR, Ketawai, S, Kunabuli, V, Taoi, M, Biribo, S, Sedudua, A, Mulholland, EK

Virological and bacteriological examination of nasopharynx of healthy servicemen in Russia
Zhogolev, SD, Ogarkov, PI, Jogolev, KD, Sologub, TS, Yakovleva, NV, Rezsova, YV

Does 7-valent pneumococcal conjugated vaccine (pcv7) influence Staphylococcus aureus (SA) nasopharyngeal (NP) carriage in 6 to 24 months old children with acute otitis media (AOM)?
Cohen, R, Levy, C, De La Rocque, F, Bonnet, E, Fritzell, B, Tetelboum, R, Boucherat, M, Varon, E

Serotype 1 MLST sequence types associated with an epidemic of serotype 1 carriage in remote Aboriginal communities of northern Australia

Epidemiology of pneumococcal carriage among Indigenous and non-Indigenous children in the Delta Amacuro in Venezuela
Rivera-Olivero, I, Bogaert, D, Bello, T, del Nogal, B, Sluijter, M, Hermans, P, de Waard, JH

Cooperation of Streptococcus pneumoniae and Chlamydia pneumoniae in the development of pneumonia and acute respiratory disease
Ogarkov, PI, Zhogolev, SD, Jogolev, KD, Sologub, TS, Zueva, NV, Suchanov, BS

Negative association between Streptococcus pneumoniae and Staphylococcus aureus in the nasopharynx of Aboriginal and non-Aboriginal children in the Kalgoorlie-Boulder area, Western Australia
Watson, K, Bowman, J, Murphy, D, Jacoby, P, Riley, TV, Lehmann, D on behalf of the Kalgoorlie Otitis Media Research Project Team

Probing the association between serotype-specific pneumococcal nasopharyngeal colonization and efficacy of the pneumococcal conjugate vaccine against pneumonia
Madhi, SA, Cutland, C, Kuvanda, L, de Gouveia, L, Von Gottberg, A, Klugman, KP

5th International Symposium on Pneumococci and Pneumococcal Diseases
Association between carriage of *Streptococcus pneumoniae* and Staphylococcus aureus in a Dutch birth cohort

Exposure to other children enhances the antibody response to a pneumococcal conjugate vaccine in 1 year old infants
Salt, PM, Banner, C, Oh, S, Yu, L, Griffiths, DT, Pan, D, Lewis, S, Ferry, BL, Pollard, AJ

Intra-familial transmission of *Streptococcus pneumoniae* and nonencapsulated *Haemophilus influenzae*
McKinnon, M, Smith-Vaughan, H, Perez, F, Shelby-James, T, Mayo, M, Leach, AJ

Multiserotype nasopharyngeal carriage of *Streptococcus pneumoniae* in infants in Fiji
Biribio, SSN, Russell, FM, Carapetis, JR, Kataiwa, S, Mulholland, EK

Nasopharyngeal carriage of *Streptococcus pneumoniae* in a displaced Asian Tsunami population in a camp setting in eastern Sri Lanka

The transition in nasopharyngeal microflora accompanying the onset of otitis media in high-risk infants

Pneumococcal carriage study in a semi-closed population of children in north-east Tanzania
Oriyo, N, Sam, N, Gillespie, SH, Charalambous, BM

Longitudinal study of antibiotic sensitivity of pneumococcal isolates colonizing the nasopharynx of Tanzanian children
Oriyo, N, Sam, N, Gillespie, SH, Charalambous, BM

Quantitative description of the carriage of multiple serotypes of *S. pneumoniae* in the nasopharynges of children in Kilifi district
Nyiro, J, Abdullahi, O, Scott, JAG

Dynamic models of pneumococcal carriage and disease, and the impact of the 7-valent pneumococcal conjugate vaccination in the UK
Melegaro, A, Choi, YH, Gay, NJ, Edmunds, W

The descriptive epidemiology of nasopharyngeal carriage of *Streptococcus pneumoniae* in Kilifi district, Kenya
Abdullahi, O, Lewa, P, Nyiro, J, Scott, JAG

Anti-capsular serum antibody concentration and protection against pneumococcal colonization Induced by 7-valent conjugate pneumococcal vaccine
Millar, EV, O’Brien, KL, Bronsdon, MA, Madore, D, Hackell, J, Reid, R, Santosham, M

Can isolates of *Streptococcus pneumoniae* carried by healthy children or children with mild or severe pneumonia predict serotypes and resistance patterns of invasive isolates?
Abdullahi, O, Lewa, P, Nyiro, J, Scott, JAG

Nasopharyngeal carriage of *Streptococcus pneumoniae* in young Papua New Guinean highland infants: Preliminary results from a neonatal pneumococcal conjugate vaccine trial
Michael, A, Phanuukoannon, S, Nale, G, Siba, P, Richmond, P, Reeder, JC, Lehmann, D

Indirect effect of heptavalent conjugate vaccine on pneumococcal colonization and invasive disease in adults
Hammit, LL, Hennessy, TW, Bruden, D, Bulkow, L, Cottle, T, Singleton, R, Hurlbut, D, Reasonover, A, Parkinson, A, Butler, JC

*Streptococcus pneumoniae* (SP) nasopharyngeal (NP) colonization: Trends over three years after introduction of 7-valent pneumococcal conjugate vaccine (PCV7) in Canada
Kellner, JD, Church, DL, MacDonald, J, Scheifele, D, Tyrrell, GJ, Vanderkooi, OG, for Calgary Area S. pneumoniae Epidemiology Research (CASPHER)

An evaluation of *Streptococcus pneumoniae* carriage rate in the nasopharynx of Filipino children attending well baby clinic in hospital and primary care center
Capeding, MRZ, Tan, R, Calimon, NC, Alpon, MM, Sepulveda, JF, Zeta, AB, Sombrero, LT

Neonatal acquisition of nasopharyngeal carriage of *Streptococcus pneumoniae*
Glass, N, Mudoga, E, Abdullahi, O, Nyiro, J, Schuchat, A, Scott, JAG
Nasopharyngeal (NP) carriage of *Streptococcus pneumoniae* (S.pn) in a cohort of healthy, Chilean new born infants (NBI) followed from age 0 to 24 months (mos)

Lagos, R, Muñoz, A, Clarke, J, Seoane, M, Maldonado, A, Hormazábal, JC, Loyola, H, Levine, M

**Symposium VIII: Pneumococcal Disease Pathogenesis - Biofilms and Viral Infection**

Clinical characteristics of children with complicated pneumococcal pneumonia caused by serotypes 1 and 3

Tan, TO and the US Pediatric Multicenter Pneumococcal Surveillance Study Group (USPMPSG)

The role of bacterial biofilm in the aetiology of chronic and recurrent ear infections in children


Pneumococcal co-Infection with human metapneumovirus

Madhi, SA, Ludewick, H, Kuwanda, L, van Niekerk, N, Cutland, C, Little, T, Klugman, KP

Haemolytic uraemic syndrome associated with invasive pneumococcal disease


Simple method to evaluate biofilm formation of *Streptococcus pneumoniae*

Tapiainen, T, Saukkoriipi, A, Kaijalainen, T, Leinonen, M, Uhari, M

*Streptococcus pneumoniae* Pili - purification and initial characterization

Hilleringmann, M, Ferlenghi, I, Giusti, F, Mercati, D, Barocchi, M, Pacchiani, N, Rappuoli, R, Covacci, A

**Symposium IX: Animal Models of Pneumococcal Disease**

Proudly Sponsored by PATH

The influence of allergic airways disease on the immune response to respiratory *Streptococcus pneumoniae* infection

Preston, JA, Horvat, JC, Wade, MA, Beagley, KW, Foster, PS, Gibson, PG, Hansbro, PM

Serum amyloid P component protects against *Streptococcus pneumoniae* in a mouse model of infection

Yuste, J, Botto, M, Brown, JS

Isolation and characterization of *Streptococcus pneumoniae* strains from animal models

Al-Lahham, A, Nicklas, W, Reinert, RR
A surveillance system for pneumococcal disease following the successful trial of the 9-valent pneumococcal vaccine in The Gambia

Hill, PC, Sambo, S, Lloyd-Evans, N, Manneh, K, Greenwood, BM, Jallow, M, Adegbola, RA PO10.01

Impact on respiratory tract infections of heptavalent pneumococcal conjugate vaccine (Pcv-7) administered at 3, 5 and 11 months of age


Immunogenicity of pneumococcal 23-valent polysaccharide and 7-valent conjugate vaccines used in combination in adults 55-70 years old


Immunogenicity following one, two, or three doses of the 7-valent pneumococcal conjugate vaccine and booster response of the 23-valent pneumococcal polysaccharide vaccine at 12 months of age


Inferior humoral response in elderly versus young adults to the 23-valent polysaccharide vaccine

Devaster, J-M, Leroux-Roels, I, Leroux-Roels, G, Vandepapellère, P, Horsmans, Y, Henckaerts, I, Poolman, J PO10.05

Specificities of immune responses against a serotype 3 pneumococcal conjugate

Schuerman, L, Prymula, R, Poolman, J PO10.06

The Immunogenicity of a Nine-valent pneumococcal conjugate vaccine in Gambian children under one year of age


Functionality of antibodies against serotypes 6A and 19A induced by three different pneumococcal conjugate vaccines (PCV) in infants

Nurkka, A, Lehtonen, H, Vuorela, A, Ekström, N, Käyhty, H PO10.08

Impact of 7vPCV and 23vPPV booster in eligible children in the Northern Territory of Australia: Impressive, but not the total answer

Krause, VL, Cook, H, Selvey, CE PO10.09

Elevated and persistent functional antibody responses in adults ≥65 years of age after a second dose of a 23-valent pneumococcal polysaccharide vaccine


Previous pneumococcal polysaccharide vaccine impacts immune response to subsequent pneumococcal conjugate vaccine in the elderly


Functional activity of antibodies against serotype 19F evoked by pneumococcal conjugate vaccines


A case-control study to investigate opsonophagocytic activity (OPA) of antipneumococcal antibodies as a serological correlate of protection against acute otitis media (AOM)


Pneumococcal vaccination and nasopharyngeal bacterial carriage in Australian Aboriginal infants


Implementation of a 7 valent pneumococcal vaccination program in Germany based on an at risk strategy

Arenz, S, Kalies, H, Toschke, AM, Al-Lahham, A, Siedler, A, Reinert, RR, von Kries, R PO10.15
Long term effects of a 9-valent pneumococcal conjugate vaccine (PCV) on nasopharyngeal colonization with pneumococcal serotypes included in the vaccine
Adrian, PV, van Niekerk, N, Jones, S, Cutland, CL, von Gottberg, A, de Gouveia, L, Klugman, KP, Madhi, SA

Response to a conjugate pneumococcal vaccine administered at five years of age in the presence and absence of a primary series of three-doses of vaccine given during early infancy
Madhi, SA, Adrian, P, Jassat, W, Kohler, M, Jones, S, Cutland, C, Kuwanda, L, Klugman, KP

Reinert, RR, Al-Lahham, A, Siedler, A, van der Linden, M, Toschke, AM, von Kries, R

Pneumococcal population biology of vaccine effectiveness and serotype replacement in S. pneumoniae (SP) isolated during the American Indian clinical trial of the 7-valent pneumococcal conjugate vaccine (PCV)

Post-PCV7 expansion and emergence of sequence types among serotype 19A and serogroup 15 Streptococcus pneumoniae (SP) in Massachusetts children 1999-2004

Safety and immunogenicity of the 13-valent pneumococcal conjugate vaccine in healthy adults
Scott, DA, Komjathy, SF, Ruckle, J, Supan, L, Dar, M, Razmpour, A, Hu, B, Lockhart, S, Gruber, W, Siber, G

Post-marketing adverse event (AE) reports in patients receiving ≥ three doses of pneumococcal polysaccharide vaccine (PPV)
Dana, A, Wilson, E, Goss, MA, Olivero, K, Benson, J

Salivary and serum responses to 7-valent pneumococcal conjugate vaccine (PCV) in HIV-infected adults.

Serotype replacement in invasive Streptococcus pneumoniae (ISP) infections after introduction of 7-valent pneumococcal conjugate vaccine (PCV7) in Canada: Implications for an expanded 13-valent conjugate vaccine
Hellner, J, Church, D, MacDonald, J, Scheifele, D, Tyrrell, G, Vanderkooi, OG for Calgary Area S. pneumoniae Epidemiology research (CASPER)

Efficacy estimates for a 7-valent pneumococcal conjugate vaccine: Impact of case definition used for acute otitis media
Palmu, AA, Jokinen, J, Mäkelä, H, Kilpi, TM

Pneumococcal conjugate vaccines in preventing vaccine-type invasive pneumococcal disease and pneumonia with consolidation on X-ray in children under two years of age
Lucero, MG, Dulalia, VE, Pannero, RAN, Lim-Quianzon, DM, Nohynek, HM, Mäkelä, H, Williams, G

Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia
Cutts, F, Zaman, SMA, Enwere, G, Jaffar, S, Levine, OS, Biney, EEO, Pierce, NF, Greenwood, BM, Adegbola, RA

Opsonophagocytic activity of antibodies against type 6B and 19F Streptococcus pneumoniae after vaccination of HIV-infected Malawian adults with 7-valent pneumococcal conjugate vaccine (PCV)
Käyhty, H, Haikala, R, Gordon, S, Mwalukomo, T, Munthama, N, French, N

Global review of pneumococcal serotype distribution in children Under 5 years of age in the era of conjugate pneumococcal vaccination
Rodgers, G, Schranz, J

Antimicrobial resistance among Streptococcus pneumoniae isolates in the era of pneumococcal conjugate vaccine: A review of the literature
Center, K, Schranz, J

Evidence of non-PCV vaccine serotypes replacement invasive pneumococcal disease in 5 counties in Tennessee in children <2 years of age
Halasa, N, Talbot, T, Arbogast, P, Schaffner, W, Griffin, M, Craig, A

Infections in children with sickle cell disease in the pneumococcal conjugate vaccine era
Adamkiewicz, TA, Brown, K, Silk, BJ, Strayhorn, G, Farley, MM
**Symposium XI: New Vaccines and Other Alternative Prevention and Intervention Strategies**

- **S. pneumoniae** (Pnc) surface exposed glutamyl tRNA synthetase (Gts), a putative adhesin, is able to induce protective immune response in mice

- DNA vaccines based on genetically detoxified derivatives of pneumolysin fail to protect mice against challenge with *Streptococcus pneumoniae*
  - Ferreira, DM, Arêas, AP, Darrieux, M, Leite, LCC, Miyaji, EN

- Solubility and carrier potential of three non-hemolytic pneumolysin proteins

- Vaccination with non-toxic pneumolysin conjugated to capsule polysaccharide protects against IPD
  - Kirkham, L-A, Douce, G, Dilts, D, Koster, M, Mitchell, TJ

- Differential regulation of memory and primary B cell responses to pneumococcal protein antigens in children by TLR-2 against bacterial lipopeptide
  - Zhang, Q, Bernatoniene, J, Bagrade, L, Clarke, E, Paton, J, Mitchell, T, Finn, A

- Cellular responses to a candidate pneumococcal whole cell vaccine in human nasopharyngeal tonsils
  - Bagrade, L, Zhang, Q, Malley, R, Finn, A

- Efficiency of polysaccharide pneumococcal vaccine and alternative means for preventing pneumonia in recruits in Russia
  - Jogolev, KD, Zhogolev, SD, Ogarkov, PI, Dobritsa, VP, Petrov, LN

- DNA vaccines expressing PspA (Pneumococcal surface protein A) elicit protection levels comparable to recombinant protein
  - Ferreira, DM, Miyaji, EN, Oliveira MLS, Darrieux, M, Arêas, AP, APM, Ho, PL, Leite, LCC

- Expression of pneumococcal conserved protein antigens in *S. typhi* live vectors
  - Barry, EM, Santiago, AE, Davis, T, Herrera, A, Singh, S, Levine, MM

- Comparative immunogenicity of different protein D-polysaccharide conjugated vaccine formulations in rat and mouse animal models
  - Kryl, JM, Cripps, AW, Deneo, P, Godfroid, F, Poolman, J

- Intranasal immunization with cholera toxin B-pneumococcal surface antigen a fusion protein induces protection against colonization with *Streptococcus pneumoniae* and has negligible impact on the nasopharyngeal and oral microbiota of mice
  - Arêas, AP, Pimenta, FC, Miyaji, EN, Oliveira, MLS, Ho, PL, Leite, LCC

- Pneumococcal surface protein A (PspA) diversity among Indian clinical isolates

**Symposium XII: Otitis Media**

- Middle ear fluid (MEF), nasopharyngeal (NP) and oropharyngeal (OP) *Streptococcus pneumoniae* (Spn) serotype distribution in Costa Rican (CR) children with otitis media (OM)

- Antibodies to PspA families 1 and 2 in saliva but not in serum of children were associated with a lower risk of pneumococcal AOM
  - Simell, B, Melin, M, Jaakkola, T, Jousimies, K, Lahdenkari, M, Kilpi, TM, Briles, D, Hollingshead, S, Kayhty, H

- Comparison of *S. pneumoniae* (sp) carriage and penicillin resistance between vaccinated and non-vaccinated young children with acute otitis media (AOM)
  - Cohen, R, Levy, C, De La Rocque, F, Bonnet, E, Fritzell, B, Tetelboum, R, Boucherat, M, Varon, E

- Microbiology of acute otitis media with perforation (AOMwiP) in Aboriginal children living in remote communities - monitoring the impact of 7-valent pneumococcal conjugate vaccine (7vPCV)

- Effect of pneumococcal vaccination in otitis-prone children during influenza epidemics
  - Hak, E, Veenhoven, R, Uiterwaal, C, Schilder, A, Sanders, E
Pneumococcal infection in preschool children: Incidence of acute otitis media and pneumonia related to influenza and respiratory syncytial virus circulation
Jansen, AGSC, Sanders, EAM, Hoes, AW, van Loon, TM, Rovers, MM, Hak, E

Use of ototopical antibiotic treatment on chronic suppurative otitis media: A randomised controlled trial and feasibility study

Otitis media research. The Aboriginal ear and hearing health web resource and national network
Hopkins, S, Brands, J, Thompson, N, Eikelboom, RH, Nelson, S, Dearing, S, Morris, P

Middle ear fluid Streptococcus pneumoniae serotype distribution and antimicrobial susceptibility patterns in Mexican children with acute otitis media
Lopez-Enriquez, C, Espinosa-Monteros, LE, Rojas, LV, Gomez-Barreto, D

Pneumococcal vaccination and otitis media in Australian Aboriginal infants

Acute otitis media in Denmark; symptoms, treatment and control
Lous, J

Otitis media research. The Aboriginal ear and hearing health web resource and national network
Hopkins, S, Brands, J, Thompson, N, Eikelboom, RH, Nelson, S, Dearing, S, Morris, P

Middle ear fluid Streptococcus pneumoniae serotype distribution and antimicrobial susceptibility patterns in Mexican children with acute otitis media
Lopez-Enriquez, C, Espinosa-Monteros, LE, Rojas, LV, Gomez-Barreto, D

Pneumococcal vaccination and otitis media in Australian Aboriginal infants

Acute otitis media in Denmark; symptoms, treatment and control
Lous, J

The impact of pneumococcal conjugate vaccine on otitis media in the outpatient setting
Martin, SW, Cohn, A, Szilagyi, P, Griffin, M, Poehling, K, Edwards, K, Schwartz, B, Nuorti, P

Symposium XIII: Chemotherapy and Alternative Treatment Interventions

Telithromycin resistance in clinical isolates of pneumococci from Europe: Resistant mechanisms and time-kill kinetics
Reinert, RR, van der Linden, M, Al-Lahham, A

The transition in nasopharyngeal microflora accompanying the onset of otitis media in high-risk infants
Yamanaka, N, Fijihara, K, Hotomi, M, Furukawa, M, Furuya, N, Totsuka, K, Ubukata, K, Baba, S

Molecular characterization of tetracycline-resistant isolates of Streptococcus pneumoniae in Poland
Izdebski, R, Sadowy, E, Hryniewicz, W

History of prior antibiotic use before acute hospital admission for invasive pneumococcal disease in South Africa in 2003 and 2004
Quan, V, Von Gottberg, A, De Gouveia, L, Klugman, KP and GERMS-SA

Epidemiology of Streptococcus pneumoniae resistance to antimicrobial agents in Taiwan
Shieh, GJ, Hwang, B, Tang, RB

Antibiotic susceptibility of pneumococci from acute otitis media in Denmark
Lous, J, Hansen, BL, Hansen, JG, Molstad, S, Nielsen, HUK, Konradsen, HB, Frimodt-Moller, N

Hellenic study on the susceptibility of Streptococcus pneumoniae isolated from the nasopharynx of healthy children in 2004

Antibiotic consumption and the recovery of nonsusceptible pneumococci from the nasopharynx of healthy children in Greece

Serotype distribution and antibiotic resistance patterns among invasive Streptococcus pneumoniae isolates in Saudi Arabia
Shibi, A

Role of efflux pumps in fluoroquinolone-resistant Streptococcus pneumoniae and failure of current CLSI breakpoints in identifying fluoroquinolone-susceptible strains with parC mutations
Kim, YS, Park, SJ, Jun, JB, Choi, SH, Jeong, JY
### Serotype-specific real-time PCR for identification of *Streptococcus pneumoniae*
Hu, A, Colella, M, Li, F, Zhao, P, Tam, J, Rappaport, R, Cheng, S-M

<table>
<thead>
<tr>
<th>PODT.02</th>
</tr>
</thead>
</table>

### Rapid differentiation of *Streptococcus pneumoniae* serotypes 6A and 6B using multiplex real-time PCR
Hu, A, Li, F, Zhao, P, Tam, J, Rappaport, R, Cheng, S-M

<table>
<thead>
<tr>
<th>PODT.03</th>
</tr>
</thead>
</table>

### Rapid diagnosis of invasive pneumococcal infections Using Binax® Now™ Immunochromatographic test
Lalitha, MK, Aarongeyaseelan, S, Jesudason, MV, Thomas, K, Steinhoff, MC

<table>
<thead>
<tr>
<th>PODT.04</th>
</tr>
</thead>
</table>

### Comparison of citrated sheep and human blood with defibrinated horse and sheep blood as culture media supplements for the isolation and antibiotic susceptibility testing of *Streptococcus pneumoniae*

<table>
<thead>
<tr>
<th>PODT.05</th>
</tr>
</thead>
</table>

### Purification and structure characterization of the active component in the pneumococcal 22F polysaccharide capsule used for absorption in pneumococcal enzyme-linked immunosorbent assays
Skovsted, IC, Kern, MB, Sonne-Hansen, J, Sauer, LE, Nilesen, AK, Konradsen, HB, Petersen, BO, Nyberg, N, Duus, JØ

<table>
<thead>
<tr>
<th>PODT.06</th>
</tr>
</thead>
</table>

### Ability of brain heart infusion broth as a holding medium to support the growth of *Streptococcus pneumoniae* and *Haemophilus Influenzae*
Lalitha, MK, Aarongeyaseelan, S, Thomas, K, Steinhoff, MC

<table>
<thead>
<tr>
<th>PODT.07</th>
</tr>
</thead>
</table>

### Development of a four-serotype, multiplexed opsonophagocytic killing assay for detecting opsonic antibodies against *Streptococcus pneumoniae*
Burton, RL, Nahm, MH

<table>
<thead>
<tr>
<th>PODT.08</th>
</tr>
</thead>
</table>

### The impact of bacterial strains, bacteria/PMNLs ratio, complement source, and complement concentration on opsonophagocytic activity for pneumococcal serotype 19F
Yu, XH, Hu, BT, Belanger, K, Fernsten, P

<table>
<thead>
<tr>
<th>PODT.09</th>
</tr>
</thead>
</table>

### Parameters that influence the performance of HL-60 cells for *S. pneumoniae* opsonophagocytic assay
Yu, XH, Hu, BT, Belanger, K, Fernsten, P

<table>
<thead>
<tr>
<th>PODT.10</th>
</tr>
</thead>
</table>

### Effect of addition of heterologous pneumococcal polysaccharide 22F to the Wyeth/WHO pneumococcal polysaccharide ELISA on IgG assignments for infant sera
Baker, S, Hu, BT, Hackell, J, Razmpour, A, Scott, DA, Tam, JS, Fernsten, P

<table>
<thead>
<tr>
<th>PODT.11</th>
</tr>
</thead>
</table>

### Equivalence of differentiated HL60 cells and human polymorphonuclear leukocytes as effector cells in pneumococcal opsonophagocytic assay
Hu, BT, Yu, X-H, Harvey, H, Kirch, C, Fernsten, P

<table>
<thead>
<tr>
<th>PODT.12</th>
</tr>
</thead>
</table>

### Improved pneumococcal opsonophagocytic assay for increased throughput and automation
Shivaprakash, SB, Shah, RN, Jones, TR, Nahm, MH, Tam, JS, Fernsten, PD

<table>
<thead>
<tr>
<th>PODT.13</th>
</tr>
</thead>
</table>

### Apoptosis of differentiated HL60 cells and effects on *Streptococcus pneumoniae* opsonophagocytic assays
Hu, BT, Harris, SG

<table>
<thead>
<tr>
<th>PODT.14</th>
</tr>
</thead>
</table>

### Evaluating the cross-reactive immune responses to pneumococcal polysaccharide conjugates of serotypes 6A, 6B, 19A & 19F by ELISA and opsonophagocytic assay
Hu, BT, Yu, X-H, Baker, S, Fernsten, P

<table>
<thead>
<tr>
<th>PODT.15</th>
</tr>
</thead>
</table>

### Thermodynamics and stoichiometry of binding of a panel of antibodies to pneumococcal polysaccharides
Harris, SL, Fernsten, P

<table>
<thead>
<tr>
<th>PODT.16</th>
</tr>
</thead>
</table>

### Molecular identification and typing of *Streptococcus pneumoniae* direct from nasopharyngeal aspirates using mPCR/RLB
Brown, M, Fanrong, K, Wang, Y, Gilbert, L

<table>
<thead>
<tr>
<th>PODT.17</th>
</tr>
</thead>
</table>

### Validation of a multiplex pneumococcal serotyping assay (multhead typing) with clinical samples

| PODT.18 |
A multiplexed assay for simultaneously detecting multiple pneumococcal capsular polysaccharides in urine
Yu, J, Nahm, MN

Enzyme-linked immunosorbent assay for quantitative determination of capsular polysaccharide production in *Streptococcus pneumoniae* Cuban clinical isolates
Cruz-Leal, Y, Menéndez, T, Coizeau, C, Espinosa, R, Canaan, L, Blanco, F, Carmenate, T, Chang, J, Quiñones, D, Tamargo, I, Cremata, J, Vezzoni-Bencomo, V, Guillén, G

Comparison of antimicrobial sensitivity results on citrated sheep blood Mueller Hinton and citrated human blood Mueller Hinton for invasive *Streptococcus pneumoniae* clinical isolates
Seduadua, A, Mulholland, K, Carapetis, J, Buadromo, E, Russell, F

Blood cultures in pneumococcal bacteraemia: The effect of blood and broth volume
Rele, M, Buttery, J, Daley, A, Carapetis, J

Improving the sensitivity of blood culture for pneumococcus

Characterization of differentiated cell lines and implications of use in the pneumococcal opsonophagocytic killing assay
Care, RS, Gillet, M, Feavers, IM, Fleck, RA

Effect of swab composition and comparison of the use of swab and swab-containing STGG media on the culture and PCR detection of pneumococci from simulated nasopharyngeal specimens
Rubin, LG, Dayan, NE, Rizvi, A

Use of the Binax NOW® immunochromatographic test for the rapid detection of pneumococcal meningitis
Plouffe, JF, Graham, AK, Murdoch, DR

The rapid spontaneous death of *S. pneumoniae* (SP) is caused by H2O2 and can be inhibited by catalase
Regev-Yochay, G, Thompson, C, Trzcinski, K, Maalley, R, Lipsitch, M

Characterization of pneumococcal polysaccharides and serotyping of clinical isolates from *Streptococcus pneumoniae* by Fourier-transform infrared spectroscopy
Menéndez, T, Bosch, A, Rodriguéz, ME, Cruz-Leal, Y, Serra, D, Prieto, C, Canaan, L, Chang, J, Guillén, G, Yantorno, O

Interlaboratory comparison of the specific IgG response to serotypes in Prevenar

A novel method for serotyping pneumococcal strains using quantitative immunoabsorption as measured by ELISA
Eastham, V, van Niekerk, N, Little, T, von Gottberg, A, de Gouveia, L, Adrian, PV, Madhi, SA

Usefulness of dot blot test for the serotyping of *Streptococcus pneumoniae*
Brandao, AP, Gorla, MCO, Lemos, APS, Yara, TI, Zanella, RC, Almeida, SCG, De Cunto Brandileone, MC

Development of a multiplex, rapid non-culture bead based assay for serotype identification of pneumococci
Laher, G, Balmer, P, Harrison, T, Borrow, R

Assessment of anti-6A antibody response induced by serotype 6B in pneumococcal polysaccharide vaccine in Koreans
Kim, KH, Seoh, JY, Nahm, M
Symposium XIV: Strategies Towards Affordable Pneumococcal Vaccines

PneumoADIP’s strategic framework to establish the value of pneumococcal vaccination in developing countries
Lee, EH, Muhib, FB, Wonodi, CB, O’Brien, KL, Cherian, T, Moisi, J, Knoll, MD

PO14.01

PneumoADIP-driven communications efforts lead to a marked increase in media coverage around pneumococcal disease
Kvist, H, Keeling, E, Griffin, C

PO14.03

The case for routine use of pneumococcal conjugate vaccine (PCV) for HIV-Infected children in the developing world
Bliss, SJ, Levine, OS, O’Brien, KL

PO14.04

Coordinated communications efforts between 2003 and 2005 lead to a marked increase in media coverage around pneumococcal disease
Kvist, H, Haylock, S, Griffin, C

PO14.05

Global, regional, and country estimates of Streptococcus pneumoniae disease burden

PO14.06
Establishment of a randomised controlled pneumococcal vaccine trial among pregnant indigenous women in the Northern Territory of Australia

Andrews, R1,2, Moberley, S1, Raye, S1, Dunbar, M4, Leach, A1, Balloch, A1, Hopkins, S1, Hare, K1, Tang, M1, Morris, P1, Carapetis, J2, Mulholland, K1

1Centre for International Child Health, University of Melbourne, VIC, Australia
2Centre for Clinical Research Excellence in Child and Adolescent Immunisation, Murdoch Children’s Research Institute, VIC, Australia
3Menzies School of Health Research, Charles Darwin University, NT, Australia
4Department of Immunology, Royal Children’s Hospital, VIC, Australia

Background: Australian Indigenous children have the highest rates of acute and chronic ear infections in the world, leading to permanent ear damage, hearing and educational disadvantage for many children. *Streptococcus pneumoniae* is the predominant pathogen. Colonisation and infection begins within days of birth, months before any potential immunological protection from infant pneumococcal conjugate vaccine (PCV) may be expected. Previous studies of maternal pneumococcal polysaccharide vaccination (PPV) have shown elevated antibody levels in breast milk, cord blood and in infant serum but no trial has evaluated clinical outcomes in a population at high risk of early onset ear disease.

Methods: We developed a study protocol for a randomised controlled trial to assess efficacy of maternal PPV against infant ear disease and nasopharyngeal carriage of vaccine type pneumococci at 7 months of age. Utilising the results of a feasibility study from another trial that did not proceed, we opted to apply for NHMRC funding prior to undertaking community consultation. We then sought ethics approval for the initial community consultation and appointed our project team, including an Aboriginal doctor and Aboriginal Health Worker who led the community consultation.

The study design was for 210 Indigenous women to receive the 23 valent PPV either during the third trimester of pregnancy, shortly after delivery, or 7 months after child birth (control group). Adult diphtheria-tetanus-acellular pertussis (dTpa) will be used as the control vaccine for the delivery dose. The sample size is designed to detect a 23% reduction in infant ear disease and 45% reduction in carriage of 23vPPV types. Interactions with childhood PCV will be assessed immunologically.

Progress: Following receipt of NHMRC funding, consultation conducted over an eight-month period has resulted in the formation of an independent Data Safety Monitoring Board (DSMB), an Indigenous Reference Group (IRG), and trialling of community information. The DSMB and IRG have endorsed the study protocol and adverse events monitoring and ethics approval has now been obtained to commence recruitment (planned for January 2006).

Conclusions: Benefits from therapeutic and preventative strategies for otitis media in this high risk population have been small. Maternal immunisation offers the potential to reduce early and therefore recurrent infection. This study will contribute data to meta-analyses and potentially lead to a larger trial with additional pneumococcal disease outcomes.

Activities of Reference Laboratory in South Asian Pneumococcal Alliance Network

Lalitha MK1, Ajoykumar P1, Thomas K2, Steinhoff MC3 and the SAPNA Networking Centres

1Department of Clinical Microbiology, 2Department of Medicine, Christian Medical College and Hospital, Vellore, India. 3Department of International Health, Johns Hopkins University Baltimore USA

Background

South Asian Pneumococcal Alliance (SAPNA) was established since January 2004 to monitor the change in serotype distribution and the Antimicrobial Resistance (AMR) patterns of *S. pneumoniae* in South Asian countries. There is a marked difference in the serotype distribution and the AMR pattern of *S. pneumoniae* from India when compared with the data available from the Western and American countries. The currently used 7-valent vaccine does not cover the major pneumococcal serotypes found in India. Hence this study was initiated to monitor the distribution of serotypes and AMR patterns in the South Asian region.

Methodology

Participating centers from Srilanka and Nepal prospectively recruit patients less than 5 years old with suspected invasive pneumococcal disease. The participating laboratories process the clinical specimens and isolate pneumococci. These centers send the isolates to the reference laboratory for further confirmatory tests and phenotypic and molecular characterization. In order to monitor the quality of service provided by the participating laboratories, a continuous ongoing External Quality Assessment Scheme (EQAS) was conducted to focus on problem finding and provide data on the accuracy of the test performance/interpretation at microbiology laboratories. Twelve unknown coded strains including *S. pneumoniae* (Penicillin susceptible and Resistant), *H. influenzae* (Ampicillin susceptible and Resistant), *H. parainfluenzae*, *S. aureus* (MRSA), *K. pneumoniae* (ESBL producer) β haemolytic Streptococci Group A and C (Penicillin Susceptible) were sent to the collaborating laboratories once in every 3 months as part of the EQAS program. *S. pneumoniae* and *H. influenzae* were repeatedly sent.

Results

As part of the reference laboratory activities 31 isolates of pneumococci from invasive sources including blood and CSF were confirmed, serotyped and their antimicrobial susceptibility pattern determined. It was observed that the laboratories maintained the quality and interest in the participation of the EQAS program. The laboratories could identify the isolates sent; however, only one laboratory could detect penicillin resistance in pneumococci. Similarly ampicillin resistance in *H. influenzae* was detected by only one laboratory. It was also clear that the participating laboratories generate disk diffusion results than determining the MIC values for the resistant isolates.

Conclusion

The study has generated important information on the pneumococcal data in Nepal and Srilanka. This information suggests distinct serotypes in each of the participating countries. It is important to establish the proficiency testing of laboratories and also, to promote accuracy of the antimicrobial susceptibility data generated.
Preliminary data on invasive pneumococcal disease in Nepal by Sapna Network

Singh AS, Sharma PR, Tuladhar N, Gami FC, Dahal M, Thomas K, Lalitha MK, Steinhoff MC

1Institute of Medicine and Kanti Children Hospital, Kathmandu, Nepal
2Christian Medical College Vellore, India
3Johns Hopkins Bloomberg School of Public Health, Baltimore, USA

Background: SAPNA, the Sanskrit word meaning 'Dream' is the acronym for the "South Asian Pneumococcal Alliance". Our dream is to protect children from invasive pneumococcal disease by generating local epidemiological data on serotypes and antimicrobial resistance, and to assist in development of national policy for the control of pneumococcal disease. This project is supported by GAVI through the Pneumococcal ADIP.

Method: Children from 2 months to 5 years with suspected invasive pneumococcal disease were recruited from Kanti Children Government Hospital, Kathmandu, the largest children hospital of Nepal with 7,000 admissions per year. Blood, CSF and other relevant normally sterile body fluids were collected and evaluated with cell counts, bacterial culture, antigen testing (latex agglutination test (LA) & Binax test), antimicrobial susceptibility testing and serotyping. Three monthly newsletters were released to disseminate study data and regular meetings with policy makers and other important stakeholders were conducted.

Results: From November 2004 to November 2005, a total of 1,168 children with suspected invasive pneumococcal disease were recruited: 913 (78.2%) were pneumonia, 123 (10.5%) were meningitis, 24 (2%) were sepsis and 118 (9.2%) had other diagnoses. 25.3% (296) were less than 6 months of age, 53.8% (629) were >6 months to 2 years, 20.8% (243) were >2 years to 5 years. A total of 27 patients had Streptococcus pneumoniae identified as the etiological agent; 15 were positive by culture and 12 additional cases were detected by LA and/or Binax test. In 5/27 (19%) cases both culture and LA were negative, but Binax was positive.

The most common serotypes were 1, 39, 5, 18F and 23F. Among all the invasive isolates 8% were resistant to Penicillin and 80% were resistant to Cotrimoxazole.

Conclusion: The SAPNA project has for the first time generated standard information on serotypes and antimicrobial resistance pattern of invasive pneumococcal isolates from Nepal. Additional serotype information is needed to determine the best combination of serotypes for a pneumococcal vaccine for Nepalese children.

Ethnic disparity in the burden of invasive pneumococcal disease in children aged less than 5 years in Fiji

Colquhoun SM, Russell FM, Carapetis JR, Tikodadua L, V L, Pryor J, Waqatakiwrea L, Mulholland EK

1Centre for International Child Health, Department of Paediatrics, University of Melbourne, Melbourne, Victoria, Australia
2Fiji Ministry of Health, Suva, Fiji Islands
3Fiji School of Medicine, Suva, Fiji Islands
4London School of Tropical Medicine, London, United Kingdom

Because there are no invasive pneumococcal disease (IPD) burden data available for children in Fiji, a retrospective laboratory based study was undertaken at the Colonial War Memorial Hospital, Suva. We included all children aged <5 years age living in the Central Medical division and fitting the IPD case definitions developed for this study. The incidence of IPD was calculated by three methods to adjust for potential missed cases due to poor laboratory technique.

The annual incidence of laboratory confirmed IPD was 45.7/ 100,000 children <5 years of age. The incidence of S. pneumoniae meningitis was 24.2/ 100,000 children <5 years of age, but could be as high as 44.7/100,000 if potential purulent and clinical cases were included. The case fatality rate in children < 5 years was 20% for IPD and 26.9% for S. pneumoniae meningitis. S. pneumoniae made up 45.6% of positive CSF and sterile site isolates identified. In the three year study period there were 47 indigenous Fijian IPD cases and three among Indo Fijian or other children giving an incidence rate ratio of 21.6 (95%CI 6.93-108.66, p< 0.001). The incidence of IPD was 104.5/100,000 children <5 years of age in the Indigenous Fijian population compared to 4.8/100,000 in the combined Indo-Fijian and other population.

Differences in disease incidence and case fatality between indigenous Fijians and Indo-Fijian’s were evident in all analyses of this study. This disparity in pneumococcal disease burden by ethnicity has also been demonstrated with radiologically confirmed pneumonia in children in Fiji, and in other Pacific island countries and New Zealand. This ethnic disparity may be due to genetic factors, differences in health seeking behaviour, overcrowding or other unknown factors.
Multicentre bronchiectasis study
A collaborative and international study of bronchiectasis in Indigenous children

International Collaboration of Bronchiectasis Network

Arctic Investigations Program – CDC, Alaska, US - Singleton, R, Thomas, T
Menzies School of Health Research, Darwin, Australia - Morris, P, Leach, A
Northern Territory Health Service, Alice Springs, Australia - Roseby, R, White, A
Queensland Institute of Medical Research, Brisbane, Australia - Valery, PC, Stirling, J
Starship Children's Hospital, Auckland, New Zealand - Edwards, EA, Byrnes, CA
Royal Children's Hospital, Brisbane, Australia - Chang, AB, Masters, IB, Masel, J
Royal Darwin Hospital, Darwin, Australia - Bauert, P
Royal Prince Alfred Hospital, Sydney, Australia - Torzillo, PJ
University of Washington School of Medicine, Seattle, US - Redding, G
Wellington School of Medicine and Health Sciences, Wellington, New Zealand - Grimwood, K, Murdoch, J, Leadbitter, P

Background
Chronic suppurative lung disease (CSLD) and bronchiectasis (Bx) still contribute to the high burden of respiratory disease in Aboriginal Australians and Indigenous children worldwide. The risk factors associated with progression to Bx, the clinical course and optimal treatment in this population are not known. Australian Aboriginal children have high rates of pneumococcal carriage, otitis media and invasive pneumococcal disease. The significance of this in terms of the natural history of Bx is unknown. The high rates of CSLD among Indigenous populations in affluent countries has resulted in the first collaborative and international study (Aboriginal and Torres Strait Island, New Zealand Pacific Island and Maori and Alaskan Native people).

Aims
Two studies are proposed. The aims of the observational study are to: (1) define the natural history of chronic moist cough and Bx; (2) identify the risk factors associated with progression to Bx.

The aims of the intervention study are to evaluate maintenance azithromycin (30 mg/kg once a week) compared to placebo on (1) the prevention of pulmonary exacerbations (2) pneumococcal and H. influenzae carriage and (3) antibiotic resistance.

Methods
The study design is identical in the participating countries.

Observational: a prospective cohort study (2005-2010) of Indigenous children aged 6 months to 8 years with Bx or chronic moist cough (with and without CXR infiltrates).

Interventional: a randomised double-blind placebo controlled trial comparing maintenance azithromycin for 24 months versus placebo in Indigenous children aged 12 months to 8 years with Bx. Primary outcomes are: number of pulmonary exacerbations and time to pulmonary exacerbation.

Results
The Observational study has commenced in all sites; to date Australia and Alaska have enrolled 12 children; we expect to identify 100-150 eligible children. NHMRC funding has been approved to start the Australian Interventional study site in 2006. We expect to randomise over 100 children (including all sites).

Conclusion
This will be the first study to prospectively document the clinical course of chronic moist cough and Bx in Indigenous children. The associated clinical trial will provide urgently needed information about the potential benefits and harms of maintenance antibiotic treatment. The project has the potential to improve health outcomes for Indigenous children and other disadvantaged children with CSLD throughout the world.

Funding: TELSTRA Foundation (seeding grant 2004-2005) and NHMRC grant 389837 (5-year grant for 2006-2010) fund the Australian study. National Health and Medical Research Council Public Health (Australia) Training Fellowship (NHMRC ID 339461)(PCV). NARCH (2005-2008) funds the Observational study of Alaska Native Children. NZ funding applications pending from HRC IIOF and ARFNZ.
A Study of Invasive Pneumococcal Disease at the University College Hospital (UCH), Ibadan, Nigeria

PO2.05

Falade, AG, Adegbola RA, Bakare RA, Odekunmi A

1 College of Medicine, University of Ibadan and University College Hospital, Ibadan, Nigeria
2 Medical Research Council Laboratories, Fajara, The Gambia

Background: Streptococcus pneumoniae remains the biggest cause of child ill health and death in the world. Successful introduction of Pneumococcal Conjugate Vaccine in the developing countries will depend on clear definition of bacterial causes of pneumonia and prevalent serotypes of S. pneumoniae.

Aim: To develop capability for isolating pneumococcus and other fastidious bacteria from samples of blood and cerebrospinal fluid (CSF) and determine the serotypes and antimicrobial resistance pattern of S. pneumoniae that is commonly found from patients presenting at the University College Hospital (UCH), Ibadan, Nigeria.

Methods: During an 8-month period (February 1 through September 30, 2005), children aged between 2 and less than 60 months, seen at the UCH, Oni Memorial Children Hospital and Adeoyo Maternity Hospital, Ibadan with possible pneumococcal syndrome were enrolled into the study. For all the children, at least 1.0ml of blood was placed in each of the 2 blood culture bottles for aerobic and anaerobic cultures. Processing of these blood cultures and CSF obtained from children with possible meningitis was according to standard procedures. Pneumococci and Hib were transported to the Medical Research Council (MRC) Laboratories for confirmation, further characterization and serotyping.

Results: There were 133(21.6%) pathogenic isolates from the 617' blood cultures. S. pneumoniae, 8(6%) and Haemophilus influenzae type b (Hib), 6(4.5%) were recovered from the blood. Major isolates from the blood were Staphylococcus aureus, 43(32.3%) and Salmonella species, 29(21.8%). As for the CSF, there were only 12 isolates (4.2%) from the 285 samples. These comprised Hib, 7(2.5%) and S. pneumoniae, 2 (0.7%); and 1 isolate each of Klebsiella species, Pseudomonas aeruginosa and Salmonella sp. One of the two isolates from blood characterized as S. pneumoniae in the UCH Laboratory was confirmed by MRC Laboratories to be S. pneumoniae (non-typeable) while the Hib from the CSF was regarded as a contaminant. The remaining isolates of S. pneumoniae (8) and Hib (12) were lost during storage in the period April through September, 2005 and status could not be confirmed.

Conclusion: The study has demonstrated the capability of this centre in culturing fastidious organisms although, there was a problem in keeping them. The apparent low rate of isolation of pneumococcus and Hib may not be unconnected with high rate of antibiotic abuse.

Dynamics of pneumococcal carriage among Warao children in the Delta Amacuro in Venezuela.

PO2.06

Rivera-Olivero, I, Bogaert D, Bello, T, del Nogal, B, Sluijter, M, Hermans, P, de Waard, JH

1 Instituto de Biomedicina, Caracas, Venezuela
2 Department of Paediatrics, Erasmus MC-Sophia, Rotterdam, The Netherlands

Aims: To determine the temporal stability of serotypes and genotypes of Streptococcus pneumoniae carriage in Warao children in 3 distinct geographically isolated communities.

Methods: In two investigation periods, separated for at least 6 months, a nasopharyngeal swab was cultured from a total of 327 children aged 0–5 years old months living in 3 villages. S. pneumoniae isolates were serotyped and their susceptibility for penicillin assessed by disk diffusion and broth microdilution method. The isolates were genotyped by restriction fragment end labeling (RFEL) analysis and the data were compared with the 26 RFEL types represented by the Pneumococcal Epidemiology Network and approximately 1200 RFEL genotypes present in the Dutch RFEL data library.

Results: In total, 131 pneumococcal isolates were collected in the two investigation periods. The overall nasopharyngeal carriage rate for S. pneumoniae in the first and second period was 46.5% and 32.9% respectively. 41 % of the isolates was intermediate susceptible to penicillin in the first sample and 28% in the second sample. The most important capsular serotypes in first period were 23F (21.6%), 6A (18.1%), 15B (16.2%), 6B (10.8%) and in the second period 23F (15.6%) and 6A/6B (17.8%) each. The theoretical coverage by the 7-valent conjugate vaccine including the cross-reactive serotype 6A in two investigation periods was 55.4% and 69% respectively. 115 isolates were available for genotyping. In the first investigation period 75 isolates showed 42 different genotypes, with 52 isolates (69%) in 14 distinct genetic clusters. In the second period 40 isolates showed 26 different genotypes with 31 isolates (76.9%) in 8 distinct genetic clusters. In total 68 genotypes were found and only 13 genotypes were present in both periods. 19 isolates of the most important genotype serotype 23F with RFEL type 4, was found in both periods (15 and 4 isolates respectively). The other genotypes that maintained in time were only found once or twice in each investigation period.

Conclusions: The theoretical coverage by the 7-valent conjugate vaccine differed significantly in each period. Clustering in both periods was high indicating ongoing transmission. Only 13 genotypes maintained in time in these geographically isolated communities which probably indicates that an important unknown reservoir not has been sampled.
Evolution of highly lethal epidemic pneumococcal meningitis in Burkina Faso

PO2.07

Yaro, S1, Koeck, JL2, Lourd, M4, Nacro, B4 for the Clinical Group, Ouedraogo, M1, Idouhou, R3, Lafourcade, B1, Hien, A1 for the Laboratory Group. Traore, Y1, Gessner, BD1

1Centre Muraz, Bobo-Dioulasso, Burkina Faso
2Laboratoire de Biologie Clinique, Bordeaux, France
3AMP, Paris, France and Bobo-Dioulasso
4Centre Hospitalier Universitaire Sanou Souro, Bobo-Dioulasso, Burkina Faso
5Université de Ouagadougou, Ouagadougou, Burkina Faso

Background. Historically, empiric case management and public health response strategies to meningitis epidemics in sub-Saharan Africa have been based on the assumption that the great majority of disease is due to the meningococcus.

Methods. During March 2002 through February 2003 and May 2004 through April 2005, we collected clinical and laboratory information for suspected bacterial meningitis cases from three districts of Burkina Faso. Streptococcus pneumoniae (Sp) was identified by culture, polymerase chain reaction, or antigen detection on cerebrospinal fluid (CSF). Pneumococcal genotyping was performed on strains using multiple loci variable number tandem repeat typing.

Results. Of 1,686 persons from whom CSF was obtained, 249 (15%) had Sp identified (annual incidence, 14 per 100,000) making Sp the most commonly identified organism. During the epidemic meningitis season (December-April), an average of 38 Sp cases per month were identified compared to 8.7 during other months; this occurred despite no seasonal change in the proportion of lumbar punctures yielding cloudy CSF or of cloudy CSF yielding Sp. Of 45 tested pneumococci, serotype 1 was identified for 21 (47%), including 19 during the epidemic seasons. Persons aged ≥5 years were at greater risk of serotype 1 infection than children <5 years (RR, 3.6; 95%-CI, 1.3 to 11). The genotypes of serotype 1 isolates were closely related but diversified over time. Sp incidence decreased from 16 to 12 per 100,000 persons during 2002-3 to 2004-5 while the proportion due to serotype 1 decreased from 58% to 29%. The case fatality ratio for pneumococcal meningitis was 46%. Sp was responsible for 68% of all acute bacterial meningitis deaths including 60% and 89% among persons during 2002-3 to 2004-5 while the proportion due to serotype 1 decreased from 58% to 29%. The case fatality ratio for pneumococcal meningitis decreased from 16 to 12 per 100,000 persons during 2002-3 to 2004-5 while the proportion due to serotype 1 decreased from 58% to 29%. The case fatality ratio for pneumococcal meningitis was 46%. Sp was responsible for 68% of all acute bacterial meningitis deaths including 60% and 89% among persons <5 and at least 15 years of age, respectively.

Conclusions. Intervention strategies during the epidemic season in Burkina Faso and perhaps elsewhere must now account for highly lethal epidemic Sp disease. Almost half of cases were due to serotype 1 that appeared to preferentially cause disease among older children and adults; however, the proportion of cases due to other serotypes increased substantially over time despite relatively small decreases in disease incidence.

Rates of hospitalization and mortality for pneumonia and respiratory illness in China

PO2.08

Zhao, GM1, Black, S2, Shinefield, H3, Eskola, J4

1School of Public Health, Fudan University, Shanghai, P.R. of China
2The Kaiser Permanente Vaccine Study Center, Oakland, California, USA
3University of California San Francisco, USA
4KTL, Helsinki, Finland

Acute respiratory tract infection (ALRI) is a major public health disease burden killing approximately 200 million children annually, most of these less than one year of age in developing countries. However, the burden of this disease is not well understood in children in China. In order to understand rates of hospitalization and mortality for pneumonia and respiratory illness, we conducted surveillance at sites established between 1989 and 2003 in the Suzhou district of Jiangsu province. Pneumonia accounted for 25-37% of the total disease burden for children admitted to the medical wards each year and was highest in those under two years of age. The number of admissions for pneumonia each year has remained relatively constant from 1999-2003 for which complete data are 2924, 3459, 3464, 3468 and 2584 respectively. The corresponding numbers of deaths with pneumonia in the same periods were 17, 14, 10, 3 and 6. This includes mortality in the hospitals in the province as well as mortality identified in rural clinics. Data is also available from an earlier national respiratory disease mortality study conducted from 1991 to 1993. This study was based upon a national stratified sample of children less than five years of age in both rural and urban areas. In this study the pneumonia related mortality was 1562 cases/100,000 person-years in the rural area and 266.4 cases/100,000 person-years in urban areas with 85% of these deaths occurring in the first year of life. Overall, pneumonia was ranked as the leading cause of death in the first five years of life. In a roughly concurrent study in Shuan Feng County sponsored by UNICEF in children four years of age, pneumonia incidence was 3370 cases/100,000 children in one year with mortality of 469 deaths per 100,000 children. Importantly this study was felt to underestimate mortality since patients with pneumonia were not reliably reported if they died at home. It would appear, therefore, that over the intervening decade that while pneumonia still remains very common, mortality has decreased substantially. We conclude that pneumonia is a major problem in young children in China and that a vaccine program successful in preventing a significant proportion of respiratory illness in these children would provide substantial benefit.
Pneumococcal meningitis: surveillance data for Buenos Aires Province, Argentina

Verzeri, LN1, Rosa, S1, Brizuela, L2, González Ayala, SE2
1Surveillance System, Buenos Aires Province, Argentina
2Infectious Disease Department, School of Medicine, La Plata National University, Argentina

Background and aims: The surveillance of meningitis based on notification reports is useful in order to know the occurrence (age group, area, causative agent) and to detect shifts in the prevalence. Meningitis is a communicable disease and must be notified within 24 hours of admission according to the national law.

Methods: The Surveillance System, Ministry of Health, Buenos Aires Province, has electronic data since 1993. Population was 12,594,974 in 1991 and 13,827,203 in 2001. The meningitis case is notified immediately by phone, fax or e-mail, and a Form is completed at the patient’s discharge from hospital. Data is processed by EpiInfo 6.04. We present data for period 1995-2004.

Results: The number of cases ranged from 30 to 147 and the global incidence rate / 100,000 inhabitants from 0.3 (1999) to 1.1 (1995, 1996). Children < 1 year had the highest incidence rate / 100,000 which varied between 20.0 (2003) and 5.8 (1999) followed by age group 1 – 4 years, 1.7 (2003), and, 0.5 (1999). The incidence rate / 100,000 for children age 5-9 years ranged from 0.5 (1999) to 1.3 (1995); for children 10-14 years, 0.1 (1999) to 0.7 (1996, 2002); for adolescents and adults 15-49 years 0.1 (1999) to 0.8 (1996); and for persons ≥ 50 years, 0.2 (1999) to 0.9 (1997, 2003, 2004). The mortality rate had a wide range from 20% (1998) to 4% (2000), average 12.1%. Streptococcus pneumoniae was the first causative agent of bacterial meningitis in children < 1 year for the last three years, and the incidence rate for year 2003 was similar to that for H. influenzae b before universal vaccination.

Conclusion: Pneumococcal meningitis is a significant health problem. The information on the burden of disease by age group will help to assess for intervention strategies.

Pneumococcal disease – a major burden in children: could the introduction of pneumococcal conjugate vaccine (PCV) help to reduce this burden in Papua New Guinea?

Francis, JP1, Phuanukoonnon, S1, Pomat, WS1, Reeder, J1, Lehmann, D2
1Papua New Guinea Institute of Medical Research, Goroka, Eastern Highlands Province, Papua New Guinea
2Telethon Institute for Child Health Research, Centre for Child Health Research, The University of Western Australia, Perth, WA, Australia

In Papua New Guinea (PNG), pneumonia is the most common cause of death and reason for hospitalisation of children. In the Asaro Valley, Eastern Highlands Province, the infant mortality rate for pneumonia is 2500/100,000 annually; 56% of deaths aged <6 months are due to pneumonia. The pneumococcus (Pnc) accounts for 46% of bacteraemic pneumonia in children aged <5 years, 26% and 63% occurring before age 3 and 6 months, respectively. Pnc also causes ~45% of bacterial meningitis, 69% of cases being <6 months of age. The very early onset of upper respiratory tract (URT) Pnc carriage (median age of acquisition = 17 days) puts young infants at high risk of early invasive pneumococcal disease (IPD) and may have long-term effects on the development of protective immunity. Intermediate resistance to penicillin is common and increasing. The serogroups included in the licensed 7-valent Pnc conjugate vaccine (PCV) (4, 6, 9, 14, 18, 19 and 23) account for 52% of bacteraemic Pnc pneumonia and 32% of Pnc meningitis; equivalent figures for the 23-valent Pnc polysaccharide vaccine (PPV) are 80% and 78%, respectively. Studies in PNG during the 1980s showed that PPV given at age 6 months to 5 years prevents death from pneumonia (efficacy 59% and 50% in children aged <5 and <2 years, respectively) and affords some protection against severe disease. Serotype-dependent maturation of antibody responses support efficacy data with 2-fold rises in IgG titres one month post-PPV at ages 5, 6, 9 and 12 months for serotypes 2, 7F, 23F and 5, respectively, but only after 18 months for serotypes 6B, 14 and 19F. To overcome the enormous burden of IPD, particularly in very young infants, the use of Pnc vaccines must be considered. Pnc vaccines are not yet available in PNG. In May 2005 we began a randomised controlled trial to determine the safety and immunogenicity of PCV in early infancy: 100 infants receive PCV at 0-1-2 months, 100 at 1-2-3 months and another 100 children are not given PCV; all children receive PPV at age 9 months. To date more than 60 children have been enrolled. This study will provide proof of principle of the safety and immunological feasibility of neonatal PCV immunization, which is essential before progression to larger-scale studies in high-risk populations.
Pan Asian review of pneumococcal disease: Regional patterns of disease burden among children and adults to better understand the value of vaccination

Kilgore, PE1, Nyanbat, B2, Han, SH2, Jodar, L3, Chang, JH4, Clemens, JD1
1Division of Translational Research, International Vaccine Institute, Seoul, South Korea
2Division of Laboratory Sciences, International Vaccine Institute, Seoul, South Korea

Background. Globally, pneumococcal disease accounts for an estimated 1.6 million deaths each year including ~841,000 deaths among children <5 years of age. Populations living in Asian countries have 3.2 billion persons representing approximately 53% of the world’s population. We undertook a systematic review of published literature to describe our present understanding of pneumococcal disease among Asian countries.

Methods. We accessed English and non-English scientific literature using available computerized databases, Medline, the National Library of Medicine (PubMed), publisher-specific databases and other sources within the Asian region for the period 1966 through 2004. Studies were described by country, year of study, patient characteristics, and pneumococcal-associated laboratory findings.

Results. A total of 57 Asian countries and territories were identified from World Bank, Asian Development Bank and World Health Organization databases for inclusion in this review. Among countries in this group, Australia, Japan, China, Hong Kong SAR, New Zealand, Papua New Guinea, India, South Korea, Thailand, VietNam and Malaysia had the greatest number of published reports on pneumococcal disease. The majority of studies were hospital-based clinical investigations or laboratory providing analysis of pneumococcal isolates. S. pneumoniae was a leading cause of bacterial meningitis in children as well as a common isolate among children with acute respiratory tract infections. Pneumococcal resistance and serotype distribution is well-documented in several locations but substantial gaps remain in our understanding of these issues in Asia. Relatively fewer studies were identified describing patients with pneumococcal bacteremia or otitis media. Despite the growing epidemic, very few studies have systematically assessed the impact or extent of pneumococcal infections among Asian children or adults with HIV/AIDS.

Conclusions. Since the late 1980s, substantial growth has occurred in our knowledge base of pneumococcal disease in Asia. However, given the enormous global annual toll of this pathogen in both children and adults, our current understanding of pneumococcal disease in several lower income countries is limited. Regional expertise in pneumococcal disease should be leveraged to provide capacity-strengthening to Asian countries that urgently need development of national laboratory-based surveillance and disease burden studies.

Epidemiology of pneumococcal meningitis in children in the district of Colombo, Sri Lanka: A prospective, population based surveillance study

Batuwanthudawe, BKR1, Rajapakse, LC2, Abeyesinghe, MRN2, Somaratne, P3, Dassanayaka, M4
1Epidemiology Unit, Ministry of Health, Colombo, Sri Lanka
2Department of Community Medicine, University of Colombo, Colombo, Sri Lanka
3Department of Microbiology, Medical Research Institute, Colombo, Sri Lanka
4Department of Microbiology, Lady Ridgeway Hospital for Children, Colombo, Sri Lanka.

Abstract

Objective To describe epidemiology of childhood invasive pneumococcal infections in the District of Colombo (Population – 2,250,000), Sri Lanka

Methodology

All children under 5 years of age with a probable diagnosis of meningitis and septicemia admitted to all 12 Consultant Paediatrician units in the Colombo District were studied in 2004. A latex agglutination (LA) test (Wellcogen, Murex for Remel – UK) which detected five bacterial antigens in CSF was performed on all CSF specimens to identify infections. CSF culture and blood culture also was performed. The estimated population of under five children in Colombo district for the year was 179,103.

Results

During the year 2004, 1146 lumbar punctures (LP) were performed in under fives in participating units (LP rate of 1.89%). Incidence of CSF suggestive of meningitis was 333 cases or 90.5 cases per 100,000 under fives, out of which 108 (32%) had a confirmed aetiological agent by LA and / or by culture. Haemophilus influenzae type b (Hib) accounted for 50% (54 cases) of the meningitis cases. Streptococcus pneumoniae was detected in 14 (13%) positive CSF specimens, 6 by both culture and latex, and 8 by latex only. The study reports an incidence rate of 7.8 pneumococcal meningitis cases per 100,000 under fives (95% CI = 3.5 – 15.76). Nine cases (64%) were in 4 -12 months group and no cases under 4 months of age, an incidence rate of 26.5 (95% CI = 77.6 – 105.6) cases per 100,000 under 12 months. Out of 57 isolates from a total of 2,374 blood cultures, there were 19 Hib and only 3 (0.13%) pneumococci.

Conclusions

A relatively low proportion of the specimens are culture-positive for pneumococcus, and LA test detected more pneumococcus in CSF than culture alone. However, relatively high sampling rates in ill children, suggest that clinical specimen handling and microbiological procedures can be modified to achieve higher rates of bacteriological isolation. Continuing the use of antigen detection in CSF, and addition of PCR detection of bacteria and viruses will improve disease burden estimates for this population, including pneumococcal vaccine-preventable disease.

Acknowledgments: WHO/SEARO for funding the study under Hib surveillance. PneumoADIP through South Asian Pneumococcal Network (SAPNA) for technical and financial assistance in continued Pneumococcal surveillance in Sri Lanka

Key words: Streptococcus pneumoniae, meningitis, epidemiology, incidence, Sri Lanka
Clinical and Laboratory characteristics of pneumonia with radiological consolidation in Gambian children

Enwere G1, Zaman A2, Akano A2, Oluwalana C3, Okoko JB1, Vaughan A1, Biney EEO1, Greenwood BMG1, Adegbola RA1, Cutts FT4

1Medical Research Council Laboratories, The Gambia
2National Hospital Abuja, Nigeria
3London School of Hygiene and Tropical Medicine, London

Background: The WHO radiology working group has standardised the definition of radiological pneumonia as the presence of consolidation or pleural effusion on radiograph for use in epidemiological studies and vaccine trials. We compared the clinical and laboratory characteristics of children with consolidation/effusion (henceforth called consolidation) according to the WHO definition with those of children with clinical pneumonia without consolidation.

Methods: During the course of a large phase 3 trial of pneumococcal conjugate vaccine in The Gambia, we recorded detailed clinical information from ill children. A chest x-ray was obtained for those with WHO-defined clinical pneumonia (raised respiratory rate for age or lower chest wall indrawing). The films were read following the WHO radiology working group procedures and definitions.

Result: Nine thousand and seventy clinical pneumonia cases were x-rayed, out of which 1156 (12.8%) had radiological consolidation. Children with consolidation were younger than those without (median age 13.2 months versus 14.4 months). Cough, fast and difficult breathing, reduced feeding and vomiting were more frequently reported in children with consolidation on x-ray than those without. Children with radiological consolidation were more likely to have diarrhoea or convulsions, and laboratory evidence of malaria infection. A primary diagnosis of pneumonia was made more often in children with consolidation than those without (72.6% versus 51.5%).

Conclusion: Children with consolidation on chest x-ray had more marked respiratory signs and were more seriously ill than those without. The higher proportion of laboratory evidence of malarial infection in children without consolidation further highlights the place of malaria as a confounder in the diagnosis of clinical pneumonia.

A cohort study to assess quality of life in young Fijian children who have a history of bacterial meningitis

Colquhoun, S M1, Russell, F M2, Carapetis, J R2, Tikoduadua, L V3, Pryor, J1, Wake M5, Mulholland, EK1,4

1Centre for International Child Health, Department of Paediatrics, University of Melbourne, Melbourne, Victoria, Australia
2Fiji Ministry of Health, Suva, Fiji Islands
3Fiji School of Medicine, Suva, Fiji Islands
4London School of Tropical Medicine, London, United Kingdom
5Centre for Community Child Health, Royal Children’s Hospital, Melbourne, Australia

A cohort study was undertaken to pilot the Paediatric quality of life questionnaire (PedsQL) in Fiji to validate its use in a developing country and to document the health related quality of life in children over two years of age with a history of bacterial meningitis, with specific reference to children with pneumococcal meningitis.

Children with a history of bacterial meningitis identified in a previous retrospective bacterial meningitis study undertaken at the Colonial War Memorial Hospital (CWMH), Suva, Fiji were included as the exposed group. Medical records were examined and local maternal child health clinics were checked to ascertain if the children identified in this study were still living. Each child with a history of bacterial meningitis was matched with 4 non-exposed children for age and ethnicity that were recruited from the locality as the exposed child. All parents were asked a validated standardized quality of life questionnaire regarding their child’s functioning (PedsQL). A score was calculated for each participant using an internationally validated Standard PedsQL scoring system.

Of the 84 cases identified in the original study 35 had since died, 2 could not be located, 1 declined to participate and 9 had not yet reached 2 years of age. There were 37 exposed and 148 non-exposed participants included in this study. In exposed group 24% (n=9) had a history of S. pneumoniae meningitis, 24% had culture positive laboratory result from another pathogenic organism. The remaining 52% (n=19) had a sterile but purulent cerebrospinal fluid laboratory result.

The PedsQL score calculated was found to be lower in the exposed group (median 86, IQR 71-97), compared to non-exposed group (median 97, IQR 91-100) The children with a laboratory proven history of Pneumococcal meningitis showed a median score of 71 with an IQR 55-81.

These preliminary results show that the health related quality of life score for children in Fiji who have a history of bacterial meningitis is diminished compared to the score obtained from their healthy peers. The scores obtained from the non-exposed group in this study correlate with internationally validated scores suggesting that the PedsQL is a valid tool to measure health related quality of life in a developing country setting.
Epidemiological and clinical characteristics of community acquired invasive bacterial infections in children aged 2-29 months in The Gambia

Enwere G1, Biney EEO1, Cheung Y1, Zaman A1, Okoko JB1, Oluwalana C1, Vaughan A1, Greenwood BMG3, Adegbola RA1, Cutts FT1

1Medical Research Council Laboratories, The Gambia
2London School of Hygiene and Tropical Medicine, London

Background: The incidence of Community Acquired Bacteremia (CAB) in Africa is 10-20 fold higher than in industrialised countries. We report here the incidence of invasive bacterial infections in rural Gambia and compare the clinical characteristics of children with pneumococcal infection with those of children with extra-intestinal non-typhoid salmonella infection (NTS) or other bacterial infections.

Methods: As part of a pneumococcal vaccine trial, we investigated children who presented with signs suggestive of invasive bacterial infections.

Results: The incidence of invasive bacterial infections in all subjects was 1009 (903, 1124) cases per 100,000 person years. It was 1108 (952, 1282) among children who had not received pneumococcal conjugate vaccine. Incidence decreased with age but remained high in 24-29 month olds for pneumococcal infections. Pneumococcal infection was more frequent than NTS in the hot dry season. Respiratory symptoms and signs were more frequent in children with pneumococcal infection than in those with NTS or other infections but diarrhea was commoner in children with NTS infections. Consolidation on chest x-ray was more frequent in children with pneumococcal infection than in those with NTS or other bacteria infections. Laboratory evidence of malaria was less common in pneumococcal than in NTS or other infections. A diagnosis of clinical pneumonia was made more frequently in pneumococcal than in other infections, while malaria was diagnosed more frequently in NTS infections.

Conclusions: Bacterial infections continue to cause significant morbidity in rural Africa. While vaccines could greatly reduce the pneumococcal burden, a high index of suspicion and appropriate use of antimicrobials are needed to manage other causes of invasive bacteria infections.

Epidemiology of invasive pneumococcal disease among children in Kilifi District, Kenya

Ndiritu M1, Nyiro J1, Njenga S1, Lewa P1, Mwarumba S1, Lowe BS1, Bauni E1, Scott JAG1,2
1Wellcome Trust/KEMRI Centre for Geographic Medicine Research - Coast, Kilifi, Kenya
2University of Oxford, John Radcliffe Hospital, Oxford, UK

Background: Pneumococcal conjugate vaccination in Africa is highly effective against invasive pneumococcal disease (IPD). Knowledge of the local epidemiology of IPD is central to the development of a regional vaccine strategy yet epidemiological data from Africa is scarce.

Methods: We identified children with IPD by isolation of Streptococcus pneumoniae from normally-sterile body sites and/or a positive antigen test of CSF at Kilifi District Hospital, Kenya in 1994-2004. The isolates were serotyped by Quelling reaction. Confirmed bacterial meningitis was defined as a positive CSF culture/antigen test; probable meningitis as CSF leucocyte count of >50/mcl or CSF:blood glucose ratio <0.1. Children were classified by clinical syndrome; pneumonia was defined by WHO criteria, malnutrition by a weight-for-height z-score of ≤-3. Population estimates were derived from a defined geographical area under continuous demographic surveillance during 2000-2004. We analysed the frequency, outcome and temporal trends of IPD, pneumococcal serotype distribution and vaccine serotype coverage.

Results: Among 51,931 admissions 669 IPD cases were identified; 515 (76%) from blood cultures, 17 (3%) from CSF, 141 (21%) from blood and CSF and 5 (<1%) from other specimens. IPD presentations peaked in the first month of life; 64 cases (10%) were aged <60 days, 347 (52%) <2 years, 488 (73%) <5 years. Fifty-eight percent were male. The commonest clinical syndromes were meningitis (29%), pneumonia (22%), malnutrition (17%) and bacteraemia without focus (10%). IPD cases comprised 35% of probable bacterial meningitis cases and 3% of severe pneumonia cases. The commonest serotypes were 1 (27%), 14 (11%), 6B (9%), 6A (7%), 5 (7%), 23F (6%) and 4 (5%). Serotype coverage of the 7-valent conjugate vaccine (including also 6A) was 43% at overall but 60% among children aged 6-29 months. Overall coverage increased to 77% by the addition of serotypes 1 and 5. IPD showed moderate seasonal variation rising during the dry seasons and falling markedly with the appearance of the rains. Inpatient mortality was 26% and a further 4% died within 9 months of discharge. The annual incidence of IPD was 239 and 137 per 100,000 for children aged <2 and <5 years, respectively.

Conclusion. The incidence and case-fatality of IPD in Kilifi are high. Disease risk is concentrated in children aged <2 years and more than half of this disease is caused by serotypes in the pneumococcal conjugate vaccine.
Nasopharyngeal carriage of pneumococci among children in Greenland

Koch, A1, Hjuler, T1, Krause, TG1, Friberg, J, Melbye, M1, Olsen, OR2, Kaltoft, MS3
1Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark
2Sisimiut Health Center, Sisimiut, Greenland
3Department of Microbiology, Statens Serum Institut, Copenhagen, Denmark

Rates of invasive pneumococcal disease and of otitis media among Inuit of the Arctic are among the highest in the world. Little information exists however, of underlying factors for this. To obtain knowledge of nasopharyngeal carriage and serotype distribution of pneumococci among Inuit children, we carried out a population-based cross-sectional study among unselected children in Sisimiut, the second-biggest town of Greenland.

Children eligible for enrolment were all non-acutely ill children attending 1-5th school class (5-11 years). A single nasopharyngeal specimen was obtained by a calcium alginate-tipped aluminium swab immediately inserted into serum broth and kept refrigerated until incubated after returning to Denmark within nine days. A pilot study had shown no loss of pneumococci with such an approach. Serotyping was done directly on the overnight incubated serum broth, using both the Pneumotest-latex kit and Neufelt’s test. After plating, single colonies were picked for antibiotic susceptibility testing by disk diffusion and E-test.

Of 313 participating children 291 had a nasopharyngeal swab taken. Pneumococcal carriage was demonstrated in 133 children (46%), 118 (41%) children with one strain and 15 (5%) with two. There was no sex difference in carriage rates, but a tendency of decreasing carriage with age from 60% among children aged 5-6 years to 41% among children aged 10-11 years. In total 22 different serotypes were identified with 7 serotypes accounting for 2/3 of isolates (in rank order 3, 4, 35B, 33F, 19F, 6A, and 18C). Thirty-six percent of isolates were covered by the 7-valent pneumococcal conjugate vaccine, 50% by the 11-valent, and 70% by the 23-valent polysaccharide vaccine. All isolates were penicillin-susceptible.

Compared with native children from Alaska and Denmark the carriage rate among the Greenlandic children seemed high. Carriage of two serotypes was found at an equal rate (5%) among the Greenlandic children as among younger Danish children. Although 18 of the 22 serotypes (84% of isolates) among the Greenlandic children were found among Danish children, serotype distributions in the two populations were somewhat different.

Nine of the 22 carried serotypes (55% of isolates) were similar to serotypes from invasive disease cases in Greenlanders, serotypes previously found to be responsible for 43% of invasive cases.

In conclusion, pneumococcal carriage among Greenlandic children is high and almost half of the carried serotypes are associated with invasive pneumococcal disease.

Invasive S. pneumoniae (Pnc) Infections in Infants and Children less than 5 years old admitted to a Tertiary Hospital in Central Philippines

Ladesma PEA1, Lupisan S1, Simoes EAF3, Sombrero L1, Quiambao B1, Lucero M1, Gozum L1, Arcay J2, Herva E4, Ruutu P4
1Research Institute for Tropical Medicine (RITM), Manila, and
2Gov. Celestino Gallares Memorial Hospital, Bohol, the Philippines,
3University of Colorado Denver and Health Science Center, Denver, USA,
4National Public Health Institute (KTL), Helsinki and Oulu, Finland

Aim: To describe the clinical and laboratory profile of infants and children less than 5 years old admitted to a tertiary hospital in the Philippines with invasive Pnc infection.

Methods: Infants and children admitted with pneumonia or suspected meningitis/sepsis were prospectively enrolled in an etiologic study of serious infections at a tertiary general hospital in Central Philippines from April 1994 to May 2000. On admission, blood and CSF (if indicated) were drawn for culture and antigen (Ag) detection by latex agglutination using standard techniques. Typing of Pnc strains was done at RITM and KTL.

Results: Blood culture was obtained in 3013 patients, 1983 with pneumonia, 745 with suspected meningitis and 285 with suspected sepsis; and CSF in 833 (28%) patients. Pnc was found in 30 patients, 8 from both blood and CSF, 21 from blood only, and 1 from CSF only. Most common serotypes were 14 (n=7), 1 (n=6) and 6B (n=5). Fifty percent of the isolates had serotypes included in the 7-valent Pnc conjugate vaccine licensed for use. CSF Ag positive cases were all culture positive. Three out of 5 CSF culture positive cases tested were positive for Ag. Nineteen (63%) of the Pnc infections were in children < 1 yr and 5 (17%) in those older than 2. Eight children (26%) died.

Conclusion: The majority of invasive S. pneumoniae infections in a rural area in the Philippines occurred in children less than 1 year old. One-half of cases were caused by strains covered by the 7-valent Pnc conjugate vaccine.
Microbial surveillance for Streptococcus pneumoniae in rural Thailand: Lessons learned, laboratory capacity, and the case for quality control


1International Emerging Infections Program, Thai MOPH-US CDC Collaboration, Nonthaburi, Thailand
2National Institute of Health, Ministry of Public Health, Nonthaburi, Thailand
3Coordinating Office for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, USA

Although Streptococcus pneumoniae is a significant cause of pneumonia and sepsis in the United States and Europe, the incidence of invasive pneumococcal disease in other regions remains unclear. A positive hemoculture, the current standard for definitive diagnosis of S. pneumoniae infections, can be difficult to obtain even under optimal laboratory conditions. The cumulative impact of many small variations in microbiological practices commonly encountered in resource-constrained settings (such as the use of human blood agar, lack of environmental temperature controls, or limited laboratory operating hours) remains difficult to quantify, but historical reports of S. pneumoniae incidence in developing nations – particularly in semi-tropical and tropical climates – likely represent serious underestimates.

We implemented a comprehensive microbial quality control/quality assurance (QC/QA) system as part of a laboratory capacity-building partnership to enhance active population-based surveillance for pneumonia in two rural Thai provinces. In the previous 2 years, neither province isolated S. pneumoniae from blood cultures. Measures included instituting dedicated specimen storage and transport systems with temperature monitoring, standardizing media and protocols, training in blood collection and processing techniques, and requiring stringent laboratory QC/QA for growth conditions and microbiological testing, implemented with sensitivity to local practices and sustainability. Clinician orientations focused on encouraging routine blood cultures for all patients with clinical signs and symptoms of pneumonia and for children ≤5 years of age with suspected sepsis. Between July and December 2005, the provincial microbiology laboratory in Sa Kaeo Province tested 2478 specimens by blood culture. Out of 368 (14.9%) blood cultures positive for any clinically significant pathogen, 6 (1.6%) were positive for S. pneumoniae, with growth detected in both aerobic and mycobacteria media of a paired-bottle system in an average of 19.1 hours in the Bac/T ALERT 3D system. In Nakhon Phanom Province, 912 blood cultures (including 709 from patients with suspected pneumonia and 77 from children with possible sepsis) obtained between November and December 2005 yielded 4 S. pneumoniae isolates, or 8.5% of 47 pathogen-positive specimens. Continual refinements in protocols and processes have led to incremental improvements in microbial surveillance sensitivity. Preliminary data suggest that lessons learned from implementing QC/QA practices in rural Thailand, as well as from other middle- and low-income nations, could improve accuracy and comparability of invasive pneumococcal disease incidence data among regions.

Bacteremia and bacterial meningitis among children 2 months to 5 years attending to out patient and observation services of Kanti children hospital, Kathmandu

Steinhoff, M, Lalitha, MK, Thomas, K, Sherchan, JB, Gauchan, P, Gami, FC, Sharma, PR, Shah, A, Tuladhar, NR

1John Hopkins University, School of Hygiene and Public Health, Baltimore, USA
2International Emerging Infections Program, Thai MOPH-US CDC Collaboration, Nonthaburi, Thailand
3Christian Medical College, Vellore, India
4Institute of Medicine, Kathmandu, Nepal

Aims: This is to know the incidence of invasive bacterial etiologic agents for bacteremia and meningitis among the children attending to tertiary Kanti children hospital during Nov. 23, 2004 to Jan. 18, 2006

Methods: The children 2 months to 5 years fulfilled the enrolment criteria for suspect of bacteremia, bacterial meningitis or both were enrolled for an etiologic study of serious infections. Blood and CSF were drawn and cultured to isolate the etiologic agent by WHO procedure manual. Antibiotic susceptibility test was done following NCCLS methodology. From CSF specimen latex agglutination tests for Streptococcus pneumoniae, Haemophilus influenzae and other pathogens were performed. Total leucocyte count was also carried out from CSF.

Result: Of the 1436 blood specimens 15 were culture positive for Streptococcus pneumoniae and one Neisseria meningitidis. Four Streptococcus pneumoniae, three Haemophilus influenzae and one Neisseria meningitidis were culture positive from 212 CSF specimens. Of the five different antibiotic discs tested in vitro for Pneumococcal isolates showed Chloromphenicol was the most effective and Cotrimoxazole was the least; Penicillin, Erythromycin and Cefotaxime were on resistance trend. Latex agglutination tests of CSF specimen by two different kits showed Binax positive in 8.9% (13 / 146) of the cases and BD Directogen showed positive in 6.3% (11/175). CSF cell count (> 10/ml) showed in 98/212 (46.23%).

Conclusion: Streptococcus pneumoniae was the most common etiologic agent of bacteremia and meningitis.
Invasive pneumococcal disease in children <5 years of age living a rural area of Mozambique

**PO3.02**

Sigaúque B\(^1,2\), Roca A\(^1,2\), Quintó LL\(^1,2\), Mandomando I\(^2,3\), Vallés X\(^1,2\), Espasa M\(^2,4\), Sacarlal J\(^2,4\), Macete E\(^2,3\), Nhacolo A\(^2,3\), Levine M\(^5\), Alonso P\(^1,2\)

\(^1\)Centre de Salut Internacional (CSI), Hospital Clinic/IDIBAPS, Universitat de Barcelona, Barcelona, Spain; \(^2\)Centro de Investigação em Saúde da Manhiça, Ministerio de Saúde, CP1929 Maputo, Mozambique; \(^3\)Instituto Nacional de Saúde and Direcção Nacional de Saúde, Maputo, Mozambique; \(^4\)Facultade de Medicina, Universidade Eduardo Mondlane, Maputo, Mozambique; \(^5\)Center for Vaccine Development, Baltimore MD, USA.

**Background:** Despite the importance of the pneumococcus as leading cause of morbidity and mortality worldwide, information on the epidemiology of pneumococcal infection in Africa is very scarce - especially in rural areas where much of the population lives. Information is urgently needed to assess the possible impact of pneumococcal vaccines in these countries.

**Objectives:** To estimate the incidence and epidemiological characteristics of invasive pneumococcal disease (IPD) in children <5 years of age living in a rural area of southern Mozambique.

**Methods:** As part of the clinical management of children admitted to the Manhiça District Hospital, prospective surveillance for invasive bacterial disease was conducted during a two-year period (June 2001 – May 2003). The level of antibiotic resistance of the isolates was also analysed.

**Results:** The pneumococcus was the most commonly isolated bacterium, accounting for 212 episodes. The estimated crude incidence rate of IPD among children <5 years of age was 416/100,000 per child-year-at-risk. The youngest age group (<3 months) had the highest incidence (778/100,000). Cases were detected in both rainy and dry season. The most common clinical diagnosis was pneumonia, made in 146/212 (69%) of the episodes of IPD. Overall case-fatality-rate was 10%, being highest among children with pneumococcal meningitis (5/8=63%). Pneumococcal isolates were highly sensitive to penicillin (86% sensitive and 14% with intermediate resistance) and cloramphenicol (98% sensitive). In contrast, up to 37% of the isolates tested were non-sensitive to cotrimoxazol.

**Conclusions:** Rates of pneumococcus and associated mortality shown in this study highlights the need for pneumococcal vaccines in rural Africa that must be effective in infants and young children.

Severity and outcome of invasive pneumococcal diseases in an industrialised country (Finland)

**PO3.03**

Koskikallio, E\(^1\), Salo, E\(^1\), Kaijalainen, T\(^2\), Peltola, H\(^1\)

\(^1\)HUCH Hospital for Children adn Adolescents, Helsinki, Finland

\(^2\)Public Health Institute, Oulu, Finland

Severity of disease and the outcome are of paramount importance when a vaccine is to be incorporated into an immunisation program, and especially so if the vaccine is costly. We analysed all documented pneumococcal (Pnc) diseases diagnosed during 16 years (1989-2004) in the largest paediatric (0-14 years) centre of Finland, Helsinki University Central Hospital. A case was included if Pnc grew from a normally sterile body site. The series was divided in previously healthy children, and those with an illness likely to predispose to Pnc infection. The clinical manifestations were grouped in five: bacteraemia (continuously good general condition, only 2-3 days in hospital), sepsis (seriously ill or succumbed patient, positive blood culture), meningitis, pneumonia (consolidation in radiograph, positive blood culture), and other infections (local inflammation with positive culture from the site or blood).

In all, there were 121 cases in 117 patients (51% boys). The median age was 1.5 years; 11% were younger than 5 months, 62% younger than 2 years. Bacteraemia was most common – 60 cases (50%) - , whereas sepsis was rare: 2 patients (<2%). Pneumonia was found in 31 (26%) children, meningitis in 20 (17%), and other manifestations (3 arthritis, 1 cellulitis) in 4 (4%) children. Most cases were treated with parenteral penicillin (N=43) or cefuroxime (N=39). Six children succumbed, the overall fatality rate being 5%. The fatal entities were meningitis (N=3, all previously healthy), sepsis (N=2), and pneumonia (N=1). Except meningitis, no sequelae were detected. Serotyping (79% of cases) disclosed that the diseases were caused by at least 24 types, the 3 most common being types 14, 6B, and 23 F. Assuming a 100% effectiveness for the 7-valent conjugate vaccine, approx. 60% of cases would theoretically have been prevented, regardless of the manifestation.

In summary, only 7-8 cases of invasive Pnc infections a year are proven in the hospital that serves 1/5 of the country’s (5.2 Mi) children. Especially pneumonia no doubt remains often unidentified etiologically, but even then severe Pnc infections are surprisingly rare. The only truly dangerous manifestation is meningitis, but its yearly incidence is very low (<3/100,000 at age 0-1 years).
Two nontypable *Streptococcus pneumoniae* clones associated with acute conjunctivitis in Southern Israel

Pediatric Infectious Disease Unit, Soroka University Medical Center, Ben-Gurion University of the Negev, Beer-Sheva, Israel

**Background:** In a recent epidemiological study nontypable *S. pneumoniae* strains were found to be highly associated with sporadic cases of acute conjunctivitis (AC) in southern Israel. The purpose of this study was to evaluate the relative importance of the absence of capsule versus genotype properties in causing AC.

**Methods:** DNA typing by pulsed field gel electrophoresis (PFGE) was carried out on nontypable organisms isolated from 3 sites: nasopharynx (NP) of healthy children, middle ear fluid (MEF) and conjunctiva.

**Results:** A total of 162 isolates were recovered in 5 years (2000-4) from children < 3yrs: 90 from the NP, 23 from MEF, and 49 from AC. Analysis of the PFGE patterns revealed the presence of 6 major clones, which were arbitrarily named clones A to F. Almost half of the strains (65/148), belonged to two clones (A and B), while the 4 other clones (C to F) included only 21% (31/148) of the strains. The remaining 35% of the isolates (52/148) showed 44 different PFGE patterns. Clones A and B had a significantly higher prevalence in the AC group, suggesting an association of these two clones with AC. The other 4 clones C to F were frequently isolated from the NP of healthy carriers but did not show a predilection to AC. The nontypable strains showed high rates of resistance to various antibiotic drugs: 74% of the strains were penicillin nonsusceptible and 30% were resistant to 3 or more antibacterial agents. Particularly, clones A and B, which were associated with AC, showed high rates of penicillin nonsusceptibility (70% and 96% of all strains respectively), and 96% of the strains belonging to clone B strains were multidrug resistant.

**Conclusion:** These data suggest that in addition to the absence of capsule which may facilitate colonization in conjunctival tissue, some genetic factors expressed in specific clones (A and B) contribute to virulence of *S. pneumoniae* in AC. These clones are also antibiotic resistant. Widespread vaccination to date may lead to increased incidence of AC by the unencapsulated pneumococcal strains.

Disease potential of individual *Streptococcus pneumoniae* (SP) serotypes in invasive disease (IPD), acute otitis media (AOM) and acute conjunctivitis (AC) in infants and young children

Greenberg, D, Shouval, DS, Givon-Lavi, N, Porat, N, Dagan, R
Pediatric Infectious Disease Unit, Soroka University Medical Center and Ben Gurion University of the Negev, Beer Sheva, Israel

**Objective:** To compare the disease potential of individual SP serotypes causing invasive pneumococcal disease (IPD), acute otitis media (AOM) and acute conjunctivitis (AC) in infants < 12 months and children ≥ 12 months.

**Methods:** Serotype distribution of SP isolates from children with IPD, AOM, and AC were compared to those carried in the nasopharynx (NP) by healthy children. All children were < 3 years of age, resided in the same area and were studied during the period 2000-2004. Odds ratios (ORs) with 95% confidence interval (95% CI) were calculated for each disease in 2 age groups: ≤ 12 months and >12 months, with multivariate analyses for each serotype including gender, age, ethnicity (Jews vs. Bedouins), antibiotic treatment during last month and year variability.

**Results:** A total of 5,500 isolates (3081 [56%] from children <12m and 2,419 [44%] in children ≥12m) were collected: IPD- 189 (89 [47%] <12m); AOM- 3,200 (1,980 [62%] <12m); AC- 348 (273 [74%] <12m); NP- 1,763 (739 [42%] <12m). Using multivariate analyses in children <12m the following serotypes were significantly higher when compared with NP carriage (ORs; 95% CI): IPD-1 (135.0; 13.7-1,342.0), 5 (31.7; 9.0-111.7) and 12F (9.0; 1.4-58.6); AOM- 1 (13.7; 1.8-103.4), 3 (15.1; 4.7-55.9), 5 (8.1; 2.9-23.0), 19A (1.7; 1.2-2.4) and 19F (1.9; 1.4-2.5); and AC- 3 (5.0; 1.1-23.5) and nontypeable SP (2.7; 1.5-4.7). In children ≥12m in IPD serotypes: 1 (18.5; 8.6-39.9) and 12F (8.6; 1.4-52.0) were significant; AOM-1 (2.1; 1.1-4.1), 3 (28.3; 3.7-215.8), 5 (2.8; 1.4-5.6), 14 (1.3; 1.0-1.7) and 19A (1.5; 1.1-2.2); AC- only nontypeable (8.2; 4.2-15.7) was significant.

**Conclusions:** Disease potential by IPD and AOM of serotypes 1 and 5 was more impressive in children < 12 months. Serotypes 12F in IPD, 3 and 19A in AOM and nontypeable SP in AC showed similar disease potential in both age groups. These findings suggest that the disease potential of the more aggressive serotypes is similar when comparing infants to toddlers.
Differences in clinical characteristics of invasive pneumococcal infections related to serotype

Trollfors, B1, Berg, S1, Backhaus, E2, Kaltoft, MS3, Bossen Konradsen, H4
1Department of Pediatrics, Sahlgrenska University Hospital, Goteborg, Sweden
2Department of Infectious Diseases, Skaraborg Hospital, Skovde, Sweden
3Statens Serum Institut, Copenhagen, Denmark

827 pneumococcal isolates from blood and cerebrospinal fluid obtained during a 4-year period in 2 counties in south-west Sweden (population of 1.8 million) were serotyped with the capsular swelling test. Clinical information was obtained from individual hospital records. The table shows some clinical characteristics for the 8 most common serotypes.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Number of strains</th>
<th>Manifestation</th>
<th>Fatal cases (%)</th>
<th>Underlying disease (%)</th>
<th>Median age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>119</td>
<td>Pneumonia 111</td>
<td>2 (2 %)</td>
<td>37 (31 %)</td>
<td>44</td>
</tr>
<tr>
<td>7F</td>
<td>89</td>
<td>Unknown focus 4</td>
<td>2 (2 %)</td>
<td>32 (36 %)</td>
<td>50</td>
</tr>
<tr>
<td>9V</td>
<td>71</td>
<td>Meningitis 51</td>
<td>4 (6 %)</td>
<td>46 (65 %)</td>
<td>61</td>
</tr>
<tr>
<td>14</td>
<td>70</td>
<td>Unknown focus 13</td>
<td>2 (3 %)</td>
<td>42 (60 %)</td>
<td>64</td>
</tr>
<tr>
<td>4</td>
<td>69</td>
<td>Pneumonia 61</td>
<td>4 (6 %)</td>
<td>42 (61 %)</td>
<td>56</td>
</tr>
<tr>
<td>12F</td>
<td>49</td>
<td>Unknown focus 9</td>
<td>3 (6 %)</td>
<td>27 (55 %)</td>
<td>61</td>
</tr>
<tr>
<td>6B</td>
<td>35</td>
<td>Unknown focus 20</td>
<td>4 (11 %)</td>
<td>20 (57 %)</td>
<td>73</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>Unknown focus 28</td>
<td>5 (14 %)</td>
<td>24 (69 %)</td>
<td>71</td>
</tr>
<tr>
<td>All other serotypes</td>
<td>290</td>
<td>Pneumonia 183</td>
<td>51 (18 %)</td>
<td>221 (76 %)</td>
<td>67</td>
</tr>
<tr>
<td>All serotypes</td>
<td>827</td>
<td>614</td>
<td>77 (9 %)</td>
<td>491 (59 %)</td>
<td>61</td>
</tr>
</tbody>
</table>

Patients with serotype 1 and 7F infections were significantly younger, had a significantly lower incidence of severe underlying diseases and a lower case-fatality rate than patients with infections caused by other serotypes. Serotypes 1 and 4 caused significantly higher proportions of pneumonia than of nonpulmonary infections compared to other serotypes. Patients with type 19A infections had a higher proportion of severe underlying diseases and higher case-fatality rate than other patients.

In conclusion, there were differences in manifestations, risk factors, age and case-fatality rate in infections caused by different serotypes, which should be considered when polyvalent pneumococcal vaccines are formulated.

Serotype and antimicrobial susceptibility patterns of Streptococcus pneumoniae causing invasive disease in The Gambia

Adegbola RA1, Hill PC1, Secka O1, Ikumapayi UN1, Lahai G2, Greenwood BM2, Corrah T1
1Medical Research Council Laboratories, P O Box 273, The Gambia, West Africa
2London School of Hygiene and Tropical Medicine, UK

Aims: Little information is available on the serotype and antimicrobial susceptibility patterns of pneumococcal isolates causing invasive disease in developing countries. We report on the characteristics of pneumococcal isolates obtained from patients with invasive pneumococcal disease in The Gambia.

Methods: Pneumococcal isolates were obtained from children aged ≤6 years with invasive pneumococcal disease (1997-2002) and from patients admitted to the MRC hospital, Fajara, for routine care (1996-2003). Isolates were identified, serotyped and tested for antibiotic susceptibility using standard methods.

Results: 531 pneumococcal isolates were obtained from 518 patients; 55 (10.6%) patients died. 415 isolates (79%) were from blood culture, 84 (16%) from CSF, and 42 (8%) from lung aspirates. 40 different serogroups/serotypes were identified; 6 accounted for 64% and 16 for 86% of all episodes. 33.7% were of serotypes 1 and 5. 23.5% were of a 7-valent vaccine serotype, 57.1% were of a 9-valent vaccine serotype; 56% were of a 7-valent serogroup and 78% were of a 9-valent serogroup. There was a significant increase in the proportion of isolates of non-vaccine serogroup with increasing age (p<0.0001). There were no significant increases in antibiotic resistance over time; there was increased intermediate non-susceptibility to penicillin and decreased resistance to chloramphenicol in isolates of vaccine serotype compared to those of non-vaccine serotype.

Conclusions: The majority of invasive pneumococcal disease in The Gambia is caused by pneumococci of relatively few serogroups. Pneumococci with high resistance to penicillin are not yet prevalent in The Gambia. A conjugate vaccine would be expected to reduce the pneumococcal disease burden substantially and should have a beneficial effect on emerging pneumococcal antibiotic resistance to penicillins.
Incidence of clinically significant pneumococcal bacteraemia among children presenting to a hospital outpatient department in Kenya

**Brent, AJ\(^1\), Ahmed, I\(^1\), Ndiritu, M\(^1\), Lewa, P\(^1\), Ngeta, C\(^1\), Lowe, B\(^1\), Bauni, E\(^1\), English, M\(^1\), Berkley, JA\(^1\), Scott, JAG\(^1\)**

\(^1\)Wellcome Trust/KEMRI Centre for Geographic Medicine Research - Coast, Kilifi, Kenya
\(^2\)University of Oxford, John Radcliffe Hospital, Oxford, UK

**Background:** Despite the success of 9-valent pneumococcal conjugate vaccine in South Africa and The Gambia, a lack of data on pneumococcal disease burden hampers vaccine implementation in sub-Saharan Africa. Previous incidence data are scarce and are limited by their focus on inpatients. We previously estimated the annual incidence of admission to a rural Kenyan hospital with bacteraemia of any cause among children aged <2 and <5 years was 1,081 and 505 per 100,000 respectively; the incidence of admission with pneumococcal bacteraemia was 111 per 100,000. There are no previous bacteraemia incidence data among children presenting to outpatient departments in sub-Saharan Africa.

**Methods:** We cultured blood from 1,093 children attending a rural Kenyan hospital outpatient department. Using a Demographic Surveillance System we derived conservative estimates of bacteraemia incidence. We also investigated the clinical significance of bacteraemia among children who were not admitted using standardised criteria for admission, and the capacity of clinical signs to identify bacteraemia cases.

**Results:** The annual incidence of bacteraemia of any cause per 100,000 children aged <2 and <5 years was 2,440 (95% CI 1,307-3,573) and 1,192 (95% CI 692-1,693) respectively. Pneumococcal bacteraemia incidence was 597 (95% CI 416-778) per 100,000 children under 5 years. Three-quarters of bacteraemia episodes had a clinical focus and/or required admission and one in six was fatal. Excluding children with 'occult' bacteraemia the incidence of clinically significant bacteraemia of any cause was 1,741 (95% CI 790 to 2,692) and 909 (95% CI 475 to 1,343) per 100,000 children aged <2 and <5 years respectively; the incidence of clinically significant pneumococcal bacteraemia was 436/100,000/year (95% CI 132-739). Malnourished and HIV positive children were more likely to be bacteraemic. The predictive value of clinical signs alone in identifying bacteraemia was poor.

**Conclusions:** Clinically significant pneumococcal bacteraemia among Kenyan children is four times as common as previously estimated among inpatients at the same hospital. Studies confined to selected patient groups, such as inpatients, are likely to underestimate the burden of invasive pneumococcal disease including bacteraemia. Malnutrition, HIV, and barriers to healthcare access may explain why self-limiting ‘occult’ bacteraemia accounts for a smaller proportion of cases compared with Europe and America. The findings reinforce the public health case for the accelerated introduction of effective pneumococcal vaccine in sub-Saharan Africa.

---

Invasive pneumococcal disease in Singapore children

**Chong C-Y, Mok Y-H, Tee N**

KK Women’s And Children’s Hospital, Singapore

Our objective was to determine the epidemiology, antibiotic susceptibility, morbidity and mortality of invasive pneumococcal disease in children admitted to KK Women’s and Children’s Hospital, Singapore. Methods: A retrospective survey was done for all pneumococcal isolates from blood, cerebrospinal fluid (CSF), pleural, peritoneal fluid from January 1997 to December 2004. Demographic data, clinical presentation, prior antibiotic usage and antibiotic susceptibility were studied. Results There were 147 positive *S. pneumoniae* isolates:80.3% blood, 8.2% pleural, 4.1% CSF, 3.4% each for blood and CSF/pleural, 0.7% blood and sputum. Diagnoses at presentation were: Pneumonia 63.3% (includes 14.3% empyema), bacteraemia 17%, meningitis 15.6% (includes 2.8% meningitis and pneumonia), 4.1% others (endocarditis, orbital cellulitis, mastoiditis, peritonitis). The overall mean age was 45 months. The morbidity rate was 25.2%, mortality rate was 6.1% (9 deaths) of which the majority occurred in meningitis patients. Overall antibiotic resistance was: Penicillin 40%, ceftriaxone 11%, erythromycin or trimethoprim-sulfamethoxazole 63-69%. A separate analysis of serotypes (n=68) from 2001-2004 showed that the Prevenar vaccine would cover 83.8% of all invasive serotypes. Conclusions: Pneumococcal invasive disease causes a significant morbidity rate of 25.2% and mortality of 6.1% in Singapore. Prevenar vaccine would cover 83.8% of all invasive serotypes in Singapore.

**Background**
Globally, pneumonia causes 19% of 10.6 million annual deaths among children < 5 years, mostly in developing countries. WHO estimate that *Streptococcus pneumoniae* (pneumococcus) infections account for ≥ 0.8 million deaths. Most pneumococcal deaths are due to pneumonia and meningitis. Additionally, single and multi-drug resistance is reported from Asia and elsewhere, complicating treatment. Invasive pneumococcal disease (IPD) prevention among young children with protein-conjugate vaccines requires knowledge of both pneumococcal disease burden and circulating serotypes. Urban Bangladesh has documented high pneumonia burden in young children. However, earlier reports from Bangladesh among children hospitalised with primarily meningitis suggest poor serotype coverage with existing conjugate vaccines.

**Design and Methods**
We conducted active morbidity surveillance once weekly among 5,000 children < 5 years in a Dhaka urban slum, and obtained blood cultures on children with fever (≥38°C), or any upper or lower respiratory illness, including pharyngitis, otitis, bronchiolitis, or pneumonia. Children with lower respiratory illness also had a chest X-ray. All pneumococcal isolates were serotyped.

**Results**
Between April 2004 to October 2005, we obtained 4,366 blood cultures from 4,819 eligible children (91%). Of these, 246 (6%) yielded bacteria, of which 23 (9%) were pneumococcus. There were 5,137 child-years of observation during the period, for an incidence of culture-proven IPD of 4.5 episodes/1000 child-years. Clinically, 19 (83%) had upper respiratory infections, 2 (9%) had lower respiratory infections, one (4%) had sepsis and one (4%) had fever with bacteraemia. Nineteen isolates (83%) were resistant to cotrimoxazole, 4 (17%) were resistant to chloramphenicol, 2 (9%) were resistant to ciprofloxacin, and one (4%) was multidrug resistant. There was no penicillin or macrolide resistance. Nine serotypes (41%) are represented in the 7-valent, and 13 (59%) are represented in the 9-valent vaccines. All patients with vaccine-covered serotypes had either a respiratory or febrile illness.

**Conclusion**
These IPD findings reflect substantial respiratory disease burden from vaccine serotypes, and are a minimal estimate, as they are based on single cultures and blood culture sensitivity for pneumococcus is low. The community-based IPD serotype distribution differs from that of hospitalised children, who primarily have meningitis. Given this population’s high pneumonia burden, these results suggest a role for conjugate pneumococcal vaccine in Bangladesh. There is no penicillin resistance, and other AMR rates, excluding cotrimoxazole, are low but need monitoring.
Streptococcus pneumoniae invasive isolates from Uruguayan children and adults: epidemiological differences among serotypes.

Camou T1, García Gabarrot G2, Pérez Giffoni G1, Felix V1, Hortal M4 and the Pneumococcal Study Group
1National Reference Laboratory. 2National Child Health Program. Ministry of Health, Uruguay.

Pneumococcal conjugated vaccines are directed at specific serotypes, therefore their specific epidemiological properties are of vital importance when selecting the most adequate vaccine for a national immunization program.

Objective. To analyze specific serotype-properties related to time, age, syndromes, resistance to antibiotics and mortality.

Methods. Invasive Streptococcus pneumoniae surveillance was carried out from 1994 to Sept.2005. Serotyping was performed by “quellung” reaction. Susceptibility to antibiotics was assessed according to NCCLS. International quality controls endorsed laboratory results.

Results. Out of 1,626 isolates (1,034 children, 592 adults), 43 serotypes were identified. In children the most frequent were 14, 5, 1, 7F, 3, 6B, 19A; and 1, 5, 3, 7F, 12F, 14, 9N in adults, representing 80% and 64% respectively. Serotypes 6B and 19A were infrequent in adults, as 12F and 9N in children. Temporal variation was observed for the three most common serotypes among children: serotype 14 (21.7% to 44.3%), serotype 5 (7.5% to 27.4%) and serotype 1 (7.1% to 26.9%). Serotype frequencies among adults correlated well in time for serotypes 1 and 5, while serotype 14 remained stable about 10%. These three serotypes caused mainly pneumonia, except for serotype 5 that caused meningitis at all ages but almost exclusively meningitis among children less than 6 months of age. Interestingly, this serotype was notoriously prevalent among adults less than 35 years old, in contrast with older adults. Serotype 1 had a low frequency among children less than 23 months of age, and was evenly distributed in patients older than 5 years. Among the 11 most frequent serotypes involved in 91 fatal cases, only serotype 7F was significantly associated. Most penicillin-resistant isolates were serotype 14 (93.7%). Resistance to trimethoprim/sulfamethoxazol for serotype 5 was 18.3% during 1994/2002, but it reached 85.4% during 2003/2005.

Conclusions. Differences in frequency for the most common serotypes were observed over time, temporarily changing the rank order. In contrast, age-assOCIated distribution of serotypes remained unchanged during the study period, as it was the association with clinical syndromes. Resistance to penicillin and trimethoprim/sulfamethoxazol are related to serotypes 14 and 5 respectively and varies according to the frequency of these serotypes. Continued surveillance allowed identifying serotype 7F as an increased risk for mortality. This as other serotype properties may be crucial for vaccine choice, underlining the value of surveillance.

Poor potential coverage of pneumococcal conjugate vaccine for pneumococcal meningitis in Papua New Guinean highland children

New Guinean highland children

Phuanukoonnon, S1, Michael, A1, Nale, G1, Johannes, M1, Orami, T1, Murphy, D2, Alpers, M1, Lehmann, D1, Siba, P1, Reeder, JC1
1Papua New Guinea Institute of Medical Research, Goroka, EHP, Papua New Guinea
2Queensland Health Pathology Services, Brisbane, Australia
3Curtin University of Technology, Perth, WA, Australia
4Telethon Institute for Child Health Research, Perth, WA, Australia

Background: This study was undertaken to determine the prevalence, serotype distribution and antimicrobial susceptibility of Streptococcus pneumoniae isolated from children hospitalized in Goroka, Eastern Highland Province (EHP), Papua New Guinea (PNG) with suspected meningitis.

Methods: From August 1996–June 2005, prospective surveillance was conducted at Goroka Hospital on cerebrospinal fluids (CSF) collected from children aged <15 years with suspected meningitis. All invasive S. pneumoniae (Pnc) isolated were serotyped, serogrouped by the Quellung reaction and tested for antimicrobial susceptibility by disc diffusion and E-test.

Results: A total of 181 Pnc were isolated from 1916 CSF samples. Fifty-three percent and 75.6% of all cases of Pnc meningitis were age <6 months and <12 months, respectively. We identified 25 different Pnc serogroups, the most common being serogroups 2 (15 %), 5 (12.2%), 46 (8.3%), 6 (6.7%), 7 (6.1%), 12 (5%), 18 (5%) and 24 (5%). Eighty-two percent of serotype 5, 63% of serotype 2 and 60% of serotype 46 were found in children aged <6 months, while 75% of serogroup 6 and 88% of serogroup 18 were found among children aged 6 months or more. Intermediate penicillin resistance (MIC 0.12–1 mg/L) was found in 25.7% of Pnc isolates. The most frequently resistant serogroups were 6 (17.4%), 24 (15.2%) and 19 (10.9%). The licensed 7-valent Pnc conjugate vaccine (PCV) would only have provided 32% coverage of cases of Pnc meningitis and 42% of penicillin-resistant isolates.

Conclusion: The licensed 7VPCV would provide only limited coverage for prevention of Pnc meningitis in Papua New Guinean highland children. Alternative immunization strategies are urgently needed. The high level of intermediate penicillin resistance among invasive isolates amplifies the potential impact of a childhood pneumococcal vaccination programme in PNG.
Monitoring antimicrobial resistance and serotypes (the MARS project): Pneumococcal carriage in child care centres in the Northern Territory of Australia, 2003 to 2005

Hare, K1,2, Morris, PS1,2,3, Beissbarth, J1,2, Kennedy, M1,2, Halpin, S1,2, Mellon, G1,2, Stubbs, E1,2, Wilson, C1,2, Smith-Vaughan, H1,2, Leach, AJ1,2
1Menzies School of Health Research, Darwin, Northern Territory, Australia
2Charles Darwin University, Institute of Advanced Studies, Darwin, Northern Territory, Australia
3Flinders University, Adelaide, South Australia, Australia

Background: Non-Aboriginal children in central Australia experience higher rates of invasive pneumococcal disease (IPD) than their counterparts in other regions. In July 2001, 7-valent pneumococcal conjugate vaccine (7vPCV, Prevenar) was made available through a Federal Government initiative for all Aboriginal infants and non-Aboriginal infants born in central Australia. In January 2005, funding was extended to include all Australian infants. An anticipated outcome is selective pressure on pneumococcal antimicrobial resistance and serotypes.

Aim: To monitor the serotype distribution and prevalence of azithromycin and penicillin resistance among S. pneumoniae carriage isolates from children attending child care centres (CCCs) in Alice Springs (central Australia) and Darwin, where 7vPCV has been available since July 2001, or January 2005, respectively.

Methods: The largest CCCs in Alice Springs (2004, 2005) and Darwin (2003-2005) were invited to participate in this study. All consenting children had a standardised nasal swab and parents provided (or consented to access to) data on antibiotic use and vaccination status. Swabs were transported and processed according to WHO recommendations for pneumococcal carriage studies.

Results: Pneumococcal carriage rates were similar (around 50%) in both regions and over time. Carriage of vaccine-type (VT) pneumococci was consistently ~25% in Darwin CCCs where vaccine coverage was less than 10% until 2005, compared to ~8% VT carriage in Alice Springs where vaccine coverage was over 50% until 2005. Carriage of penicillin- and macrolide-resistant pneumococci were each around 10% in Darwin CCCs. While most resistant pneumococci are VTs such as 6B and 19F, penicillin resistance is also found in the vaccine-related types 6A and 19A and the frequently isolated non-VT 11A, while macrolide resistance has been observed in types 15B/C.

Conclusions: Provision of free 7vPCV vaccine in a region with high rates of IPD, was associated with vaccine uptake of over 50% and very low VT carriage (7%) suggesting an indirect effect in non-immunised children. Vaccine uptake of 25% in Darwin was not associated with a decline in VT carriage. A threshold vaccine coverage may be required to achieve indirect effects. There was an association between higher vaccine uptake and lower antimicrobial resistance despite similar prescribing. Continued monitoring is advised, particularly for the emergence of antimicrobial resistance in non-VT pneumococci.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Children consented</td>
<td>316</td>
<td>419</td>
<td>341</td>
<td>122</td>
</tr>
<tr>
<td>Children swabbed</td>
<td>302</td>
<td>377</td>
<td>317</td>
<td>94</td>
</tr>
<tr>
<td>Penicillin given</td>
<td>17/302 (6%)</td>
<td>23/377 (6%)</td>
<td>26/317 (8%)</td>
<td>11/94 (12%)</td>
</tr>
<tr>
<td>Macrolide given</td>
<td>3/302 (1%)</td>
<td>0/377 (0%)</td>
<td>5/317 (2%)</td>
<td>1/94 (1%)</td>
</tr>
<tr>
<td>&gt;1 dose 7vPCV given</td>
<td>22/302 (7%)</td>
<td>33/377 (9%)</td>
<td>77/317 (24%)</td>
<td>49/94 (52%)</td>
</tr>
<tr>
<td>Spn positive</td>
<td>151/302 (50%)</td>
<td>183/377 (49%)</td>
<td>164/317 (52%)</td>
<td>46/94 (49%)</td>
</tr>
<tr>
<td>VT Spn</td>
<td>75/302 (25%)</td>
<td>100/377 (27%)</td>
<td>86/317 (27%)</td>
<td>7/94 (7%)</td>
</tr>
<tr>
<td>PenR Spn</td>
<td>30/302 (10%)</td>
<td>40/377 (11%)</td>
<td>36/317 (11%)</td>
<td>2/94 (2%)</td>
</tr>
<tr>
<td>MacR Spn</td>
<td>19/302 (6%)</td>
<td>33/377 (9%)</td>
<td>32/317 (10%)</td>
<td>0/94 (0%)</td>
</tr>
</tbody>
</table>

Spn: S. pneumoniae, VT: vaccine-type, PenR: penicillin-resistant (MIC>0.12), MacR: macrolide-resistant (azithromycin MIC>8, erythromycin MIC>2).
Serotypes and sequence types of *Streptococcus pneumoniae* isolated from blood in Poland  

Sadowy, E. and Hryniewicz, W.  
National Institute of Public Health, Warsaw, Poland

**Aim:** to study the serotype distribution in Poland among *Streptococcus pneumoniae* isolates from blood and their clonal structure by multilocus sequence typing (MLST).

**Methods:** 163 consecutive *S. pneumoniae* isolates from blood (1995-2004) were subjected to serotyping by the capsule swelling method and to MLST according to the standard procedure. Alleles and sequence types corresponding to particular allelic profiles were identified using the database [www.mlst.net](http://www.mlst.net).

**Results:** the investigated group contained 29 different serotypes and a single rough isolate. The 7-valent conjugated vaccine coverage was 85% in the group of children below 24 months old; the 23-valent polysaccharide vaccine coverage was 95% for patients older than 2 years and 97% for the patients older than 65 years (including cross-protecting serotypes). Seventy-six STs were found; these included 17 newly identified STs. The most numerous were ST180 and ST306, with 10 (6.1% of the group) and 9 (5.5%) isolates, respectively. Representatives of international multiresistant clones Spain23F-1, Spain9V-3, Taiwan23F-15, Poland23F-16, Columbia23B-19, Poland23F-20 and Greece6B-22 accounted for 18% of the isolates.

**Conclusions:** Although there is high diversity among the blood isolates of *S. pneumoniae* in Poland, both at the serotype and MLST level, vaccines can provide satisfactory protection against this pathogen. A significant proportion of multiresistant international clones may represent a factor compromising therapeutic options in this type of infection.

---

Increase in macrolide resistance among invasive isolates of *Streptococcus pneumoniae* in Norway, caused by the England14-9 clone  

Sogstad, MKR1, Litauer, P2, Auberger, JS2, Caugant, DA1, Høiby, EA1  
1Norwegian Institute of Public Health, Oslo, Norway  
2University Hospital of North Norway, Tromsø, Norway

**Background:**  
In the past four years there has been an increase in macrolide resistance among invasive isolates of *Streptococcus pneumoniae* in Norway. To get a better understanding of the reasons for this increase all erythromycin-resistant systemic isolates of *S. pneumoniae* received at the Norwegian Institute of Public Health (NIPH) in the period from 2001 – 2004 were characterized using phenotypic and genotypic methods.

**Materials and Methods:**  
Isolates from cases of systemic pneumococcal disease are routinely sent to NIPH for analysis. All strains were screened for their susceptibility to six different antibiotics by the agar diffusion method; strains with reduced inhibition zone size were further tested by Etest (AB Solna, Sweden), to determine MICs for penicillin, erythromycin, doxycycline, cefotaxime, ceftriaxone, clindamycin and ciprofloxacin. All strains were serotyped by the quellung reaction using antisera from Statens Seruminstitutt, Danmark. A selection of serotype 14 strains was genotyped using multilocus sequence typing (MLST) and examined for macrolide resistance genes by *erm* and *mef* specific PCRs, *mef*(A) and *mef*(E) were separated by Bamh I digestion.

**Results:**  
A total of 200 (6.1 %) of the strains were resistant to macrolides. The main serogroups among the resistant strains were 14 (85 %), 6 (7 %), 19 (2 %), 23 (1.5 %) and 9 (1.5 %). Serotype 14 showed an increase in numbers from 16 isolates in 2001 to 75 isolates in 2004. More than 90 % of the serotype 14 strains had low level resistance to erythromycin (MIC 2 µg/ml – MIC 64 µg/ml), and they were susceptible to all other antimicrobials tested. MLST analysis of a selection of these serotype 14 strains revealed that all strains were sequence type (ST) -9, the PMEN England14-9 clone. All the strains carried the macrolide resistance gene mef(A).

**Conclusions:**  
The increase in macrolide resistance in Norway was related to the PMEN England14-9 clone. This is the first of the internationally PMEN clones that successfully has spread in our country. The consumption of macrolides in Norway is low; indicating that this clone might be extra virulent. The mechanism responsible for the spread of this macrolide resistant clone susceptible to other commonly used antimicrobial agents remains to be elucidated.
Invasive pneumococcal disease in England and Wales 1996-2004

George RC, Gungabissoon U, Slack MPE, Martin S, Broughton K, Weight P, Miller E
Health Protection Agency Centre for Infections, Colindale, London, UK

Surveillance of invasive pneumococcal disease (IPD) in England and Wales was enhanced from 1996 onwards in advance of wider use of 23-valent plain polysaccharide vaccine (PPV) and the introduction of 7-valent conjugate vaccine (PnC). Laboratory reports of IPD and sterile site isolate referrals to the Health Protection Agency Centre for Infections (predominantly blood and CSF isolates) were reconciled and de-duplicated to produce a single dataset by epidemiological year (July to June). Lab reports in the absence of a serotype prompted a request to submit that isolate for typing. The mean number of IPD cases each year was 5059 (range 4586-5497) reported from around 215 labs. Culture-confirmed meningitis cases numbered between 260 to 363 each year with a mean of 308 per annum. Overall IPD incidence per 100,000 ranged from 8.8 to 10.5 in each year with higher rates in children and the elderly. E.g. incidence by age for 2003/04 was <2 months (mo) 21.8, 2-5mo 39.1, 6-11mo 72.6, 1-2 years (y) 33.8, 2-4y 9.6, 5-9y 2.5, 10-14y 1.0, 15-44y 3.7, 45-64y 7.9, 65-74y 17.8, 75-79y 32.3, 80+y 51.3. The overall proportion of isolates serotyped increased steadily from 36% in 1996/97 to 50% or higher of all IPD cases from 2000/01 onwards and for children aged < 5 years exceeded 70% in each of these years. A grand total of 60 different serotypes was observed over the 4 years but 93% of the total (N=11670) fell within 19 serotypes in the following rank order (%), 14 (18.1), 9V (9.1), 8 (7.7), 23F (6.9), 6B (6.0), 3 (5.4), 4 (5.3), 19F (5.1), 1 (4.8), 18C (3.5), 6A (3.4), 19A (3.2), 7F (3.2), 22F (3.2), 12F (2.0), 9N (2.0), 20 (1.5), 11A (1.3), 33F (1.0) all other serotypes were <1% of the total, 3% were nontypable. The rank order (RO) of the top 4 serotypes did not change by year but there was some variability in RO below this with a notable increase in serotype 1 from 3.6% in 2000/01 (RO 11) to 6.5% (RO 5) in 2003/04. PnC potential “coverage” was 76% for children < 5 years, 49% for all other ages combined and 53% for those ≥ 65. As expected PPV coverage exceeded 96%. These data will allow evaluation of the impact of pneumococcal immunisation initiatives in England and Wales.

Chronic pulmonary disease and risk of invasive pneumococcal infections

National Public Health Institute (KTL), Helsinki, Finland

Background Chronic obstructive pulmonary disease (COPD) is an established risk factor for invasive pneumococcal infections (IPI), but studies evaluating the risk associated with asthma have been contradictory. In Finland, chronic pulmonary disease (CPD), i.e. asthma and COPD, is the second most common reported underlying condition among patients with IPD (8.6%) after alcohol abuse. We conducted a population-based case-control study to evaluate the association of CPD and IPI among persons aged 18-64 years.

Methods IPI cases were identified through population-based laboratory surveillance during 1995-2002. Information on underlying illness was obtained from the National Hospital Discharge Registry (NHDR), Cancer Registry, National Social Insurance Institution (NSII), and National Infectious Disease Registry. For each case, ten controls matched on age, sex and place of residence were selected randomly from the Population Information System. Stable CPD was defined as entitlement for special reimbursement of medications for asthma or COPD in NSII data; unstable disease was defined as at least one hospitalization for asthma or COPD during 12 months before IPI episode according to ICD-10 codes.

Results A total of 2216 persons aged 18-64 years with IPI were identified; 791 (35.7%) had an underlying condition for which pneumococcal polysaccharide vaccine (PPV23) is recommended. Of cases and controls 123 (5.6%) and 666 (3.0%) had CPD, 57 (2.6%) and 36 (0.2%) had asthma and 43 (1.9%) and 19 (0.1%) had COPD. In a conditional logistic regression model adjusted for other independent risk factors, cases were more likely than controls to have CPD (OR, 2.0; 95%CI, 1.7-2.5), unstable asthma (OR, 20.0; 95%CI, 12.7-31.6) and unstable COPD (OR, 24.7; 95%CI, 3.5-45.3).

Conclusions CPD is an independent risk factor for IPI. The risk among persons with a recent hospitalisation for asthma or COPD was ten times as high as among those with stable disease. The risk for IPI was similar in persons with unstable asthma and unstable COPD suggesting that unstable asthma should be considered for inclusion in the list of conditions for which PPV23 is recommended.
The burden of pneumococcal disease and other invasive bacterial infections in children in urban Nepal

Maskey, M1, Mahat, S1, Kelly, DF2, Thorson, SM2, Adhikari, N1, Werno, AM3, Hamaluba, MM2, Pollard, AJ2, Murdoch, DR3
1Patan Hospital, Kathmandu, Nepal
2University of Oxford, Oxford, UK
3Christchurch School of Medicine & Health Sciences, Christchurch, New Zealand

Encapsulated bacteria cause a major burden of illness and death among children in resource-poor countries. Vaccines that provide protection against infection with *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* type b could prevent many of these cases today. However, the absence of information about rates of disease, affected age groups and local serotypes makes planning for implementation of these expensive vaccines difficult, even if the funding becomes available through global initiatives.

We aimed to define the burden of invasive bacterial infections and pneumonia among children admitted with febrile illnesses to Patan Hospital, Kathmandu, Nepal during 2005-6. In the first 8 months of the study we recruited 616 patients (aged 2 months to 13 years) with suspected bacterial infection. 48% had pneumonia, 10% had enteric fever and 4.4% had meningitis/encephalitis. 9.6% of blood cultures were positive (*Salmonella* species, 4.7%; *S. pneumoniae* 2%; *H. influenzae* type b, 0.2%; other pathogens, 2.7%). Of the 33 children with encephalitis/meningitis, 7 (21%) had CSF samples positive for *S. pneumoniae* by culture and/or antigen detection, and 1 (3%) had a positive CSF culture for *H. influenzae* type b. The greatest burden of invasive pneumococcal disease was in children <1 year old. The most common serotype of *S. pneumoniae* isolated was serotype 1. Preliminary testing showed an HIV seroprevalence of <3% among the study population.

These preliminary data highlight the large numbers of children presenting to an urban paediatric unit with serious bacterial infections that might be prevented by immunization. The currently available 7-valent pneumococcal vaccine does not provide protection against the most common isolates found in bacteraemic infections in Nepal but recently studied vaccines that cover serotype 1 could be used. Development of vaccines that prevent enteric fever should be a priority for this population.

This project was funded by the Pneumococcal Vaccines Accelerated Development and Introduction Plan (PneumoADIP) and contributes to the South Asian Pneumococcal Network Alliance (SAPNA).

Streptococcus pneumoniae serotypes isolated from Argentinian children with invasive disease

Vescina, CM1, Regueira, M2, González Ayala, SE3, Gatti, B1, Cecchini, DM3, Agosti, MR1
1Sor Maria Ludovica Children’s Hospital, La Plata, Argentina
2National Institute for Infectious Diseases Carlos G. Malbrán, Buenos Aires, Argentina
3Francisco J. Muñiz Hospital, Buenos Aires, Argentina

Background: Invasive pneumococcal disease (IPD) is frequent in children under 5 years of age. The increasing number of *S. pneumoniae* strains resistant to penicillin is an emergent problem in our country.

Objective: To study the distribution of serotypes isolated from normally sterile sites in children with IPD.

Material and methods: Prospective direct observational study of 557 children aged 0 - 14 years old with IPD undertaken at Sor Maria Ludovica Children’s Hospital, La Plata, Argentina, 1993-2003. *S. pneumoniae* strains were isolated from blood, cerebrospinal fluid, and articular, peritoneal and pleural effusions and studied for capsular serotype (capsular swelling method, antisera from Statens Serum Institute, Denmark) at the National Institute for Infectious Diseases Carlos G. Malbran.

Results: During the period of the study, forty serotypes were recognized and 1.8% strains were not typable. The number of serotypes per year ranged from 7 to 19. The overall frequency distribution for the ten more common serotypes was for 14, 29.5%; 5, 12.7%; 1, 10.1%; 6B, 5.3%; 7F, 5.1%; 19A, 4.1%; 9V, 3.9%; 23F, 3.7%; 19F, 3.4%; and 18C, 2.8%. Serotype 14 was the most frequent (15.8-40.0%) and the only one present every year. Serotypes 1, 5, 6B and 19A caused IPD in ten years of this period. Other serotypes (2, 3, 4, 6A, 7C, 8, 9N, 10A, 12F, 15A, 15B, 15C, 16F, 17F, 18A, 18B, 19F, 21, 22F, 23A, 23B, 33F) were also identified. Some serotypes (10, 11A, 13, 14C, 15, 24F, 27, 28A and 35F) were recognized just in a year and accounted with a case for each one.

Comment: We have observed shifts in the prevalence of individual serotypes causing invasive disease. It is very important to have the knowledge of the circulating serotypes in order to recommend accurate intervention strategies such as the use of the conjugate vaccines.
Severity of pneumococcal disease among hospitalized children in Bangladesh

Naheed A1, Saha SK2, Khatoon F1, Arifeen SE1, Brooks, WA1, Sack DA1, Breiman RF1, Luby SP1
1ICDDR,B: Centre for Health and Population Research, Dhaka, Bangladesh
2Dhaka Shishu Hospital, Dhaka, Bangladesh

Objective

Streptococcus pneumoniae is an important cause of illness, hospitalization, and death worldwide. We conducted surveillance in seven hospitals in Bangladesh for invasive pneumococcal disease in children < 5 years of age to document the severity of illness in this setting.

Methods

For patients admitted into a pediatric medicine ward in each of the participating hospitals who met broad case definitions of pneumonia, severe meningitis, or very severe disease, hospital clinicians were encouraged to obtain blood and or CSF culture. Samples were sent to the hospital laboratories for culture and antimicrobial susceptibility testing.

Results

Between May 2004 and April 2005, 8,802 eligible children were identified and 4,610 children (52%) had appropriate cultures obtained and were enrolled. Blood samples were obtained from 4,407 children and CSF from 1,047 children. A bacterial pathogen was isolated from 356 children (7%); 39 (11%) were S. pneumoniae. Each hospital isolated S. pneumoniae at least once (range 1 – 21). Among children with S. pneumoniae in blood or CSF, 67% were self referred, 26% reported use of an antibiotic prior to admission, 74% were infants and 62% male. The clinician’s diagnosis in children with culture positive S. pneumoniae was meningitis (n=29, 74%) pneumonia (n=10, 29%), febrile convulsion (n=5, 8%), and septicemia, severe malnutrition, upper respiratory tract infection and appendicitis in one patient each. The median duration between illness onset and hospital admission was 4 days (range 1-37 days). Despite treatment with an antimicrobial that the isolate was sensitive to, 23% of children with culture positive S. pneumoniae died (one of the three pneumonia patients and 24% of meningitis patients). Children, who died of S. pneumoniae infection, were predominately infants (78%); 67% of deaths occurred within the first two days of hospitalization.

Conclusion

Isolation of Streptococcus pneumoniae in all hospitals demonstrates that pneumococcal illness in children is a wide spread problem in Bangladesh. The high mortality despite children presenting to leading hospitals in the country and treatment with appropriate antimicrobials suggests that further efforts at prevention are important.

Prospective meningitis burden of disease study and rapid assessment of neurological outcomes in children in Fiji

Kunabuli, VL1, Mulholland, EK2, Tikoduadua, L1, Seduadua, A1, Pryor, J1, Russell, FM5
1Fiji Pneumococcal Project, Ministry of Health, Suva, Fiji
2Infectious Disease Epidemiology Unit, London School of Hygiene and Tropical Medicine, London, UK
3Ministry of Health, Suva, Fiji
4Fiji School of Medicine, Suva, Fiji
5Centre of International Child Health, Department of Paediatrics, University of Melbourne, Melbourne, Victoria, Australia

Aims: To document the incidence, morbidity and mortality of pneumococcal (Pnc) meningitis in children aged one month to less than 5 years in the Central Medical division of Viti Levu, Fiji. In addition, antibiotic susceptibility patterns and serotype distributions of the Pnc isolates will be documented.

Methods: Children aged one month to less than 5 years presenting to the Colonial War Memorial Hospital with suspected meningitis had cerebrospinal fluid (CSF) and blood cultures taken. The cases included fitted the bacterial meningitis (BM) definite and probable case definitions developed for this study. Cases had paired sera collected 10 days apart for anti-Hib ELISA and anti-Pnc surface adhesin A (PsaA) serology. Additional tests on CSF include Binax Streptococcus pneumoniae test, Pnc specific PCR, generic bacterial 16S PCR, and enterovirus PCR. Cases were reviewed post discharge for a neurological and developmental assessment.

Results: Over the first 13 months there were 40 children with confirmed meningitis. 2/40 did not have CSF collected as a LP was contraindicated. One had clinical signs of meningitis, and the other had BM confirmed on autopsy. 11/40 (27.5%) were definite BM cases, 27/40 (67.5%) were probable BM cases and 2/40 (5%) were non-BM. There were 7/40 causative organisms including 5 Pnc (45.5%), 2 (18.2%). Group B streptococcus, and 1 case each of Haemophilus influenzae, Group A streptococcus, Staphylococcus aureus, Neisseria meningitidis and Eschericia coli. 12/40 (30%) cases were pretreated with antibiotics. Results of PCR and serological tests will be presented at the meeting. All Pnc isolates were penicillin susceptible. The Pnc isolates have yet to be serotyped. The annual incidence of Pnc meningitis was 14.2 per100,000 children aged one month to < 5 years. The case fatality rate (CFR) for BM was 15% (6 cases). The CFR for non-BM was 0% (0/2). 3/36 (9.1%) BM cases and 0/2 non-BM cases had neurological sequelae. The remaining 4 cases of BM are alive but have not been reviewed.

Conclusion: The annual incidence for Pnc meningitis is lower than what is found in other developing countries yet higher than developed countries. CFR and neurological sequelae are lower than what is found in other developing countries yet higher than developed countries. Meningitis does account for a significant morbidity and mortality in the paediatric population in Fiji.
Overall rates of resistance (defined as the rate of intermediate resistance plus the rate of resistance) were as follows: penicillin, 62.55% against these isolates according to the Clinical and Laboratory Standards Institute (CLSI before NCCLS). Overall rates of resistance (defined as the rate of intermediate resistance plus the rate of resistance) were as follows: penicillin, 62.55% (intermediate and resistant categories were 45.37% and 17.18%, respectively); amoxicillin, 7.93%; cefaclor, 32.16%; CRO, 4.85%; cefuroxime, 28.29%; cefotaxime, 6.9%; cefazidime, 5.14%; erythromycin, 26.43%; clindamycin, 20.71%; azithromycin, 73.57%; tetracycline, 5.73%; imipenem, 41.4%; meropenem 13.22%; trimethoprim-sulfamethoxazole (TMP-SMX), 69.6%; and vancomycin, 1.32%. No resistance was observed with amoxicillin/clavulanate, chloramphenicol or teicoplanin; Trend analysis indicated that rates of resistance to beta-lactams, macrolides, tetracyclines, TMP-SMX, and multiple drugs are becoming more prevalent.

The isolated pneumococci were of 33 serotypes, of which the most frequent were 23F, 17.53%; 6B, 11.85%; 14, 10.43%; 19F, 9.06%; 6A, 7.11%; 9V, 6.16%; 19A, 5.69%; 18C, 4.26%; 7F, 3.79%; 15, 3.32%; and 5, 2.84%. The site of isolated were: bronchial secretion of bronchoalveolar lavage (in children with chronic obstructive pulmonary disease and pneumonia), 17.6%; blood cultures, 35.54%; CSF 30.33 %, peritoneal fluid, 11.85%; pleural fluid, 3.39%; Joint fluid, 1.42%. The age (in months) of patients were < 1, 0.47%; 1-2, 5.21%; 3-24, 36.97%; 25-59, 28.91%; and >60, 28.44%.

CONCLUSIONS: Trend analysis indicated that rates of resistance to beta-lactams, macrolides, tetracyclines, TMP-SMX, and multiple drugs are becoming more prevalent. Knowledge of serotypes is crucial for prevention with conjugated vaccines.

A multi-centre retrospective analysis of empyema and pleural effusion in hospitalized Asian children

**Background:** In Asia, there are limited data that describe the burden of pneumococcal empyema and pneumococcal serotypes that are associated with empyema or pleural effusions in children.

**Methods:** We conducted a retrospective review of existing hospital microbiologic and discharge data to identify children aged ≤15 years with bacterial pathogens isolated from parapneumonic pleural effusion or empyema fluid collections in seven collaborating centres located in Australia, China, Indonesia, South Korea, Taiwan, Thailand and VietNam. For the period 1995 through 2004, each centre conducted a systematic search for information on pneumococcal isolates. Stored pneumococcal isolates underwent culture and serotyping using standardized methods.

**Results:** Over the 10-year period, 1,373 empyema and pleural effusion specimens from children ≤15 years of age were identified from centres in China, Indonesia, Korea, Taiwan, Thailand and VietNam. Empyema and pleural effusion specimens were distributed in a bi-modal pattern (one peak among infants and a second in children 6-15 years of age). In New South Wales, hospital discharge data analysis identified 1,008 children with empyema or pleural effusion—these children were also distributed in a similar bi-modal age distribution. A total of 57 empyema and pleural fluid cultures were positive for *Streptococcus pneumoniae* (including 37 from Taiwan). Serotyping performed to date on available isolates identified the following pneumococcal serotypes: 14 (n=3), 19 (n=3) and 1 (n=1). Based on specimens from China, Korea and VietNam, preliminary analysis suggests the presence of two seasonal peaks in the occurrence of empyemas and pleural effusions in May—June and December—January. No pneumococcal isolates were recovered from empyema or pleural effusion specimens in Australia, China and Indonesia.

**Conclusions:** Overall, less than 5% of empyema and pleural effusion specimens were positive for *S. pneumoniae*. Two pneumococcal serotypes (14 and 19) isolated from empyema and pleural effusion specimens are available in the 7-valent pneumococcal conjugate vaccine. Our results suggest that robust data are available in centres across Asia but clinical microbiologists do not have sufficient laboratory resources to maintain local repositories of bacterial isolates. Future studies using polymerase chain reaction and antigen detection testing may enhance detection of *S. pneumoniae* in culture-negative empyema and pleural fluid specimens. Systematic clinical and laboratory-based surveillance for children who manifest empyema or pleural effusion is needed to better understand the spectrum of bacterial pathogens and pneumococcal serotypes associated with these conditions.
Longitudinal carriage study of *Streptococcus pneumoniae* serotype or group in Tanzanian children

Orvio, N1, Sam, N, Gillespie, SH2, & Charalambous, BA2.
1Tumaini University, Moshi, Tanzania
2Royal Free & University College Medical School, UCL, London, U.K.

**Background:** Despite the availability of vaccines *Streptococcus pneumoniae* kills annually around one million children world-wide. Prevenar™ costs $150/child and provides partial protection against a geographically limited sub-set of serotypes. This has highlighted the need for a vaccine that is globally affordable particularly for resource limited countries where the burden of disease is greatest. As nasopharyngeal carriage usually precedes disease a vaccine that reduces carriage would not only protect, but also decrease transmission and the emergence of drug resistant strains.

**Aim:** To study the temporal dynamics of pneumococcal carriage in Tanzanian children.

**Methods:** Pneumococcal carriage was followed over 12 months in 24 Tanzanian children from a semi-closed closed community. Healthy children under 6 years of age carrying pneumococci at the first sample point were recruited to the study. Pernasal and oropharangeal swabs were taken at monthly intervals and archived in 1.0mL STGG medium at -70°C1. Up to 10 pneumococci were serotyped or serogrouped (ST). Non-typable isolates (NTs) were either not in the 23-valent vaccine or not typable.

**Results:** Although the baseline carriage rate was 32.5%, 87.5% (21/24) of children were found to be carrying multiple STs, and only 3 children (12.5%) carried single STs. Two children carried the same ST consecutively for several months with a child carrying ST 6 for 3 months, which was carried again after a 4 month gap. The other child carried ST 17 for four consecutive months. The proportion of NTs was high and frequently carried for more than a month.

**Conclusions:** Pneumococcal carriage in these children ranged from short duration of carriage of single STs to complex multi-serotype carriage with the same ST carried up to 4 months.

**Further work:** Work is in progress to genotype by MLST the isolates with the same ST that were carried for more than one month. A PCR-RFLP method for serotyping will be used to analyse the NT isolates, and those with the same RFLP profile will be genotyped. To assess why some serotypes colonise the nasopharynx for longer than others an analysis of host pneumococcal immune responses to Pneumolysin and to capsular polysaccharide of various serotypes/ groups will be investigated.

**References:**

---

*Streptococcus pneumoniae* nasopharyngeal colonization in day-care centers of central Greece

Grivea, IN, Chryssanthopoulou DC.
The Hellenic Antibiotic-resistant Respiratory Pathogens (HARP) Study Group, Syrogiannopoulos, GA
University of Thessaly, School of Medicine, Larissa, Greece

**Background:** Greece is a European country with high prevalence of antibiotic-resistant (A-R) *Streptococcus pneumoniae* (Sp). In our country the 7-valent pneumococcal conjugate vaccine (PCV7) became available in Oct 2004.

**Methods:** In an ongoing study, initiated in February 2005, we investigated nasopharyngeal (NP) colonization with Sp in children living in Central Greece. Isolates were studied by disk diffusion test and Etest in CO2 for their susceptibility to penicillin G (Pen), cefotaxime (CTX), erythromycin (Ery), clindamycin (Cli), chloramphenicol (Chl), tetracycline (Tet), ciprofloxacin (Cipro), and cotrimoxazole (SXT).

**Results:** Between February and June 2005, NP cultures were obtained from 769 children (median age 4.2 yrs, range 0.5-6.3 yrs) attending 12 day-care centers in the cities of Larissa and Volos, Central Greece. More than half (52.8%) of the children had received antibiotics during the last 3 months. The percentage of children aged <5 yrs vaccinated with PCV7 was 17.1%. Of the 769 children, 374 (48.7%) carried Sp isolates. Of these 389 isolates, 29.8% were Pen-non-susceptible (NS); 19% were intermediate (I) and 10.8% resistant (R) to Pen. 6.6% of the Sp isolates was CTX-NS (I=1.5%, R=5.1%). 29.6% of the Sp isolates were Ery-R. 5.9% of the Sp isolates had decreased susceptibility to Cipro (MIC=2 µg/ml). The two most frequently recovered serogroups were 6 and 19, accounting for 24.7% and 13.6% of the total isolates, respectively. A-R were 53 (55.2%) of the 96 serogroup 6 isolates; 6B (n=28) and 6A (n=25). The predominant resistance patterns in A-R 6B isolates were SXT (n=14), Cli-SXT (n=7), and Cli-Tet-Ery-Cli-SXT (Greece 6B-22 clone, n=6), whereas in 6A were SXT (n=15) and Pen-Ery (n=9). A-R were 50 (94.3%) of the 53 serogroup 19 isolates; 19F (n=38) and 19A (n=12). The predominant resistance patterns in A-R 19F isolates were Pen-Tet-Ery-Cli-SXT (n=21) and Pen-Tet-Ery-SXT (n=14), whereas in 19A were Pen (n=7) and Pen-SXT (n=3). A recent emergence and spread of Pen-NS 19F isolates with high MICs to CTX was noted in Greece.

**Conclusions:** During 2005 we found high resistance rates in Sp NP isolates; 29.8% were Pen-NS, 5.1% had MICs to CTX of 4-8 µg/ml, and 29.6% were Ery-R. The universal administration of PCV7 should be a priority for Greece. Significant resistance was identified in serotypes 6A and 19A, a point that should be taken into consideration in the development of new vaccines.
The invasive disease potential of Icelandic pneumococci

**Brueggemann, AB**<sup>1</sup>, Dhillon, SS<sup>2</sup>, Erlendsdottir H<sup>3</sup>, Kristinsson, KG<sup>4</sup>, Spratt, BG<sup>2</sup>

<sup>1</sup>University of Oxford, Oxford, United Kingdom
<sup>2</sup>Imperial College, London, United Kingdom
<sup>3</sup>Landsnálitli University Hospital, Reykjavik, Iceland

Understanding the population biology of the pneumococcus is important for many reasons, one of which is to understand potential effects of widespread vaccination on the pneumococcal population. Previous work suggests that serotype is a key determinant in the ability of pneumococci to cause invasive disease, which is important in the context of vaccines that offer serotype-specific protection. In this study we explored whether the observations originally made from studies of children in Oxford were generalisable to a population of pneumococcal isolates recovered from children in Iceland. Pneumococci from children <7 yrs of age in the Reykjavik area were characterised. Carriage isolates (n = 1049) were collected during 1992, 1995-97, 1999, and 2004-5 from healthy children attending day care centres. Invasive isolates (n = 105) were recovered during 1993-2005. Pneumococcal identification and serotyping was performed in Reykjavik, and genotyping by multilocus sequence typing (MLST) was performed in Oxford on all invasive isolates and a selected group of carriage strains (total n = 394) based on serotypes of interest. Serotype estimates of invasive disease potential were calculated using a standard odds ratio (OR), comparing a particular serotype with all other serotypes. An OR > 1 indicates an association with invasive disease and an OR < 1 an association with carriage. Serotype-specific ORs (95% confidence intervals) were statistically significant for serogroup 7, OR = 5.6 (2.8-11.2) and serotypes 14, OR = 5.1 (3.0-8.5), 9V, OR = 2.4 (1.1-5.3) and 23F, OR = 0.4 (0.2-0.9). One-third (n = 33) of invasive disease isolates were from one of two serotype 7F clones (n = 15, ST191 and 218) or two serotype 14 clones (n = 18, ST9 and ST36). Other major clones included global clone Spain<sup>6</sup>-2 (ST90), all serotype 6B, 10% of invasive strains; ST199 (n = 69, serotypes 19A and 15; all 6 invasive strains were serotype 19A); ST156 and 162 (33/35 serotype 9V strains, including 7/8 invasive serotype 9V strains); and ST37 (all serotype 23F, from carriage). The serotype-specific ORs in Iceland are consistent with those calculated for other datasets and support the serotype as an important predictor of invasive disease potential. Major invasive disease-causing clones found among children in Iceland include strains of serotypes 7F, 14, 9V and 6B. A vaccine selected for use in Iceland should include these serotypes.

Variations in prevalence of serotypes of *Streptococcus pneumoniae* isolated from nasopharynx in Indian children

**Wattal C**<sup>1</sup>, Mathur M<sup>2</sup>, Kulkarni M<sup>2</sup>, Prabhi PK<sup>2</sup>, Oberoi JK<sup>3</sup>, Patil S<sup>2</sup>, Rambhad G<sup>2</sup>

<sup>1</sup>Department of Microbiology, Sir Gangaram Hospital, New Delhi, India
<sup>2</sup>Department of Microbiology, L.T.M. Medical College, Sion, Mumbai, India
<sup>3</sup>Department of Pediatrics, L.T.M. Medical College, Sion, Mumbai, India
<sup>4</sup>Department of Pediatrics, Sir Gangaram Hospital, New Delhi, India
<sup>5</sup>Wyeth Limited, Mumbai, India

**Objectives:** Nasopharyngeal (NP) carriage is a major factor in the transmission and pathogenesis of pneumococcal disease. This study was conducted in two different centers to evaluate the NP carriage and prevalent serotypes of *Streptococcus pneumoniae* in healthy children. The antibiotic-resistance of NP carriage isolates was also assessed.

**Methods:** Nasopharyngeal swabs were obtained in children aged 3 months to 3 years, attending 2 study settings, a public hospital and a private hospital. Isolates of *Streptococcus pneumoniae* were identified using standard methods. Pneumococcal serotyping was performed by Quellung reaction using pneumococcal antiserum supplied by the Statens Serum Institute, Copenhagen, Denmark. Antimicrobial susceptibility to penicillin, amoxicillin, erythromycin, cefotaxime, cotrimoxazole and ciprofloxacin was performed by Kirby Bauer disk diffusion and E-test method.

**Results:**

**Hospital 1 - Public Hospital**

300 healthy children attending a public hospital urban health care center were enrolled. NP colonization was noted in 222/300 (74%) of the children. 4 serogroups/serotypes(ST) were identified. Serotype distribution was as follows: ST 6 (59/134, 44%), ST 1 (45/134, 33.6%), ST 19F (21/134, 15.7%) and ST 10 (7/134, 5%). Two isolates were untypable. Antimicrobial susceptibility testing demonstrated that 24.8% of isolates were resistant to cotrimoxazole (55/222), 4.5% to amoxicillin and penicillin (10/222) and 0.9% to cefotaxime (2/222).

**Hospital 2 - Private Hospital**

200 healthy children attending a private hospital pediatric outpatient were enrolled. NP colonization was noted in 13/200 (6.5 %) of the children. 4 serotypes were identified. Serotype distribution was as follows: Serogroup 19 (8/13, 61.5%), followed by 6, 2/13 (15.4%), ST14 (1/13, 7.7%) and ST 1 (1/13, 7.7%). One isolate (7.7%) was not typable. All the isolates were resistant to co-trimoxazole. 2 isolates showed penicillin non-susceptibility, one with intermediate resistance and one with high level resistance. All isolates were sensitive to amoxicillin and cefotaxime. Erythromycin and ciprofloxacin resistance was observed in 1 (7.6%) and 2 (15.3%) isolates, respectively.

**Conclusion:** There was a significant variation in the colonization of *Streptococcus pneumoniae* as well as the serotypes isolated in the public setting versus the private setting. The results stress the need for further evaluation of *Streptococcus pneumoniae* serotypes in differing setting. The 7-valent coverage ranged from 59.7% in the first setting to 84.6% in the second setting. Data on serotype prevalence can serve as a basis for recommendations for the use of the pneumococcal conjugate vaccine in India.
Impact of antibiotic use on clearance and acquisition of pneumococcal carriage in UK families

Centre for Infections, Health Protection Agency, London, UK

Aim: The impact of antibiotic use on the acquisition and clearance of pneumococcal carriage in families was studied using a UK based longitudinal pneumococcal carriage study.

Data: A ten-month longitudinal study of pneumococcal carriage in 121 families analysed 3,753 swabs including 230 swabs (6%) with antibiotic use between consecutive swabs at an interval of one month.

Analysis: Previous studies (Melegaro 2004) have used a family-based first order Markov model to derive estimates of pneumococcal transmission parameters (transmission rates between family members; acquisition rates from outside the household; and carriage clearance rates) from longitudinal data. This model was adapted to incorporate the effect of antibiotic usage on acquisition and clearance of pneumococcal carriage, and other risk factors such as age, gender, smoking and duration of child care attendance. Maximum likelihood techniques were used to identify risk factors with significant effects on the transmission.

Results: Antibiotic use significantly affected clearance of pneumococcal carriage in all age groups; antibiotic use cleared pneumococcal carriage 2.13 times faster (95% CI: 1.33, 3.33) among children under 5, and 3.47 times faster (95% CI: 1.44, 10) for those aged 5 and above. Antibiotic use showed a significant effect on pneumococcal acquisition only among children under 5 (relative risk of acquisition 0.44, 95% CI: 0.18, 0.89) (adults: 1.89, 95% CI: 0.66, 4.28). Significant effects of age (p=0.0001) and duration of child care attendance (p=0.018) were also found, while gender (p=0.22) and smoking (p=0.2) were not significant.

Invasive pneumococcal disease among children in rural Bangladesh: Results from a population-based surveillance

Arifeen SE1, Saha SK2, Rahman SM1, Bari S1, Naheed A1, Mannan I1, Seraji HR1, Ahmed N1, Hassan MS1, Huda N1, Rahman S1, Quasem F1, Islam M1, Fatima K1, Brooks WA1, Breiman RF2, Sack DA2, Laby S2
1ICDDR,B: Centre for Health and Population Research, Dhaka, Bangladesh
2Dhaka Shishu Hospital, Dhaka, Bangladesh
3Kumudini Hospital, Mirzapur, Bangladesh
4Centers for Disease Control and Prevention, USA

Background
Pneumonia accounts for almost 2 million (19%) of the 10.6 million childhood deaths annually in the world. *Streptococcus pneumoniae* (pneumococcus) infections are estimated to be responsible for a substantial proportion of these pneumonia deaths, though population-based data is largely lacking for most parts of the developing world.

Methods
We initiated population-based surveillance in a rural Bangladesh community of Mirzapur, 60 kilometres north of the capital, Dhaka. Trained village health workers (VHWs) made weekly visits to approximately 12,000 children aged 1-59 months in the study area. Children with reported fever, cough or difficult breathing were assessed by the VHWs using a clinical algorithm, and referred to the hospital when required. Sick children were hospitalized, as determined by the attending physician, and subjected to clinical and laboratory work-up. We present here interim results from 37 weeks of surveillance from February to October 2005.

Results
Assuming weekly visits as equivalent to 7-days of observation, there were 7,987 child-years of observation; VHWs identified 1,236 cases of possible severe pneumonia/very severe disease (15 per 100 child-years) and 1,840 cases of possible pneumonia along with 3,002 cases of high fever, and 636 cases of suspected meningitis/very severe disease (8 per 100 child-years). A total of 883 under-5 children from the surveillance area were admitted in the hospital with most common diagnoses of acute bronchitis (23%), upper respiratory tract infection (14%) and pneumonia (8%). *Streptococcus pneumoniae* was isolated from 5 of 308 blood cultures and 1 of 13 CSF cultures; non-pneumococcal bacteria were isolated from 9 other specimens. All 6 pneumococcal isolates were susceptible to penicillin, chloramphenicol, erythromycin and ciprofloxacin; 5 (84%) isolates were non-susceptible to cotrimoxazole.

Conclusions:
The surveillance findings suggest substantial burden of pneumonia and meningitis among under-5 children in rural Bangladesh. We may have underestimated the role of *S. pneumoniae* as a cause for these syndromes as blood cultures have low sensitivity and the weekly surveillance likely missed some cases. The burden of pneumococcal disease and the potential beneficial impact of pneumococcal vaccines in this community would be best evaluated via a prospective “vaccine probe” study. The low resistance to most antibiotics is heartening; however, the national ARI control programme may need to re-evaluate its choice of cotrimoxazole as the first line drug for management of pneumonia.
Clonal and capsular type decides whether pneumococci will act as a primary or opportunistic pathogen

Sjöström K1,2, Spindler C3,4, Ortqvist A4, Kolin M2, Sandgren A1,2, Kühlmann-Berenzon, S5, Henriques-Normark B4,2

*Contributed equally

1Dep of Bacteriology, Swedish Institute for Infectious Disease Control, Solna, Sweden
2Microbiology and Tumorbiology Center, Karolinska Institutet, Stockholm, Sweden
3Unit of Infectious Diseases, Department of Medicine, Karolinska Institutet, Karolinska University Hospital, Solna, Sweden;
4Unit of Infectious Diseases, Department of Medicine, Karolinska Institutet, Karolinska University Hospital, Solna, and Department of Communicable Diseases and Prevention, Stockholm County, Sweden.
5Dep of Epidemiology, Swedish Institute for Infectious Disease Control, Solna, Sweden

Streptococcus pneumoniae is an important contributor for invasive disease among adults. Recent epidemiological studies, comparing the distribution of invasive isolates with carriage isolates, have shown that the potential for pneumococci to cause invasive disease differs with serotype. Certain serotypes, such as types 1 and 7F, are mainly found causing invasive disease and are rarely found among carriers, while others are predominately found to cause carriage (such as type 19F and 23F). The role of the different capsular and clonal types in invasive disease severity remains to be defined.

Aim: The aim of the study was to investigate existence of correlations between severity and type of disease with age, underlying diseases as well as capsular serotype and clonal type of the microbe in adult patients with invasive pneumococcal disease (IPD). The patient material and pneumococcal isolates were assembled during two prospective clinical studies of IPD in adult patients.

Material and methods: The first clinical study was performed during 1993-1995, where hospitals in 5 countries participated and 354 isolates were available for characterization. The second clinical study was performed in the Stockholm area during 1999-2000, where 140 patients were included. Totally 494 patients were included in the study. Treatment routines and clinical parameters were recorded. APACHE II scores were used for estimation of severity of disease. Three molecular typing methods were used for characterizing the clinical isolates, Box fingerprinting, pulsed-field gel electrophoresis and multilocus sequence typing.

Results: Serotype 1 and 7F, respectively, were genetically homogenous and had the highest potential to infect previously healthy individuals and were not causing deaths. Also, type 1 isolates were only found among younger adults while other serotypes were mainly found among elderly, for example type 23F. Some clones were more prone to cause severe disease as observed by high APACHE II scores at admission, as well as a high mortality (such as clones of type 3 and 11A).

Conclusion: We suggest that clones with capsular types 1 and 7F, known to have a high invasive disease potential, behave as primary pathogens. Clones with other capsular types, with lower relative risk of causing invasive disease, are more opportunistic primarily affecting patients with underlying diseases. Disease caused by the latter group was however more severe even in previously healthy individuals.

Secular variations in age incidence (Inc) and serotypes (St) causing invasive pneumococcal disease (IPD) in children 0 to 59 months of age (MoA) in the Metropolitan Region (MR), Chile.

Lagos R1,2, Muñoz A3, San Martin O1, Ingrid Heitmann3, Loyola H2, Levine MM4

1Centro para Vacunas en Desarrollo-Chile; Santiago, Chile
2Hospital de Niños Roberto del Rio; Santiago, Chile
3Instituto de Salud Pública de Chile (ISP); Santiago, Chile
4Center for vaccine Development, University of Maryland; Baltimore, USA

Background: We have systematically monitored paediatric hospitalizations due to culture-proved IPD in the MR since 01/1994, detecting cases by regular visits to the laboratories of 11 hospital that account for >80% of all MR paediatric admissions. Clinical and demographic data extracted from the patient’s medical records are archived in an anonymous database. By national norm, all invasive S.pn isolates are referred to the Institute of Public Health (ISP) for confirmation of species and serotyping.

Methods: CVD-Chile IPD database, ISP database of serotyped S.pn isolates, and 1994 – 2004 MR population (ppn) were used to calculate, per 1000 children 0 to 59; 0 to 5; 5 to 35 and 36 to 59 MoA: i) annual age-specific of IPD; ii) annual Inc of IPD caused by S.pn of the 4 foremost common Sts identified during the study period. Trends and coefficients of variation (CV, %) of overall and St-specific Inc of IPD were examined. Pearson’s coefficients (r) were calculated to assess correlation between overall and St-specific Inc of IPD in each age stratum.

Results: The overall Inc of IPD in ppn 0 to 59 MoA varied from 16,6x105 in 1994 to 35,3x105 in 2004. Significantly increasing trends occurred among children 0-59; 6-35 and 36-59 MoA (χ2 trends < 0.001); Inc in infants 0-5 MoA diminished (p = 0.008). 1169 of the 1465 (79,8%) invasive S.pn isolates were detected; 49.7% of the isolates were Serotype 14 (n=284), Serotype 5 (n=125), Serotype 6B (n=89) and Serotype 1(n=83). Overall CV of IPD was 22,6%, with St-5 and St-1 disease exhibiting highest (83,2 and 61,5%, respectively), and St-14 the lowest variability (40,4%). Annual, overall IPD Inc correlated significantly with the St-5 specific Inc in infants 0 to 5 MoA (r=0.6; p = 0.04); with St-14 Inc in the 6-35 MoA group (r = 0.9; p = 0.0001), and with the Inc of St-1 disease in children 36 to 59 MoA (r = 0.8; p = 0.004).

Conclusions: The recorded Inc of pediatric IPD in the MR rose during the 11-year study period, probably due to more sensitive bacteriological methods for investigation of blood and other sterile fluids. Improvements in socioeconomic and environmental conditions might have reduce early life exposure to S.pn, resulting in diminution of Inc among young adults.
Little is known about the epidemiology of pneumococcal disease in children after the implementation of PCV7. The objective of this study was to determine trends in invasive pneumococcal disease (IPD) and serotypes of *S. pneumoniae* among children in Barcelona. This is an observational cohort study including all paediatric patients diagnosed of IPD who were seen at Hospital Sant Joan de Deu in Barcelona from Jan 1998 to Jun 2005. IPD was defined as the presence of clinical findings of infection together with isolation of *S. pneumoniae* in sterile fluids. PCV7 was introduced in Spain in June 2001. Isolates of *S. pneumoniae* were serotyped at the Centro Nacional de Microbiologia, Madrid.

A total of 139 episodes of IPD (in 135 patients) were diagnosed during the study period (44 pneumonias, 27 meningitis, 32 bacteremia, 12 arthritis, 8 mastoiditis and 16 others). The mean age of the patients was 3.1 yrs (range 1-17 yrs). Of the 139 episodes, 55 were diagnosed in the period 1998-2001 and 84 in 2002-2005. The rate of IPD increased from 0.85 episodes/1000 admissions in the first period to 1.48 episodes/1000 admissions in the second period. Overall, 133 strains were available for serotyping and 23 different serotypes were identified. The serotype distribution varied between the two periods (1998-2001 vs. 2002-2005): vaccine serotype (7VS) (65% vs. 34%, P<0.01), vaccine-related serotypes (7VRS) (9% vs. 25%, P=0.02) and non-vaccine serotype (7NVS) (26 % vs. 41%, P=0.08). A significant increase was observed in 19A serotype (1.8% vs. 17.7%, P<0.01) and in serotype 7 (0% vs. 6.3 %, P=0.08); all 7VS except serotype 14 decreased in the second period. Of the 46 episodes caused by 7NVS, 24 had pneumonia (58% with empyema), 8 meningitis, 7 bacteremia and 7 others. Of the 139 episodes, 28 (20%) were admitted to paediatric intensive care unit (PICU): 12 in the first period (8 were 7VS, 2 were 7VRS and 2 were 7NVS), and 16 cases in the second period (6 were 7VS, 3 were 7VRS, and 7 were 7NVS).

Invasive pneumococcal disease caused by vaccine-related and non-vaccine serotypes of *S. pneumoniae* among children is increasing in Barcelona, Spain.

---

**Pneumococcal clones causing invasive disease in Iceland, 1990-2004**

**Kristinsson, KG, Jensdottir, H, Erlendsdottir, H, Gumarsdottir, T**

Landsfópoli University Hospital and University of Iceland, Reykjavik, Iceland

The propensity of pneumococci to cause invasive disease varies between serotypes, and there appear to be differences in virulence within serotypes. Our aim was to study the clonality of serotypes causing invasive disease in the Icelandic population and temporal changes over a 15-year period.

Invasive pneumococcal disease has been recorded for the whole Icelandic population since 1975. Since 1990 most of the invasive pneumococcal isolates (blood and CSF) have been serotyped and stored (-80 °C). We subcultured all viable isolates for antimicrobial susceptibility testing to oxacillin, erythromycin, chloramphenicol, trimethoprim-sulphamethoxazole, tetracycline and chloramphenicol using disc diffusion and to penicillin and ceftriaxone using the E-test (oxacillin resistant strains). Serotyping was performed on all isolates and molecular typing using pulsed field gel electrophoresis (PFGE, after DNA restriction with the Smal enzyme) on the majority of isolates. Of the 698 pneumococci isolated from invasive infections, 492 could be located and were viable. Overall 41 (8.3%) had reduced susceptibility to penicillin but only one of these was resistant (0.2%). Non-susceptibility to chloramphenicol, erythromycin, tetracycline and trimethoprim-sulpha was 5.5%, 8.9%, 6.7% and 21.2% respectively. For the periods 1990-4, 1995-9 and 2000-4, penicillin non-susceptibility was 7.7%, 9.4% and 7.8% and erythromycin non-susceptibility 8.8%, 8.8% and 9.1%, respectively. The most frequent serotypes were 7F (108), 9V (44), 6B (41), 14 (39), 23F (29), 4 (27) and 19A (22). PFGE typing has been performed on 343 (70%) isolates, focusing on the common serotypes. The number of clones within serotypes varies. Serotypes 4 (8 of 27 typed), 19A (14 of 22 typed) and 3 (all 20 typed) only have a single clone each, whereas serotype 14 has 8 different clones (38 of 39 typed). For serotype 7F all 108 isolates belong to only two clones, where one of the clones increases in incidence with time and the other decreases and disappears in 2003. Of the 7 serotype 1 isolates, all but one belong to the same clone that appeared in 2002.

The diversity within serotypes varies between serotypes. Clonal incidence can change with time, which may not be reflected in serotype incidence. This may have important implications for future vaccines. The PFGE typing is in progress and enables a comparison with carriage isolates collected from the same geographical area during the same period.
Clinical characteristics, antibiotic resistance and serotype distribution of invasive pneumococcal disease in Metropolitan Atlanta after introduction of pneumococcal conjugate vaccine

Albritch, WC 1, Baughman, W 2, Schmotzer, B 3, Farley, MM 1, 2, 4
1Division of Infectious Diseases, Emory University School of Medicine, Atlanta, GA, USA
2Georgia Emerging Infectious, Atlanta, GA, USA
3Rollins School of Public Health, Emory University, Atlanta, GA, USA
4Veterans Affairs Medical Center, Atlanta, GA, USA
*Current affiliation: Respiratory and Meningeal Pathogens Research Unit, Chris Hani Baragwanath Hospital, Soweto, South Africa

Background: Invasive pneumococcal disease (IPD) has decreased among children and adults since licensure of 7-valent pneumococcal conjugate vaccine (PCV7) for infants in the USA in 2000.

Methods: Temporal trends of incidence, antibiotic resistance, clinical syndromes and underlying conditions were analysed, separately for adults (age ≥18 years) and children (age <18 years). Active laboratory-based surveillance data from the CDC-sponsored Emerging Infections Program for the 20-county Metropolitan Atlanta area from 1 January 1997 until 30 July 2004 was used for IPD, defined as isolation of Streptococcus pneumoniae from sterile sites. Clinical information was obtained through chart reviews since 2000. We compared serotype (ST)-coverage of the current PCV7 (ST: 4, 6B, 9V, 14, 18C, 19F, 23F) with that of candidate 11- and 13-valent conjugate vaccines (additional ST: PCV11: 1, 3, 5, 7F; PCV13: 6A, 19A). Comparative time periods were chosen from 1 July to 30 June of the subsequent year.

Results: The incidence of IPD for adults decreased from 22.8/100,000 in 1997/98 to 14.9/100,000 in 2003/04 (Cochran-Armitage trend test p < 0.0001; for children: 45.0/100.000 to 10.7/100.000; p < 0.0001). Penicillin resistance in adults increased from 10.5% in 1997 to 16.9% in 2000/01 before decreasing to 9.3% in 2003/04 (RR: 0.55, 95% CI: 0.38-0.79), with similar trends for children. Among adults, the proportion of non-PCV7 ST increased from 45.3% in 1999/2000 to 79.1% in 2003/04 (p < 0.0001). PCV11 and PCV13 would extend adult 'coverage' from 20.9% to 22.9% and 31.8% in the 2003/04 season (p = 0.069, p = 0.0001 for comparisons PCV7 to PCV11 and PCV13, respectively). Since 2000 there were significant declines of rates of adult bacteremic pneumonia and meningitis (p for trend = 0.0002 and p = 0.0003, respectively), and rates of pediatric primary bacteremia, bacteremic pneumonia and meningitis (p for trend < 0.0001, p = 0.003 and p = 0.0092, respectively). An increasing proportion of IPD patients aged ≥40 years had at least one underlying condition since 2000 (p for trend = 0.0038).

Conclusions: The declining incidence of IPD in Atlanta since introduction of PCV7 was associated with decreases in both adult and pediatric pneumonia and meningitis, and in primary bacteremias in children. The proportion of IPD patients aged ≥40 years with underlying conditions has increased. Introduction of new PCV with extended ST-coverage may lead to further benefits.

Pneumococcal epidemiology in Sevilla and Malaga, Spain

Brueggemann, AB 1, Arroyo, LA 2, Hausdorff, WP 3, Sanchez-Tatay D 2, Moreno D 2, Torronteras R 2, Mateos F, Fenoll A 1, Obando, F
1University of Oxford, Oxford, United Kingdom
2Hospital Virgen del Rocio, Sevilla, Spain
3Glaxosmithkline Biologicals, Rixensart, Belgium
4Hospital Carlos Haya, Malaga, Spain
5Centro Nacional Microbiología, Madrid, Spain

The heptavalent vaccine was licensed in Spain in June 2001. Initial uptake was low but increased from 2002. Estimated 2004 coverage in Sevilla/Malaga was ~40%. The aim of this study was to examine the epidemiological characteristics of paediatric invasive pneumococci. This analysis focused on isolates recovered from 2001-2005, and represents part of a larger study to compare these with isolates recovered before 2001. Identification and serotyping was performed in Spain and multilocus sequence typing in Oxford. From July 2001 – June 2005, 83 children <14 yrs of age (85 episodes) were diagnosed with invasive pneumococcal disease (IPD) at Sevilla/Malaga hospitals: meningitis (31%), bacteremic pneumonia (22%), empyema ≠ bacteremia (19%), bacteremia/septicemia (14%), occult bacteremia (11%), and arthritis (4%). Mean age was 44 months; children with nonvaccine-serotype (NVT) disease were older (mean 66 months) versus those with vaccine-serotype (VT) disease (mean 18 months, p<0.01). There were no obvious changes in referral patterns, paediatric population, or number of cases over time. 61 isolates were available for serotyping and 20 different serotypes were identified: 44% VT, 44% NVT, and 11% vaccine-related serotypes (VRT). The serotype distributions differed significantly between two time periods, (i) July 2001 – December 2002 and (ii) January 2003 – June 2005: (i) 72% (16/22) VT versus 19% (4/22) NVT, compared to (ii) 59% (23/39) NVT versus 28% (11/39) VT, p=0.01. Nearly one-quarter of invasive pneumococci were serotype 1 (n = 14) and were virtually exclusively associated with pulmonary disease: bacteremic pneumonia (11%, n = 7) and empyema (10%, n = 6). Isolation of serotype 1 also differed significantly between time periods: i. 14% (3/21), versus ii. 28% (11/40). The serotype 1 pneumococci are members of a recognised serotype 1 genetic lineage that includes major sequence types (ST) 306 and 228. The global clones SpainV-2 (ST90) and SpainV-3 (ST156) were detected over these time periods, but SpainV-3 was circulating as the serotype 14 variant. The overall prevalence of serotypes 14 and 6B was 18% and 11%, respectively, but both serotypes have virtually disappeared since 2003 (14, n = 2; 6B, n = 0). Remaining serotypes each accounted for <5% of cases of IPD. The overall burden of invasive pneumococcal disease in Sevilla/Malaga has remained stable since 2001, but the serotype distribution has shifted toward NVT, in particular the highly invasive serotype 1 pneumococcus.
Family and perinatal risk factors of invasive pneumococcal disease

Hjuler T, Koch A, Kaltoft MS, Wohlfarth J, Melbye M
Statens Serum Institut, Copenhagen, Denmark

Context:
Risk factors for IPD relating to perinatal and family-related exposures have not been investigated on a population-basis. Studies of the importance of perinatal factors on the risk of IPD are few and of small sample-size. Albeit crowding is an established risk factor for IPD the effect of family contacts is uncertain. The aim of the present study is to identify perinatal and family-related risk factors for IPD on a population-basis.

Methods:
Population-based nested case-control study based on Danish register data. IPD cases (defined as isolation of Streptococcus pneumoniae from a normally sterile site) were identified from national surveillance of IPD in Denmark from 1968 to 2005. Ten age and sex-matched controls per case were drawn among all Danish residents. Exposure information for cases and controls was gathered from a number of population-based person-identifiable Danish registers.

Results:
During the study period 19,073 cases of IPD were identified. Low birth weight, low gestational age, and being born by a young mother all significantly increased the risk of IPD among young (0-½ years of age) but not older children. Whereas infants aged <½ years were at increased risk of IPD when having older compared to no siblings (RR 3.09 95% CI 2.22-2.19) we observed a significantly decreased risk of IPD among children ≥½ year with older siblings. No association between having younger siblings and IPD was found.

Conclusions:
Low birth weight, a preterm birth, and having a young mother are all significant risk factors for IPD. Of particular interest, we found a protective effect of having older siblings among infants more than 6 months of age whereas there was an increased risk of IPD for those below that age. This suggests that immunity protecting against invasive pneumococcal disease is mounted only after approximately 6 months of life.

Pneumococcal conjugate vaccine and pneumococcal community-acquired pneumonia in hospitalized children: A retrospective epidemiologic study from 2000 to 2003

Huang LM1, Lin HC2, Huang YC3, Ho YH4
1National Taiwan University Hospital, Taipei, Taiwan
2China Medical University Hospital, Taichung, Taiwan
3Chang-Gung Medical Hospital, Kaohsiung, Taiwan
4Buddhist Tzu Chi General Hospital, Hualien, Taiwan

Objective: To assess the frequency and severity of pneumococcal community-acquired pneumonia (Sp.CAP) in hospitalized children.

Methods: A retrospective review of computer-generated hospital records of children, aged 3 months to 15 years old, with diagnosis of pneumonia from January 2000 through December 2003 was conducted in 4 hospitals in Taiwan. Children with radiographic findings of lobar or focal pneumonia requiring hospitalization were eligible for analysis as the population with suspected Sp.CAP. Children fulfilling one of the following criteria were considered to have likely Sp.CAP: WBC >20,000/mm³, neutrophils >10,000/mm³, C-reactive protein ≥60mg/L, positive Sp urinary antigen or positive Sp in sputum and/or throat swab. Children with either Streptococcus pneumoniae (Sp) identification from blood or pleural fluid (PL), and/or positive Sp antigen in PL were considered to have definite Sp.CAP. Hospital record review was performed to obtain demographic and hospitalization information. Medical costs of Sp.CAP management were calculated.

Results: Diagnosis of all categories of CAP was most common in children between 2 to 5 years old. No sex predilection was seen. Frequency of suspected Sp.CAP was 7.9% (1519/19165) from 2000 to 2003; 6.5% (322/4953) in 2000, 37.5% (84/5107) in 2001, 7.7% (387/5020) in 2002, and 10.4% (426/4085) in 2003. Frequency of definite/likely Sp.CAP was 3.2% (620/19165) from 2000 to 2003, 2.9% (142/4953) in 2000, 3.1% (157/5107) in 2001, 3.5% (175/5020) in 2002, and 3.6% (146/4085) in 2003. Only 1% of children had received any previous pneumococcal vaccination. Sp.CAP was more prevalent from January to March, coinciding with influenza season. Penicillin, cephalosporins, and beta-lactam/plus beta lactamase inhibitor were the most frequently used parenteral antibiotics to treat Sp.CAP. Most of the patients with Sp.CAP, including those admitted to ICU (18.0% in suspected Sp.CAP and 24.2% in definite and likely Sp.CAP) stayed in the hospital for less than 7 days. Mean cost from 2000 to 2003 for management of Sp.CAP was NT$33,053 per patient for suspected Sp.CAP and NT$44,630 per patient for definite/likely Sp.CAP.

Conclusion: Pneumonia is a frequent cause of hospitalization in Taiwan. About 3% of pneumonia among children attending a hospital from 2000 to 2003 was secondary to definite/likely pneumococcal CAP. Vaccination against pneumococcus may decrease burden of pneumonia disease in children in Taiwan.
Serotype distribution and antimicrobial susceptibility of pneumococcal bacteremic infections in adults – An international prospective study

Chiu CC1, McGee L2, Jackson D3, Yu V1 and Klugman KP3
1University of Pittsburgh, PA, USA
2Emory University, Atlanta, GA, USA
3Centers for Diseases Control and Prevention, Atlanta, GA, USA

A prospective, international, observational study was conducted to determine the serotypes and antibiotic resistance patterns of pneumococcal isolates from hospitalized adult (≥15 yrs) patients with blood cultures positive for S. pneumoniae. From December 1998 to December 2000, 844 consecutive hospitalized adults with pneumococcal bacteremia were enrolled in 21 hospitals in 10 countries on 6 continents (South Africa, 29.6% of patients; United States, 13.6%; Sweden, 12.1%; Spain, 11.7%; New Zealand, 11.7%; Taiwan, 5.7%; Argentina, 5.2%; Brazil, 3.9%; Hong Kong, 3.9%; and France, 2.6%). The mean patient age was 52.6 years (median, 52 years; range, 15–97 years). The most common serotypes causing bacteremia were 1 (10.7%), 14 (10.4%), 4 (9.6%), 3 (7.8%), 23F (6.4%), and 6B (5.0%) which accounted for 50.2% of isolates. The majority of isolates (n=684, 86.3%) were included in the serotypes represented in the 23-valent pneumococcal vaccine. Geographic differences in serotype distribution were clearly evident. For example, types 1 and 5 were not isolated from France, Taiwan and the United States and type 5 accounted for 42.3% of all strains from Argentina. Serotype 14 accounts for a significant amount (6.1%-25.6%) of invasive disease in a number of countries but only 3.6% of strains in South Africa were serotype 14. One hundred sixteen (14.6%) of the 793 available pneumococcal isolates had intermediate susceptibility to penicillin, and 76 (9.6%) were resistant by Etest. Thirteen pneumococci had an MIC of penicillin of ≥3 µg/mL and were considered to be highly resistant. The predominant serotypes of penicillin-nonsusceptible S. pneumoniae were types 14 (20.8%), 19A/F (19.8%), 23F (17.2%), 6A/B (16.7%), and 9N/V (15.1%). All isolates were susceptible to linezolid and telithromycin and only 1 isolate (MIC µg/ml) was resistant to levofloxacin. Resistance rate to erythromycin was 15.8%, tetracycline, 18.4%, clindamycin, 9.8% and trimethoprim/sulfamethoxazole, 15%. Geographic differences in antibiotic non-susceptibility were also evident, for example, resistance rates to most antibiotics were relatively low in Sweden and Argentina and higher rates were determined from Taiwan and Hong Kong.

Respiratory syncytial virus hospitalisation and invasive pneumococcal disease: True true but unrelated

Stensballe LG1, Hjuler T1, Koch A1, Simoes EAF2
1Statens Serum Institut, Copenhagen, Denmark
2University of Colorado, Denver, US

Background: Earlier studies (Talbot, T. Am J Med. 2005 Mar;118(3):285-91) have shown a temporal association between Severe Respiratory Syncytial Virus (RSV) infection and Invasive Pneumococcal disease (IPD), strongly suggesting causality. We tested this hypothesis.

Methods: Using nationwide population-based register data linkage by personal identifier in the Danish National RSV (Stensballe. Scand J Inf Dis. In Print) and the Danish Streptococcus pneumoniae database we examined the temporal association between RSV related hospitalization and IPD between 1996 and 2003.

Results: In Denmark, there were 15.068 RSV hospitalizations, 41.179 RSV negative tests among hospitalized patients and 9.457 cases of IPD during the study period. Stratifying by age (Group I: < 12 months, Group II: 12-24 months, Group III: >2-16 years, and Group IV; > 16 years), the distribution of RSV and IPD hospitalizations in Groups I was 43% & 3%, II was 19% & 3%, III was 14% & 3%, and IV was 24% & 91%, respectively. Commensurate with earlier studies, overall there was a striking temporal association between IPD and RSV hospitalization; however the association was just as clear between IPD and RSV negative tests. When stratified by age, we found no causal association. Not surprisingly, most severe RSV disease was in young children (Groups I & II) 62%, while most IPD was observed among adults (Group IV) 91%. In Groups I & II most RSV disease occurred during winter season from November to April, but there was little correlation with IPD, which was not as seasonally correlated. In contrast in Groups III & IV, there was little RSV disease but most IPD occurred in months 1-4 and 10-12. Furthermore, analyses at a personal level revealed only 8/15.060 persons with an RSV positive test up to 30 days prior to an IPD event compared to 13/41.166 persons with a negative RSV test, P=0.32.

Conclusions: The seasonality of RSV disease and IPD are almost identical, but there is no causal relationship, as a) the disease occurs in different age groups b) The seasonality of non-RSV hospitalization was as correlated to IPD as was RSV c) There was no difference in IPD frequency among RSV positive and RSV negative patients.
Nasal *Staphylococcus aureus* (SA) is inversely related to nasopharyngeal *Streptococcus pneumoniae* (SP) carriage in children and their parents

Regev-Yochay G^1,2, Dagan R^3, Raz M^4, Shainberg B^1, Pinco E^1, Derazne E^1 and Rubinstein E^1

^1Sheba Medical Center, Tel-Aviv University, Israel, ^2Harvard School of Public Health, Boston, MA, USA ^3Soroka Medical Center, Ben-Gurion University, Beer-Sheva, Israel, ^4Macabbi-Healthcare Services, Israel

We have previously reported an inverse relation between nasopharyngeal SP carriage and nasal SA carriage in children. We now extend our observations to include a larger population of children and their parents. Our aims were to: reconfirm the previous findings and extend them to adults.

**Methods**: During 2002-2004, 5 surveillances were carried out in pediatric clinics of Maccabi-Healthcare Services, in Israel in an unvaccinated population. Children and their parents attending the clinics were recruited after signing an informed consent. Nasopharyngeal swabs for SP and nasal swabs for SA were obtained from each child and the accompanying parent. Data collected from the parent and the physician included demographic and medical information.

**Results**: 5031 children and 4881 parents were recruited, the analysis included 3978 independent observations of children and their parents. SA carriage was significantly lower in SP carriers vs. non-carriers (children: 4.13% vs. 10.74% p<.0001, adults: 14% vs. 22% p=.005) and SP carriage was significantly lower in SA carriers vs. non-carriers (children: 25% vs. 48.2%, adults: 2.4% vs. 4.1%). In a multivariate analysis, the 5 most significant factors associated with child SP carriage were: DCC (OR-2.88 95%CI 2.43-3.40), age (OR-1.92, 2.89, 3.11, 2.48 for ages 3-6m, 6m-1y, 1-2y, 2-3.4y, respectively), child SA carriage (OR-0.49 95%CI 0.35-0.66), parent SP carriage (OR-1.80 95%CI 1.22-2.64) and current breastfeeding (OR-1.44 95%CI 1.09-1.90). 397 (9.98%) children were breastfeeding at the time of screening. SP carriage but not SA carriage was significantly higher in breastfed infants of all age groups. The only factors associated with parental SP carriage were: child SP carriage (OR-2.14 95%CI 1.49-3.06), parent SA carriage (OR-0.49 95%CI 0.29-0.81), smoking (OR-1.44 95%CI 0.97-2.14) and a child-care associated job (OR-1.64 95%CI 0.98-2.75).

**Conclusion**: These data re-confirm that inverse association between SA-SP is not unique for children but is also observed in adults. The significance of this finding in SP vaccinated population is not yet known. The intriguing result regarding the association of breastfeeding with SP carriage requires further investigation.

---

Twelve months after beginning universal vaccination with 7vPCV. Have we made a difference?

Murphy DM^1, Hicks VA^2, Smith HV^1, Bates JR^1 and Hanna JN^2

^1Queensland Health Scientific Services, Brisbane, Queensland Australia ^2Tropical Public Health Unit Network, Queensland Health, Cairns, Queensland, Australia

Invasive pneumococcal disease has been notifiable in Queensland since 1996 with enhanced surveillance beginning in 2001. Laboratories throughout the state have been encouraged to send all invasive isolates to the Queensland Pneumococcal Reference Laboratory for serotyping and sensitivity testing. While all laboratories are encouraged to send isolates it is not mandatory and not all notified cases are received. Since enhanced surveillance began the ratio of received isolates to notified cases exceeds 95%. Knowledge of the serotype has become more important since the introduction into the vaccination schedule of the 7-valent conjugated vaccine Prevenar™ in January 2005 for children under two. This vaccine was suitable for use in young children in contrast to the previous 23-valant polysaccharide vaccine which was not suitable for use in children under two years of age. Government funded vaccination with the 7-valent Prevenar was only available to Australian Indigenous children and other selected children from mid-2001. The data on non-Government funded vaccinations of children under five is not available however it is known that this occurred. Six years of Queensland serotyping and sensitivity data pre-universal vaccine and one year post-universal vaccine were reviewed. This study was restricted to children under five as these cases were the most likely to have been impacted by the introduction of the universal vaccine.

In 1999 there were 70 invasive isolates received from children under five. 87% of these were Prevenar™ vaccine strains. In 2000 there were 121 invasive isolates received from children under five with 82% being vaccine strains. Having 80% or more vaccine strains among the invasive isolates received from children under five has been consistent for 1999-2004 with 148 invasive isolates serotyped from children less than five with 81% being vaccine strains in 2004. To the first of December there were 52 invasive isolates from children under five and of these 50% were vaccine strains. While it is too early to make conclusive judgements and the 2005 winter has been mild, this decrease is very promising. It will take at least two more years to determine whether this decrease will be sustained.
Risk factors for pneumonia and hospitalisation with pneumonia in Auckland, New Zealand

Grant CC1, Emery D1, Milne T1, Coster G1, Forrest C1, Wall CR1, Scragg R1, Aickin R4, Crengle S1, Leversha A4, Huakau J3, Nosa V1.
1University of Auckland, Auckland, New Zealand
2Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
3Massey University, Auckland, New Zealand
4Starship Children’s Hospital, Auckland, New Zealand

Background: New Zealand has a high paediatric pneumonia hospitalisation rate compared with other developed countries.

Objective: To identify risk factors for pneumonia and hospitalisation with pneumonia in children.

Methods: Subjects: Cases were children admitted to the Starship Children’s Hospital with pneumonia. The control groups were (1) children diagnosed with pneumonia in the Starship Children’s Hospital Emergency Department but not hospitalised, and (2) a random sample of well children who did not have pneumonia (community controls). Pneumonia was defined by the WHO case definition (tachypnoea or indrawing and no wheeze). Community controls were a clustered sample identified from random residential address start points. From these identified children a probability matched sample was obtained with the intention of the probability of selection being to achieve an age/ethnic distribution similar to that of the hospitalised pneumonia cases. Exclusions: Children with acute asthma, with chronic disease or hospital admission in past 2 weeks.

Data collection: Interview with caregiver, child’s anthropometry and audit of child’s medical records. Measurements: Demographics, socioeconomic status, child’s sunlight exposure, child’s immunisation status, household crowding, dampness, coldness and cigarette smoke exposure, health care received by child.

Data analysis: Logistic regression. Odds ratios estimates involving the community control group adjusted for clustering.

Results: 326 pneumonia cases, 179 Emergency Department and 315 community controls enrolled from 2002 to 2005. After adjustment for season of admission, age and ethnicity risk factors for pneumonia were weight-for-height less than -1 s.d. (OR 2.46, 95% CI 1.39, 4.36), less than 30 minutes sunlight exposure per day (OR 3.64, 95% CI 1.99, 6.68), any previous chest infections (OR 1.81, 95% CI 1.26, 2.61) and living in a house that was always/almost always damp (OR 2.54, 95% CI 1.35, 4.80). Risk factors for hospitalisation with pneumonia were weight-for-height less than -1 s.d. (OR 3.27, 95% CI 1.52, 7.32), living in a household with cigarette smokers (OR 1.94, 95% CI 1.13, 3.45) and in a more crowded house (OR 2.75, 95% CI 1.40, 5.58).

Conclusions: Among children in New Zealand poorer nutrition and lower quality internal living environments are risk factors for pneumonia and hospitalisation with pneumonia.
Monitoring antimicrobial resistance and serotypes (the MARS project): Pneumococcal carriage in remote Aboriginal communities in the Northern Territory of Australia, 2003 & 2005

PO3.47

Mellon, G1,2, Leach, AJ1,2, Morris, PS1,2,3, Beissbarth, J2, Halpin, S1,2, Hare, K1,2, Kennedy, M1,2, Stubbs, E1,2, Wilson, C1,2, Smith-Vaughan, H1,2
1Menzies School of Health Research, Darwin, Northern Territory, Australia
2Charles Darwin University, Institute of Advanced Studies, Darwin, Northern Territory, Australia
3Flinders University, Adelaide, South Australia, Australia

Background: Aboriginal people in remote Northern Territory (NT) communities experience high rates of invasive pneumococcal disease (IPD). In July 2001, Prevenar was made available through a Federal Government initiative for Aboriginal newborns in the NT. A catch up program was also supported. Per capita azithromycin use is expected to be high in remote areas of the NT, where rates of STDs and trachoma are extremely high and eradication programs using azithromycin are ongoing. An anticipated outcome of these combined initiatives is a unique selective pressure on pneumococcal antibiotic resistance and serotypes.

Aim: To monitor the serotype distribution and prevalence of azithromycin and penicillin resistance among S. pneumoniae carriage isolates from Aboriginal children living in remote Aboriginal communities across the NT.

Methods: Antibiotic use and vaccination data were obtained from the medical records of consenting participants and the NT Childhood Immunisation database. Swabs were collected and processed according to WHO recommendations for pneumococcal carriage studies.

Results: In each region, vaccine uptake was approx. 75% in 2003 and approx. 85% in 2005. Although carriage of vaccine-types (VT) was very low (13% overall) and was similar in both regions and in both years, pneumococcal carriage was very high (81% overall). The number of prescriptions for penicillin, and the proportion of isolates with penicillin resistance, were consistently higher in central Australia than the Top End. Macrolide resistance was also higher but macrolide prescribing was not. The proportion of resistant isolates was higher in central Australia than expected for such a low carriage of vaccine types.

<table>
<thead>
<tr>
<th></th>
<th>Central Australia 2003</th>
<th>Central Australia 2005</th>
<th>Top End 2003</th>
<th>Top End 2005 to date</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of communities</td>
<td>11</td>
<td>2</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>No. children</td>
<td>164</td>
<td>65</td>
<td>752</td>
<td>698</td>
</tr>
<tr>
<td>No. swabbed</td>
<td>142</td>
<td>61</td>
<td>725</td>
<td>570</td>
</tr>
<tr>
<td>Penicillin given</td>
<td>40 (28%)</td>
<td>21 (34%)</td>
<td>149 (21%)</td>
<td>125 (22%)</td>
</tr>
<tr>
<td>Macrolide given</td>
<td>0 (0%)</td>
<td>1 (2%)</td>
<td>8 (1%)</td>
<td>47 (8%)</td>
</tr>
<tr>
<td>&gt;1 dose PCV7 given</td>
<td>108 (76%)</td>
<td>51 (84%)</td>
<td>558 (77%)</td>
<td>496 (87%)</td>
</tr>
<tr>
<td>Spn positive</td>
<td>120 (85%)</td>
<td>52 (85%)</td>
<td>595 (82%)</td>
<td>423 (74%)</td>
</tr>
<tr>
<td>VT Spn</td>
<td>16 (11%)</td>
<td>9 (15%)</td>
<td>93 (13%)</td>
<td>30/241 (12%)*</td>
</tr>
<tr>
<td>PenR Spn</td>
<td>37 (26%)</td>
<td>25 (41%)</td>
<td>112 (15%)</td>
<td>64 (11%)</td>
</tr>
<tr>
<td>MacR Spn</td>
<td>16 (11%)</td>
<td>12 (20%)</td>
<td>30 (4%)</td>
<td>24 (4%)</td>
</tr>
</tbody>
</table>

Spn: S. pneumoniae, VT: vaccine-type, PenR: penicillin-resistant (MIC>0.12), MacR: macrolide-resistant (azithromycin MIC>8, erythromycin MIC>2).

* Darwin Rural Only, Top End includes East Arnhem communities

Conclusions:
Pneumococcal carriage rates remain extremely high in children living in remote Aboriginal communities. Despite high vaccine coverage and low carriage of vaccine types, pneumococcal resistance to penicillin and macrolides can by high, presumably because replacement (non-vaccine) types are (or have become) resistant. Plans for mass treatment to eradicate trachoma are likely to increase macrolide resistant pneumococci (and penicillin resistance if cross resistant strains are present). Monitoring pneumococcal populations for emergence of antimicrobial resistance in non vaccine types is warranted.
S. pneumoniae (SP) infections cause substantial morbidity and mortality especially in young children and prevention is therefore of importance. Knowledge of the prevailing pneumococcal serotypes is necessary to formulate recommendations regarding use of pneumococcal conjugate vaccine.

**Aims:** To evaluate the serotype distribution of invasive SP strains isolated from Filipino children.

**Methods:** SP isolates from children admitted to the Research Institute for Tropical Medicine, one of the tertiary care center for infectious diseases of the Department of Health, during the years 2000–2005 were serotyped. Serotyping was done using the Quellung reaction using specific antiserum from the Statens Serum Institut (Denmark). Hospital medical records of children with invasive SP infections were reviewed.

**Results:** Fifty-four invasive isolates were identified. The median age of patients was 6 months (range 1 month to 11 years), 22 (73%) were male. Twenty seven (90%) of the patients were <2 years of age and 77% were <1 year of age. Diagnoses were as follows: pneumonia in 52%, measles complicated by pneumonia in 11%, meningitis in 30% and bacteremia in 7%. Nine of the 27 patients died for a mortality rate of 33%. Thirty of the 54 isolates were available for serotyping and represented 11 serotypes. Three serotypes/serogroup (6, 18, 14) were responsible for 50% of infections. Sixty seven percent of the serotypes were included in the 7-valent pneumococcal conjugate vaccine.

**Conclusions:** Invasive pneumococcal disease was an important cause of infections particularly in children less than 2 years of age. The most common diagnosis was pneumonia, in 11% of cases measles infection. There was significant mortality associated with IPD. The serotype distribution of SP isolates in this study varied from our previous study in which serotype 1 which was the most common invasive serotype but was found in only one patient in the present study. Immunization with conjugate pneumococcal conjugate vaccine will help prevent infections in Filipino children.

---

**Pneumonia among young infants in Bohol Island, Philippines**

**Chiaumboo BP**, Ruatu P.F., Ladesma EA, Gozum L, Lapisan SP, Sombrero LT, Romano V, Simoes EAF, ARIVAC Consortium

**Background:** Pneumonia is responsible for 19% of deaths among young infants. The WHO young infant study categorized pneumonia as a mild and uncommon disease among infants younger than 3 months of age. Local data showed otherwise. We describe the clinical presentation and etiology of neonatal pneumonia and define criteria among young infants that can be used to diagnose pneumonia and differentiate it from sepsis.

**Methods:** Infants with severe pneumonia or suspected sepsis meningitis had a clinical evaluation, CBC, blood culture, and chest radiograph and CSF in those with suspected meningitis.

**Results:** Of 767 infants enrolled from April 1994 to May 2000, 303 had pneumonia: 185 radiographically confirmed and 118 clinically diagnosed (wheezing and/or crackles). Pneumonia increased with age and was significantly more frequent after 1 month of age. S. pneumoniae comprised 4 of 15 (27%) culture positive cases and 3 of 10 (30%) culture positive radiographic pneumonia; case fatality rate was 25%. Other pathogens: S. pyogenes (2), Klebsiella sp (2), P. aeruginosa (2), S. typhi (1), E. coli (1), Enterobacter sp (2) and P. vulgaris (1). Respiratory rate ≥ 60/min (88.4%), crackles (75.6%) and chest indrawing (60%) were the most common clinical signs. 235 of the 303 pneumonia cases were assessed to have WHO pneumonia. Of the remaining 68 diagnosed as either sepsis or meningitis, 62 (91%) had radiographic pneumonia. The WHO algorithm was able to correctly identify 66.5% of infants with radiographic pneumonia. Our algorithm, which included apnea, grunting, cyanosis, inability to drink and RR ≥ 70/min, identified 72% of radiographic pneumonia but could not differentiate it from sepsis.

Death from pneumonia was more likely among those with age <7 days (OR 9), inability to drink (OR 6.4), radiographic infiltrates (OR 4.98), apnea (OR 4.5), cyanosis (OR 3.6) and grunting (OR 2.4). On multivariate analysis, age <7 days, radiographic infiltrates and inability to drink were independent predictors of death.

**Conclusion:** Our study emphasizes the importance of neonatal pneumonia as a contributor to mortality with S. pneumoniae as a significant pathogen in this age group. Any algorithm assessing pneumonia in a young infant should include apnea, cyanosis and grunting.
Predictors of death among children with community-acquired pneumonia in a referral hospital in Bohol, Philippines

Lupisan, S1, Lucero, MG2, Ruatu, P2, Quiambao, B3, Abucejo-Ladesma, E4, Gozum, L5, Sombrero, L5, Simoes, EAF5, Riley, I6 and the ARIBAC Consortium
1Research Institute for Tropical Medicine, Philippines, 2National Public Health Institute, Finland 3Bohol Regional Hospital, Philippines, 4Imperial College, UK and University of Colorado and 5University of Queensland, Australia

Objective: To determine the predictors of death among children 2-59 months old admitted for pneumonia

Methods: Children hospitalized with severe or very severe pneumonia according to the WHO classification were prospectively enrolled from April 1994 to May 2000 in a study of serious pediatric infections at Bohol Regional Hospital (BRH), a tertiary care general hospital in rural Central Philippines. Blood cultures and chest radiographs were obtained from all patients and CSF cultures when indicated. Meningitis was diagnosed in children with meningeal signs and definite pathogen in CSF or blood or a strong clinical suspicion of meningitis based on signs and symptoms.

Results: 8% of 1667 children with pneumonia had concomitant meningitis. Overall case fatality rate (CFR) was 6.5%. CFR was 29% in those with meningitis vs 4% in those without. 358 were referrals (21.5%) and 1309 walk-ins (78.5%). Proportion of meningitis cases was greater in the referred group (4.2%) compared to the walk-ins (0.8%). S. pneumoniae (17), H. influenzae (14) S. typhi (4) and other salmonella (2) were definite bacterial pathogens isolated. Using a multivariate analysis model, which included all variables, independent predictors of death among those referred were: age 12-23 months (OR=2.915 95% CI 1.128, 7.534), inability to drink (OR=3.087 95% CI 1.284, 7.42), altered sensorium (OR=6.305 95% CI 2.615, 15.201). Among walk-in patients, the predictors included age 2-11 months old (OR=2.056 95% CI 1.051, 4.024), inability to drink (OR=2.897 95% CI 1.568, 5.354), weight for age z-score <-2SD (OR=3.607 95% CI 1.051, 4.024), dense infiltrates on chest x-ray (OR=2.559 95% CI 1.433, 4.57).

Conclusion: Meningitis as a complication of pneumonia is a significant predictor of death. S. pneumoniae and H. influenzae were the major pathogens isolated in patients with pneumonia with or without meningitis. Vaccines directed against these pathogens need to be considered in a national control program.

The evolution of macrolide resistance in Streptococcus pneumoniae isolates over a 20-year period

Syraropoulou VP1, Tsiodras S2, Koutrasiotou A3, Braoudaki M4, Theodoridou M4, Soulis K5, Charissiadou A6, Pangalis A4, Daikos GL6
1First Department of Pediatrics, Aghia Sophia Children’s Hospital, Athens Greece 2Fourth Department of Internal Medicine, Athens University Medical School 3Department of Microbiology Aghia Sophia Children’s Hospital, Athens Greece 4First Department of Internal Medicine, Athens University Medical School

Objectives: To study the evolution of macrolide resistance in invasive and non-invasive S. pneumoniae isolates.

Methods: Five hundred seventy seven invasive and non-invasive S. pneumoniae isolates that had been collected over a 20 year period in a tertiary care Children’s Hospital were examined. All isolates were identified as S. pneumoniae by conventional microbiological techniques and ltaA PCR. Serotyping was performed by the latex agglutination test and the Quellung reaction using specific antisera (Statens Serum Institute, Copenhagen, Denmark). Susceptibility testing to commonly used antibiotics was performed by Etest. The presence of mefE and ermA genes were detected by PCR. Data on outpatient macrolide and lincosamide use over the last 15 years were available from a market research company and antibiotic consumption was expressed in DDD per 1000 inhabitants daily (DID).

Results: A progressive increase in macrolide resistance rate (MR) was noted from 7.1 % of isolates collected from 1985 to 1996 to 52.2% of those collected from 2003 to 2004. Notably, MR rate was higher among non-invasive than invasive isolates throughout the study period. A parallel increase of macrolide and lincosamide consumption had been recorded from 3.68 in 1990 to 8.38 DID in 2004. mefE gene was present in 50.3% of MR isolates, ermA in 21.5% and both genes in 22.6%, whereas in the remaining 5.6% of the isolates neither gene was detected. The serotypes 19F and 14 were most commonly encountered among the MR isolates (38% and 36.9% respectively).

Conclusions: A significant increase in macrolide resistance was noted in S. pneumoniae over the last 20 years in our geographic region. Outpatient macrolide consumption may be associated with the evolution of macrolide resistance in S. pneumoniae. mefE and ermA genes were the predominant genetic determinants conferring resistance to macrolide antibiotics.
A national review of the epidemiology of pneumococcal disease in children in Singapore  
Chan, FLF1, Low, S1, Cutter, J1, Ma, S1, Goh, KT1, Chew, SK1
1Ministry of Health, Singapore

Aim: This study describes the epidemiology and burden of pneumococcal disease in children in Singapore.
Method: Retrospective review of national hospitalization data from all public and private hospitals in Singapore on pneumococcal related disease in children aged under 15 years based on a list of diagnosis coding for pneumococcal related diseases using the ninth revision of the international classification of disease (ICD 9).
Results: In a ten year period from 1995 to 2004, a total of 2693 hospitalisation episodes (or an average of annual incidence rate of 36.5 per 100 000) of pneumococcal related disease in children aged under 15 years were identified. The annual incidence rate was more than two times higher in those under the age of 5. The average annual incidence rates were 69.7, 72.7 and 20.3 per 100 000 children aged under 1, 1 to 4 and 5 to 14 years respectively. Males accounted for 56.9% of all cases. Over 90% of all cases was discharged on or before day 7 of hospitalisation, with a mean length of stay of 3.7 days (SD 4.1). However, the mean length of stay for those aged under 1 year was the highest among all ages at 4.6 days (SD 7.5). The overall case fatality rate was the highest among the under 1 year age group, at 1.6%, and the overall case fatality rates for the 1-4 years age group and 5 to 14 years age group were at 0.3% and 0.5% respectively.
Conclusion: Pneumococcal disease presents a significant burden of disease in children in Singapore, especially to those under 5 years of age.

Epidemiology of pneumococcal disease in Singapore - A national study  
Low S1, Chan FLF1, Cutter J1, Ma S1, Goh KT1, Chew SK1
1Ministry of Health, Singapore

Objective: To describe the epidemiology of pneumococcal disease for all age-groups in Singapore
Methods: A retrospective study of records of 9472 pneumococcal cases admitted to all hospitals in Singapore from 1995 to 2004 was conducted. In this study, we included pneumonia, meningitis, peritonitis and otitis media as infections associated with Streptococcus pneumoniae. Extraction of records was based on the International Classification of Diseases, Ninth Revision (ICD-9) codes for pneumococcal disease. We analysed the annual incidence, and distribution by age, gender, ethnicity, type of pneumococcal disease, episode outcome and length of stay.
Results: A decrease of 41.8% in annual incidence of pneumococcal diseases from 1995 to 2004 was observed. The average annual incidence of pneumococcal disease is 24.2 per 100,000 population. Persons 65 years old and above have the highest average annual incidence per 100,000 population of pneumococcal disease (65.6 per 100,000), followed by those under 15 years old (36.5 per 100,000). The incidence in persons 15 to 64 years old was 17.5 per 100,000 population. Males have a higher average annual incidence per 100,000 population than females (24.6 versus 23.7 per 100,000). The distribution of average annual incidence per 100,000 population of pneumococcal-related diseases by ethnicity is as follows: Indians (27.9 per 100,000), Malays (26.8 per 100,000) and Chinese (22.6 per 100,000). Pneumococcal pneumonia (ICD-9 code 481) is the predominant type of pneumococcal disease, accounting for 38.8% of the pneumococcal diseases analysed. The average length of stay for pneumococcal-related cases is 3.9 days. The overall case fatality for 1995 to 2004 is 1.4%.
Conclusions: Our study has shown that people who are 65 years old and above have a higher incidence of pneumococcal disease. This is consistent with the finding in studies conducted overseas.
Invasive pneumococcal disease burden in Hong Kong children

Ho PL1, Susan S Chiu1, Chris HY Cheung2, Rodney Lee3, Theodore F Tsai3, Lau YL1
1University of Hong Kong, and 3Pamela Youde Hospital, Hong Kong SAR, CHINA; 2Global Medical Affairs, Wyeth, United States

Pneumococcal infections are an important vaccine-preventable cause of paediatric morbidity and mortality. However, little information is available on the incidence of paediatric pneumococcal disease in this area. Therefore, this study was conducted to estimate the burden of invasive pneumococcal diseases (IPD) among in-patients in Hong Kong.

The Hong Kong Special Administrative Region (SAR) is divided geographically into the New Territories, Kowloon peninsula and Hong Kong island. A retrospective population-based study was conducted to estimate the incidence of IPD in the Hong Kong island region for a ten-year period, 1995-2004. In the region, two acute care hospitals, the Queen Mary Hospital (QMH) and the Pamela Youde Hospital (PYNEH) provide in-patient service for approximately 90% of the 1.4 million population in the region. Both hospitals have all the clinical disciplines and emergency departments, and have capacities of 1400 and 1700 beds in QMH and PYH, respectively. Healthcare funding policy in the SAR requires residents to seek inpatient medical care at hospitals in their neighbourhood healthcare cluster hospital. Unique cases were identified by reviewing the computer database and record books in the two hospital microbiology departments. A case of IPD was defined by the isolation of S. pneumoniae in blood, cerebrospinal fluid (CSF), and/or other normally sterile sites (e.g. pleural fluid, joint fluid, peritoneal fluid). The mean of the population figures for the region in 1996 and 2001 was multiplied by ten years to obtain the population at risk and was used for calculation of the mean annual age-specific rates.

The overall mean annual incidence for the Hong Kong island region was 2.3 per 100,000 persons for IPD and 0.11 per 100,000 persons for pneumococcal meningitis, for all ages. The rates of pneumococcal meningitis were 2.2 and 1.3 per 100,000 for children aged ≤2 years and ≤ 5 years, respectively. The age-specific incidence rates for IPD are shown in table 1. There were 3 IPD-associated deaths in children aged ≤ 5 years. The findings indicate a significant disease burden.

Table 1. Estimation of invasive pneumococcal disease stratified by age, Hong Kong, 1995-2004

<table>
<thead>
<tr>
<th>Age</th>
<th>Rate (per 100,000 per year)</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5 y</td>
<td>15.6</td>
<td>12.8-18.6</td>
</tr>
<tr>
<td>0-2 y</td>
<td>18.3</td>
<td>13.9-23.3</td>
</tr>
<tr>
<td>3-5 y</td>
<td>12.8</td>
<td>9.5-16.8</td>
</tr>
<tr>
<td>6-14 y</td>
<td>1.0</td>
<td>0.5-2.1</td>
</tr>
<tr>
<td>15-34 y</td>
<td>0.5</td>
<td>0.3-0.7</td>
</tr>
<tr>
<td>35-64 y</td>
<td>1.2</td>
<td>1.0-1.6</td>
</tr>
<tr>
<td>≥65 y</td>
<td>10.2</td>
<td>8.6-12.0</td>
</tr>
<tr>
<td>m, month; y, year</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SAPNA Sri Lanka: Pneumococcal surveillance at the largest tertiary paediatric care facility in South Asia – Lady Ridgeway Hospital, Colombo Sri Lanka

Batusenthudawe, K2, de Silva, S3, Karunaratne, K3, Lalitha, MK3, Thomas, K3, Steinhoff, M4, Abeeysinge, N4
3Epidemiology Unit, Ministry Of Health, Colombo, Sri Lanka, 2Lady Ridgeway Hospital for Children, Colombo, Sri Lanka, 4Christian Medical College and Hospital, Vellore, India, 3Johns Hopkins University, Maryland, USA

Acknowledgments: PneumoADIP through SAPNA for technical and financial assistance for Pneumococcal surveillance in Sri Lanka

Key words: Streptococcus pneumoniae, serotypes, antibiotic resistance, Sri Lanka

Objective
To describe invasive pneumococcal infections among children admitted to the largest Paediatric Hospital (Bed strength 850) of South Asia: Lady Ridgeway Hospital (LRH) for Children - Colombo, Sri Lanka

Methodology
Sri Lanka is a member of South Asian Pneumococcal Surveillance Network (SAPNA) and uses standard procedures in patient recruitment and laboratory testing along with India and Nepal. Admitted children aged 2 months – 5 years presenting with fever and signs and symptoms suggestive of infections in nervous, respiratory and circulatory systems were studied. CSF and blood culture were carried out and a latex agglutination (LA) test (Wellcogen, Murex for Remel – UK) which detected five bacterial antigens in CSF was also performed on all CSF specimens to identify infections.

Results
During the year 2005, 41,398 children were admitted to the six Consultant Paediatric Units of the hospital out of which 25,847 (62%) were between 2 months and 5 years of age. A total of 3183 blood cultures (rate ≥ 8%) were carried out over the year and 1259 were included in the study. Of the 920 lumbar punctures (LP) performed (LP rate of 2.2%) 402 were included in the study. Thirteen Strepptococcus pneumoniae isolates were found (10 from blood, 2 from CSF and one from pus culture). Latex detected 23 additional pneumococcal infections in the 402 CSF specimens. Out of the eight serotypes detected the commoner were 23F (3 isolates), 19F (3 isolates) and 14 (2 isolates). All 13 isolates were resistant to penicillin and 10 (75%) were resistant to Erythromycin.

Conclusions
This is the first description of Sri Lankan pneumococcal serotypes from invasive disease with serotypes and antibiotic resistance data. An increase (60%) of pneumococcus isolations from 2004 (8 isolates) to 2005 (13 isolates) is evident mainly due to infrastructure and procedural improvements in the microbiology laboratory of LRH through SAPNA. Low yield rates with standard procedures and high sampling rates indicates the necessity of continuing the use of latex antigen detection and addition of other antigen detection tests (e.g.: Binax) in CSF, and PCR detection of bacteria and viruses will improve disease burden estimate, including vaccine-preventable pneumococcal disease.
Serotype distribution of *Streptococcus pneumoniae* among children in Bamako, Mali

PO3.57

Sow, SO1, Hormazabal, JC2, Tapia, MD3, Diallo, S4, Campbell, JD1, Kotloff, K3, Levine, M3
1Centre pour le Développement des Vaccins - Mali, Bamako, Mali
2Instituto de Salud Pública, Santiago, Chile
3University of Maryland School of Medicine, Baltimore, MD, USA
4Hôpital Gabriel Touré, Bamako, Mali

**Background**: Based on assessment of disease burden and predicted impact, policymakers in industrialized countries are increasingly incorporating conjugate pneumococcal vaccine into their routine infant immunization programs. In developing countries the paucity of similar analysis and the lack of data on serotype distribution have impeded decisions about the need for routine immunization and the specifications of a suitable vaccine to prevent this infection.

**Methods**: Children age 0-15 years admitted to the principal pediatric hospital in Bamako with a fever ≥ 39°C or a syndrome compatible with an invasive bacterial infection were invited to participate in a study in which blood and relevant body fluid (e.g. cerebrospinal fluid (CSF)) were cultured to identify bacteria using standard microbiologic techniques.

**Results**: From June, 2002 to May, 2005, 5514 children were enrolled in the study (91% of eligible). *S. pneumoniae* was isolated from 374 children (8%), 73 of whom died (case fatality 19.5%). Among the159 isolates that have been serotyped to date (42.5%), the most common types were 5 (n=52, or 32.7%) and 2 (n=30, or 18.8%). 7-valent, 9-valent and 11-valent vaccine coverage was 19%, 40.5% and 42.9% among 0-3 month olds, 27.9%, 55.8% and 58.1% among 4-11 month olds, 24.3%, 78.4%, 83.8% among 1-4 year olds and 21.6%, 62.2 and 67.6% among 5-15 year olds.

**Conclusions**: These preliminary results demonstrate that pneumococcal infections are an important cause of morbidity and mortality among Malian children and suggest that multivalent conjugate pneumococcal vaccines have the potential to substantially reduce this disease burden.

---

Invasive pneumococcal disease among children in Bamako, Mali

PO3.58

Tapia, MD1, Sow, SO2, Diallo, S3, Campbell, JD1, Keita, M2, Doumbia, MM2, Keita, MM3, Sylla, M4, Levine, M1, Kotloff, K1
1University of Maryland School of Medicine, Baltimore, MD, USA
2Centre pour le Développement des Vaccins - Mali, Bamako, Mali
3Hôpital Gabriel Touré, Bamako, Mali

**Background**: Development of a multivalent conjugate vaccine against *Streptococcus pneumoniae* has been a remarkable public health achievement and its implementation in developed countries has met with great success. Due to cost, paucity of data on the invasive pneumococcal disease (IPD) burden and serotype distribution, this vaccine has not been introduced in developing countries.

**Methods**: Hospitalized children aged 0-15 years and ambulatory patients aged 0-35 months evaluated at Hôpital Gabriel Touré, the main pediatric hospital in Bamako, with fever ≥ 39°C or syndrome compatible with invasive bacterial infection were invited to participate in a study in which blood and relevant body fluid (e.g. cerebrospinal fluid (CSF)) were cultured to identify *S. pneumoniae* using standard techniques. Hospitalized children were followed until discharge and outpatients were contacted if the culture result was positive.

**Results**: From June 2002 to November 2005, 6001 hospitalized children were enrolled (>90% of eligible); from June 2005 to November 2005, 1265 ambulatory patients (>90% of eligible) were enrolled. *S. pneumoniae* was isolated from 389 inpatients (6.5%) and 29 outpatients (2.3%). Among inpatients, 214 cases (55%) occurred in 0- to 11-month old infants, 103 (26.5%) in 1- to 4-year olds and 72 (18.5%) in 5- to 15-year olds. 171 isolates (44%) were from blood only, 201 (51.7%) from CSF (+/- blood), 16 from pleural fluid (+/- blood) (4.1%) and 1 from peritoneal fluid (0.2%). 70 inpatients died, including 39 infants (overall case fatality = 18%). Among 0- to 3- and 4- to 11-month olds, the incidence of hospitalized IPD was 137.6/105 and 111.6/105. Among outpatients, 12 (41.4%) cases occurred in infants and 17 (59%) in 12- to 35-month olds. All isolates were recovered from the blood (including one from blood and CSF). Follow-up of ambulatory bacteremias revealed that 17 children had improved, 2 had not, 2 had died, 4 were hospitalized (2 of whom later died), and 4 could not be located (case fatality = 14%).

**Conclusions**: Surveillance for IPD among hospitalized children in Bamako confirms the high incidence of disease and death, particularly among the youngest infants. Recent extension of these studies to outpatients has uncovered another important source of IPD, indicating the burden is larger than previously estimated. The introduction of pneumococcal conjugate vaccines for routine immunization of infants would be of great benefit.
Invasive pneumococcal disease in adults in North-Rhine Westphalia, Germany, 2001-2004

van der Linden, M.P.G., Al-Lahham, A. and Reinert, R.R.
National Reference Centre for Streptococci and Institute of Medical Microbiology, RWTH Aachen, Germany

Objectives: Elderly adults are at increased risk for invasive pneumococcal disease (IPD). We conducted a population-based survey of IPD among adults in North-Rhine Westphalia, Germany. Our study included 202 of the 386 hospitals and the 27 microbiological laboratories that submitted reports of IPD in these hospitals to the National Reference Center for Streptococci. We also reviewed comprehensively the reports of 16 laboratories.

Methods: Surveillance for our current study focused on North-Rhine Westphalia, the largest federal state in Germany (18 million inhabitants). 202 (52.2%) acute care hospitals 27 microbiological laboratories serving these hospitals agreed to participate. We studied hospitalized patients ≥ 16 years of age. A case of IPD was identified by the isolation of *S. pneumoniae* from an otherwise normally sterile site. Isolates were sent for confirmation of species diagnosis by optochin testing, bile solubility and for serotyping by the Neufeld Quellung reaction. MICs of penicillin G, amoxicillin, cefotaxime, cefpodoxime, cefuroxime, clarithromycin, clindamycin, gatifloxacin, levofloxacin, telithromycin, tetracycline, and trimethoprim/sulfamethoxazole were determined using the microdilution method according to the latest CLSI guidelines.

Results: We found that 95.4% of IPD isolates were susceptible to penicillin G and 15.2% were clarithromycin resistant. Serotypes 14 (14.8%), 3 (8.8%), 7F (7.4%), 4 (6.9%) and 1 (5.6%) were the most common serotypes. The serotype coverage of the of the 7-valent pneumococcal conjugate vaccine was 40.1%, the coverage of the 23-valent pneumococcal polysaccharide vaccine was 80.2%. 774 (77%) isolates were obtained from blood, 61 isolates (6%) from CSF. Among the CSF isolates no cefotaxime intermediate and resistant strains were found. Between 2001 and 2004 penicillin resistance increased from 0.7% to 1.5%. Clarithromycin resistance increased from 12.1% to 14.8%.

Conclusions: The percentage of isolates from IPD in adults that are resistant to penicillin is increasing but remains low. The level of resistance to clarithromycin is now 14.8%. The coverage of both the 7-valent pneumococcal conjugate vaccine and the 23-valent pneumococcal polysaccharide vaccine remains high.

Serotype distribution and antibiotic susceptibility of invasive and respiratory pneumococcal isolates from children < 5 admitted for pneumonia, suspected sepsis or meningitis in Bohol, Philippines

Sombrero, L1, Esparar G1, Girasol F1, Herva E2, Lupsan, S1, Ruatu, P1, Quiambao, B1, Gozum, L1, Lucero M1, Nissinen A2

1Research Institute for Tropical Medicine, Philippines,
2National Public Health Institute, Finland,
3Bohol Regional Hospital, Philippines

Objective: To determine the serotype distribution and antibiotic susceptibility of invasive and respiratory pneumococcal (Pnc) isolates from children < 5 years old admitted for pneumonia, suspected meningitis or sepsis.

Methods: In preparation for a Pnc conjugate vaccine trial, children hospitalized with severe or very severe pneumonia according to WHO classification and suspected meningitis or sepsis were prospectively enrolled from April 1994 to May 2000 at Bohol Regional Hospital (BRH), a government referral hospital in rural Central Philippines. Blood cultures were obtained from all patients and CSF cultures when indicated. Nasal swab for Pnc carriage was likewise collected. Identification of *S. pneumoniae* isolates were confirmed by colony morphology on blood agar plates, Grams stain and susceptibility to optochin disc. Antibiotic susceptibility test was done based on CLSI. Serotyping was done by chess board method employing Quellung reaction, using specific antisera from Staten Seruminstitut (Copenhagen). Quality of clinical and laboratory work was monitored through monthly visits by consultants from RITM and semiannual visits from KTL. Serotype of all invasive and 10% of respiratory Pnc isolates was confirmed in KTL.

Results: 30 out of 3013 cases (1%)were positive for *S. pneumoniae* in blood and/or CSF. Serotypes 14, 1 and 6B were the most common. 77% of all invasive isolates are included in the 11-valent Pnc conjugate vaccine. 1357 (45%) were positive for *S pneumoniae* in nasal swab. Of the 1199 respiratory samples with serotyping result, 19F, 6A, 6B, 15, 23F and G were common. 404 (33.7%) were vaccine types. Of the 30 invasive isolates, 10 had the same serotype in the respiratory sample. All invasive Pnc isolates were sensitive to penicillin. 4 % of 712 respiratory Pnc isolates tested for susceptibility were penicillin non-susceptible.

Conclusion: Monitoring of invasive and respiratory Pnc serotypes is crucial for choice of the ideal Pnc conjugate vaccine formulation for specific country use.
Evolution of pneumococcal serotype distribution over a 5 year period in 1-35 month old children in the city of Cordoba, Argentina

Tregnaghi M1; Ceballos A1; Ruttimann R2; Hausdorff W2; Ussher J1 Yudouski S1
1Centro de Desarrollo de Proyectos Avanzados (CEDEPAP) Córdoba, Argentina
2GSK

Streptococcus pneumoniae (SP) is the principal cause of community-acquired pneumonia and invasive bacterial disease. Following introduction of childhood vaccination against this microorganism, there have been reports of dramatic changes in and even replacement of the most prevalent disease-causing serotypes.

Objective: To determine in a population of children aged 1-35 months if temporal variations naturally exist among the different strains of SP and if specific clinical presentations are associated with certain serotypes.

Methods: This study was carried out in 2000-2005 in a population not immunized with the heptavalent pneumococcal conjugate vaccine [PCV] and which was under active surveillance for suspected invasive pneumococcal disease (IPD: fever ≥ in the previous 24 hours, pneumonia, bacterial meningitis, arthritis, soft tissue infections, peritonitis, empyemas, etc). This population comprised 216,617 children (average 39,385 yearly) in Cordoba, Argentina.

Results: The annual incidence of IPD was 170.3/100,000 based on a total of 257 isolates, all of which were included in this analysis. The serotypes were 14 (43.6%), 5 (11.7%), 6B (10.1%), 1 (8.9%), 18C (5.4%), 19F (2.7%), 9V (2.3%), 12F (2.3%), 4 (2.3%), 23F (1.9%), 7F (1.9%), 6A (1.6%), 33F (1.2%), 9A (0.8%), 3 (0.8%), 23B (0.8%), 13C (0.4%), 7B (0.4%), 20 (0.4%) and 7C (0.4%). We observed statistically significant fluctuations over this time period for serotypes 14, 6B, 5 and 1 (p > 0.05). The relative distribution of some serotypes varied according to pathology and age, mostly notably serotype 14 (p < 0.001). This was rarely isolated from children <6 months old, but in older infants represented an important cause of pneumonia that were frequently complicated with empyemas beginning in the 2nd year of life. Serotype 5 produced meningitis in the first 6 months of life and had a pattern similar to 14 with respect to pneumonias and empyemas. Serotype 1 produced pneumonias in infants and empyemas in older children, and 6B was isolated in all age groups; in children <6 months it only produced bacteremias.

Conclusions: We observed significant changes over the years for some serotypes in a population that has not been immunized with PCV. These observations raise the question as to whether PCV has been solely responsible for all apparent serotype changes and “replacement” described in other countries, or whether some changes are simply part of the natural history of certain serotypes.

The epidemiology of Streptococcus pneumoniae in Scotland between 2003 and 2005

Diggle, MA, Edwards, GSF
Scottish Meningococcus and Pneumococcal Reference Laboratory (SMPRL), Greater Glasgow Health Board, Glasgow, Scotland, UK.

Streptococcus pneumoniae (the pneumococcus). The most significant of the alpha-haemolytic streptococci is S. pneumoniae. It is responsible for acute lower respiratory tract infections, septicemia and meningitis. The principle virulence determinant is the anti-phagocytic polysaccharide capsule of which at present there are over 90 different serotypes.

As part of a national reference service provided by the Scottish Meningococcus and Pneumococcus Reference Laboratory (SMPRL), all invasive pneumococci, primarily isolated from blood and cerebral spinal fluid (CSF) are serotyped using latex agglutination techniques, antibiotic sensitivity profiles performed and genotyped using Multi-Locus Sequence typing (MLST). Here we present the distribution of serotypes and sequence types (STs) from disease-causing pneumococci, isolated between 2003 and 2005.

In summary, over 3 years between 2003 and 2005, a total of 1798 isolates were received and typed at the SMPRL. From these 97% were from blood and the remaining from CSF. In total 6% of all pneumococci were isolated from person’s aged 4 and below and over 17% were isolated from ages 65 and above. There were 55 different serotypes identified and these were further characterised by 269 different sequence types. The total number of pneumococci each year fluctuated significantly with a variance of between 15 – 20%. Characteristics that remained consistent over this three year period included, distribution between males and females and pneumococci isolated from ages 4 and below and 65 and above. Serotype 1 increased year on year finally representing 14% of all disease-causing pneumococci in 2005. Sequence types 306 and 162 increased year on year finally representing 25% and 10% respectively of all disease-causing pneumococci in 2005.

It is important to continue to monitor the distribution of pneumococci. Especially to observe the impact current and future polysaccharide and conjugate vaccines may have on targeted serotypes. In addition, consequences of these vaccines should continue to be observed to identify the possible emergence of vaccine non-targeted pneumococci.
Relative invasive disease (ID) potential of Streptococcus pneumoniae (S.pn) of specific serotypes and serogroups, in Chilean infants and children from 0 to 24 months of age (MoA).

1Centro para Vacunas en Desarrollo-Chile (CVD-Chile); Santiago, Chile
2Hospital de Niños Roberto del Río; Santiago, Chile
3Instituto de Salud Pública de Chile (ISP); Santiago, Chile
4GlaxoSmithKline Biologicals; Rixensart, Belgium
5Center for vaccine Development, University of Maryland; Baltimore, USA

Aims: To examine the potential of specific S.pn serotypes to cause ID, by comparing their distributions among invasive (IN) and nasopharyngeal (NP) isolates obtained from infants and children 0-24 MoA residing in the Metropolitan Region (MR), Chile.

Methods. During 12 consecutive months, 524 newborn infants served by the government health care system (HCS) were recruited in 18 primary care clinics of the MR, to participate in a 24-months-long follow-up study aimed at assessing the burden of acute respiratory illnesses (ARI). Nasopharyngeal (NP) cultures were performed at ages 2, 6, 12, and 18 months and for investigation of S.pn. 794 of 1923 (41.3%) NP specimens collected between 10/23/2001 and 07/27/2004 tested positive for S.pn (Spn+). During the same 33-month period, 428 cases of invasive pneumococcal disease (IPD) affecting MR children 0-24 MoA were detected by regular visits to the bacteriology laboratories where blood and sterile fluids from children affiliated with the government HCS are processed. Both NP and invasive (IN) S.pn isolates were referred to the Institute of Public Health of Chile (ISP-Chile) for confirmation of species and serotyping according to the Danish nomenclature, by Quellung reaction with antisera from the Statens Seruminstitut (Copenhagen). S.pn isolates were stratified according to the child's age (0-5; 6-11; 12-17 and 18-23 mos) at the time of collection of the NP or normally sterile body fluid. Within each age stratum, risk ratios (RRs) were calculated for each of the capsular serotypes represented in a developmental 10-valent vaccine and for pooled vaccine serotypes, non-vaccine serotypes/serogroups and nontypeable S.pn.

Results: RR estimates significantly different from 1, denoting increased (RR > 1) or reduced (RR < 1) representation of the specific serotype/serogroup among the set of IN isolates are shown in bolditalics.

Conclusions: Several recent studies have assessed the invasiveness of various S.pn serotypes or MLST clones by comparing their distributions among IN vs NP isolates. The size of our study allowed ranking the IPD potential of most of the examined S.pn serotypes/serogroups on the basis of statistically significant (yet empirical) RR estimates. Serotype 5 exhibited the highest RR in each of the age strata and in all ages; none of the 794 NP Spn isolates was serotype 5.

Relative invasiveness of Streptococcus pneumoniae serotypes / serogroups, among Chilean children 0-24 MoA.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>IN 10-valent Vaccine Serotypes</th>
<th>0 to 5 MoA</th>
<th>6 to 11 MoA</th>
<th>12 to 17 MoA</th>
<th>18 to 23 MoA</th>
<th>0 to 23 MoA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolates (N)</td>
<td>IN NP RR</td>
<td>IN NP RR</td>
<td>IN NP RR</td>
<td>IN NP RR</td>
<td>IN NP RR</td>
<td>IN NP RR</td>
</tr>
<tr>
<td>10-valent vaccine serotypes</td>
<td>67 160</td>
<td>152 247</td>
<td>146 222</td>
<td>63 165</td>
<td>428 794</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>2.4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>7.2</td>
<td>6</td>
<td>1</td>
<td>9.8</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>6B</td>
<td>2</td>
<td>9</td>
<td>0.5</td>
<td>12</td>
<td>16</td>
<td>1.2</td>
</tr>
<tr>
<td>7F</td>
<td>3</td>
<td>1</td>
<td>7.2</td>
<td>4</td>
<td>4</td>
<td>1.6</td>
</tr>
<tr>
<td>9V</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>9</td>
<td>10</td>
<td>2.1</td>
<td>60</td>
<td>22</td>
<td>4.4</td>
</tr>
<tr>
<td>18C</td>
<td>2</td>
<td>4</td>
<td>1.2</td>
<td>12</td>
<td>2</td>
<td>9.8</td>
</tr>
<tr>
<td>19F</td>
<td>8</td>
<td>12</td>
<td>1.6</td>
<td>4</td>
<td>18</td>
<td>0.4</td>
</tr>
<tr>
<td>23F</td>
<td>1</td>
<td>9</td>
<td>0.3</td>
<td>4</td>
<td>12</td>
<td>0.5</td>
</tr>
<tr>
<td>All</td>
<td>40</td>
<td>47</td>
<td>2.0</td>
<td>116</td>
<td>76</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Other 10-valent vaccine-related serotypes | 3 | 25 | 0.3 | 15 | 56 | 0.4 | 29 | 49 | 0.9 | 7 | 38 | 0.5 | 54 | 168 | 0.6 (0.4, 0.8) |

Non-vaccine related serotypes | 18 | 51 | 0.8 | 15 | 65 | 0.4 | 9 | 54 | 0.3 | 2 | 37 | 0.1 | 44 | 207 | 0.4 (0.3, 0.5) |

Non-Typeable S. pneumoniae | 6 | 37 | 0.4 | 6 | 50 | 0.2 | 3 | 23 | 0.2 | 0 | 20 | 0 | 15 | 130 | 0.2 (0.1, 0.4) |
Evaluation of co-morbidity on the incidence of pneumococcal infection in the far east of Russia

Martynova AV, Turkutyukov VB, Skurikha Yu V, Sheparyov AA
Epidemiology Department, State Vladivostok Medical University, Russia

Despite of the instant study pneumococcal infections are still remaining the frequent reason of morbidity and mortality among the population of the Far East of Russia. The incidence of community-acquired pneumonia of *S. pneumoniae* etiology is about 1.4 per 1000 of population, every second child younger of 5 years suffers from the pneumococcal otitis, etc. Also the epidemiological study of pneumococcal infections in our region is still remaining actual problem and needs careful planning, but the complex structure of co-morbidity of all groups of population allow to imply that the epidemiology of *S. pneumoniae* should be studied more carefully especially with data on the co-morbidity prevailing in population of our region.

We used 2002 and 2005 data from the State Medical Information Archive to determine rates of invasive pneumococcal disease in healthy adults (≥ 20 years old) and in adults with various high-risk conditions. The risks of invasive pneumococcal disease in persons with specific chronic illnesses was compared with that in healthy adults, controlling for age, professional status, and the other chronic illnesses. Overall incidence rates, in cases/1000 persons, were 0.9 in healthy adults, 8.29 in adults with chronic lung disease, 8.37 in adults with chronic heart disease, and 9 in adults who abused alcohol. Among the high-risk groups evaluated, risk was highest in adults with cancer (50.4), HIV/AIDS (40.9), and hemotological cancer (60.1). Incidence rates increased with advancing age in adults with chronic lung disease, diabetes, and solid cancer. On professional status the main groups were people working in marine industry (120), military (180) service, teachers (70) of the primary and secondary schools.

Also these data would be useful for organizing of vaccination against pneumococcal infection especially in the high risk groups.

Incidence of respiratory tract infections (RTI) in children < 2 years of age: Impact of environmental risk factors and effectiveness of a pneumococcal conjugate vaccine (PCV7)

Adam, D1, Helmerking, M2
1Children's Hospital University of Munich, Munich, Germany; 2German Society for Pediatric Infectious Diseases (DGPI) Study Group, DGPI Office Munich, Strasslach - Munich, Germany

**Background and Aim:**
*Streptococcus pneumoniae* is a common cause of respiratory tract infections (RTI) and acute otitis media (AOM) in infancy and early childhood. The German Society of Pediatric Infectious Diseases (DGPI) Study Group conducted a large-scale study to evaluate the impact of environmental risk factors and the effect of vaccine immunization with 7-valent pneumococcal conjugate (PCV7) vaccine in children with RTI in the first two years of life.

**Methods:**
Children aged from three to six months were enrolled to receive, in a 1:3 ratio, either the standard immunization (control group) alone or in combination with the pneumococcal conjugate vaccine. PCV7 was given to infants 2, 3, 4 and 12 to 15 months of age. Children who had received all four doses of PCV7, including a follow up time of 12 months were evaluable for efficacy.

**Results:**
By two years of age, 35.9 % of 5,984 children had at least one episode of RTI, including AOM (25.8 %). PCV7 reduced the number of children with RTI of any cause by 4.4 % (P = .001); otitis media by 5.2 % (P = <.001). 41.4 % of all children had at least one environmental risk factor. In children with risk factors immunization with PCV7 was associated with substantial reduction of respiratory tract infections by 8 % (P = <.001) compared to the control group. Day care attendance of siblings (more than 1 sibling) was the most important risk factor. PCV7 was effective in reducing the number of children with AOM in this high risk group by 12 %. 87.5 % of children with infections were treated with antibiotics and 13.5 % were hospitalized during the study period. Fewer children received antibiotics in the PCV7 group.

**Conclusion:**
Immunization with PCV7 was effective and safe in reducing RTI that may be caused by *Streptococcus pneumoniae* in young children.
Measuring the immunogenicity of the PNEUMOVAX®23 vaccine

Marchese, RD, Esser, M, Schlottman, S, Jain, N, Mallette, L, Raub, J, Butterfield-Gerson, K, Norris, M, Sikkema, D, Chirmule, N
Merck Research Laboratories, Wayne, PA, USA

PNEUMOVAX®23 is a sterile, liquid vaccine which consists of a mixture of highly purified capsular polysaccharides from 23 of the most prevalent or invasive serotypes of Streptococcus pneumoniae. The Merck Pneumococcal (Pn) ELISA’s were validated to measure IgG antibodies to 12 serotypes (1, 3, 4, 6B, 7F, 8, 9V, 12F, 14, 18C, 19F and 23F) included in the PNEUMOVAX® 23 vaccine and uses C-polysaccharide, Pn polysaccharide (Ps) 25, and Pn Ps 72 for pre-adsorption of samples, standards and controls. This assay does not utilize serotype 22F to pre-absorb non specific reactivity, since this polysaccharide is a component of the PNEUMOVAX®23 vaccine. The Merck Pn assay utilizes a pool of post-immunization sera obtained from subjects immunized with PNEUMOVAX® 23 as standards for measuring IgG concentrations in sera in vaccine clinical trials. This material was calibrated to the U.S. FDA Pneumococcal Reference standard serum, 89SF, in such a way to include adsorbent material in the standard, controls and test serum thus improving assay specificity and simplifying automation efforts. Furthermore, ELISA’s have been recently developed and validated to measure antibodies to the additional 11 serotypes in the vaccine. Experimentation performed to compare the Merck Pneumococcal ELIA’s to the International EIA methodology shows moderate agreement between the two assay formats. In order to significantly improve the throughput of testing large numbers of samples from pediatric clinical trials, which need to be tested on an array of antigens for concomitant use vaccines, we have also recently developed a Luminex-based assay to measure antibodies to all 23 Pneumococcal serotypes in the vaccine. The method uses 4-(4,6-dimethoxy[1,3,5]triazin-2-yl)-4-methyl-morpholinium (DMTMM) to conjugate the Pn antigens to amino or carboxyl Luminex microspheres, producing stable PnPs-microspheres when used in the serological assay. The method was shown to be robust and to produce minimum cross-reactivity while maintaining sensitivity and antigenicity of the polysaccharides. Using the DMTMM chemistry, we developed Pn-microspheres to use in a serology assay to simultaneously quantitate IgG responses to all the Pneumococcal serotypes in Pneumovax®23 and demonstrated assay linearity with increased dynamic range.

Uptake of Pneumococcal Conjugate Vaccine Among Children in the United States, 2000-2004

Nuorti JP, Martin S, Smith P, Moran J, Schwartz B
1National Immunization Program, Centers for Disease Control and Prevention (CDC)
2National Vaccine Program Office, Atlanta, GA, USA

Background In 2000, a 7-valent pneumococcal conjugate vaccine (PCV7) was recommended for universal vaccination of children in the U.S. Vaccine shortages in August 2001-May 2003 and February-September 2004 caused the CDC to recommend abbreviated schedules for healthy children. By 2003, the incidence of invasive pneumococcal disease (IPD) had decreased substantially in children and adults. We evaluated patterns of uptake and factors associated with undervaccination among the first six birth cohorts eligible to receive PCV7.

Methods The U.S. National Immunization Survey (NIS) collects vaccination histories of children aged 19-35 months by random-digit dialed household telephone interviews validated by mail survey of vaccination providers. We combined data from four survey years (2001-2004) to measure receipt of PCV7 doses by ages 12 and 24 months according to birth cohort among children born in 1998-2003. Survey estimates were adjusted to account for complex sampling design.

Results The proportion of children receiving at least one dose of PCV7 by age 12 months increased from 72.4% among children born in 2000 to 92.8% among those born in 2003; receipt of three or more doses increased from 44.9% to 73.9%. Among children born in 1998 and 1999, 7.0% and 15.2% received one and 3.0% and 23.3% received two catch-up doses by age 24 months, respectively. Among children born in 2000, 2001 and 2002, 30.0%, 37.4% and 47.9%, respectively, had received a fourth dose by age 24 months. In each cohort, blacks were as likely as whites to receive one or two doses but significantly less likely to receive three or four doses. Other factors associated with undervaccination included living in the Southern Census Region, poverty, and receiving vaccinations from a public provider.

Conclusions After PCV7 introduction, the initial uptake was rapid. A substantial proportion of children born in 1998-1999 received catch-up vaccinations. Few children born in 2000-2002 received the complete 4-dose series and there were racial differences in coverage. Despite shortages and incomplete coverage, post-licensure surveillance has documented significant reductions in IPD incidence, especially among black children, during 2001-2003 due to direct and indirect vaccine effects.
Invasive pneumococcal disease and the impact of the 7-valent pneumococcal conjugate vaccine in the greater Sydney region, NSW 1998 – 2005

McIntyre, PB1, Gilmour, R1,2, Brown, M1,4, Watson, M4, Bartlett, M2 and Gilbert, GL4

1National Centre for Immunisation Research and Surveillance, Sydney, NSW, Australia
2New South Wales Health Department, Sydney, NSW, Australia
3The Children’s Hospital at Westmead, NSW Australia.
4Institute of Clinical Pathology and Medical Research, Westmead, NSW Australia

Introduction
Enhanced surveillance data (ESD) for invasive pneumococcal disease (IPD) commenced in the greater Sydney region (GSR) in 1997, a population of approximately 4 million. A targeted immunisation program with 7-valent pneumococcal conjugate vaccine (7vPCV) for high-risk children commenced in 2001 and became universal for those born after January 2005.

Aims
To evaluate the impact of the 7vPCV one year post introduction on the incidence of IPD by age, vaccine serotype and focus of infection in the GSR.

Methods
Age-specific incidence over 8 years (1998 - 2005) was calculated for the GSR using census denominators based on data January to September for each year by type of infection, serotype, outcome and presence of underlying illness.

Results
IPD incidence in children < 5 years of age in the GSR fell from a 7 year mean of 70.7 cases per 100 000 population 1998 – 2004 to 38.2 per 100 000 population in 2005. The largest decline was in children under 2 years of age where the incidence was 53% (95% CI 43% - 62%) lower in 2005 (55.5 per 100 000 vs 120.1 per 100 000; p<0.0001). IPD caused by vaccine serotypes declined by 64% (p<0.001) while non-vaccine serotypes increased by 17% (95% CI 0.4% - 40%). IPD with pneumonia and meningitis incidence declined by 67% and 42% respectively in 2005. There was no change in IPD among high-risk children following the targeted immunisation program in 2001-4 but IPD in high-risk children decreased by 25% (95% CI 7% - 42%) in 2005. Deaths from IPD fell by 70% (95% CI 20% - 120%). Overall, a decline of 27% in IPD occurred in 2005, varying by age group from 7% (15-49 years) to 43% (5-14 years), with the exception of those aged 50-64 years where there was a 13% (95% CI 5% - 20%) increase.

Conclusion
One year post introduction of universal 7vPCV into the Australian immunisation schedule, a significant reduction in IPD in children under five years was seen in the GSR despite early evidence of replacement disease with non-vaccine serotypes. Evidence of herd immunity in other age groups was observed with moderate reductions in notifications of IPD. Continued surveillance of IPD and serotype replacement is necessary to monitor these trends in all age groups.

An electronic system to simplify the WHO process for the radiological diagnosis of pneumonia in research

O’Grady, K1, Taylor-Thomson, D2, Ruben, A2

1University of Melbourne, Melbourne, VIC, Australia
2Menzies School of Health Research, Darwin, NT, Australia

Background
The World Health Organization (WHO) has developed guidelines for the radiological diagnosis of paediatric pneumonia in epidemiological studies and vaccine trials. As such studies will often be multi-centre with potentially large numbers of subjects and chest radiographs to be reviewed, data collection and management may present significant challenges. To simplify this process, we designed an electronic, distributable data and image management program.

Methods
The system was developed to facilitate a large epidemiological study of radiologically diagnosed pneumonia in Aboriginal children in the Northern Territory Australia. It was a collaborative process between the investigators and a commercial database design company. The structure and functionality was based on key data requirements specified in the WHO protocol and was designed to take account of the diverse location and resources of co-investigators responsible for the reading and diagnosing of films.

Results
The program was designed in an ORACLE platform with an ACCESS front-end. Once digitised, images are imported into the database. The program randomly generates batches of 100 films to be burnt to compact disc and sent to readers (2 independent readers receive each batch of 100) for diagnosis. A distributable version of the ACCESS database accompanies the images. Images are simultaneously viewed on the same screen that data entry occurs. Data are emailed back to the central database where the program assigns a final diagnosis and identifies discordant films to be reviewed by an independent panel. Radiograph data are then relationally linked to core study data.

Discussion
The PICTURE distributable database has enabled over 18000 images corresponding to approximately 10000 subjects and 26 000 episodes of care to be stored, read and analysed by over 10 readers in various locations around Australia and overseas. This program is simple to use, flexible and readily adaptable to a variety of research centres. It will be a valuable resource for organisations using the WHO criteria in respiratory disease research in both developed and developing settings.
The WHO guidelines for the radiological diagnosis of pneumonia in children: Outcomes of investigator training in the Northern Territory, Australia

Ruben A1, O’Grady, K2, Chang A3, Bauert P4, Wheaton G5, Morris P1, McKenzie G1, Roseby R6, Martin L7, Torzillo P8

1Menzies School of Health Research, Darwin, NT, Australia
2University of Melbourne, Melbourne, VIC, Australia
3University of Queensland, Brisbane, QLD, Australia
4Royal Darwin Hospital, Darwin, NT, Australia
5Womens & Childrens Hospital, Adelaide, SA, Australia
6Alice Springs Hospital, Alice Springs, NT, Australia
7Gove District Hospital, Nhulunbuy, NT, Australia
8Sydney University, Sydney, NSW, Australia

Background
In 2003, we commenced a large epidemiological study of the incidence of hospitalised, radiologically diagnosed pneumonia in Aboriginal children in the Northern Territory, Australia. Radiological pneumonia is classified according to the World Health Organization (WHO) guidelines. We describe the outcomes of investigator training using the WHO training films, and discuss issues that impact on achieving high inter-observer agreement with the use of this methodology.

Methods
A panel of 10 investigators including paediatricians, general physicians and respiratory physicians were assembled. Two calibration training sessions using the WHO training films were held over two two-day periods. Films were viewed initially as a group, then individually and then as a group again. Inter-observer agreement between the investigators themselves, and between our team and the WHO panel was calculated for each lung and for the final diagnosis.

Results
For the initial 100 films, inter-observer agreement within the study team was 95% on the left lung and 92% on the right lung. Agreement with the WHO diagnosis was significantly lower (69% on the left lung, 79% on the right lung). All investigators jointly disagreed with the WHO panel on 9% of films.

The most common reason for disagreement with the WHO was the interpretation of infiltrates and film quality. Disagreements between investigators were predominantly related to appearances on the left lung, particularly behind the heart.

Discussion
The differences between our investigators and the WHO panel are likely a result of the predominance of clinicians on our team and the differences between the interpretation of a chest radiograph for clinical and epidemiological purposes. However there was uniform disagreement with WHO on a significant number of films. Potential reasons for this include film quality issues, the prevalence of acute and chronic respiratory disease in the populations from whom the images were sourced and that our initial team did not include radiologists. Study sites should feedback the results of calibration training to allow further evaluation of the WHO films.

Public health potential of a 13vPnC vaccine for immunization of adults in the US

Hackell, JG, Paradiso, PR, Siber, G
Wyeth Vaccines Research, Pearl River, NY, USA

The 13-valent pneumococcal conjugate (13vPnC) vaccine covers fewer serotypes than the 23-valent polysaccharide (23vPS) vaccine, but potentially has the additional benefits of a conjugate vaccine. This includes the ability to extend protection throughout the high-risk period by allowing revaccination, if necessary, without risk of induction of hyporesponsiveness (blunting of subsequent immune response).

Fry et al1 at CDC developed a model to look at the relative potential public health impact of various pneumococcal conjugate formulations, as compared to the currently available 23vPS vaccine in adults >65 years of age. The authors found a significant benefit of the conjugate vaccines based on the potential for more durable immunity and perhaps higher efficacy. We updated this model, using the rate of invasive pneumococcal disease (IPD) observed in 2004 (significantly lower than the 1998 data used in Fry et al). We also expanded the analysis to include 50-64 year olds. We assumed that 13vPnC had the same level of efficacy for the serotypes in the vaccine as 23vPS, but a longer duration of immunity, that could be sustained either through the induction of memory or through re-immunization, if needed. We assumed vaccine uptake to be 60%, comparable to the current estimates for 23vPS uptake in >65 year olds.

Similar to the original Fry et al estimates, the model predicts that more cases of IPD could be prevented with the 13vPnC vaccine compared to the cases currently prevented with the 23vPS vaccine (5544 vs 2979). The same is true for deaths due to IPD (895 vs 489). This is due in part to the ability to extend the age of initial vaccination down to 50 years of age without the risk of diminished immune responsiveness later in life, and in part due to the ability to maintain immunity throughout the entire high-risk period.

A new method of assessing frailty in older patients recruited into pneumonia vaccination trials

Lindley, R1, MacIntyre, R2, Ridda, F1, McIntyre, P2, Sullivan1, J, Gilbert, L4, Koovor, P5, Manolios, N6

1Discipline of Medicine: Geriatric Medicine. The University of Sydney, NSW, Australia
2Discipline of Medicine: Geriatric Medicine. The University of Sydney, NSW, Australia
3The Australian Red Cross blood services, Sydney, NSW, Australia
4Centre for Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research Westmead Hospital
5Discipline of Medicine: Cardiology department. The University of Sydney, NSW, Australia
6Discipline of Medicine: Rheumatology department. The University of Sydney, NSW, Australia

Introduction: Frail older people are usually excluded from randomised controlled trials (RCT’s) due to concerns about co-morbidity and difficulties in obtaining informed consent due to problems such as dementia. However, due to the ageing population, increasing numbers of frail elderly patients present to hospital and it is important to establish an appropriate evidence base for such patients. Pneumonia is an important cause of mortality for older people and it is important to recruit frail older people in new trials of pneumococcal vaccination (or treatment).

Aim: To design a new RCT to include frail older people and, for the first time, incorporate a new method of measuring frailty – the Frailty Index. The RCT is evaluating the immunogenicity of the 7-valent conjugated pneumococcal vaccine versus the polysaccharide vaccine. The total sample size for the trial is 400 patients. We present initial findings from the trial describing the frailty of patients recruited to this study.

Methods: Frail hospitalised older people (60 years or older) are screened for recruitment. Eligible patients must not have had previous pneumococcal vaccine and have an estimated life expectancy of at least 12 months. The New South Wales Guardianship Tribunal permits consent by a relative or friend if the patient lacks mental capacity and the study has appropriate ethics approval. The baseline assessment includes Mini-Mental Status Examination (MMSE), Barthel Index (BI) and Frailty Index. The Frailty Index consists of a 40 item (Yes/No) list of problems.

Results: The trial started recruitment on the 16th of May 2005. Data is available from the first 60 patients recruited. Patients are aged between 62 and 100 years; the MMSE ranges from 7 to 30, median 26.50. The Barthel Index at baseline ranges from 30 (moderately disabled) to 100 (functionally independent) with median of 85. The Frailty Index ranges from 2 to 24 (maximum frailty score 40).

Conclusions: The study is including appropriately frail patients as illustrated by the low MMSE scores and BI scores representing moderate to severe disability. The results from the Frailty Index do not show any “ceiling effect” and demonstrate a wide range of scores from 2 through to 24. The Frailty Index is easy to measure, has previously been shown to correlate with subsequent mortality and morbidity and may be a new method of measuring frailty in RCT’s.

Vaccination uptake in hospitalised geriatric patients 4-6 months after commencement of a funded national pneumococcal vaccination program for >65’s

Ridda, F1, MacIntyre, R1, Lindley, R2, McIntyre, P1, Sullivan, J1, Gilbert, L4, Koovor, P5, Manolios, N6

1National Centre for Immunization Research and Surveillance Sydney, NSW, Australia
2Discipline of Medicine: Geriatric Medicine. The University of Sydney, NSW, Australia
3The Australian Red Cross blood services, Sydney, NSW, Australia
4Centre for Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research Westmead Hospital
5Discipline of Medicine: Cardiology department. The University of Sydney, NSW, Australia
6Discipline of Medicine: Rheumatology department. The University of Sydney, NSW, Australia

Background: Pneumococcus is the most common cause of community-acquired pneumonia, leading to hospitalisation for many elderly and demented patients. The mortality rate in the elderly is up to 57%. Studies show that uptake was <30% prior to 2005. In January 2005, a publicly funded program commenced, offering free vaccination for people aged 65 years or over.

Aims: To determine vaccination uptake in hospitalised geriatric patients, in the period immediately following funding of the national pneumococcal vaccination program.

Methods: Study population: all patients admitted to the geriatric department aged >65 years from 16/5/05-17/6/05. (n= 350). Regardless of self reported vaccination status, GP’s were contacted to verify vaccination status against influenza and pneumococcus. We validated self-reported vaccination status against the GP’s records. A descriptive analysis was performed.

Results: A total of 350 patients were inpatients between 16/05/05 and 17/07/05. There were 203/350 (58%) females, and 68 patients who with dementia. GP vaccination records were obtained for 292/350 (83%). These records contained meaningful data for 265 patients of 292 (91%). Of these, 163/265 (62%) were vaccinated against pneumococcus. The majority of patients who had been vaccinated against pneumococcus were vaccinated in 2005. Only 8/68 (12%) demented patients were vaccinated.

Discussion: This study shows that in a highly vulnerable subset of the targeted population, the commencement of a publicly funded vaccination program resulted in a significant increase in vaccination uptake in the first months of the program, from less than 30% to 62%. Patients with dementia were identified as an under-vaccinated group.
Effect of 2 versus 3 pneumococcal conjugate vaccinations Prevnar® on nasopharyngeal carriage, transmission and herd immunity; a randomized, controlled study

van Gils, E1M, Veenhoven, RH1, Hak, E1, Lijmer, EPF1, Rijkers, GT1, Sanders, EAM1
1University Medical Center/ Wilhelmina Children’s Hospital, Utrecht, the Netherlands
2Sparnpe Hospital, Hooftdorp, the Netherlands
3Julius Center for Health Sciences and Primary Care, Utrecht, the Netherlands
4Regional Laboratory of Public Health, Haarlem, the Netherlands

Background. A 2+1-doses 7-valent pneumococcal conjugate vaccination (PCV7) scheme or a 2-doses scheme without booster may offer sufficient protection against invasive pneumococcal disease (IPD). However, the 2-doses scheme may lead to less carriage reduction of vaccine-type (VT) pneumococci and therefore less replacement by non-vaccine-type (NVT) pneumococci and other potentially pathogenic bacteria. Although less herd immunity and less influence on respiratory tract infections may occur, ongoing natural maturation of the antipneumococcal antibody levels by contact with VT-pneumococci may result in long-term persistence of protective antipneumococcal serum antibody levels against IPD both in infants and the community.

Aims. The primary aim of this randomized controlled study is to compare the influence of a 2+1 and 2-doses PCV7-vaccination scheme on nasopharyngeal pneumococcal carriage, transmission and herd immunity. Further, to determine serum antipneumococcal antibody levels after these vaccination schemes at the age of 12 and 24 months.

Methods. Prospective, randomized, controlled trial. 1000 healthy infants and their families will be included. The infants will be randomized in three groups: group I: PCV7 (Prevenar®, Wyeth-Lederle) at age 2 and 4 months; group II: PCV7 at age of 2, 4 and 11 months; group III: PCV7 at age 24 months. Nasopharyngeal swabs from infants and questionnaires on risk factors and (respiratory) infections are obtained before the first vaccination and during follow-up at 6, 12, 18 and 24 months and from family members at the infant’s age of 12 and 24 months. The NP swabs are cultured by conventional methods and pneumococcal serotypes are determined by Quellung reaction. Antipneumococcal serum antibody levels at 12 and 24 months are obtained from 80 children in group I and II and from 30 children in group III.

Results. 617 infants are included in the study (December 1st) since the start of the study in June 05. Preliminary results show that prior to vaccination nasopharyngeal pneumococcal carriage was 13%. S. aureus was found in 51% of NP swabs, H. influenzae in 14% and M. catarrhalis in 19%.

Conclusions. A large scale randomized controlled carriage study on the influence of 2+1 versus 2 doses PCV7 in 1000 healthy infants will provide insight in the influence on nasopharyngeal carriage, transmission and herd immunity and long-term antipneumococcal antibody development before the introduction of pneumococcal vaccination in the national vaccination program.

Multilocus sequence typing scheme for Streptococcus pneumoniae: Novel strains among Gambian carriage isolates

Sankareh KK1, Hanage WP2, Antonio M3, Obaro S1, Greenwood BM1, Adegbola RA1, Spratt BG2.
1Medical Research Council Laboratories, P.O. Box 273, The Gambia, West Africa.
2Department of Infectious Disease Epidemiology, Imperial College London. 3London School of Hygiene and Tropical Medicine.

In contrast to the developed world, the population biology of Streptococcus pneumoniae in developing countries is poorly understood. We used multilocus sequence typing (MLST) to characterise S. pneumoniae carriage isolates from the Gambia.

Method: A total of 77 pneumococcal isolates were collected from the nasopharynx of Gambian children under 2 years of age whose mothers had been immunized with the 9 valent pneumococcal polysaccharide vaccine prior to delivery during a pilot study undertaken from 1997 to 1999. All these optochin sensitive S. pneumoniae isolates were characterised by capsular serotyping and MLST.

Results: S. pneumoniae isolates belonging to serotypes 6A and 23F were found to be most prevalent. Out of the 77 DNA extracts subjected to MLST, 64 isolates were associated with the STs, which had not been previously reported. In total 51 STs were found to be novel to the MLST global database (ST no > 900). A considerable ST diversity associated with the most common prevalent carriage serotypes has been observed as 6A was found in various associations with STs 912(4), 913(1), 914(1), 919(6), 1736(4), 1737(2), 1741(1), 1742(1), 1748(1) and 1785(1) whilst 23F was found in STs 802 (5), 847 (1), 172 (1), 925 (1) and 918 (1). Although the most prevalent strain ST 802 was found most in association with the vaccine serotype 23F (5), it was also found in a non-vaccine serotype 33 (2). The presence of these multiple serotypes associated with several of the novel STs in this study strongly suggests a history of capsular switching mediated by recombination exchange at the capsular biosynthetic locus.

Conclusions: This is the first MLST analysis of carriage isolates of S. pneumoniae from The Gambia. Most of the strains found in The Gambia have not been described elsewhere. MLST of larger carriage pneumococcal samples is now underway and will provide further understanding of the population and evolutionary biology of S. pneumoniae in a community with high and early carriage of the pneumococcus.
Impact of co-trimoxazole on carriage and antibiotic resistance of *Streptococcus pneumoniae* and *Haemophilus influenzae* in HIV infected children in Zambia

Mwenya DM 1,2, Charalambous BM 2, Gibbs D 3, Nunn A 1, Mwansa JCL 1, Gillespie, SH 2.
1University Teaching Hospital, Lusaka, Zambia
2Royal Free & University College Medical School, UCL, London, U.K.
3MRC Clinical trials Unit, London, U.K.

**Background:** Recent studies indicate that co-trimoxazole prophylaxis is associated with reduced morbidity and mortality in HIV infected patients. In order to determine the long term effect of co-trimoxazole prophylaxis on nasopharyngeal carriage and resistance of potential respiratory pathogens, a study on nasopharyngeal carriage and susceptibility of *S. pneumoniae* and *H. influenzae* in HIV-infected children was carried out.

**Study design:** Sub study of a double-blind randomised placebo controlled trial of co-trimoxazole prophylaxis.

**Methods:** HIV infected children between 6 months and 14 years were recruited at University Teaching Hospital of Zambia (UTH) between 2001 and 2003 and followed up for two years. Children <5yrs received a daily dose of 5ml (240mg) of co-trimoxazole or a matching placebo and those ≥5yrs received 10mls (480mg) of co-trimoxazole or a matching placebo. Pernasal swabs were collected before commencing trial drugs and then at weeks 4, 12, 24 and thereafter every 24 weeks. Specimens were processed for isolation and antibiotic resistance of *S. pneumoniae* and *H. influenzae*.

**Results:** Co-trimoxazole prophylaxis did not affect carriage of *S. pneumoniae* and *H. influenzae*. Nevertheless, there was an overall upward trend in *S. pneumoniae* carriage (p = 0.04, slope = +0.14 ± 0.31) and no significant change in *H. influenzae* carriage. There was a significant upward trend in co-trimoxazole resistant *S. pneumoniae* in both co-trimoxazole (p = 0.0001) and placebo groups (p = 0.001). However, by week 12, resistance was higher in the co-trimoxazole group than the placebo group. This difference was greatest at 24 weeks of treatment (Odds Ratio (OR), 3.89; 95% Confidence Interval (CI), 1.62-9.30; p = 0.001) and by week 72 was no longer evident. In *H. influenzae* there was no upward trend in resistance in either co-trimoxazole or placebo group. However, there was a decrease in resistance to co-trimoxazole in the placebo group while it remained constant in the co-trimoxazole group. This resulted in higher co-trimoxazole resistance in the drug group by week 12 of treatment, which was greatest at 72 weeks (OR, 3.84; 95% CI, 0.98-15.12; p = 0.05) compared with the placebo group.

**Conclusion:** Co-trimoxazole prophylaxis did not affect carriage of *S. pneumoniae* and *H. influenzae*. However, it led to a short term increase in pneumococcal resistance and a sustained level of resistance in *H. influenzae*.

The impact of the Australian Indigenous Adult Pneumococcal Immunisation Program

Menzies, R 1, McIntyre, P 1,2.
1National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases, Sydney, NSW, Australia
2University of Sydney, Sydney, NSW, Australia

The Australian National Indigenous Pneumococcal and Influenza Immunisation (NIPI) program is unique in the world as a fully funded national immunisation program targeting Indigenous adults. Since 1999, it has provided vaccine for all Indigenous adults aged 50 years or more and Indigenous adults aged 15-49 years with medical risk factors. This study aimed to provide an estimate of the impact of the program on pneumococcal vaccination coverage and morbidity, from an analysis of routinely collected data and a review of published reports.

In those aged 50 or more years, the proportion vaccinated in the past 5 years was 25% nationally in 2001. Coverage was higher in areas with relatively larger Indigenous populations (73% in northern Queensland in 2000 and 50% in the Northern Territory in 2001), but little other regional or local data were available. Invasive pneumococcal disease notification rates in Indigenous adults decreased following immunisation in northern Queensland and the Kimberley, but not the NT. National data were not available prior to the commencement of vaccination. Post vaccination, the difference in rates (Indigenous: non-Indigenous rate ratio) was greater in those aged 15-49 years, where vaccination is recommended only for Indigenous adults with risk factors. The rate ratio was lower in those aged 50 or more years, where vaccination is for all Indigenous adults. This was also true for hospitalisations due to pneumococcal meningitis or septicaemia.

In conclusion, vaccination coverage in 2001 was moderate, with considerable room for improvement. Coverage appeared to be higher in northern areas with larger Indigenous populations and lower in southern urban areas. There appears to have been a greater impact on morbidity in the 50+ year age group than in younger Indigenous adults. Universal Indigenous adult vaccination may increase the impact of the program in the 15-49 years age group.
Yields of different pneumococcal tests in community-acquired pneumonia in elderly

Palmu, AA1, Erkkilä, L1, Saukkoriipi A1, Poolman J1, Hausdorff, WP1, Leinonen, M2, Kilpi, TM2
1National Public Health Institute, Helsinki
2National Public Health Institute, Oulu, Finland
3GlaxoSmithKline Biologicals, Rixensart, Belgium

Background. *Streptococcus pneumoniae* (Pnc) has been the bacterial pathogen most commonly identified in studies of community-acquired pneumonia (CAP), with positivity rates between 25% and 50% in CAP cases with identified microbiol etiology, and 10 to 30% for all CAP cases. However, a valid diagnosis of pneumococcal etiology is problematic. We have commenced a Finnish Community-Acquired Pneumonia Epidemiological (FinCAP Epi) study to find a case definition for pneumococcal pneumonia suitable for trials of new vaccines against pneumonia in the elderly. A major objective is to define a highly sensitive and specific algorithm for the clinical and etiological diagnosis of pneumococcal CAP.

Methods. A specific study clinic with study physicians and nurses was established at municipal emergency clinic of Tampere city in central Finland. Active recruitment efforts are underway in an attempt to enroll a high proportion of cases of CAP in non-institutionalized patients aged ≥65 for two years from May 2005 onwards. Patients with a diagnosis of pneumonia confirmed by a series of pre-existing, acute, and follow-up chest x-rays are enrolled after informed consent and samples from blood, urine, sputum, and nasopharyngeal swabs (NPS) are used for extensive microbiological analyses (culture, quantitative PCR, antigen detection, serology).

Results. During the first 5 months of the study, 75 cases of CAP have been enrolled. Pnc has been cultured from blood in one case only. This subject was also one of the two patients positive for the urine antigen test (Binax NOW). A sputum sample has been obtained from 48 of the 75 cases (64%) and was positive for Pnc culture in 13 samples (27%). The overall positivity rate for Pnc by sputum culture is thus 13/75 (17%). Of the 33 NPS cultures done so far, 7 (21%) have been positive for Pnc. Compared to culture, higher positivity rates for sputum and NPS samples were demonstrated by Pneumolysin-PCR.

Conclusions. Preliminary results on blood culture and pneumococcal urine antigen test show low yields. Sputum and NPS are potentially more suitable samples for sensitive diagnosis of pneumococcal etiology. Specificity will be assessed by comprehensive analyses of samples from healthy individuals and patients with acute respiratory infections without CAP and patients with stable chronic obstructive pulmonary disease.

Invasive pneumococcal disease in German children vaccinated with pneumococcal vaccines

Reinert, RR1, Al-Lahham, A1, Siedler, A1, van der Linden, M1, Toschke, AM1, and von Kries, R2
1National Reference Center for Streptococci and Institute of Medical Microbiology, Aachen, Germany
2Robert-Koch Institute, Berlin, Germany
3Ludwig-Maximilians-University of Munich, Institute for Social Pediatrics, Munich, Germany

Background: In Germany the 7-valent pneumococcal conjugate vaccine (PCV7) is recommended on basis of an 'at risk' only strategy defining risk by prematurity and a broad range of underlying diseases including immune defects and chronic diseases. To date, there has been no significant impact of this at risk strategy on the incidence rate of invasive pneumococcal disease (IPD) in Germany. The present report focuses on paediatric cases of IPD in vaccinated children in Germany.

Methods: In a German-population based study, all pediatric hospitals and their microbiological laboratories are independently asked to send case reports of IPD infections in children up to 15 years and send isolates to the NRCS for confirmation of species diagnosis, MICs by the microdilution method and serotyping by the Quellung reaction. Cases included were IPD in children vaccinated with the PCV7 or with the 23-valent pneumococcal polysaccharide vaccine (Pneumovax) in the study years 2003 to 2004. Multilocus Sequence Typing (MLST) was performed according to standard methods.

Results: A total of 19 cases occurred in the study years 2003 and 2004. In three vaccinated cases there were no isolates sent to the NRCS for serotyping and MLST. The following pneumococcal serotypes and MLSTs (16 cases were observed): 7F [MLST 191] (n = 3), 22F [MLST 433] (n = 2), 24F [MLST 72] (n = 1), 10A [MLST 461 and 1551] (n = 2), 38 [MLST 393 and 1849] (n=2), 35B [MLST 452] (n = 1), 19A [MLST 1611] (n=1), 15B [MLST 199] (n = 1), 9V [MLST 156] (n = 1), 4 [MLST 205] (n=1) and 23F (n = 1). 13/16 cases serotyped were caused by non PCV7 serotypes. All 3 cases of vaccine serotype disease (one case of serotype 4, 9V and 23F) occurred in patients vaccinated with the 23 valent polysaccharide. Conclusions. No cases of PCV7 serotype failure were reported sofar in children vaccinated with the PCV7 in Germany after the introduction of the PCV7.
Clinical predictors of radiological pneumonia and hypoxia in Gambian children

Zaman, SMA1, Enwere, G2, Akano, A2, Oluwalana, C1, Brown, JO1, Vaughan, A1, Biney, EEO1, Greenwood, BMG3, Adegbola, RA1, Cutts, FT1

1Medical Research Council Laboratories, Fajara, The Gambia
2National Hospital, Abuja, Nigeria
3London School of Hygiene and Tropical Medicine, London, UK

Background: Radiological pneumonia is being increasingly used as an outcome measure in vaccine efficacy trials. In the Gambia Pneumococcal Vaccine Trial (PVT), we found significant efficacy against clinical pneumonia accompanied by consolidation or effusion, but no protection against clinical pneumonia without consolidation or effusion. We aimed to assess whether clinical signs could be used to predict radiological pneumonia and hypoxia, an indicator of severe pneumonia.

Methods: We analysed data on children 2-30 months of age who presented with cough or difficult breathing for <2 weeks and had chest x-rays (n=8035). Malaria microscopy was performed when axillary temperature was $\geq 38^\circ$C. Children were considered hypoxic when oxygen saturation was $<$90% by pulse oximetry. The diagnostic performance of respiratory rates for radiological pneumonia was assessed on all children by constructing receiver operating characteristic curves (ROCs). The association between other clinical signs and radiological pneumonia or hypoxia was evaluated on admitted children only by estimating adjusted odds ratios.

Results: Overall, respiratory rates were poor predictors of radiological pneumonia (area under ROC curve: AUC=0.62). The sensitivity and specificity of $\geq50$ breaths/minute in infants was 94% and 9%. We found similar results in children 12-30 months of age. AUCs for respiratory rates with malaria parasitaemia and severe anaemia were lower compared to children without those conditions (AUC 0.5796 vs 0.6544; P=0.010 and AUC 0.5293 vs 0.6330; P=0.031 respectively). Exclusion of children with parasitaemia resulted in a substantial gain in specificity.

Grunting, bronchial breath sounds, and hypoxia were positively associated with radiological pneumonia (ORs $\geq2.0$, P<0.05), whereas rhonchi, malaria, and severe anaemia were negatively associated (ORs=0.4; P<0.05). The presence of grunting, bronchial breath sounds, or hypoxia without rhonchi, parasitaemia, and anaemia had sensitivity and specificity of 38% and 87% respectively. Respiratory rates were also poor predictors of hypoxia (AUC=0.59). The sensitivity and specificity of $\geq70$ breaths/minute for hypoxia was 36% and 79%, respectively. Very sick appearance, poor verbal response, or a respiratory rate of $\geq90$/minute were strongly associated with hypoxia (ORs $>$3.4). The presence of any of these signs had a sensitivity of 70% and a specificity of 65%. Addition of crepitations or indrawing improved sensitivity (90%) at the expense of reduced specificity (27%).

Conclusion: We found that clinical signs were poor predictors of radiological pneumonia and hypoxia even after excluding children with malaria and severe anaemia.
Replacement invasive pneumococcal disease 9 years after introduction of PCV use among a population at high risk for IPD: the Navajo experience

Johns Hopkins Center for American Indian Health, Baltimore, MD, USA
Arctic Investigations Program, CDC, Anchorage, AK.

Background: Prior to pneumococcal conjugate vaccine (PCV) use, Navajo had rates of invasive pneumococcal disease (IPD) 3-5 fold those of the general US population; moreover, only 50% of IPD cases among children <2 years were caused by PCV7 serotypes. PCV7 was introduced among Navajo children during a phase III efficacy trial (4/97-8/00) and then as a routine infant vaccine beginning 11/00. We aimed to assess whether serotype replacement has occurred in this setting of high disease burden, broad disease causing serotype distribution and extensive PCV7 use.

Methods: Active, population, and laboratory based surveillance for IPD was conducted; a case was defined as isolation of *S. pneumoniae* (SP) from a normally sterile site in a Navajo person. Isolates were serotyped (quellung reaction) and charts abstracted for clinical and demographic information. Annual Indian Health Service user population statistics (extrapolations for 2004 and 2005) were used as denominators for rate calculations. IPD cases from 1995-97 (3 years pre-trial) and 2001- Dec 2, 2005 (5 years post-trial) were compared.

Results: We identified 475 IPD cases in 1995-97 and 492 in 2001-05 (38% reduction in average annual cases). Annual rates (cases/10^5) of all serotype, VT, and NVT IPD for these periods are shown.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1</td>
<td>391</td>
<td>226</td>
<td>225</td>
<td>29</td>
<td>174</td>
<td>178</td>
</tr>
<tr>
<td>1-&lt;2</td>
<td>442</td>
<td>183</td>
<td>259</td>
<td>34</td>
<td>185</td>
<td>140</td>
</tr>
<tr>
<td>2-&lt;5</td>
<td>105</td>
<td>29</td>
<td>49</td>
<td>10</td>
<td>47</td>
<td>21</td>
</tr>
<tr>
<td>5-&lt;18</td>
<td>14</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>18-&lt;40</td>
<td>31</td>
<td>18</td>
<td>2</td>
<td>2</td>
<td>29</td>
<td>16</td>
</tr>
<tr>
<td>40-&lt;64</td>
<td>76</td>
<td>84</td>
<td>10</td>
<td>7</td>
<td>66</td>
<td>57</td>
</tr>
<tr>
<td>65+</td>
<td>162</td>
<td>152</td>
<td>16</td>
<td>14</td>
<td>137</td>
<td>126</td>
</tr>
</tbody>
</table>

Although overall NVT rates fell, the annual rate of type 3 (0/10^5 vs. 18/10^5, P=0.009), 7F (9/10^5 vs. 29/10^5, P=0.04) and 19A (9/10^5 vs. 47/10^5, P=0.002) increased while that of type 5 (58/10^5 vs. 0/10^5, P<0.001) decreased among those <2 years between 1995-97 and 2003-05.

Conclusions: The rate of all serotype and VT IPD among Navajo have fallen, especially among vaccinated age groups, since PCV7 introduction. Overall NVT IPD has also fallen in all age groups; rates of specific types (3, 7F, 19A) have increased while type 5 has decreased among those < 2 years. Evaluation and introduction of PCV with broader serotype coverage is needed in this population to address the disproportionate morbidity of IPD still observed.


1Centers for Disease Control and Prevention, Atlanta, GA, USA, 2Monroe County Department of Public Health, Rochester, NY, USA, 3Connecticut Department of Public Health, Hartford, CT, USA, 4Oregon Department of Human Services, Portland, OR, USA, 5Emory University Department of Medicine, Atlanta, GA, USA, 6Tennessee Department of Health, Nashville, TN, USA, 7Minnesota Department of Health, St. Paul, MN, USA, 8University of California, Berkeley, CA, USA, 9University of Pittsburgh, Pittsburgh, PA, USA

Background: Incidence of invasive pneumococcal disease (IPD) is highest in young children and older adults. We determined the impact of pediatric use of pneumococcal conjugate vaccine (PCV7) in the U.S. on age-specific incidence of IPD among adults.

Methods: We used population-based, laboratory-based surveillance for IPD, defined as isolation of pneumococcus from a sterile site, in 8 continuously monitored Active Bacterial Core surveillance (ABCs) sites to estimate IPD rates. Serotyping was performed by reference laboratories. We compared IPD rates among adults ≥18 years in 2004 to rates during the baseline period of 1998/1999.

Results: During 1998-2004, rates of IPD caused by all serotypes and by PCV7 serotypes decreased by 20-43% and 64-77%, respectively, depending on age group (Table). The incidence of IPD caused by serotype 19A increased among all groups. We observed no change in incidence of IPD caused by PCV7-related serotypes, exclusive of 19A, or in incidence of IPD caused by non-PCV7 types.

Table. Rates of IPD among U.S. Adults, 1998-2004

| Age Group, years | Serotype category | 1998/1999 average Rate (cases/100,000) | 2003 Rate (cases/100,000) | 2004 Rate (cases/100,000) | % change (95% CI) 2004 vs. 1998/1999 |
|------------------|------------------|----------------------------------|------------------|------------------|----------------------------------|------------------|
| 18-49            | All              | 13.3                             | 9.1              | 7.6              | -43 (-48, -38)                   |
|                  | PCV7             | 7.6                              | 2.7              | 1.8              | -76 (-80, -72)                   |
|                  | 19A              | 0.40                             | 0.67             | 0.94             | 139 (72, 231)                    |
|                  | PCV7-related le  | 1.1                              | 1.1              | 0.85             | -20 (-39, 4)                     |
|                  | NonPCV7          | 4.2                              | 4.6              | 4.0              | -5 (-17, 8)                      |
| 50-64            | All              | 24.0                             | 19.4             | 19.2             | -20 (-28, -12)                   |
|                  | PCV7             | 12.8                             | 6.2              | 4.6              | -64 (-70, -57)                   |
|                  | 19A              | 0.73                             | 1.4              | 2.2              | 210 (105, 368)                   |
|                  | PCV7-related le  | 1.9                              | 2.4              | 2.0              | 5 (-24, 46)                      |
|                  | NonPCV7          | 8.8                              | 9.4              | 10.4             | 21 (4, 40)                       |
| 65-79            | All              | 45.9                             | 32.2             | 30.3             | -34 (-41, -26)                   |
|                  | PCV7             | 25.0                             | 9.5              | 6.6              | -74 (-79, -67)                   |
|                  | 19A              | 1.7                              | 2.8              | 3.2              | 83 (22, 176)                     |
|                  | PCV7-related le  | 3.9                              | 3.7              | 4.1              | 5 (-24, 44)                      |
|                  | NonPCV7          | 14.4                             | 16.2             | 16.5             | 8 (-8, 27)                       |
| ≥80              | All              | 99.1                             | 66.9             | 58.8             | -41 (-48, -33)                   |
|                  | PCV7             | 57.4                             | 18.6             | 13.0             | -77 (-71, -82)                   |
|                  | 19A              | 3.7                              | 4.2              | 6.5              | 77 (12, 179)                     |
|                  | PCV7-related le  | 10.9                             | 12.1             | 9.5              | -13 (-37, 20)                    |
|                  | NonPCV7          | 27.2                             | 32.1             | 30.0             | 10 (-9.34)                      |

*Excluding serotype 19A

Conclusions: Incidence of overall and PCV7-type IPD among adults has continued to decline consistently across all adult age groups, suggesting a uniform, indirect effect of PCV7 use among children. Replacement disease with serotype 19A, while concerning, has not overshadowed the substantial indirect benefits among adults.
Invasive Streptococcus pneumoniae (ISP) infections continue to cause high mortality

Sanvictores, DH1, Lucero, M1, Tallo, V4, Adamson, F, Chester, A3, Nohynek, H2, Williams, G2 for the ARIVAC Consortium

1Research Institute for Tropical Medicine, Metro Manila, Philippines
2University of Queensland, Brisbane, Australia
3National Public Health Institute (KTL), Helsinki, Finland

Introduction. The phase III 11-valent PCV trial is a randomized, placebo-controlled, double-blind clinical trial conducted by the ARIVAC consortium in Bohol, Philippines. 12,194 infants were enrolled between 2000 and 2003 and were monitored until their second birthday or until end of clinical phase of the trial, i.e., December 2004.

Methods. Basic demographic data, routine vaccines administered, trial endpoints (pneumonia, sepsis, meningitis) from hospitalizations or consultations and serious adverse events (SAEs) were collected. Independent data entry persons completed a double data entry process on-site at Bohol Data Management Centre using a Relational Database Management System consisting of Microsoft SQL7 and Visual Basic front end combined with various Microsoft Access2000 client fields. Data entry error rates, classified by forms and fields, were monitored monthly for quality assurance. The compare procedure in SAS was used to validate double data entry errors. Errors were minimized with build-in validity checks applied concurrently upon data entry. Audit trails were produced to document entry and correction on the data.

Results. Double data entry error rates were 150 per 100,000 numeric and 555 per 100,000 character fields at the start, and stabilized to 22 per 100,000 numeric and 66 per 100,000 character fields after seven months of data entry. There were higher error rates in forms with more character fields, e.g., infant data, SAE and case ascertainment CRFs.

Conclusion. The combination of the identification number and name initials was critical for accurate identification of the infant and linkage of his/her various records. The use of linkage procedures ensured all hospitals episodes and SAEs were reported for the appropriate infant.
Developing a quasi-dynamic model for evaluating vaccines against \textit{Streptococcus pneumoniae} in infants

\textbf{PO4.21}


\textsuperscript{1}M-TAG Limited, a unit of IMS Health, London, United Kingdom
\textsuperscript{2}SHW Health, London United, Kingdom
\textsuperscript{3}GlaxoSmithKline Biologicals, Rixensart, Belgium

We have developed a model which depicts the natural history and epidemiology of \textit{Streptococcus pneumoniae} (\textit{S. pneumoniae}). The purpose of the model is to simulate the impact of multi-valent pneumococcal conjugate vaccines in infants in terms of costs and health effects.

The gold standard for economic models depicting conjugate vaccination strategies is the transmission dynamic model. \textit{S. pneumoniae} has a complex epidemiology which makes the development of such a model a considerable challenge. To date, the vast majority of \textit{S. pneumoniae} vaccination models have utilised a standard ‘static’ approach, which does not allow the force of infection to change over time. Our objective was to balance practical data and computational constraints against the complex nature of disease dynamics.

Our model offers an alternative to the typical dichotomy of static versus dynamic models: it is quasi-dynamic. Developed using TreeAge\textsuperscript{®} software, the model is structured as a Markov process, whereby the cohort is cycled through a series of health states. These represent the combination of serotypes to which an individual can be immune. For example, an individual can be immune to infections covered by a 10-valent vaccine, a 7-valent vaccine or no vaccine (or combinations of the above). Individuals not receiving the vaccine are included in the model, allowing for the herd immunity impact to be quantified. The risk of infection in the unvaccinated population is modelled as a direct function of the epidemiological data, of the coverage in the vaccinated cohort and of the effectiveness of the vaccine. The model also assesses the impact of vaccination of a number of different diseases caused by \textit{S. pneumoniae}, including pneumonia.

The results of our model indicate that the benefits of the vaccine extend beyond the direct benefits observed in the vaccinated cohort. This result alone appears to warrant our quasi-dynamic approach because it has captured an important effect of the vaccine (herd immunity) without the data requirements and computational burden that would have been necessary in a full transmission dynamic model.

Epidemiology of community-acquired pneumoniae caused by \textit{Streptococcus pneumoniae}

\textbf{PO4.22}

\textbf{Martynova, A.V.}, Turkutjukov, V.B., Skurshina, E.Ju., Sheparjov, A.A.

Epidemiology Department, State Vladivostok Medical University

\textbf{Background}: Despite the last achievements in diagnosis and treatment of pneumococcal infection, the problem of community-acquired pneumococcal pneumoniae at the Far Eastern region of Russia is still actual. The morbidity level on pneumococcal infections at the Far East of Russia exceed that ones at the central parts of the country twice. Moreover, the morbidity among children and aged patients is 2.5 more than in the same groups in the neighbouring regions. Aim: So, as this situation needs more careful and precise epidemiological evaluation, the aim of our project was to estimate epidemiological situation using the a simplified set of prognostic indicators and to compare the predictive values of different sets of clinical parameters, using available clinical data for pneumococcal CAP patients.

\textbf{Methods}: So, we analysed the morbidity on pneumococcal community-acquired pneumonia (CAP) of patients who admitted to Navy Vladivostok Hospital between 1 February 2001 and 31 December 2004. The aetiology was confirmed by positive sputum and/or blood cultures. The severity assessment based on the Pneumonia Outcome Research Trial (PORT) scoring systems. Primary end-points were in-hospital CAP-attributable deaths and length of hospitalisation.

\textbf{Results}: We identified 70 patients with \textit{S. pneumoniae} CAP. The mean age at the time of diagnosis was 62 (+/-15) years. Eleven patients (11.5%) were admitted to the medical intensive care unit. The mean (median) hospitalisation duration was 12.5 (+/-5) days. Most frequent antibiotics used initially were cephalosporins and fluoroquinolones. The mean (+/- standard deviation) PORT score was 105 (+/-37). The observed CAP-related mortality was 1/70 (1.42%). The mortality rate in ICU was 8.58% (6/70). Twenty five patients (35.7%) had \textit{S. pneumoniae} bacteraemia an admission. The bacteraemic and non-bacteraemic patients had length of hospitalisation (7 vs. 5 days, P = 0.41). Patients admitted with pneumococcal CAP, although severe and with multiple co-morbidities (weighted index of co-morbidity is 6.6; combined condition and age-related score is 7+/- 1.2 ) had higher in-hospital mortality rates and lengths of hospitalisation.

\textbf{Conclusion}: neither prior antimicrobial use nor revealing of antimicrobial resistance contributed to an adverse outcome, early recognition of higher risk patients and placement in ICU, use of broad spectrum antibiotics could all have combined to effect a good outcome for these patients.
Pneumococcal infections in young children in Hong Kong – the pre-vaccine era and cost-effectiveness of pneumococcal conjugate vaccine

Ng YM, Yau YS
Department of Paediatrics, Queen Elizabeth Hospital, Kowloon, Hong Kong

Introduction: Streptococcal pneumoniae infection is a common bacterial infection in Hong Kong. However, vaccination program to prevent pneumococcal infection is not included in the routine EPI scheme. Pneumococcal Conjugated Vaccine (PCV) is not available in Hong Kong until recently, in August 2005.

Method: This is a retrospective study of the incidence of streptococcal pneumoniae infections in children below 5 years of age who had been admitted to all the hospitals under the Hospital Authority in Hong Kong from 1 Jan 2002 till 31 December 2004. The incidence of invasive diseases (meningitis, septicaemia, peritonitis and pneumonia with pleural effusions) was calculated according to the data from Hong Kong Census Department. With the data, a cost effectiveness estimation and projection with the introduction of PCV is calculated and projected, using the USA experience of reduction of disease load after her vaccination program in year 2001.

Results: There were 239 episodes of streptococcal pneumoniae infections in children below 5 years of age who had been admitted to all the hospitals under the Hospital Authority in Hong Kong from 1 Jan 2002 till 31 December 2004. They are all sputum culture positive or culture positive from sterile sites like the CSF, blood and pleural fluid. Twenty-nine cases were suffering from invasive diseases, with an incidence of 27.0. These cases had positive cultures from either the CSF, blood and pleural fluid. One mortality only was recorded from Queen Elizabeth Hospital. Queen Elizabeth Hospital, one of the seven largest general hospitals in Hong Kong, with 200 paediatric beds and 8 paediatric intensive care beds, recorded 53 cases of streptococcal pneumoniae infections, 10 with invasive diseases and the one death. These cases are analysed in detail. Cost savings from different disease states such as meningitis, septicaemia and pneumonia are estimated according to the vaccine efficacy, hospital cost of each disease.

Conclusion: The incidence of invasive diseases in young children in Hong Kong is similar to the USA before the PCV vaccination program. The introduction of PCV in the Hong Kong immunization scheme, besides reducing morbidity and mortality caused by streptococcal pneumoniae infections will also be cost effective.
Effect of oxygen availability on membrane composition of \textit{Streptococcus pneumoniae}\textsuperscript{PO5.01}

Pesakhov, S\textsuperscript{1}, Sikron, N\textsuperscript{1}, Benisty, R\textsuperscript{1}, Cohen, Z\textsuperscript{2}, Khazaïn-Goldberg, F\textsuperscript{1}, Dagan, R\textsuperscript{1}, Porat, N\textsuperscript{1}.
\textsuperscript{1}Pediatric Infectious Disease Unit, Soroka University Medical Center, Ben-Gurion University of the Negev, Beer-Sheva, Israel
\textsuperscript{2}Microalgal Biotechnology Laboratory, Ben-Gurion University of the Negev, Beer-Sheva, Israel

\textbf{Background:} \textit{S. pneumoniae} possesses mainly fermentative metabolism and lacks the cytochromes and heme containing proteins involved in aerobic respiration. Oxygen-rich environments present a challenge to the survival of \textit{S. pneumoniae}. The organism is particularly susceptible to exogenous and endogenous oxidants, as it lacks the antioxidant defense mechanisms observed in aerobic organisms. In the present study we aimed to explore the ability of the cytoplasmic membrane to function as a separate oxidative-protective entity under conditions of oxidative burst, by studying the mechanism’s controlling membrane adaptation to changes in oxygen availability.

\textbf{Methods:} Two clinical isolates belonging to serotype 2 (p210) and 6A, and an isogenic \textit{spxB}-mutant of p210, lacking pyruvate oxidase activity, were used. Fatty acid composition was analyzed by gas chromatography. Fatty acid synthase-II (FASII)-mRNA expression levels were determined by RT-PCR.

\textbf{Results:} The transition from anaerobic to aerobic conditions was accompanied by a remarkable decrease in the desaturation level (23-26\%, \textit{p}<0.05) and in the average length of the fatty acyl chains (29-39\%, \textit{p}<0.05), in the wild type strains. However, in the case of the \textit{spxB} mutant, desaturation index was higher by almost 2 fold compared to the wild type strains, and it did not change when shifting from anaerobic to aerobic conditions. The reduced desaturation level under aerobic conditions may originate from either lower rate of double-bond formation and/or higher rate of double-bond breakdown by the bacterial \textit{H}_{2}\text{O}. RT-PCR data revealed a significant increase (ranging from 20\% to 66\%) in the transcript levels of the various FASII genes under aerobic conditions, indicating that the reduced desaturation level and chain length could not be attributed to lower rate of FASII transcription. On the other hand, addition of salicylate (a known scavenger of reactive oxygen intermediates) to the culture resulted in increased desaturation levels of the fatty acyl chains, similar to that observed under anaerobic conditions. No change in the fatty acyl chains was observed for the \textit{spxB} mutant when grown in the presence of salicylate.

\textbf{Conclusions:} It appears that high levels of endogenous peroxides and exogenous reactive oxygen species lead to the breakdown of membrane unsaturated fatty acids. We present data showing that under aerobic conditions \textit{S. pneumoniae} is capable of compensating this damage by the upregulation of FASII transcription.

Molecular characterisation of invasive paediatric \textit{Streptococcus pneumoniae} isolates collected during a 9-valent pneumococcal conjugate vaccine trial in The Gambia. \textsuperscript{PO5.02}

\textit{Antonio M\textsuperscript{1}, Biney E\textsuperscript{1}, Saaka M\textsuperscript{1}, Pflüger V\textsuperscript{1}, O’Callaghan J\textsuperscript{1}, Greenwood B\textsuperscript{3}, Cutts F\textsuperscript{3}, Plaschke G\textsuperscript{2}, RA Adegbola\textsuperscript{1}.}
\textsuperscript{1}MRC Laboratories Fajara, Banjul, The Gambia, \textsuperscript{2}Swiss Tropical Institute, Basel, Switzerland, \textsuperscript{3}London School of Hygiene and Tropical Medicine, London, UK.

\textit{S. pneumoniae} consists of at least 90 different serotypes, the majority of which rarely cause disease; however, a few serotypes (e.g. serotypes 1, 5 and 9V) cause >30\% of invasive disease in all age groups in The Gambia. The recently concluded trial of a 9-valent pneumococcal conjugate vaccine in a typical rural African setting in The Gambia (Basse) was highly successful; it showed protective efficacy against radiological pneumonia (37\%) and all pneumococcal disease (50\%) and reduced all cause hospitalisations and mortality by 15\% and 16\% respectively. Though the 9-valent vaccine contained glycoproteins for serotypes 1 and 9V, these serotypes caused a small number of cases of invasive disease in the trial, with no reduction in cases in vaccinated children. The very small numbers of cases detected precludes estimation of serotype-specific efficacy for these two serotypes but these findings raise the possibility that efficacy was lower against serotypes 1 and 9V than against other vaccine-type serotypes despite the fact that the vaccine elicited strong immune responses, as measured by ELISA, for all serotypes contained in the 9-valent vaccine, including serotypes 1 and 9V. Lower efficacy for serotype 1 and 9V could be explained by heterogeneity or intraspecies variations among these invasive serotypes. We, therefore, characterized a total of 153 isolates of \textit{S. pneumoniae} from cases of invasive disease collected during the PVT trial from Aug. 2000 to April 2004 by BOX-PCR and found a correlation between BOX-PCR fingerprinting and serotypes. Molecular analysis for serotypes 1 and 9V are ongoing however, preliminary results for serotype 1 showed a correlation between PFGE and MLST. In addition, there were two sequence types (STs) of serotype 1 circulating during the PVT trial period of which ST618 was common. ST618 belongs to the same clonal complex (ST 217, ST303 and ST612) of serotype 1 that has caused an outbreak of meningitis in Northern Ghana, indicating a dominant West African clone.
Role of the pneumococcal MerR-like regulator in resistance to oxidative stress

Stroeher UH¹, Kidd SP², McEwan AG², Jennings, MP² and Paton JC¹
¹University of Adelaide, Adelaide, SA, Australia
²University of Queensland, Brisbane, Queensland, Australia

For invasive disease one of the watershed events in the pathogenic process of *S. pneumoniae* is the migration from the nasopharynx were it exists asymptomatically, to the lungs and blood, where it causes pneumonia and bacteraemia, respectively. The organism is subjected to markedly different environmental conditions in these niches, one likely variable being oxidative stress. Previous studies have shown that members of the MerR-like regulator family, such as SoxR of *Escherichia coli* and more recently NmlR of *Neisseria gonorrhoeae*, are involved in response to oxidative stress. We have found that one of the MerR-like regulators in the *S. pneumoniae* strain D39 also appears to be involved in survival under stress conditions. We initially constructed an in-frame deletion/insertion mutation in *merR* by PCR overlap extension. This mutant was examined for its ability to survive and grow in the presence of a number of stress-inducing chemicals such as diamide, S-nitrosoglutathione and spermine-NO; under all of these conditions the mutant’s fitness was reduced compared to the D39 parent.

We extended our studies to an in vivo mixed infection models. In invasive disease, the *merR* mutant showed markedly reduced ability to compete with its wild type parent (its competitive index was less than 0.001). However, *merR* appears to play no role in adherence to epithelial cell lines in vitro, or colonisation of the murine nasopharynx. Thus *merR* appears to play a role only in systemic disease. We have now extended our work to look at the genetic basis for this phenotype. Examination of the DNA upstream of *merR* indicates a typical *merR*-dependent promoter; the spacing between the −10 and −35 promoter elements is 19 bp, compared to the generally accepted spacing of 17 bp. Furthermore, this region encompasses a small incomplete inverted repeat, also typical of this sort of promoter. Initial microarray data comparing the parent and the *merR* mutant grown under diamide stress has revealed a number of potential candidate genes regulated by MerR, such as those encoding alcohol dehydrogenase and thioredoxin family proteins.

The two-component signal transduction system RR/HK06

Standish AJ, Stroeher UH and Paton JC.
School of Molecular and Biomedical Science, University of Adelaide, Adelaide, SA, Australia

*Streptococcus pneumoniae* encounters a number of environmental niches in the body including the nasopharynx, lungs, blood, middle ear and brain. Recent studies have identified thirteen two-component signal transduction systems (TCSTS) in *S. pneumoniae*, which are likely to be important for gene regulation in these diverse host niches. We have investigated one of these systems, RR/HK06, and have previously demonstrated that it regulates the known virulence gene *cbpA*. However, some phenotypic changes, such as epithelial cell adherence, did not appear to correlate with expression of *cbpA*. To identify other genes regulated by the system, we have constructed a strain over-expressing RR06 from a maltose inducible promoter, and analysed gene expression using microarray. As expected, we saw increased *cbpA* expression indicating that RR06 is binding to the promoter region and increasing the expression of this virulence factor. We also found differences in a number of other genes, including putative virulence factors, phosphotransferase systems, ATP binding proteins, and another two-component signal transduction system. One operon, containing a gene showing homology to gls24 from *Enterococcus faecalis*, was down-regulated when RR06 was over-expressed. Interestingly, in a *hk06* in-frame deletion mutant, these genes were up-regulated at both the protein and mRNA level, while there was no difference in expression in mutants in which either the entire system or just *rr06* were deleted. An inverted repeat is found in the promoter region of the first gene of the operon, which may act as a possible binding site for RR06. We have also constructed mutants in RR06 containing single amino acid changes that are predicted to either mimic the phosphorylated or non-phosphorylated state, as well as changes in HK06 predicted to affect both kinase and phosphatase activities, in order to further investigate the regulation of *cbpA*. Additionally 2D-gel electrophoresis is being used to confirm results from the microarray analysis.
Evaluation and selection of tandem repeat loci for \textit{Streptococcus pneumoniae} MLVA strain typing

\textbf{Koeck, JL}1, Lafourcade, B2, Gessner, BD2, Varon, E3, Sangare, L4, Valjevac, S5, Vergnaud, G5, Pourcel, C5

1HIA Robert Picqué, Villenave d’Ornon, France
2AMP, Paris, France, and Bobo Dioulasso, Burkina Faso
3Centre National de Référence des Pneumocoques, Hôpital Européen Georges Pompidou, Paris, France
4Centre Hospitalier Universitaire Yalgado Ouédraogo, Ouagadougou, Burkina Faso
5Université Paris XI, Orsay, France

\textbf{Background}

Precise identification of bacterial pathogens at the strain level is essential for epidemiological purposes. In \textit{Streptococcus pneumoniae}, the existence of 90 different serotypes makes the typing particularly difficult and requires the use of highly informative tools. Available methods are expensive or time-consuming and cannot be used for large-scale routine typing of all new isolates. We explore here the potential of MLVA (Multiple Loci VNTR Analysis; VNTR, Variable Number of Tandem Repeats), a method of growing importance in the field of molecular epidemiology, for genotyping of \textit{Streptococcus pneumoniae}.

\textbf{Results}

Available genome sequences were searched for polymorphic tandem repeats. The loci identified were typed across a collection of 56 diverse isolates and including a group of serotype 1 isolates from Africa. Eventually a set of 16 VNTRs was proposed for MLVA-typing of \textit{S. pneumoniae}. These robust markers were sufficient to discriminate 49 genotypes and to aggregate strains on the basis of the serotype and geographical origin, although some exceptions were found. Such exceptions may reflect serotype switching or horizontal transfer of genetic material.

\textbf{Conclusion}

We describe a simple PCR-based MLVA genotyping scheme for \textit{S. pneumoniae} which may prove to be a powerful complement to existing tools for epidemiological studies. Using this technique we uncovered a clonal population of strains, responsible for purulent meningitis in Burkina Faso. The electronic portability of VNTRs data should allow the construction of an expanding database and the exchange of molecular typing data for global epidemiology via the Internet. We believe that the proposed MLVA typing scheme can become a standard for epidemiological studies of \textit{S. pneumoniae}.

---

\textbf{Differential expression of pneumococcal virulence genes in vivo}

\textbf{LeMessurier, KS, Ogunniyi AD, and Paton JC}

The University of Adelaide, SA, Australia

The gene regulatory mechanisms associated with the translocation of pneumococci from the nasopharyngeal epithelium into underlying host niches such as the lungs, blood and the CSF are poorly understood. This has primarily been due to technical difficulties in harvesting sufficient quantities of pneumococci from an animal model to perform accurate and quantitative RNA assays, particularly from niches such as the nasopharynx where bacteria exist asymptomatically and at a low level. In this study, we investigated expression of key pneumococcal virulence genes \textit{cbpA}, \textit{psaA}, \textit{ply}, \textit{psaA}, \textit{cps2A}, \textit{psaA}, \textit{nanA} and \textit{spxB} in the nasopharynx, lungs and bloodstream of mice following intranasal challenge with the serotype 2 strain D39. Bacterial RNA was extracted, linearly amplified, enriched and assayed by real time RT-PCR. At 72 h, \textit{cbpA} mRNA was present in higher levels in the nasopharynx and lungs than in the blood. At this time point, the mRNAs for PspA and PsA were most abundant in the nasopharynx, whereas no significant difference in gene expression between niches was observed for \textit{ply}, \textit{psaA} and \textit{cps2A}. Both \textit{nanA} and \textit{spxB} mRNAs were present in higher amounts in the nasopharynx than in the lungs or blood. Using microarray technology we carried out global comparative gene expression analyses between the niches. The microarray data corroborate the real-time RT-PCR results and also reveal differential expression of other genes that could potentially be important during pathogenesis of pneumococcal disease.
Characterization of two highly conserved pneumococcal proteins that are essential for \textit{in vivo} survival and virulence

Intercell AG, Vienna, Austria

The currently available pneumococcal polysaccharide-based vaccines, although highly efficacious in protecting against invasive diseases, provide only partial coverage for the >90 different serotypes causing human infections. There is a need to develop novel vaccines containing protective antigens that are conserved in all serotypes. Our Antigen Discovery Technology has identified several pneumococcal proteins that induce protection in a murine sepsis/lethality model. Two of these proteins - named ICSp1 and ICSp2 - with the highest amino acid sequence conservation across serotypes were further characterized by investigating their role in bacterial growth, \textit{in vivo} survival and virulence. Deletion of the genes for ICSp1 and ICSp2 resulted in a drastically reduced growth \textit{in vitro} and a dramatic reduction in virulence \textit{in vivo}. Electron microscope analysis revealed abnormal septum formation in the \textit{icsp}1 gene deletion mutant strain whereas the \textit{icsp}2 gene deletion mutant strain shows an elongated rather than cocci like cell shape. Recombinant forms of these antigens detected high levels of specific antibodies in human sera, suggesting that the corresponding proteins are expressed during pneumococcal disease and colonization. ICSp1 and ICSp2 induce functional antibodies in mice and rabbits as analyzed in \textit{in vitro} assays. These results support our protection data that these proteins are promising candidates for a novel subunit vaccine to prevent invasive pneumococcal diseases.

Genetic characteristic of the invasive pneumococci collected in elderly population in England and Wales during winter season 2003/04

Centre for Infection, London, United Kingdom

Invasive (predominantly blood culture) isolates collected in England and Wales during winter season 2003/04 from patients aged 80 years or more and received at the Respiratory and Systemic Infection Laboratory for serotyping were genotyped by MLST (n=542). Among 32 serotypes 144 sequence types (STs) were identified, these can be clustered into 67 clonal complexes as defined by the e-BURST method. More than one serotype was observed within 9 different STs. The origin of these “serotype variants” was probably caused by capsular switching which has occurred in the more or less recent past during recombination events between two pneumococci expressing different capsule types. Capsular switching has led to a high genetic variability particularly within serotype the common serotypes 6A and 19F where respectively 9 and 8 clonal complexes (11 and 10 STs) were observed. Eighty-two invasive isolates belonged to 8 international antibiotic resistant clones as defined by the Pneumococcal Molecular Epidemiology Network. England 14-9 (n=52) and Spain 19V-3 (N=18) were the most prevalent. Isolates of different serotype but identical ST to the clones England 14-9, Tennessee 14-18 and Spain 19V-3 were observed. The overall case fatality ratio (CFR) amongst these elderly patients was 57% but there was no overall evidence of association between ST and mortality (p=0.45). However, there was some evidence of a difference in CFR ratio between ST in clonal complex 176 where 6/8 of patient infected by isolates of ST 138 and ST 1627 (single and double locus variants of ST 176) died compared to only 1/8 patient infected by isolates of ST 176. As all isolates of clonal complex 176 were of serotype 6B it may be that pathogenicity of this serotype varies between clones. In reference to the 23PPV vaccination campaign initiated in spring 2003 in England and Wales no particular difference in STs was observed between vaccinated and non-vaccinated patients. Vaccine failure within 2 years of immunisation was observed in 28 patients infected with strains of serotype 3, 4, 6B, 7F, 9N, 14, 19A, 22F, 23F but no particular ST within serotypes was associated with vaccine failure. To date there is no evidence of emergence of new non-23v vaccine serotype clones by capsular switching.
MLST characterization of *Streptococcus pneumoniae* clones of serotypes 6B, 14, and 23F associated with invasive disease in the Czech Republic

Žemličková, H, Urbášková, P, Motlová, J
Centre of Epidemiology and Microbiology, National Institute of Public Health, Prague, Czech Republic

**Objectives**
To use multilocus sequence typing (MLST) to further characterize invasive isolates of *S. pneumoniae* serotypes most frequently associated with invasive pediatric disease in the Czech Republic. Invasive isolates from adults were also included in the study to find out whether distribution of sequence types (STs) is uniform across age groups or age specific.

**Methods**
Altogether 128 isolates of serotypes 6B, 14, and 23F, including 44 isolates from children under 6 years of age, recovered from blood and cerebrospinal fluid and referred to the National Institute of Public Health between 1996 and 2003 by 39 microbiology laboratories from 28 cities, were analyzed by MLST.

**Results**
Isolates of serotypes 6B and 23F appeared quite diverse: 10 clonal groups (16 STs) were identified among 44 serotype 23F strains and 9 clonal groups (13 STs) were recorded among 30 strains of 6B serotype. Nevertheless, two predominant distinct clonal groups represented by ST176 and ST36 were recovered each within serotype 6B and 23F strains. In contrast, isolates of serotype 14 were highly homogenous: 51 of 54 isolates belonged to one clone (ST124). Isolates harboring STs identical to those of two globally spread penicillin resistant *S. pneumoniae* clones, Spain193-1 and Poland46-20, were detected among penicillin non-susceptible isolates of serotype 23F and penicillin susceptible isolates of serotype 6B, respectively.

**Conclusion**
The continued presence of previously described penicillin susceptible invasive *S. pneumoniae* clones was demonstrated within each of pneumococcal serotypes 6B, 14, and 23F. The recovered major clonal groups contributed equally among the isolates collected from children and adults. Despite the low number of penicillin non-susceptible *S. pneumoniae* isolates in our study set, multidrug resistant clones known to be implicated in high prevalence of resistance to β-lactams in many countries, were also detected.

Development of genomic array footprinting for the identification of conditionally essential genes in *Streptococcus pneumoniae*

Bootsma, HJ, Kloosterman, TG, Burghout, P, Bijlsma, JJE, Hermans, PWM, Kuijpers, OP

1Laboratory of Pediatric Infectious Diseases, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands
2Department of Molecular Genetics, University of Groningen, Haren, The Netherlands

*Streptococcus pneumoniae* is a major cause of serious infections such as pneumonia and meningitis in both children and adults worldwide. Given the limitations of the current capsule polysaccharide vaccine as well as the increasing antibiotic resistance among circulating strains, identification of novel targets for therapy or prophylaxis remains of utmost importance. Although the genomic sequences of several *S. pneumoniae* strains have been available for some years now, as yet no high-throughput genome-wide technique to efficiently screen for genes important for survival of the bacterium during infection has been described. To be able to readily identify such conditionally essential genes, we are currently developing genomic array footprinting (GAF), a method combining random genome-wide mutagenesis with microarray technology. We have established both *in vitro* and *in vivo* transposon mutagenesis protocols to create mutant libraries. To provide suitable probes for microarray analysis, chromosomal DNA from the mutant pool is extracted and used as template for the generation of mutant-specific probes. To this end, several procedures have been tested for sensitivity and specificity of amplification of transposon-insertion sites. Finally, amplified products are labeled and hybridized to an amplicon-based microarray. Genes essential for *S. pneumoniae* under particular conditions, such as adhesion and/or invasion *in vitro* and mouse infection models, are characterized by differential hybridizations patterns obtained before and after challenge of the mutant pool. We expect GAF to be a fast and flexible method to identify conditionally essential genes of *S. pneumoniae*, which should easily be adaptable for use with other bacterial species as well.
To monitor the distribution of *Streptococcus pneumoniae* amongst the population, the UK Department of Health launched a pneumococcal vaccination campaign in 2003 targeting the elderly population (65 years and over). Simultaneously surveillance programme was established to monitor the effect of vaccination on this population. We are performing large-scale, sequence-based typing and establishing related databases for the microbiological and epidemiological investigation of invasive pneumococcal disease, particularly but not exclusively amongst the elderly population. This is in support of the surveillance programme. A Multi locus sequence typing (MLST) scheme for *S. pneumoniae* was recently described and is proposed as a suitable and effective genotyping approach. MLST characterises the alleles present at seven housekeeping genes directly by nucleotide sequencing. MLST uses variation that slowly accumulates and that is expected to be selectively neutral. MLST also achieves very high resolution by analysing multiple loci. The most important advantage of MLST over other DNA typing methods is the unambiguity and electronic portability of nucleotide sequence data. In this study MLST was applied to a large number of invasive *S. pneumoniae* isolates to determine the genetic characteristics of this bacterial population in England and Wales. Because MLST is being used for high throughput analysis it becomes a manually intensive process to generate accurate consensus sequences, assign allelic profiles and sequence types (ST). Therefore, we have developed a pipeline on the Bionumerics platform for automated sequence analysis in order to reduce the time of the analytical steps by hours, if not days. We have also used single-strand sequences as input data for the Bionumerics pipeline which, as well as saving time has been cost effective. In one year we have tested 1650 isolates and made 11,500 sequences. Of the 1650 isolates tested, 1151 have had their ST identified and 80 of those are new ST. A descriptive account of the techniques involved in the large-scale genotyping of *S. pneumoniae* by MLST will be presented.

**Comparative analysis of the pneumococcal capsular loci**

**Background/ Aims:** The evolution of pneumococcal capsular polysaccharide (cps) loci is almost certainly very complex, with a long history of gene capture and loss, and genetic re-arrangements. Rather than take a phylogenetic approach, we have sought to group cps loci according to the extent to which they share similar genes.

**Methods:** The sequences of the cps loci of all 88 pneumococcal serotypes that synthesize the capsule by the Wzx-Wzy dependent pathway and the available CPS biochemical structures were used (Accession Nos. CR931632-CR931722). All cps gene products were assigned to homology groups (HG) which correspond to proteins that share significant sequence similarity (cut-off of 1e-20) and their presence in the cps locus determined the cps profile of each serotype. A 88x88 table of the weighted pairwise comparisons of the cps profiles was produced and Agglomerative Hierarchical Clustering Analysis was performed using the XL-STAT software.

**Results:** At a level of similarity ≥0.10, serogroups 6, 9, 10, 11, 12, 15, 18, 22, 23, 24, 25, 28, 32 and 41 each cluster together, whereas the cps loci of serogroups 7, 19, 16, 17, 33, 35 and 47 do not. At a level of similarity ≥0.45, six clusters of serotypes are identified. Cluster 1 includes the cps loci of serogroups 9, 19 and serotype 36 possessing the mnaA and the N-acetylmannosamine transferase wchO genes. Cluster 2 is mostly comprised of serotypes harbouring the initial transferase gene wciE in their cps loci, whereas the fnlA-C and the N-acetylfucosamine transferase wciF genes are present in the cps loci of serogroup 12 and serotypes 4, 5, 44, 45, 46. Cluster 3 includes the cps loci of serogroups 11, 15 and serotypes 8, 14 possessing the glycosyltransferase (GT) wchJ and the GT enhancer wchK genes (except from serotype 8). The rmlA-D and the rhamnosyltransferase wchF genes are present in the cps loci of serotypes comprising cluster 4 (only serogroup 6 lacks wchF). Cluster 5 is comprised of serogroup 41 and serotypes 17A and 31 cps loci. The presence of certain GT genes and the commonalities in the 3' end of the cps loci characterise the serotypes comprising cluster 6.

**Conclusions:** The relatedness among the pneumococcal cps loci revealed by clustering analysis provides new insights for the analysis of the immunologically defined serogroups/ serotypes.
Regulation of glutamine and glutamate metabolism by GlnR and GlnA in *Streptococcus pneumoniae* PO5.13

Kloosterman, TG1, Hendriksen, WT2, Bijlsma, JJ2, Bootsma, HJ2, Kok, J1, Hermans, PWM3, Kuipers, OP1
1University of Groningen, Haren, The Netherlands
2Erasmus MC-Sophia Children’s Hospital, Rotterdam, The Netherlands
3Radboud University, Nijmegen, The Netherlands

Regulation of nitrogen metabolism in bacteria is intimately connected with the intracellular levels of glutamine and glutamate, which are the main nitrogen donors in the cell. Interestingly, a number of genes involved in glutamine metabolism play a role in the virulence of several pathogens. We studied the role of the glutamine regulatory protein GlnR in *S. pneumoniae*. We demonstrated that GlnR of *S. pneumoniae* mediates repression of its own operon *glnRA*, the glutamate/glutamine transport operon *glnPQ* and *gldA*, encoding a biosynthetic glutamate dehydrogenase. The observed regulation was dependent on the presence of glutamate, glutamine and ammonium in the growth medium. The expression of *gldA* was independently of GlnR also repressed by the pleiotropic regulator CodY. Strikingly, the gene *zwf*, encoding glucose-6-phosphate dehydrogenase, the key enzyme of the pentose phosphate pathway, was shown to form an operon with *glnPQ* and its expression was regulated by GlnR as well. Furthermore, in contrast to previous suggestions, our results demonstrate that *glnPQ* encodes the only glutamate/glutamine transport system in *S. pneumoniae*. In a deletion mutant of glutamine synthetase *glnA*, repression of the identified GlnR targets was relieved, indicating that, similar to what has been observed in *B. subtilis*, GlnA is required for the function of GlnR. In accordance, binding of GlnR to the *glnR* and *glnP* promoter regions was dependent on GlnA, suggesting a previously unidentified cooperation between these proteins. GlnA and *glnRA* and *glnP* mutants, but not the *glnR* mutant, displayed reduced adherence to Detroit 562 human nasopharyngeal cells, which indicates that GlnA and GlnP play a role in pneumococcal virulence. In conclusion, we present a thorough characterization of regulation of glutamate/glutamine metabolism by GlnR and GlnA in *S. pneumoniae*, which we propose is important for virulence. Currently, we are analysing the importance of the GlnR regulatory system in an *in vivo* murine infection model.

Regulation of gene expression in *Streptococcus pneumoniae* by two-component system 09 is strain-dependent PO5.14

Hendriksen, WT1, Silva N2, Blue, C3, Paterson, G2, Kerr, A2, Bootsma, HJ2, de Jong, A2, Kuipers, OP4, Hermans, PWM3, and Mitchell, TJ2
1Erasmus MC-Sophia Children’s Hospital, Rotterdam, the Netherlands.
2University of Glasgow, Glasgow, United Kingdom.
3Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands.
4University of Groningen, Haren, the Netherlands.

Recent murine studies have demonstrated that TCS09 of *S. pneumoniae* is virulence-associated, moreover, is strain-specific. In the present study, we used a murine model of infection to assess the virulence of a TIGR4 rr09-mutant, and found that TIGR4∆rr09 was attenuated after intranasal infection and mice infected with rr09-mutant had significantly longer survival times than the wild-type infected mice. Further, we investigated the transcriptional changes in pneumococcal mutants lacking the response regulator of TCS09 (∆rr09) by microarray analysis of two strains, D39 and TIGR4. The transcriptional pattern of D39∆rr09 and TIGR4∆rr09 displayed clear differences as compared to their parental wild type strains. Moreover, TCS09 appeared to (directly or indirectly) regulate different genes in D39 and a TIGR4. In D39∆rr09, genes involved in competence (e.g. comAB) were upregulated, while several genes involved in sugar uptake (e.g. PTS systems) were downregulated. In TIGR4∆rr09 fewer genes were found to be regulated by RR09, most prominently genes located on the rlrA pathogenicity islet. Furthermore, we found that the genes encoding a β-galactosidase, a putative mannose-specific PTS, a putative sugar isomerase and putative aldose epimerase were strongly downregulated in D39, and not regulated in TIGR4. Real-time PCR confirmed these findings and this was extended to strain 0100993 in which these genes were not regulated by RR09. In conclusion, our results indicate strain-specific regulation of pneumococcal genes by TCS09.
Contribution of Glutamine Synthetase GlnA and its transcriptional repressor GlnR to pneumococcal virulence

Hendriksen, WT1, Kloosterman, TG2, Estevão, S1, Bootsma, HJ3, de Groot, R1, Kuipers, OP2, Hermans, PWM1
1Erasmus MC-Sophia Children’s Hospital, Rotterdam, The Netherlands
2University of Groningen, Haren, The Netherlands
3Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Central nitrogen metabolism is of utmost importance in bacterial survival. In the pneumococcus, several systems involved in amino acid metabolism, such as peptide uptake systems, have been shown to contribute to virulence. Signature tagged mutagenesis studies have shown that in pneumococcus, glnA, the gene encoding Glutamine Synthetase (GlnA) and glnQ, a glutamine ABC transport system, are involved in virulence. In S. pneumoniae, GlnR controls, together with GlnA, the expression of the glnRA and glnPQ operons.

To assess the relevance of this regulatory system in vivo, we used D39 wild type, ΔglnA and ΔglnR in a murine model of colonization and infection. We observed that the glnA mutant had a reduced ability to colonize the murine nasopharynx (p < 0.03). The glnR mutant, however, did not show any reduction in colonization. In vitro adherence to human nasopharyngeal cells of these mutants correlated with these observations, since reduced adherence to Detroit 562 cells was only observed for ΔglnA. Furthermore, upon intravenous infection, mutants for both glnR and glnA showed reduced levels of bacteremia (p = 0.0182 and p = 0.0002, respectively), and mice infected with these mutants showed increased survival times (p = 0.0260 and p = 0.0043, respectively).

We hypothesize that the maintenance of a glutamine pool in the bacterium contributes to full virulence. Furthermore, GlnR-mediated regulation also plays a role in virulence, in particular during sepsis, albeit to a lower extent. Individual targets of GlnR are currently assessed for their contribution to virulence.

Molecular epidemiology of pneumococci causing invasive disease among Oxfordshire children, 1995 – 2005

Brueggemann, AB1, Foster, D1,2, Griffiths, D1, and Crook, DW3 on behalf of the Oxford Pneumococcal Surveillance Group
1University of Oxford, Oxford, United Kingdom
2Thames Valley Health Protection Unit, Health Protection Agency, Oxford, United Kingdom

The heptavalent conjugate vaccine is likely to be licensed for widespread use in the United Kingdom in the near future. This vaccine has proven to be highly effective at reducing invasive pneumococcal disease among US children, which is a major success in disease prevention. However, there are reports of serotype replacement occurring in the US post-vaccine implementation and thus epidemiological surveillance pre- and post-vaccination is vital for any country planning to implement the vaccine. An ongoing programme of invasive pneumococcal disease surveillance has been collecting pneumococci since July 1995 from adults and children in Oxfordshire. Since vaccine selective pressure will cause perturbations among the pneumococcal population, we sought to genotype all the paediatric pneumococcal isolates circulating pre-vaccine introduction so that any post-vaccine changes in molecular epidemiology could easily be detected.

Pneumococci were collected by 10 regional laboratories and sent to the John Radcliffe Hospital (JRH), where strains were identified, serotyped, and genotyped using multilocus sequence typing. 499 pneumococci were collected from children ≤5 yrs of age from 1995-2005; after removing duplicate, non-invasive, and non-viable strains, 449 strains were genotyped, 434 of which are included here. Data for 150 of the JRH isolates were reported previously. 102 sequence types (ST) were identified. Using eBURST (www.mlst.net), STs were clustered into 20 different clonal complexes (groups of closely-related genotypes; CC), nine of which included >10 strains (n = 306, 71% of the total collection). The remaining CCs were comprised of only 2-7 strains each, and there were 42 singleton STs (no closely-related genotypes). All major clonal complexes included one predominant genotype and only 1 or 2 major serotypes, (n, genotype, serotype): CCa (86, ST9, 14); CCb (45, ST162, 19F and 9V); CCc (44, ST113, 18C); CCd (39, ST124, 14); CCe (28, ST138, 6B); CCl (19, ST311, 23F); CCg (17, ST176, 6B); CCh (14, ST199, 6A). Two of the major CCs were comprised of nonvaccine serotypes 19A, 15BC and 6A; the remaining seven were of vaccine serotypes. Vaccine serotypes comprised 77% (347/449) of the collection. The heptavalent vaccine should reduce pneumococcal invasive disease in Oxfordshire. Whether any undesirable epidemiological or genotypic changes will occur among the pneumococcal population is unknown, but these data provide a baseline from which to detect and measure any changes should they occur.
Alterations in predicted primary structure of the penicillin binding protein 2B of 29 penicillin non-susceptible clinical isolates of *Streptococcus pneumoniae*

Bengtsson D, Laurell MH
Department of Medical Microbiology
University Hospital, Malmo
S-205 02 Malmo, Sweden

In Malmö, Sweden, the prevalence of penicillin non-susceptible *S. pneumoniae* (PNSP) started to increase in 1995 with the presence of several genetically different clones. 851 PNSP isolates belonging to different serogroups and genotypes have been analyzed with pbp-profiling (RFLP with *Rsa I*) of the *pbp2b* gene. In the present study, the nucleotide sequence of a segment of the *pbp2b* gene of 29 of these PNSP strains, belonging to 16 different *pbp2b* restriction profiles was determined. We also compared 16 isolates with pair wise similar genotypes, but with different MICs, for PceG to examine if alterations in the transpeptidase-encoding region (TER) of the *pbp2b* gene could explain the observed differences in MIC. The nucleotide sequence of the strain R6 was used as reference. The predicted amino acid sequences (codon 90 to 488) of the isolates were aligned.

The region comprised between 218Ser and 296Trp revealed extensive sequence divergences with up to 25% of the nucleotides altered, resulting in ~2 to 10% of the amino acids being substituted. The most striking amino acid alterations were found at codon 234 to 240 with the substitution of 6 amino acids in 9 isolates. Insertions resulted in the addition of an extra aa 238Tyr in 3 isolates and of aa 233Pro in one isolate. The most common substitutions shared by 28 of the PNSPs were 254Thr→Ala, 284Glu→Gly and 297Thr→Ala or Ser. Other common substitutions were 139Glu→Gly or Thr, 218Ser→Pro, 246Gln→Glu, 263Leu→Ile, 346Asn→Asp. None of the substitutions were shared by all the isolates. A second group of alterations were found close to the conserved amino acid motif Lys-X-Gly (codon 423 to 425). Six isolates shared the substitutions 433Asp→Gly, 435Gln→Glu and 438Thr→Asn. A group of substitutions directly adjacent to the C terminal of the PBP2B TER (codon 482-485) were Glu-Lys-Tyr→Asn or His-Gln-His which was common to 5 of the isolates. Among the paired isolates with different MICs no significant difference in the predicted primary structure was detected indicating that changes in other PBP than *pbp2b* may be the cause of the higher MICs.


Y-family polymerases of *S. pneumoniae*

Henderson-Begg, SK1, Livermore, DM2, Hall, LMC1
1Barts and The London School of Medicine & Dentistry, London, UK
2ARMRL, Health Protection Agency, London, UK

The DNA polymerase UmuC/D is capable of error-prone DNA repair in many species, and is required for UV-resistance and SOS-induced mutability in *Escherichia coli*. Pneumococci lack a classical SOS response, and the sequenced genomes lack homologues of UmuC/D. However, the pneumococcal antibiotic resistance transposon Tn5252 includes a locus encoding a Y-family DNA polymerase related to UmuC/D, and acquisition of this locus by a UV susceptible pneumococcus was reported to confer increased resistance to UV-irradiation and elevated levels of UV-induced mutagenesis.

We investigated carriage of *umuC/D* homologues among 77 clinical isolates of *Streptococcus pneumoniae* by Southern hybridization and PCR. Five isolates, out of 21 resistant to tetracycline or chloramphenicol, carried genes related to *umuC* and *umuD* of Tn5252. The *umuC* genes were on *HindIII* fragments of different size in each isolate; two isolates carried two copies of the gene. The sequence of a PCR product from one isolate revealed 91% identity to Tn5252 *umuC*.

UV killing was investigated in four *umuC/D*+ and three *umuC/D*− clinical isolates. Two *umuC/D*+ isolates were markedly more resistant to UV than the other five clinical isolates; resistance did not correlate with *umuC* copy number. The mutation frequency to rifampicin resistance was not consistently higher in *umuC/D*+ than *umuC/D*− isolates.

We conclude that carriage of *umuC/D* homologues is relatively common among pneumococci resistant to tetracycline or chloramphenicol, and may be associated with mobile elements similar to Tn5252. Their presence was associated with UV-resistance in some isolates, but not all; no consistent relationship to mutability was seen.
Serotypes and genetic characterization of a large collection of drug-susceptible pneumococci isolated from preschool children and comparison with drug-resistant pneumococcal lineages

Sá-Leão, R1, Nunes, S1, Frazão, N1, Sousa, NG1, and de Lencastre H1,2
1 Instituto de Tecnologia Química e Biológica, Oeiras, Portugal
2 The Rockefeller University, New York, NY, USA

The nasopharynx of pre-school children is a major reservoir of pneumococci. The serotypes and population structure of drug-resistant pneumococci have been well studied and several internationally disseminated clones have been identified among pre-school Portuguese children. Much less is known about the serotypes and genetic lineages of drug-susceptible pneumococci (DSPn) isolated from the same population.

We have characterized by serotype, pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) a collection of 1,164 DSPn recovered between 2001 and 2003 from children attending day-care centers in Lisbon. This collection was compared to a population of 667 DRPn recovered from the same day-care centers.

We found higher serotype diversity among DSPn - 42 types – than in the DRPn collection - 19 types. Five serotypes – 6A, 11A, 15B, 16F, and 19F - accounted for half of the DSPn. A total of 66 clones were identified among the DSPn compared to 39 clones identified in the DRPn collection. Twenty-three serotypes identified among the DSPn showed no genetic diversity: each was associated with a single PFGE clone. MLST of 74 representatives of the DSPn collection identified 58 sequence types, 55 of which had identical or closely related matches in the pneumococcal MLST database.

Our study suggests that the population structure of DSPn and/or of particular serotypes found in this population, is more homogeneous than that of DRPn probably reflecting an increased capacity for microevolution among serotypes associated with drug-resistance.

Congruence of pneumococcal population structure resolved by ribotyping and MLST in a defined community

Huot, H1, Bouchet, V1, Leroy, M1, Pelton, SI1, O’Brien, KL2, Reid, R2, Santosham, M2, O’Neill, K3, Lipsitch, M4, Goldstein, R1
1 Boston University Medical Center, Boston, MA, USA
2 Johns Hopkins University, Baltimore, MD, USA
3 Broad Institute, Cambridge, MA, USA
4 Harvard School of Public Health, Boston, MA, USA

To understand determinants of vaccine impact on Streptococcus pneumoniae (SP) transmission, SP population structure must be defined. As serotyping is insufficiently discriminatory for defining SP clones, other typing techniques such as ribotyping (RT) and multi-locus sequence typing (MLST) are often used. Here we compared RT and MLST on a large set of SP isolates from a defined community to determine degree of congruence of resulting SP population structures.

We generated RT-RFLP patterns and MLST data for 493 SP strains (60 otitis media, 63 invasive and 370 carriage isolates) collected during an efficacy trial of pneumococcal conjugate vaccine (PCV). A neighbor-joining tree was generated from the RT-RFLP data. eBURST was used to identify clonal complexes (CC) of MLST sequence types (ST), defining a CC as a group of strains sharing at least 5 out of 7 identical alleles. SP population structure by RT and MLST were compared.

~74% (17/23) of the RT-based lineages showed congruence to the CC classification. CC1 is the largest congruent clonal complex, with 74 isolates (90%) falling into a single RT lineage B (n=81). CC1 consists of 11 MLST types and 6 serogroups. In contrast, RT lineages E, F and G (n=63) are not as highly congruent to the CC breakdown, with 25 (73.5%) CC2 isolates (serogroup 6) intermixed with 18 other CCs (14 different serogroups). In addition, RT and MLST analyses demonstrate the existence of multiple clonal lineages for a given serotype (fragmented clonality).

In contrast to other studies describing the panmictic nature of SP, this dataset found RT and MLST to be surprisingly congruent. This congruence may result from: i) neutral genes being the basis of both assays, and/or ii) temporally and geographically defined character of isolates reflecting a recent evolutionary history of SP in this setting. The lesser congruence of CC2 and RT lineages EFG may indicate that CC2 is an older CC as compared to CC1 (or other highly congruent lineages) which has not yet undergone sufficient recombination to disrupt congruence. This data suggests that CC and RT lineages are sufficiently reflective of SP clonal descent to allow their use to draw epidemiologic inferences, e.g. appearance of new serotypes within a lineage, or expansion of a formerly limited serotype or MLST type within a population, following use of PCV.
The pathogen and the commensal: Comparison between the genome of *Streptococcus mitis* B6 and *Streptococcus pneumoniae*

Reinhold Brückner, Dalia Denapaite, Bernhard Henrich, 1Michael Nuhn, 1P. Reichmann, 2Wolfgang Zimmermann, 2Rolf Wambutt, and Regine Hakenbeck

Department of Microbiology and 1Nano-Bio-Center, University of Kaiserslautern, Kaiserslautern; 2AGOWA GmbH, Berlin

The genome of *Streptococcus pneumoniae* is highly variable, and 10 % and more of the genes of one single strain may not be shared by other pneumococcal strains (1). Gene transfer events between the pneumococcus and closely related commensal *S. mitis* have been shown to be important for the evolution in this species. We have sequenced the genome of a high level penicillin resistant and multiple antibiotic resistant *S. mitis* B6 strain (2) to determine the relatedness to the pathogenic *S. pneumoniae* on the DNA level, and to investigate the nature of antibiotic resistance genes.

The 2.14 Mb genome of *S. mitis* B6 reveals a surprising degree of plasticity due to gene transfer events. It contains multiple copies of the repetitive BOX element described in *S. pneumoniae*, three novel IS elements, and a prophage. Antibiotic resistance cassettes include two large regions that are apparently the result of gene transfer events. They encode determinants for tetracycline resistance and aminoglycoside resistance and show high similarity to genetic elements described in *Staphylococcus aureus* and *Enterococcus faecalis*. In addition, genes encoding surface proteins were found that indicate unusual potential in host-pathogen interaction. Several of these genes occur also in some *S. pneumoniae* strains and may be responsible for modulation of the virulence potential (3). The presence of genes encoding physiologically relevant choline binding proteins and genes involved in choline metabolism in *S. mitis* clearly documents their widespread occurrence.

References.

A functional *dlt* operon confers resistance to antimicrobial peptides in *Streptococcus pneumoniae*

Kovacs, M1, Halfmann, M1, Fedtke, F1, Heintz, M1, Peschel, A1, Vollmer, W1, Brückner, R1, and Hakenbeck, R1

1University of Kaiserslautern, Kaiserslautern, Germany
2University Hospitals Tübingen, Germany
3University Tübingen, Germany

*Streptococcus pneumoniae* is one of the few species within the group of low-GC gram-positive bacteria reported to contain no D-alanine in teichoic acids, although the *dltABCD* operon encoding proteins responsible for D-alanylation is present in the genome of two *S. pneumoniae* strains, the laboratory strain R6 and the clinical isolate TIGR4. The annotation of *dltA* in R6 predicts a protein, D-alanine-d-alanyl carrier protein ligase Dcl, that is shorter at the amino terminus than all Dcl of TIGR4 and all other Dcl proteins. Thus, *S. pneumoniae* R6 could be a *dltA* mutant, calling the generally accepted structure of teichoic acids of *S. pneumoniae* into question, since absence of D-alanine in teichoic acids has been mainly deduced from structural studies using strain R6. To determine which of putative start codons is used, translational fusions of *dltA* to *Escherichia coli* lacZ were constructed applying a novel integrative lacZ translation probe plasmid. The result of these experiments clearly demonstrated that *dltA* translation starts at a GTG upstream the annotated start codon. Consequently, *S. pneumoniae* R6 is indeed a *dltA* mutant. Sequencing *dltA* of *S. pneumoniae* D39, the parental strain of R6, and of Rx, another derivative of D39, revealed intact *dltA* genes. Repair of the stop codon in *dltA* of R6 and insertional inactivation of *dltA* in D39 and Rx resulted in pairs of *dltA*-proficient and *dltA*-deficient strains. Subsequent phenotypic analysis showed that *dltA* inactivation resulted in enhanced sensitivity to the cationic antimicrobial peptides nisin and gallidermin, a phenotype fully consistent with *dltA* mutants of other gram-positive bacteria. In addition, mild alkaline hydrolysis of heat-inactivated whole cells released D-alanine from *dltA*-proficient strains but not from *dltA* mutants. The results of our study strongly suggest that teichoic acids of *S. pneumoniae* contain D-alanine like many other low-GC gram-positive bacteria.
Difference response with antibodies to PPV and PCV in asplenic children

Bernatowska, E1, Kayhty, H2, Mikoluc, B3, Pac, M4, Berglof, A4
1The Children’s Memorial Health Institute, Department of Immunology, Warsaw, Poland, 2National Public Health Institute, University of Helsinki, Finland, 3Medical University in Bialystok, Poland, 4Clinical Research Center, Karolinska Institute, Huddinge, Sweden

Aims: The aim of the study was to determine the level of pneumococcal antibodies in children vaccinated with one dose of 7-valent conjugate pneumococcal vaccine (Prevenar –Wyeth) (PCV) in two groups of asplenic patients vaccinated or not vaccinated with 23 polysaccharide vaccine (PPV).

Methods: Thirty asplenic children aged 6 months to 19 years were analysed. Fifteen patients were vaccinated only with PCV. In the second group of 15 patients PCV vaccination was secondary to PPV. The specific antibodies against 4, 6B, 9V, 14, 18C, 19F, and 23F serotypes were detected by ELISA. Antibodies > 0.35 µg/dl. were considered to be protective.

Results: Five out of 15 children not vaccinated with PPV demonstrated protective antibodies against 4 and 6B serotypes after splenectomy. In another five, the level of antibodies was below the protective value for 9V, in six children for 23F, in four for 18C and in the remaining two respectively for 14 and for 19F. Two patients from this group did not produce anti-6B antibodies in response to PCV and in the next four patients didn’t produce anti-4, 9V, 14, 23F antibodies. Nine of 15 children vaccinated with PPV didn’t develop protective antibodies to serotype 6B, eight to 23F, six to 4, four to 14 and 18C serotypes.

Following vaccination with PCV 80% of patients synthesised antibodies level within protective values, except three: one with the low response to 6B serotype, one patients with un-protective level to serotypes 6B and 23F and the third one to 4, 6, 14, 18C, 23F serotypes.

Conclusion: Efficacy of PPV vaccine was relatively low especially for 6B serotype, with protective value achieved by 40% of the patients. PCV vaccine inducing protective level all 100% patients to serotypes 18C and 19F and in 90% of children to 4,9V,14 and 23F. Lack of the response to serotypes 4,6,14,18C and to 23F following PCV and PPV was observed in two patients with spherocytosis.

Polysaccharide capsule formation is crucial for Streptococcus pneumoniae to evade neutrophil extracellular traps

Beiter, K1, 2, Wartha, F1, Albiger, B1, Normark, S1, Zychlinsky, A1, Henriques-Normark, B1, 2
1Dep. of Bacteriology, Swedish Institute for Infectious Disease Control, Solna, Sweden.
2Microbiology and Tumor Biology Center, Karolinska Institutet, Stockholm, Sweden.
3Max-Planck Institute for Infection Biology, Berlin, Germany

Upon activation, neutrophils release Neutrophil Extracellular Traps (NETs) that bind and kill bacteria. NETs contain granular proteins bound to a DNA scaffold. Abundant neutrophil infiltration is found in bacterial infections such as pneumonia, caused by Streptococcus pneumoniae (pneumococcus). We found that NET formation is observed in mouse pneumococcal pneumonia where high numbers of neutrophils are present in the tissue. Employing confocal microscopy as well as a quantitative assay based on bacterial counts, we studied trapping of pneumococci by NETs in vitro. We found that NETs were inefficient at capturing encapsulated pneumococci, but very efficiently trapped non-encapsulated variants. Therefore we conclude, that the pneumococcal polysaccharide capsule limits trapping by NETs.

These data suggest that Neutrophil Extracellular Traps play an important role for pneumococcal pathogenesis since formation is observed during bacterial challenge but the major pneumococcal virulence factor, the capsule, can limit trapping by NETs. Thus encapsulated pneumococci might be able to evade NETs, thereby spreading in the host, whereas the non-virulent, non-encapsulated, variants are not only phagocytosed but also trapped and held back by NETs.
Background: NanA, a cell surface-associated enzyme with sialidase activity, is a major neuraminidase produced by all clinical isolates of Streptococcus pneumoniae. NanA cleaves the terminal sialic acid residues from a wide variety of glycoproteins, glycolipids and oligosaccharides present on host cell surfaces. Such activity has potential to cause great damage to the host and to enhance pneumococal (pnc) colonization by unmasking new receptors for pnc adherence. The data from animal models suggest that NanA plays a crucial role in pnc nasopharyngeal colonization and in the development of AOM. In the present study, we have evaluated the natural development of serum antibodies to NanA in children in relation to age and to previous culture-confirmed pnc colonization.

Methods: Serum anti-NanA IgG antibodies were measured with enzymeimmunoassay (EIA) in 50 children at the ages of 6, 12, 18 and 24 months, and in 50 adults from the Finnish Otitis Media (FinOM) Cohort Study. In the FinOM Cohort Study, the children were followed for pnc carriage and pnc AOM from 2 to 24 months of age with the bacterial cultures of nasopharyngeal and middle ear fluid samples. According to their previous pnc culture findings, the children were at each time point grouped into two categories: pnc culture negative (pnc-) and pnc culture positive children (pnc+).

Results: All serum samples from the children collected at 6, 12, 18 and 24 months of age and the adults contained detectable anti-NanA IgG antibodies. The geometric mean concentration (GMC) of anti-NanA antibodies increased with age during the first 2 years of life. The increase was associated with prior pnc contacts. There were significantly higher concentrations of anti-NanA in pnc+ than in pnc- children (p < 0.001 at all ages). The anti-NanA concentration of adults was significantly higher than that of children at 6 and 12 months of age (p < 0.001). At 24 months, the GMC of anti-NanA of children was comparable to that of adults.

Conclusions: Pnc carriage and pnc AOM induce the production of serum anti-NanA antibodies early in life. The anti-NanA concentrations in the sera of children reach the adult levels by the age of 24 months. All serum samples tested were positive for anti-NanA antibodies, which may indicate the cross-reactivity of NanA with the neuraminidases produced by other streptococci.

Mannan-binding lectin levels and polymorphisms in children with recurrent Acute Otitis Media

Background: Mannan-binding lectin (MBL) is a key mediator of innate immunity that can bind highly conserved structures on a wide range of micro-organisms, leading to initiation of the lectin pathway of complement activation. Aberrant functional MBL serum levels, caused by single nucleotide polymorphisms (SNPs) in the MBL2 gene, are a possible risk factor for recurrent infections. Based on three MBL2 exon 1 SNPs (Arg52Cys, Gly54Asp, Gly57Glu) and three promoter SNPs (C-550G, C-290G, C-66T), seven common MBL haplotypes are currently recognized. Yet, within these 7 common haplotypes, considerable variation in MBL activity exists.

Objective: To associate MBL haplotypes with functional serum MBL levels and number of AOM episodes.

Methods: Functional and molecular MBL analyses were performed in a cohort of 244 children with a history of recurrent acute otitis media. Haplotypes of the three MBL2 exon 1 SNPs and the C-290G SNP were constructed using DGGE. Additionally, 12 SNPs were determined using SSP-PCR. Haplotypes of these 12 SNPs were constructed using computer modelling (PHASE2.1). Haplotypes were associated with functional serum MBL levels and number of AOM episodes.

Results: Highest mean functional MBL levels were observed in carriers of a wild-type MBL haplotype. The 7 common MBL2 haplotypes mainly determine the level of functional MBL in serum. Additionally, the 3130G>C SNP, located in exon 4, further influenced functional MBL levels within the LXPA haplotype. Carriers of 3130G showed a significantly lower geometric mean functional MBL serum level of 0.19 g/ml compared to 0.70 g/ml in 3130C carriers (p=0.026). Clinically, DGGE non-wild type MBL2 carriers between 12-24 months of age had a significantly increased number of acute otitis media episodes (5.1) compared to wild type MBL2 carriers (4.1) (p=0.027). In older children this association was not found anymore.

Conclusion: Additional SNPs within the seven common haplotypes can further explain the observed variation in functional MBL serum levels. MBL seems to be of particular clinical importance during early childhood, when maternally derived antibodies have waned, and protective adaptive immunity is not fully developed.
Enhanced adaptive immune maturation in neonates born in a high-risk area for invasive pneumococcal disease in Papua New Guinea

**PO6.08**

**Van den Biggelaar, AHJ**1, Nadal, MA1, Pomat, WS2, Francis, JP2, Prescott, SL1, Richmond P1, Lehmann D1, Holt PG1.

1Centre for Child Health Research, Perth, WA, Australia
2Papua New Guinea Institute of Medical Research, Goroka, EHP, Papua New Guinea

In developing countries, children experience bacterial colonization of their upper respiratory tract at a very young age, which is associated with an increased risk for invasive pneumococcal disease. Little is known about the maturation status of the early immune system and risk of susceptibility to pneumococcal colonization and disease in high-risk infants.

In a first step to address this possible association, the immune phenotype and function of neonatal cells in a high-risk versus low-risk area were compared. Hereto cord blood mononuclear cells (CBMC) were isolated from cords obtained from deliveries in Goroka, Papua New Guinea (PNG), where all children acquire S. pneumoniae before the age of 3 months (n = 10), and Perth, West-Australia (WA), where acquisition rates for (non-indigenous) children are considerably lower (n = 10). Cells were phenotyped by flow cytometry, but no major differences were found between PNG and WA: the proportions of CD4+-, CD4+CD25+- and CD8+ T-cells, NK- and NK-T cells, and B-cells were compared. Hereto cord blood mononuclear cells (CBMC) were isolated from cords obtained from deliveries in Goroka, Papua New Guinea (PNG), where all children acquire S. pneumoniae before the age of 3 months (n = 10), and Perth, West-Australia (WA), where acquisition rates for (non-indigenous) children are considerably lower (n = 10). Cells were phenotyped by flow cytometry, but no major differences were found between PNG and WA: the proportions of CD4+-, CD4+CD25+- and CD8+ T-cells, NK- and NK-T cells, and B-cells.

**Results:** After primary course of PCV, 20%, 90%, 70% and 30% of Aboriginal children had IgA antibody to 6B, 14, 19F and 23F, respectively, compared with 20%, 57%, 63% and 33%, respectively, in unvaccinated children. At 15-25 months, 64%, 79% and 21% of children post-PCV priming had IgA to 6B, 14, 19F and 23F, respectively, compared with 0%, 40%, 60% and 50%, respectively, of unimmunised children of the same age, while equivalent figures for those who had received a PPV booster were 89%, 100%, 100% and 56%. IgA antibody titres to serotypes 6B and 14 were significantly higher in children who had received a booster of PPV at 18 months (GMC 0.017 and 0.03 ng/ml, respectively) compared with those who had only received PCV (GMC 0.009 and 0.01, respectively) as were proportion of subjects who were positive for IgA for these serotypes. There were no significant differences in GMC or proportion saliva’s positive for serotypes 19F and 23F.

**Conclusion:** PCV primes for mucosal immune responses in Aboriginal Australian children for boosting by PPV.
**MyD88-dependent signalling controls bacterial growth during colonization and systemic pneumococcal disease in mice**

Albiger, AB\(^1,2\), Sandgren, A\(^1,2\), Katsuragi, H\(^3\), Meyer-Hoffert, U\(^1,2\), Beiter, K\(^1,2\), Wartha, F\(^2,2\), Hornef, M\(^4\), Normark, S\(^2\), Henriques Normark, B\(^1,2\)

\(^1\)The Swedish Institute For Infectious Disease Control, Karolinska Institutet, Stockholm, Sweden  
\(^2\)Microbiology and Tumorbiology Center, Karolinska Institutet, Stockholm, Sweden  
\(^3\)Nippon Dental University, Niigata, Japan  
\(^4\)Institut für Medizinische Mikrobiologie und Hygiene, Freiburg, Germany

The Toll-like receptors (TLR) and the myeloid differentiation factor 88 (MyD88) are key players in the activation of the innate immune defence during microbial infections. Using different murine infection models, we show that MyD88-dependent signalling is crucial for the activation of the innate immune defence against *Streptococcus pneumoniae*. Our data demonstrate that both local and systemic inflammatory response to *S. pneumoniae* depends on the presence of MyD88 to clear bacterial colonization of the upper respiratory tract and to prevent pulmonary and systemic infection in mice. Finally, we described a strong correlation between enhanced bacterial growth in the bloodstream of MyD88 deficient mice and the inability to lower the serum iron concentration in response to infection.

**Immunity to Streptococcus pneumoniae in a healthy blood donor population**

Pererva P\(^1,2\), Keating J\(^1,2,1\), Ashton A\(^1\), Bryant J, Yuan F F\(^1,2,4\), Dyer W\(^1,2,4\), Watson, S\(^1\) & Sullivan JS\(^1,2,4\)

\(^1\)Australian Red Cross Blood Service, \(^2\)CRC-Vaccine Technology, 153 Clarence St, Sydney, NSW 2000, \(^3\)Children’s Cancer Institute, Randwick, NSW 2031 & \(^4\)Transfusion Medicine and Immunogenetics Research Unit, Central Clinical School, Faculty of Medicine, University of Sydney.

The correlates and extent of protective immunity to *Streptococcus pneumoniae* in a normal healthy population are unknown. In the present investigation we have identified plasmapheresis donors displaying high-titre functional antibodies reactive against the most prevalent pneumococcal serotypes. Sera from 820 plasmapheresis donors were tested for total antibodies to a mixture of 23 pneumococcal capsular polysaccharides (Pneumovax 23) using an enzyme-linked immunosorbent assay. The reactivity of international reference serum 89-SF was used as selection criteria with reactive donors defined as having antibody titres > the reference hyperimmune serum. Elevated titres of capsular antibodies were detected in sera from fifty-one (6.2%) plasmapheresis donors. Female donors had higher incidence of pneumococcal antibodies than male donors. Antibody reactivity against 24 individual capsular polysaccharides and three pneumococcal proteins were further characterised in this group of pneumococcal-reactive donors. The study group showed a wide variation in the level of serotype-specific antibody. The highest concentration was found for serotype 5 followed by 19F, 33F, 15B and 20. When classified by the number of serotypes, 36% of reactive donors had elevated titres of pneumococcal antibodies to all 24 capsular polysaccharides. Antibodies to three pneumococcal virulence proteins were detected in all samples, but the concentrations varied greatly among individuals. The functional activity of pneumococcal antibodies was measured against three pneumococcal serotypes 6B, 9V, and 23F (serotypes commonly associated with invasive pneumococcal disease) using a flow cytomteric opsonophagocytic assay. Sixty four percent of reactive donors demonstrated functional antibody activity against either a single serotype or a number of serotypes. The correlation of opsonophagocytic titre and the total IgG concentration varied according to serotype; i.e. being stronger for serotype 6B ($r = 0.7$), than for serotype 23F ($r = 0.6$) or serotype 9V ($r = 0.5$). This study demonstrated that a small number of healthy individuals have high titre functional antibodies against pneumococcus but in the absence of a clear history of exposure, it is difficult to explain the reasons why healthy donors have major differences in the quantitative and qualitative antibody response to *Streptococcus pneumoniae*. 
The role of CbpA on complement deposition and opsonophagocytosis of *Streptococcus pneumoniae*

**PO6.11**

Yuste J, Ansari N, Khandavilli S, Paton JC, Botto M, Brown JS

1Centre for Respiratory Research, University College London, London, United Kingdom
2School of Molecular and Biomedical Science, University of Adelaide, Adelaide, Australia.
3Rheumatology Section, Faculty of Medicine, Imperial College London, London, United Kingdom

Choline binding protein A (CbpA) is an important virulence factor for *Streptococcus pneumoniae* and a protein vaccine candidate. CbpA is a surface protein which interacts with the complement components C3 and factor H, therefore affecting innate immunity. The high allelic variation found in the highly polymorphic CbpA locus by other authors suggests that the function of CbpA may vary between strains. To characterise the role of CbpA on complement immunity we used cbpA− *S. pneumoniae* strains belonging to the serotypes 2, 3, 6A, 6B 9V and 23F and analysed complement deposition on the *S. pneumoniae* surface in human serum and phagocytosis using flow cytometry assays. As CbpA binds to factor H, which is an important regulator of the alternative complement pathway, we also investigated the importance of the alternative pathway on complement immunity for CbpA using human serum depleted in factor B.

Loss of CbpA had different effects on complement deposition depending on the serotype of *S. pneumoniae* analysed, both in wild-type and factor B depleted serum. In serotypes 2 and 23F, cbpA− strains had decreased C3b deposition compared to the wild-type strains, while in cbpA− strains belonging to the serotypes 3, 6A, 6B and 9V there was an increased C3b deposition compared to the wild-type strains. In the phagocytosis assays, loss of CbpA in serotypes 2 and 3 resulted in decreased phagocytosis whereas the loss of CbpA in serotypes 6A, 6B, 9V and 23F resulted in increased phagocytosis compared to the wild-type parental strain after incubation in both wild-type and factor B depleted serum. Our results suggest that CbpA has variable effects in complement deposition and opsonophagocytosis depending on the serotype, but that this surprisingly seems to be independent of alternative pathway activity. For the majority of the serotypes analysed, the role of the surface protein CbpA is to reduce complement deposition on the pneumococcal surface therefore decreasing the efficiency of phagocytosis and suggesting why cbpA− strains are attenuated in virulence. Further investigation is required to identify why loss of CbpA has varying effects on the interaction of *S. pneumoniae* with complement depending on the strain background.

The use of pneumococcal conjugate vaccine in children with nephrotic syndrome

**PO6.12**

Wood N, Richmond P, McIntyre P

1National Centre for Immunisation Research & Surveillance
2The Children’s Hospital at Westmead, NSW, Australia
3Princess Margaret Hospital for Children, WA, Australia

**Introduction:** Children with nephrotic syndrome have an increased risk of pneumococcal disease due to immune suppression from their disease and drug therapy. No published data exist on antibody responses and duration, and influence of immune suppressant therapy following pneumococcal conjugate vaccination, especially for older children. This study compared responses to pneumococcal conjugate vaccine (7vPCV) with polysaccharide pneumococcal vaccine (PPV23) by age and level of immune suppression.

**Method:** Eight children under 5 years old received 2 doses of 7vPCV at 6 week intervals. Thirteen children over 5 years old were randomised to receive 7vPCV followed by PPV23 (n=7) or PPV23 followed 7vPCV (n=6) at 2 month intervals, stratified by degree of immune suppression. Serum for IgG anti-pneumococcal antibody assay (7 serotypes) were obtained at baseline, 2 months, 6 months and 1 year after vaccination and local and systemic reactions monitored for 15 days after each vaccination.

**Results:** All eight children under 5 years old, (clinically nephrotic and on immune suppressant therapy at time of vaccination) had geometric mean (GMT) antibody titres >0.99ug/ml for all serotypes one year post 2 doses of 7vPCV. In children over 5 years old there was no significant difference in antibody level six weeks post 7vPCV versus PPV23, except for serotype 6B, (7vPCV>PPV23, p=0.02) and no difference one year post vaccination. Pneumococcal antibody levels for high (n=5) and low (n=8) immunosuppression in over 5 year olds one year post vaccination were not significantly different and GMT in both groups was >1.5ug/ml for all serotypes. There were no injection site reactions and no serious adverse events. Non fatal pneumococcal peritonitis (not typed) occurred in one child (age 4 years) who had been completely vaccinated.

**Conclusion:** Immune suppression did not appear to influence antibody response in nephrotic children over 5 years old and order of vaccination (7vPCV versus PPV23) did not influence antibody levels except for 6B.
Opsonophagocytic responses to pneumococcal conjugate vaccine in the elderly

PO6.13

de Roux, A1, Schmöle-Thoma B2, Siber, G1, Laudat, F3, Lockhart, S1, Gruber, W1, Baker, S1, Fernsten, P1, Welte, T4, Lode, H1

1Dept. of Chest and Infectious Diseases; HELIOS Klinikum Emil von Behring, Berlin, Germany
2Wyeth Vaccines Research, Wyeth Pharma GmbH, Münster, Germany
3Wyeth Vaccines Research, Wyeth Pharmaceuticals, Pearl River, USA
4Dept. of Pneumology, Medizinische Hochschule, Hannover, Germany

Introduction: Protective immunity to encapsulated organisms induced by polysaccharide or conjugate vaccines, and at least partly by natural exposure, is mediated by antibodies to the capsule. However, capsular IgG measured by ELISA may not always reflect functional opsonophagocytic antibody (OPA).

Methods: 217 ambulatory immunocompetent elderly subjects ≥ 70 years without previous pneumococcal vaccination were randomized to receive 7vPnC (Prevnar™, Wyeth) or 23vPS Pneumovax™, Aventis Pasteur MSD. Antibody responses after 1 dose of 7vPnC in the elderly are informally compared to antibody responses after 3 doses of 7vPnC in infants (at 2, 4, 6 mo) in a previous study.

Results:

<table>
<thead>
<tr>
<th>Type</th>
<th>ELISA GMC (µg/ml)</th>
<th>OPA GMT</th>
<th>ELISA GMC</th>
<th>OPA GMT</th>
<th>ELISA GMC</th>
<th>OPA GMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.19</td>
<td>14.2</td>
<td>3.27</td>
<td>1503.8</td>
<td>3.29</td>
<td>1571.2</td>
</tr>
<tr>
<td>6B</td>
<td>1.04</td>
<td>58.1</td>
<td>8.02</td>
<td>1351.2</td>
<td>5.18</td>
<td>1887.6</td>
</tr>
<tr>
<td>9V</td>
<td>0.87</td>
<td>128.8</td>
<td>9.82</td>
<td>2914.6</td>
<td>1.88</td>
<td>3551.3</td>
</tr>
<tr>
<td>14</td>
<td>1.87</td>
<td>93.5</td>
<td>17.54</td>
<td>2164.8</td>
<td>7.13</td>
<td>3016.8</td>
</tr>
<tr>
<td>18C</td>
<td>1.15</td>
<td>46.9</td>
<td>12.97</td>
<td>1317.5</td>
<td>2.88</td>
<td>1558.6</td>
</tr>
<tr>
<td>19F</td>
<td>1.17</td>
<td>13.6</td>
<td>5.50</td>
<td>182.2</td>
<td>4.17</td>
<td>203.2</td>
</tr>
<tr>
<td>23F</td>
<td>0.95</td>
<td>42.4</td>
<td>12.4</td>
<td>1309.3</td>
<td>2.16</td>
<td>4845.5</td>
</tr>
</tbody>
</table>

Infants: N = 33 - 34; Elderly: N = 104 - 107 (23vPS), 110 (7vPnC)

Even prior to immunization elderly adults have substantial IgG concentrations relative to infants who have received a primary series of 7vPnC. However OPA is remarkably lower relative to immunized infants. After a single dose of 7vPnC the elderly increase OPA antibody titers to levels similar to infants after 3 doses.

Conclusion: Low opsonic activity explains why unimmunized elderly are at risk of invasive pneumococcal disease despite the presence of capsular IgG. This suggests that in older adults OPA is a more appropriate correlate of protection than IgG.
Complement deposition on pneumococcal capsular types 6B and 19F

Melin M1, Jarva H2, Meri S3, Käyhty H2.
1National Public Health Institute, Helsinki, Finland
2University of Helsinki, Helsinki, Finland

Background: Capsular polysaccharide shields pneumococcus from being recognised by phagocytes, but anticapsular antibodies in combination with the complement system enhance phagocytic killing. Pneumococcal conjugate vaccines protected children equally from invasive infections caused by types 6B and 19F in the US and South Africa. Protection against acute otitis media was good against type 6B but poor against 19F in the FinnOM Vaccine Trial. Using the in vitro assay for the opsonophagocytic activity, we have shown that 5 times more anti-19F antibody is needed for 50% killing than anti-6B antibody. In this study, we tested whether differences in the capsular structure might influence the deposition and degradation of C3 on the surface of pneumococcus, which would affect the level of opsonophagocytosis. Our preliminary data with western blot and EIA-based methodologies suggested that less C3 would be deposited on strains with capsule type 19F than on 6B (Melin et al ISPPD-4).

Methods: Pneumococcal nasopharyngeal, middle ear and blood isolates (N=16) of serotypes 6B and 19F were chosen for comparisons. Isogenic capsular variants 6B and 19F from TIGR4 (received from Dr Lipsitch, Harvard School of Public Health, Boston) allowed analysis of the role of the capsule type alone on C3 deposition. Bacteria were incubated for 30 minutes with human serum containing equal concentrations of IgG against capsular polysaccharides 6B and 19F. The amount of total C3 (C3b, C3c, iC3b) and its degradation fragment C3d bound on the bacterial surface was detected with FITC-labelled antibodies and the percentage of bacteria binding the fluorescent label was measured with FACS-analyser.

Results: The bacterial strains from serotypes 6B and 19F that were analysed showed variance in the binding of C3 and in the degradation of C3 to C3d. On average, there were no differences between serotypes 6B and 19F in resistance to complement deposition.

Conclusions: The binding and degradation of complement protein C3 on pneumococcal surface appears not to be related to the serotype. Other pneumococcal virulence factors besides the capsule in combination with antibodies to pneumococcal surface proteins are likely to play major roles in the sensitivity or resistance of pneumococcal strains to complement deposition. Differential deposition of complement on pneumococcal surfaces does thus not explain the differences observed in phagocytic killing of the serotypes 6B and 19F.

The role of surface tethered mucins in pneumococcal nasopharyngeal carriage using a primary cell model

Hendy E, Erlewyn-Lajeunesse M, Finn A
University of Bristol, Bristol, UK

The mechanisms by which pneumococci are acquired and adhere to the mucosal surface of the nose are poorly understood. Acquisition of carriage has been associated with a coryzal illness suggesting both the presence of a cytokine mediated immune response and alteration of mucin gene expression during acquisition. Mucins are complex, heavily O-glycosylated proteins that exist as either secreted (MUC2, 5AC and 5B) or surface tethered forms (MUC1, 4 and 8). Secreted mucin forms a gel that acts as a protective barrier to the mucosa, whilst the role of tethered mucins is not known. Some tethered mucins, such as MUC 4, undergo proteolytic processing once targeted to the plasma membrane. This releases part of the protein into the mucus layer, whilst a portion of the protein remains embedded in the plasma membrane. The significance of this action for bacterial carriage is currently unknown.

Aim: The aim of this project is to investigate the effects of pneumococcal proteins on mucin gene expression in respiratory epithelial cells.

Methods: Human Nasal Epithelial Cells (HNEC) were cultured from brushings taken from the noses of healthy children undergoing anaesthetic for orthopaedic procedures. These HNEC primary cells and Detroit 562 cells were cultured in the presence of wild type pneumococcal culture supernatants taken from exponential phase culture of D39 strain Streptococcus pneumoniae. mRNA was extracted at time intervals and RT PCR performed for a range of respiratory mucin genes. Real time PCR was then conducted for mucin genes MUC1 and MUC4.

Results: Detroit 562 cells constitutively express MUC1, MUC4 and MUC8 and do not produce secreted mucin genes MUC2, 5AC and 5B. HNEC constitutively express MUC1 and MUC4 but not MUC8, MUC2, 5AC and 5B at this stage in submerged culture. Preliminary results suggest that pneumococcal proteins may modulate mucin gene expression.

Conclusions: Using HNEC we have developed a unique model for exploring the mucosal interactions of pneumococcal carriage. The mucin phenotype of Detroit cells has not been described previously and its constitutive expression of MUC1, 4 and 8 is consistent with a respiratory epithelial phenotype. MUC8 expression has not been investigated in HNEC previously. The use of primary HNEC cultures provide a higher level of evidence concerning bacterial-epithelial interactions and a potential model to investigate the pathophysiology of pneumococcal carriage.
**Human immune response to meningitis-causing Streptococcus pneumoniae**

Pereira D, Barroso DE, Brasil P, Rebelo MC, Jessouroun E

1 BioManguinhos, Fiocruz, Rio de Janeiro, RJ, Brazil
2 Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, RJ, Brazil
3 Infectious Diseases State Institute São Sebastião, Rio de Janeiro, RJ, Brazil

*Streptococcus pneumoniae* invasive disease is a significant cause of community acquired bacteremia world-wide. Young children are particularly affected to this bacterium followed by elderly people. Meningitis is not the most frequent clinical manifestation but so far is associated with high chance to develop permanent sequelae or a fatal outcome. We conducted a serological study to evaluate the human immune response to meningitis-causing *S. pneumoniae*.

Meningitis cases were defined in this study as the isolation of *S. pneumoniae* from CSF. Paired serum samples were obtained with acute phase sera drawn within 24h after hospital admission and convalescent serum 15-25 days later. Total IgG antibody concentrations (µg/ml) against *S. pneumoniae* polysaccharide (PS:4;6B;9V;14;18C;19F;23F) in human serum were determined by means of a standard ELISA procedure (optimal concentrations of polysaccharide mixed with methylated human serum albumin, optimal concentration for each type). PS were selected on the base of serotypes covered by the 7-V pneumococcal conjugate vaccine. Serum samples are diluted two-fold in serum conjugate buffer containing 1.0 µg/ml C PS and 2.0 µg/ml Ph 22F.

We included fourteen patients with no previous episodes of meningitis and four with recurrent pneumococcal meningitis (RPM) due to head injury. Ages of cases were distributed as follow: 4y (2); 10y (1); 14y (1); >39y (10). All four RPM cases were teenagers (12-16y). Four patients were infected with serotypes included in the tested panel (14, 19F). All patients but three showed an acute phase IgG antibody concentration to at least one of the PS tested higher than 1 µg/ml. There was no difference observed in the group with recurrent meningitis cases. Most patients did not showed an increase in antibody level in their convalescent sera. Patients with low acute phase IgG did not present a positive response to the homologous PS of the strain-causing disease, but cross-reactivity was seen. Alternatively, a high acute phase IgG was indicative of a decrease in the convalescent phase IgG level.

The human immune response to *S. pneumoniae* disease, measured by the PS ELISA seems to be non-polysaccharide specific. Nevertheless, a proportion in both groups of patients did not show a significant increase in antibody level during the convalescent phase. Our data indicate that patients may not develop an adequate PS immune response and an impaired antibody response can be involved in adult patients.

---

**Effect of aging and gender on naturally acquired antibodies to pneumococcal polysaccharides**

Väkeväinen, M, Simell, B, Grönholm, S, Reunanen, A, and Käyhty, H

National Public Health Institute, Helsinki, Finland

**Introduction:** Infections caused by *Streptococcus pneumoniae* (*Pnc*) remain an important cause of morbidity and mortality in the elderly. The increasing incidence of pneumococcal diseases by age in otherwise healthy individuals suggests that the immune system of elderly functions differently from that of younger adults. Aging is known to have widespread effects on the immune system leading to a diminution of immune responsiveness of the elderly. Limited information exists on the concentration and function of natural antibodies to *Pnc* acquired during the periods of pneumococcal carriage and infection in unvaccinated elderly population.

**Methods:** Serum IgG antibody concentrations were measured by specific enzyme immunoassay with 22F neutralization to pneumococcal polysaccharides 3, 4, 6B, 9V, 14, and 23F. These are, on average, the most common serotypes causing invasive pneumococcal diseases in developed countries. The sera were received from 240 younger adults aged 30 to 64 years (115 men, 125 women) and 240 elderly adults aged 65 to 97 years (119 men, 121 women) in Health 2000 study carried out in Finland in 2000-01. The results are expressed as geometric mean concentrations (GMC) with 95% confidence intervals.

**Results:** The concentrations of natural IgG antibodies to pneumococcal polysaccharides, especially to serotype 4, were low both in younger and elderly adults. Elderly subjects had significantly lower GMCs of antibodies than the younger adults to serotypes 3, 6B, and 23F. The effect of aging was different in men and women. The GMCs of elderly women were significantly lower than those of younger women to serotypes 3 (0.27 vs. 0.47), 6B (0.28 vs. 0.45), and 23F (0.57 vs. 0.96), whereas elderly men had significantly lower GMC of antibodies than younger men only for serotype 3 (0.21 vs. 0.34). However, the concentrations of antibodies to all serotypes were similar in elderly men and women. At younger age, the antibody concentrations to 6B, 14, and 23F were significantly higher in women than in men.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Younger adults</th>
<th>Elderly adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.41 (0.34-0.48)</td>
<td>0.24 (0.20-0.28)</td>
</tr>
<tr>
<td>4</td>
<td>0.13 (0.11-0.15)</td>
<td>0.13 (0.11-0.15)</td>
</tr>
<tr>
<td>6B</td>
<td>0.37 (0.30-0.45)</td>
<td>0.26 (0.21-0.32)</td>
</tr>
<tr>
<td>9V</td>
<td>0.39 (0.33-0.46)</td>
<td>0.44 (0.37-0.53)</td>
</tr>
<tr>
<td>14</td>
<td>1.18 (0.94-1.48)</td>
<td>1.21 (1.02-1.50)</td>
</tr>
<tr>
<td>23F</td>
<td>0.76 (0.63-0.91)</td>
<td>0.57 (0.48-0.70)</td>
</tr>
</tbody>
</table>

**Conclusions:** The age-associated reduction of natural antibody concentrations to pneumococcal polysaccharides is serotype-dependent and mostly subtle. It may be a factor, but probably not a major one. However, the concentrations of antibodies to all serotypes were similar in elderly men and women. At younger age, the antibody concentrations to 6B, 14, and 23F were significantly higher in women than in men.
Production of cytokines after stimulation of peripheral blood mononuclear cells with pneumococcal bacteria, polysaccharides, proteins, and vaccines

Vuorela, A, Käyhty, H, Valtonen, H, Julkunen, I, and Väkeväinen, M
National Public Health Institute, Helsinki, Finland

Background: The increasing incidence of pneumococcal diseases by age in otherwise healthy individuals suggests that the immune system of elderly functions differently from that of younger adults. Studies with aged mice suggest that alterations in the cytokine secretion patterns may be associated with the reduced immune function against pneumococcal antigens in the elderly. To set up conditions for studying cytokine secretion in the elderly as compared to younger adults, we performed a preliminary assay using different antigens and blood samples of younger adults.

Methods: Human peripheral blood mononuclear cells (PBMC) were isolated from buffy coats (N=9; donors aged 18 to 64 years) from the Finnish Red Cross Blood Transfusion Service. The PBMC were stimulated with pneumococcal bacteria (Pnc) of serotypes 4 and 14, pneumococcal capsular polysaccharides 4 and 14, C-polysaccharide, nontoxic derivative of pneumolysin, PdB, pneumococcal polysaccharide vaccine, and Prevenar. As controls, the cells were stimulated with LPS and Haemophilus influenzae type b (Hib). The optimal concentrations of antigens used in the stimulation were titrated. The cytokines (IL-6, TNF-α, IL-12, IL-2, IFN-γ, IL-10, IL-4) present in cell culture supernatants were quantitated by a sandwich enzyme immunoassay (EIA). The results are expressed as geometric mean concentrations (GMC). The EIA was sensitive to 0.02 ng/ml.

Results: No stimulation of IL-2, IL-12, and IL-4 production was detected with any antigen. A lot of variation was found in cytokine production between blood donors. Strong production of IL-6 was stimulated with Prevenar and PdB, followed by pneumococcal bacteria. Polysaccharides and PPV induced low concentrations of IL-6. Pneumococcal bacteria were the best inducers of TNF-α, followed by PdB and Prevenar with low concentrations induced by the other antigens. Concentrations of IL-10 were low, except after stimulation of PdB and Prevenar. None of the antigens was a good inducer of IFN-γ production. Hib, as a positive control, stimulated strong production of all four cytokines. Cytokine production stimulated by LPS varied from strong to weak.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>IL-6</th>
<th>TNF-α</th>
<th>IL-10</th>
<th>IFN-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pnc 4</td>
<td>1.67</td>
<td>0.36</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>Pnc 14</td>
<td>1.65</td>
<td>0.44</td>
<td>0.09</td>
<td>0.04</td>
</tr>
<tr>
<td>Pnc PS 4</td>
<td>0.21</td>
<td>0.02</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Pnc PS 14</td>
<td>0.08</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>C-polysaccharide</td>
<td>0.26</td>
<td>0.01</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>PdB</td>
<td>10.28</td>
<td>0.27</td>
<td>2.99</td>
<td>0.06</td>
</tr>
<tr>
<td>PPV</td>
<td>0.36</td>
<td>0.07</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>Prevenar</td>
<td>13.06</td>
<td>0.14</td>
<td>0.49</td>
<td>0.04</td>
</tr>
<tr>
<td>Hib</td>
<td>40.12</td>
<td>13.7</td>
<td>3.02</td>
<td>2.30</td>
</tr>
<tr>
<td>LPS</td>
<td>25.02</td>
<td>0.69</td>
<td>5.05</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Conclusions: From PBMC, no stimulation of IL-2, IL-12, and IL-4 production could be detected with any antigen tested. The best inducers of cytokine production, excluding the positive controls, were Prevenar and PdB, followed by pneumococcal bacteria. Pneumococcal polysaccharides and PPV were weak inducers of cytokine production.
Dynamics of the immune response in children to the 23-valent pneumococcal capsular polysaccharide vaccine (Pneumovax)

Mlacha SZK, Warira A, Scott JAG

1Wellcome Trust/KEMRI Centre for Geographic Medicine Research - Coast, Kilifi, Kenya
2University of Oxford, John Radcliffe Hospital, Oxford, UK

Introduction: This study tests the hypothesis that there is significant natural variation in the speed of IgG responses following exposure to pneumococcal capsular antigens. If the variation is significant - a matter of days - it may influence pneumococcal disease susceptibility and outcome.

Methods: Healthy HIV-seronegative children, aged 24-36 months, were immunized with 23-valent polysaccharide vaccine (Pneumovax). Serum samples were collected before, and 5, 7, 9, 11, 14 and 28 days after vaccination and assayed by WHO-standardized ELISA for IgG to polysaccharides 1, 6B, 14, 19F and 23F. Response time was defined as time to achieve half of the antibody rise between baseline and maximum antibody concentration. Differences in mean response times were tested by analysis of variance.

Results: Forty children were recruited and four were lost to follow-up. Half of all children had antibody concentrations against serotypes 1, 6B, 14 and 19F that were above the vaccine-derived protective threshold (0.4 mcg/ml). All but two children responded with a 22-fold increase in antibody for all serotypes tested. The greatest responses were to serotypes 1 and 19F and the smallest to serotype 23F. Antibody responses, measured as a ratio-rise, were negatively correlated with baseline antibody concentrations (r=0.61, r<0.35, p<0.04 for all serotypes).

Response times were normally distributed and mean response times for each serotype ranged from 6.4 to 7.3 days. Response times varied by subject (p<0.0005) and by serotype (p=0.007). Responses to serotype 6B were fastest followed by those to serotypes 23F, 1, 19F and 14. Standard deviations were in the range 0.91-1.85 for each serotype suggesting a response time range of 4-7 days. The table shows the cumulative percentages of children who had reached the response threshold against time since vaccination.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>60</td>
<td>94</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>6B</td>
<td>30</td>
<td>72</td>
<td>97</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>11</td>
<td>33</td>
<td>92</td>
<td>97</td>
<td>100</td>
</tr>
<tr>
<td>19F</td>
<td>14</td>
<td>39</td>
<td>75</td>
<td>89</td>
<td>100</td>
</tr>
<tr>
<td>23F</td>
<td>17</td>
<td>50</td>
<td>97</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion: Most children mount IgG responses to polysaccharide antigens 6 to 7 days after exposure but there is reasonable natural variation in the rapidity of these responses over a period of days. On this basis we next hypothesize that children who succumb to invasive pneumococcal disease respond more slowly to stimulation with pneumococcal capsular antigens than do healthy children.

Children with invasive pneumococcal disease have low levels of antibodies to virulence proteins and develop poor antibody responses compared to age-matched children who carry pneumococci in their nasopharynx

Jonsdottir I, Ingolfsdottir, G, Paton, JC, Kristinsson, KG, Gudnason, T.
1Landspitali University Hospital and University of Iceland, Reykjavik, Iceland,
2University of Adelaide, Adelaide, Australia and 3 Directorate of Health, Seltjarnarnes, Iceland.

In order to identify immunological host factors associated with increased risk of developing invasive pneumococcal infections in children we studied children <7 years of age with invasive pneumococcal disease and two controls per case, matched for age, sex and nasopharynx carried serotype. Specific IgG antibody levels against pneumococcal polysaccharides and the virulence proteins Choline binding protein A (CbpA), pneumolysin (Ply), pneumococcal surface adhesin A (PsaA) and pneumococcal surface protein A (PspA) were measured by ELISA. To assess the immunological status of the children we measured serum levels of immunoglobulin isotypes by nephelometry, IgG subclasses by radial immunodiffusion and mannann binding lectin (MBL) by ELISA. Eight children with invasive disease and 15 controls living in the Greater Reykjavik area have been analysed sofar. All children in both groups had normal levels of IgM, IgG and IgA for their respective age, but 2/8 invasive cases and 1/15 controls had slightly elevated IgG3. MBL levels were comparable between the cases and controls (p=0.455), but 1/8 cases and 3/15 controls had low MBL levels (<50 mg/L). A time of diagnosis all the children with invasive disease had very low levels of IgG antibodies to the pneumococcal virulence proteins tested compared with their controls, with differences in geometric mean titers of IgG antibodies of 26-fold for CbpA (p<0.005), 4-fold for PsaA (p<0.005), 4-fold for Ply (p<0.009) and 13-fold for PspA (p<0.001). Although the invasive infection caused significant rise in IgG antibodies to all four proteins in the convalescent sera (1 month after disease onset), the levels were still markedly lower than in the controls. Collection of cases and controls continues and antibodies to the polysaccharides and additional virulence proteins will be measured. Our results indicate that low serum antibodies to the pneumococcal virulence proteins CbpA, PsaA, PspA and Ply are associated with increased risk for invasive disease in children. These findings suggest that effective immune responses to several pneumococcal virulence proteins may contribute to protection against invasive pneumococcal disease. Lack of immunity reflected in low Ab in acute sera from children with invasive pneumococcal disease may aid the identification of important virulence factors, which may be considered potential pneumococcal vaccine candidates.
Functional antibody responses to PCV-PPV regimen in HIV infected children on HAART categorized by entry and nadir CD4 percentage (PACTG 1024)

Pelton, SI1, Abzug, MJ2, Borkowsk, W4, Nachman, SA1, Levin, M5, Song, L1, Fenton, T4 for the P1024 Team.

Pediatric AIDS Clinical Trial Group.

1Boston Univ. Sch. of Med and Boston Medical Center, Boston, MA,
2Univ. of Colorado Sch. of Med and Children’s Hospital, Denver, Co,
3Statistical and Data Analysis Center, Harvard Sch. of Public Health,
4NYU Medical Center and Bellevue Hospital, NY, NY
5SUNY at Stonybrook, Stony Brook, NY.

HIV+ children were recruited for participation in PACTG 1024. Children were 2 through 18 years; on stable HAART regimen > 3 months; and had plasma viral load < 60,000 copies/ml. Subjects were stratified into 4 cohorts based on entry CD4% and nadir CD4%.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening CD4%</td>
<td>&lt; 15</td>
<td>&gt; 15</td>
<td>&gt; 15</td>
<td>&gt; 25</td>
</tr>
<tr>
<td>Nadir CD4%</td>
<td>&lt; 15</td>
<td>&lt; 15</td>
<td>15-25</td>
<td>≥ 25</td>
</tr>
</tbody>
</table>

Subject received PCV7 at entry, week 8 and 23V PPV at week 16. Ab response to serotypes (ST) 1, 6B, 14, 19F and 23F were measured in eligible subjects (225) by ELISA and for ST 1, 6B and 14 by OPA in 20 best responders (ELISA) from strata 2, 3 & 4 and all 12 from group 1.

Results as follows:

ENTRY:
1. GMC (ELISA) for 225 subjects were comparable for each ST regardless of prior history or interval between prior PPV and entry (none;<2yrs;>2yrs). No difference in entry GMC was observed among strata.
2. In subset of children with OPA, GMC and OPA activity were discordant. For ST 6B, GMC (ug/ml) and proportion with GMC> 0.35ug/ml were 1.81 and 95%, 1.34 ug/ml and 80%; 1.41 ug/ml and 85% and .75 ug/ml and 75% for group 4 thru 1, yet <35% of any group had OPA >1:16.

POST-VACCINATION:
1. PCV-PPV elicited ELISA responses at week 24 comparable to healthy infants. Immune status correlated with response to PCV7 for all 4 STs in PCV7 but not ST 1 (23V PPV); multivariate analysis identified the presence of plasma viremia at entry decreased response and higher baseline ab correlated with better responses.
2. At week 24, OPA greatest in Group 4 vs 6B (mean 1:374; ≥ 1:16=100%) vs. Group 3 & 2 (1:181;95% and 1:108;90%, respectively) vs. Group 1 (1:21; 42%).

Based on discord between GMC ab concentration by ELISA and OPA among high ELISA responders, and comparability in GMC among children with and without prior PPV at entry, ab to 6B, 14,19F and 23F elicited by 23V PPV does not persist and/or is low quality. In contrast, PCV-PPV elicited ELISA and OPA ab responses. Functional ab response was greatest in children with CD4% at or above 25%. Functional ab persisted for ≥ 80 wks. in 72% of children with ≥15% CD4. The data demonstrate PCV7 is immunogenic in HIV+ children with CD4% ≥15% and suggests maintaining CD4 ≥25% best preserves immune function as measured by ELISA and functional response.
Population-based surveillance for suspected (S) and radiologically-confirmed (RxC) community acquired pneumonia (CAP) in children 1-35 months of age (MoA), in 6 municipalities (Mn) of the metropolitan region (MR), Chile

Lagos R1,2, Muñoz A1, Espinoza A3, Moëne K4, Hausdorff W1, Ruttimann R6, Levine M7
1Centro para Vacunas en Desarrollo-Chile; Santiago, Chile
2Hospital de Niños Roberto del Río; Santiago, Chile.
3Unidad de Imagenología, Hospital San Borja Arriarán; Santiago, Chile
4Unidad de Imagenología, Clínica Las Condes; Santiago Chile.
5GlaxoSmithKline Biologicals; Rixensart, Belgium.
6GlaxoSmithKline Biologicals; Buenos Aires, Argentina.
7Center for Vaccine Development, University of Maryland; Baltimore, USA

Aims: To examine the incidence (Inc) of S-CAP and RxC-CAP in population (ppn) 1-35 MoA affiliated with the Chilean state’s health insurance system (FONASA) in 6, mid-and-mid-low socioeconomic level Mn of the MR.

Methods: S-CAP was an Acute Respiratory Illnesses (ARI) or febrile patient sent by the attendant physician for chest radiological examination (Chest-Rx) to either of the 3 facilities (Rad-F) that serve the targeted ppn. Enrolment of S-CAP cases ensued from 12/01/2003 to 11/30/2004, from 9AM to 6PM the first 5 working days of each month in the largest, and the first 10 days in the 2 smaller Rad-F. S-CAP patients 1-35 MoA with domicile in the selected Mn and parental consent were eligible; those with chronic cardiac or pulmonary illnesses or suspected nosocomial ARI were excluded. Routine statistics were inspected to compute the universe of eligible children seen at the Rad-F. A case of RxC-CAP was a participant whose Chest-Rx revealed consolidating (C) or non-consolidating (NC) infiltrates, as defined by the WHO-Pneumonia Working Group. The total number of S-CAP, C-CAP and NC-CAP cases occurred in the targeted ppn was calculated by extrapolation from the study sample to the universe of eligible children who presented to the Rad-F during the study period. FONASA ppn data were used as denominators to estimate the mean annual Inc of the S-CAP, C-CAP, NC-CAP, as well as of S-CAP cases that proved negative on Chest-Rx.

Results: 1,088 and 1,225 S-CAP cases were enrolled during the 1st and 2nd year, respectively. Age distribution and relative frequencies of the 3 radiological endpoints among 2,282 participants with interpretable Chest-Rx images are presented in Table 1. Inc estimates of the same endpoints are shown in Table 2. A higher proportion of C-CAP than of NC-CAP patients required hospitalization (46/133, 34.6% vs.141/1408, 10.0%, respectively; p <0.0001).

Conclusions. Chest-Rx is widely used in young children with ARI in the MR. This practice explains the high Inc of RxC-CAP and the marked predominance of disease with non-consolidating Rx pattern observed this study. Recent data from vaccine studies have demonstrated that pneumococci are responsible for a considerable proportion of NC-CAP cases. RxC-pneumonia has become a key endpoint in intervention and epidemiological studies of paediatric ARI. Interpretation of such studies requires a clear understanding of the standards of Chest-Rx use prevailing in each community.

Table 1:

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age groups (months)</td>
<td>Rx/C-CAP</td>
</tr>
<tr>
<td></td>
<td>C-CAP</td>
</tr>
<tr>
<td>1 to 5</td>
<td>12</td>
</tr>
<tr>
<td>6 to 11</td>
<td>12</td>
</tr>
<tr>
<td>12 to 23</td>
<td>12</td>
</tr>
<tr>
<td>24 to 35</td>
<td>14</td>
</tr>
<tr>
<td>1 to 35</td>
<td>46</td>
</tr>
</tbody>
</table>

Table 2:

<table>
<thead>
<tr>
<th>Age Groups (months)</th>
<th>Mean, annual FONASA ppm (2004-2005)</th>
<th>Estimated, mean Annual S-CAP Cases</th>
<th>Estimated, Mean Annual C-CAP Cases</th>
<th>Estimated, Mean Annual NC-CAP Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inc x 10^3</td>
<td>Inc x 10^3</td>
<td>Inc x 10^3</td>
<td></td>
</tr>
<tr>
<td>1 to 5</td>
<td>3652</td>
<td>2924</td>
<td>800.7</td>
<td>114</td>
</tr>
<tr>
<td>6 to 11</td>
<td>4382</td>
<td>2395</td>
<td>546.6</td>
<td>125</td>
</tr>
<tr>
<td>12 to 23</td>
<td>10578</td>
<td>3078</td>
<td>291.0</td>
<td>193</td>
</tr>
<tr>
<td>24 to 35</td>
<td>10911</td>
<td>1946</td>
<td>178.0</td>
<td>161</td>
</tr>
<tr>
<td>1 to 35</td>
<td>29523</td>
<td>10342</td>
<td>350.3</td>
<td>596</td>
</tr>
</tbody>
</table>
Correlation of naturally acquired anti-pneumococcal IgG antibody levels with nasopharyngeal carriage in Gambian villagers

Sawa M1, Akisanya A. A. 1, Hill, PC3, Sankareh, K3, Jeffries, D1, Nahua, E1, Cheung, YB2, Lahai, G1, Greenwood, BM2, and Adegbola, RA1
1Bacterial Diseases Programme, MRC Laboratories, Banjul, The Gambia
2Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom

Background: Streptococcus pneumoniae can cause otitis media, pneumonia, meningitis and death in developing countries. Pneumococcal carriage in the nasopharynx is common; but poorly understood. The role of pneumococcal carriage of different serotypes in protection against invasive pneumococcal infection is also unclear; and how serotype specific antibodies relate to carriage is unknown.

Aims: We aimed to determine risk factors for pneumococcal carriage, invasive disease; and how anti-pneumococcal IgG antibodies relate to pneumococcal carriage in individuals and the community.

Method: Consenting individuals in twenty villages in the Western Division of The Gambia were enrolled and venous blood, saliva and nasopharyngeal swabs taken at a point in time. Serum was separated from each blood sample and stored at -70°C until required for testing. A standard ELISA technique based on the WHO recommended ELISA was used to quantify anti-pneumococcal IgG antibodies in the sera. Type specific antigens at 10µg/ml were adsorbed on wells of microtitre plates, sealed and incubated at 37°C for 5hours. All sera were absorbed with 10µg/ml Type F22 and cell wall polysaccharides (CWPS) for 30 minutes before testing. Preliminary antibody results for serotypes 1 and 5 (the least frequent in carriage); and serotypes 3 and 6 (most frequent) were compared to carriage for correlation; using the WHO minimum protective antibody concentration of 0.35 µg/ml, as a cut-off value (<0.35µg/ml were negative, and >0.35µg/ml positive). Carriage of any type of pneumococcus was considered positive and absence of pneumococcus was negative. Data were analysed using the Phi Correlation statistic and summary p value.

Results: Results for 150 villagers are presented. 79 (52.7%) villagers were male, 71(47.3%) female; their mean age was 23.9years (median, 12.5years; range 0-81years). Agreement between the antibody and carriage results were: 50.99%, 50.81%, 55.55% and 53.80% for serotypes, 1, 3, 5 and 6 respectively. The agreement was not significantly different when stratified by age group and sex. Correlation for each serotype and carriage was weak; but statistically significant (type1; Phi = 0.26, p=0.003; type 3; Phi=0.20, p=0.024; type5; Phi=0.27, p=0.002 and type 6; Phi=0.32, p=0.002). There was no significant difference in correlation between high and low prevalence serotypes.

Conclusions: There is weak, but significant correlation between antibody levels and carriage. Further studies are worthwhile to determine serotype specific carriage and antibody correlations in cross-sectional and longitudinal studies.

Development of antibodies to pneumococcal proteins PhtD, CbpA and LytC in Filipino pregnant women and their offspring in relation to pneumococcal carriage

Holmlund E1, Quiambao B2, Ollgren J1, Jaakkola T1, Hermand P3, Neyt C3, Poolman J3, Nohynek H1 and Käyhty H1
1The National Public Health Institute, Helsinki, Finland
2Research Institute for Tropical Medicine, Manila, the Philippines
3GlaxoSmithKline Biologicals, Rixensart, Belgium

Background: Our study focuses on three pneumococcal vaccine candidate proteins, pneumococcal histidin triad D protein (PhtD), choline binding protein A (CbpA) and lysozyme (LytC), all inducing protection in animal models.

Methods: IgG antibody concentrations to PhtD and it’s putative protective and exposed C-terminal fragment, PhtD C, CbpA and LytC were measured by EIA in 52 serum samples of pregnant women, 39 cord bloods and 6 consecutive serum samples (N=263) scheduled to be taken at 6 weeks to 10 months of age from 52 newborns. At every time point when a serum sample was taken, a nasopharyngeal swab was also taken to detect pneumococcal carriage.

Results: Antibody concentrations in cord blood were similar to those of mothers. In infant sera, the kinetics of antibody development by age was similar for the three proteins. First, the geometric mean antibody concentrations (GMC) decreased suggesting that maternal antibodies disappeared without simultaneous antibody production by infants. At 4 to 5 months of age the GMCs started to increase suggesting that infants started their own antibody production. The GMC for CbpA was for mothers 44.7 µg/ml, for infants at 16-20 weeks 4.7 µg/ml, and for infants at 38-42 weeks 8.0 µg/ml, for LytC 5.9, 1.2, and 3.2 µg/ml, for PhtD 21.4, 1.8, and 1.9 µg/ml, and for PhtD C 4.9, 0.6, and 0.9 µg/ml, respectively. The increase in GMCs by age was associated with pneumococcal carriage, most clearly for CbpA.

Conclusions: Already young infants develop antibodies to CbpA, LytC and PhtD in association with pneumococcal contacts. The good correlation between anti-PhtD and -PhtD C suggests that antibodies are directed at least partly to the supposedly exposed C-terminal part of the protein. The role of the antibodies to these proteins in defence against pneumococcal infection remains to be studied.
Nasopharyngeal carriage of Streptococcus pneumoniae in Gambian villagers

Hill, PC1, Akisanya, A1, Sankureh, K1, Cheung, Y B2, Saaka, G1, Lahai, G1, Greenwood, BM2, and Adegbola, RA1
1Bacterial Diseases Programme, MRC Laboratories, Banjul, The Gambia
2Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom

Objectives. To establish the prevalence of nasopharyngeal carriage with Streptococcus pneumoniae in Gambian villages and the serotype distribution and antibiotic resistance patterns of pneumococcal isolates.

Methods. A cross-sectional survey of pneumococcal carriage in 21 rural Gambian villages was conducted after a census. Demographic characteristics, previous medical history, presence of possible risk factors for carriage and access to treatment were recorded prior to collection of a nasopharyngeal swab for isolation of S. pneumoniae, serotyping and determination of antibiotic susceptibility.

Results. The prevalence of S. pneumoniae carriage in 2872 villagers was 72%. It was highest in infants (97%). Fifteen (93%) of 16 babies less than 1 month of age were carriers. Carriage decreased with age above 5 years (p=0.001). No individual risk factors for carriage were identified but, at the village level, there was an association with closeness to another village. Overall, 23% of pneumococcal isolates were of a 7-valent vaccine serotype and 43% were of a vaccine or vaccine-related serotype. However, in children under 5 years of age the prevalence of vaccine or vaccine-related serotypes was approximately 70%. Antibiotic resistance was higher in isolates of vaccine serotype than in those of non-vaccine serotype.

Conclusions. The prevalence of pneumococcal carriage in Gambian villagers is higher than that reported from any other location. Introduction of a pneumococcal conjugate vaccine of restricted valency should reduce the pool of antibiotic resistant pneumococci. However, there is a large reservoir of pneumococci of non-vaccine serotypes and the impact of vaccination on these bacteria will require close monitoring.

Pneumococcal nasopharyngeal carriage and penicillin resistance patterns in young children in Fiji

Russell FM1, Carapetis JR1,2, Ketawai S1, Kanabuli V1, Tani M1, Biribo S1, Seduadua A1, Maileholland EK1,3
1Centre for International Child Health, Department of Paediatrics, University of Melbourne, Victoria, Australia
2Murdoch Children’s Research Institute, Royal Children’s Hospital, Melbourne, Victoria, Australia
3Fiji Pneumococcal Project, Ministry of Health, Fiji
4Fiji School of Medicine, Suva, Fiji
5London School of Health and Tropical Medicine, London, United Kingdom

In the Pacific, little data exist on pneumococcal NP carriage prevalence or antimicrobial resistance. The aims of this study were to document pneumococcal NP carriage prevalence, document antibiotic resistance and pneumococcal serotypes in healthy children in Fiji. Risk factors for carriage were documented.

A cross sectional survey of healthy children aged 3-13 months was undertaken. Risk factors for carriage were documented. Buffered cotton swabs were collected and processed according to the World Health Organization guidelines. Each sample was inoculated onto a 2.5 mg/L gentamicin 5% sheep blood Columbia agar plate. Plates were incubated at 37°C in 5% CO2 for 18-24 hours. Isolates were initially identified by α-hemolysis, colony morphology, and optochin sensitivity. Antibiotic resistance was determined by disk diffusion on Mueller Hinton media with 5% sheep blood. The level of resistance was confirmed by minimum inhibitory concentration (MIC) using E tests. Penicillin resistant isolates on E test, had erythromycin, chloramphenicol, co-trimoxazole, and ceftriaxone E tests performed. Serotyping was performed by the Quellung reaction.

Of the 440 consecutive NP swabs taken, 195 were S. pneumoniae positive (carriage rate 44.3%). Higher rates were found in the indigenous Fijian population (55.8%) than other ethnic groups (21.3% and 30.3% for Indo-Fijian, and others respectively). Penicillin non-susceptibility was found in 11% of isolates, with one isolate demonstrating high level resistance. Co-trimoxazole resistance was common (20.3%) and no isolates were chloramphenicol resistant. Three (1.2%) isolates were multi-drug resistant. The commonest serotypes were 6A (13.2%), 23F (8.3%), 19F (7.4%), and 6B (6.2%). Thirty percent were of the 7-valent pneumococcal conjugate vaccine (PCV) type and 53.7% were represented if the potentially cross reactive strains were included. Forty-five percent of isolates had serotypes represented in the 23-valent pneumococcal polysaccharide vaccine. The most common penicillin non-susceptible isolates were serotypes 23F (34.5%), 19F (10.3%) and 14 (10.3%). The multiple logistic regression analysis showed that being indigenous Fijian or having symptoms of acute respiratory infection were independent risk factors for carriage.

In conclusion, pneumococcal NP carriage is common in Fijian children. Penicillin resistance has been documented for the first time in Fiji and as a result first line treatment for meningitis has been altered. Being indigenous Fijian is a risk factor for disease which may be related to overcrowding. There is low serotype coverage among carriage isolates for the 7-valent PCV.
Does 7-valent pneumococcal conjugated vaccine (pcv7) influence *Staphylococcus aureus* (SA) nasopharyngeal (NP) carriage in 6 to 24 months old children with acute otitis media (AOM)?

**Cohen, R**1, Levy, C2, de La Rocque, F1, Bonnet, E3, Fritzell, B4, Tetelboum, R3, Varon, E4
1CHI Créteil, France
2ACTIV, France
3Wyeth, France
4HEGP Paris, France

**Background:** A French national surveillance network on *Streptococcus pneumoniae* (Sp) NP carriage was set up since implementation of PCV7 in 2001. Negative association between NP carriage of pneumococcal vaccine serotypes and Sa has been described in recent studies. Therefore, surveillance was extended to Sa carriage since December 2003.

**Methods:** 95 French pediatricians carried out NP cultures from children 6 to 24 months of age with AOM who had not received antibiotics within 7 days. Sp isolation, serotyping, antibiotic susceptibility and Sa isolation were performed in the French National Reference Centre for Pneumococci.

**Results:** 1035 patients were enrolled. Vaccinated (n=785) and non vaccinated (n=250) children were comparable regarding sex ratio, day care attendance, number of siblings and proportion of children who received antibiotics within 3 months prior enrollment. Vaccinated children were younger (13.5 months ± 5) than non vaccinated (14.8 months ± 6.2), p=0.0006. Percentages of children carrying Sa alone or both Sa and Sp were similar in vaccinated (≥1 dose) and non vaccinated children.

<table>
<thead>
<tr>
<th></th>
<th>Non vaccinated</th>
<th>Vaccinated (≥1dose)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>250</td>
<td>785</td>
<td></td>
</tr>
<tr>
<td>Sp carriage</td>
<td>165 (66%)</td>
<td>454 (58%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Sa carriage</td>
<td>33 (13%)</td>
<td>103 (13%)</td>
<td>0.9</td>
</tr>
<tr>
<td>Sp+Sa carriage</td>
<td>17 (7%)</td>
<td>54 (7%)</td>
<td>0.9</td>
</tr>
<tr>
<td>Non vaccine serotypes+Sa</td>
<td>7 (2.8)</td>
<td>37 (4.7)</td>
<td>0.2</td>
</tr>
<tr>
<td>Vaccine serotypes+Sa</td>
<td>10 (4%)</td>
<td>17 (2%)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

In children older than one year (n=573), 414 received at least 1dose of PCV7 and 134 got the booster dose. Percentages of children carrying Sa alone were similar in the three following groups: non vaccinated 14%, vaccinated without booster dose 10% and vaccinated with booster dose 13%. Similarly, percentages of children carrying both Sa and Sp were similar in the three groups: non vaccinated 7%, vaccinated without booster dose 6% and vaccinated with booster dose 4%. In children carrying Sp (n=619), co-colonisation with Sa is comparable in children carrying vaccine serotypes (27/244 ie 11.1%) and children carrying non vaccine serotypes (44/375 ie 11.7%).

**Conclusion:** In young children suffering from AOM, PCV7 doesn’t influence Sa carriage. Moreover, no correlation for colonization of vaccine serotypes and Sa was found in our study.
Pneumococcal serotype 1 is an important cause of invasive pneumococcal disease (IPD), but is rarely detected in carriage studies. Of 16 sequence types (STs) identified worldwide in a recent survey, six STs made up 90%. Of these, ST306 and ST304 were predominantly from Europe; ST227 from UK, Canada and USA; and ST217 from Africa and Israel. STs of carriage serotype 1 isolates have not been reported.

Methods: Nasopharyngeal and ear discharge swabs were collected from infants in 3 communities during longitudinal studies between 1992 and 2003. In 2002 and 2004 cross sectional surveys in children >2yo were also conducted in these communities. In 2003, a cross sectional survey in 29 communities was conducted. Pneumococci were isolated and serotyped, and antibiotic susceptibility determined using standard microbiological techniques. All serotype 1 isolates (n=30) were BoxA typed and 6 isolates had MLST.

Results: From the 3 communities studied between 1992 and 2003, 1 ear discharge and 3 nasopharyngeal isolates were detected in 1993. The isolates were clonal and identified as ST304. During the 2002 survey in these same communities, serotype 1 was the second most common carriage serotype (11/83 (13%) 2-5yo; 10/53 (19%) 5-16yo; and 0/86 (0%) adults). These isolates were clonal and identified as ST227. No serotype 1 isolates were found in 2004.

In 2003, swabs from 625 children in 29 communities identified 520 pneumococcal carriers. Five serotype 1 isolates were found in two communities including one ear discharge isolate. These isolates were clonal and identified as ST227. Cases of serotype 1 IPD occurred in <2yo in January 1996, January 1997 and August 1998. None occurred in communities where serotype 1 carriage was found. The STs of these isolates will be determined.

Conclusions: To our knowledge, this is the first report of carriage serotype 1 ST, and the first report of ST304 or ST227 outside the Northern Hemisphere. We identified an epidemic of serotype 1 ST227 carriage one year following universal infant Prevenar immunisation. Isolation of 2 isolates from ear discharge swabs suggests the strains do not have impaired virulence for mucosal infection. Comparative studies of invasive and carriage serotype 1 isolates of the same ST are warranted.
Cooperation of *Streptococcus pneumoniae* and *Chlamydia pneumoniae* in the development of pneumonia and acute respiratory disease

Ogarkov, PF1, Zhogolev, SD1, Jogolev, KD2, Sologub, TS2, Zueva, NV1, Suchanov, BS2
1Epidemiology Department, Military Medical Academy, St Petersburg, Russia
2Microbiology Department, Research Institute of Pulmonology Pavlov Medical Academy, St Petersburg, Russia

Epidemiological study of an outbreak of respiratory infections among recruits was conducted in basic training unit during 6 months from November to May. 197 cases of acute respiratory diseases (ARD), 47 cases of acute bronchitis and 51 cases of pneumonia were recorded. 18 patients with pneumonia, 15 patients with bronchitis and 30 patients with ARD were serosurveyed. Antibodies against *Streptococcus pneumoniae* were detected using indirect immunofluorescence. Antibodies against *Chlamydia pneumoniae* were discovered by bivalent ELISA. IgG Immunocomb ORGENICS. A 4-fold and more increase in titer between paired sera or antibody titers of 1:640 and more to *S. pneumoniae* and of 1:512 and more to *C. pneumoniae* were considered diagnostic. Examination of 63 patients with affection of respiratory tract at its different level (ARD, acute bronchitis and pneumonia) revealed chlamydiosis in 36 cases (57.1 %), and pneumococcosis - in 43 cases (68.3 %). Mixed pneumococcal-chlamydial infection was diagnosed in 27 cases (42.9 %), monochlamydial infection - in 4 cases (6.4 %), and monopneumococcal infection - in 11 cases (17.5 %). Most likely, the action of *S. pneumoniae* and *C. pneumoniae* is interrelated and causes both upper- and lower-respiratory tract infections.

---

Negative association between *Streptococcus pneumoniae* and *Staphylococcus aureus* in the nasopharynx of Aboriginal and non-Aboriginal children in the Kalgoorlie-Boulder area, Western Australia

Watson, K1, Bowman, J2, Murphy, D3, Jacoby P4, Riley, TV4 and Lehmann, D5 on behalf of the Kalgoorlie Otitis Media Research Project Team
1Telethon Institute for Child Health Research, Centre for Child Health Research, The University of Western Australia Perth, Western Australia, Australia
2Division of Microbiology & Infectious Diseases, PathWest Laboratory Medicine, Perth, Western Australia, Australia
3Queensland Health Scientific Services, Brisbane, Qld, Australia
4Microbiology and Immunology, The University of Western Australia, Perth, Western Australia, Australia

**Introduction:** Recent reports suggest an inverse relationship between carriage of serotypes included in the 7-valent pneumococcal conjugate vaccine (7vPCV) and *Staphylococcus aureus*. An increase in incidence of otitis media (OM) due to *S. aureus* following 7vPCV immunisation has also been found. The relationship between *S. pneumoniae* and *S. aureus* has not been examined in Australia and may be particularly relevant given the high rates of community-acquired methicillin-resistant *S. aureus* (MRSA) reported in some Aboriginal communities.

**Methods:** As part of an investigation of causal pathways to OM, 504 and 1045 nasopharyngeal aspirates were collected from 100 Aboriginal and 180 non-Aboriginal children, respectively, over the first 2 years of life. Samples were plated onto selective media. The Quellung reaction was used to serotype 226 and 240 pneumococci isolated from 79 Aboriginal and 111 non-Aboriginal children, respectively.

**Results:** Overall isolation rates of *S. pneumoniae* and *S. aureus* in Aboriginal children were 49% and 23%, respectively, and 25% and 29% in non-Aboriginal children. *S. pneumoniae* carriage peaked at 10-14 months in Aboriginal (67%) and non-Aboriginal (37%) children. *S. aureus* carriage was highest in both Aboriginal (55%) and non-Aboriginal (61%) children <1 month of age. In Aboriginal children, 22% of *S. aureus* were MRSA, while 11% were MRSA in non-Aboriginal children. The most common pneumococcal serotypes were 6B (24%), 19F (13%), 23F (11%) and 14 (7%) in non-Aboriginal children compared with 6B (11%), 6A (8%), 19F (8%) and 16F (8%) in Aboriginal children. For all children aged <5 months, *S. aureus* was isolated more often when vaccine serotypes were absent (48%) than when present (27%) and Age and Aboriginality adjusted Mantel-Haenszel Odds Ratio=0.571, p<0.05. After adjustment for age and Aboriginality, there was a specific negative association between carriage of serotype 6B and concurrent *S. aureus* carriage in all children (Mantel-Haenszel Odds Ratio=0.398, p=0.030) with a stronger association in children <5 months (Mantel-Haenszel Odds Ratio=0.087, p=0.016). In non-Aboriginal children *S. pneumoniae* carriage was higher (p=0.01) and *S. aureus* carriage lower (p<0.05) in winter than in summer.

**Conclusions:** We have found an inverse relationship between carriage of pneumococcal vaccine serotypes and *S. aureus* in young children. The potential for replacement of *S. pneumoniae* with *S. aureus* in the nasopharynx is of concern, particularly with increasing MRSA rates worldwide. Carriage and invasive disease due to these pathogens must be monitored closely following introduction of 7vPCV immunisation for all Australian children in 2005.
Probing the association between serotype-specific pneumococcal nasopharyngeal colonization and efficacy of the pneumococcal conjugate vaccine against pneumonia

Madhi. SA1, Cutland. C1, Kuwanda. L1, de Gouveia. L1, Von Gottberg. A1, Klugman. KP1,2
1Rollins School of Public Health and Division of Infectious Diseases, School of Medicine, Emory University, Atlanta, GA.
2Respiratory and Meningeal Pathogens Research Unit, University of the Witwatersrand; Jhb, Gauteng, South Africa.

Background: The pathogenesis of pneumococcal pneumonia involves either contiguous spread of the pneumococcus from the nasopharynx or blood-borne passage into the lung.

Objective: To determine the association if any of pneumococcal conjugate vaccine impact on colonization in the nasopharynx, at the time of hospitalization for lower respiratory tract infections (LRTI), with protection from clinical disease.

Methods: The results involve a nested study among children participating in an efficacy trial of a 9-valent pneumococcal conjugate vaccine (PCV) when hospitalized for LRTI (n=1 003). Nasopharyngeal swabs (NPS) were performed upon hospitalization and cultured for pneumococcus using routine microbiologic methods. Serotyping of pneumococcal isolates was performed using the quellung method. Pneumococcal serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F, 23F; and 6A were categorized as “vaccine-serotypes (VT)” and all other serotypes were categorized as “non-vaccine types” (NVT). The efficacy (VE) of PCV in reducing hospitalization for: i any LRTI; ii. LRTI episodes associated with colonization of VT, NVT or “no-growth” did not differ by vaccination status. The incidence of bronchiolitis associated with colonization was also reduced overall in vaccinees (VE 35%; 95%C.I. 14-50), as well as in HIV infected children (VE: 44%; P=0.004) and HIV uninfected children (VE: 23%; P=0.19). The incidence of CXR-AC associated with NVT colonization, or for which no-growth of pneumococci was identified on NPS was measured.

Results: Overall, vaccinees were 31% (95%C.I. 12-46) less likely to be hospitalized for LRTI associated with VT colonization, whereas there was no difference in the incidence of LRTI associated with NVT colonization (VE: -2%; P=0.82). Similarly, the incidence of CXR-AC associated with VT colonization was reduced by 42% (95%C.I. 16-60) overall, 47% (P=0.009) in HIV infected children and 36% (P=0.12) in HIV uninfected children. There were no statistical differences (P=0.57 for all observations) overall or by HIV stratification in the incidence of CXR-AC associated with NVT colonization, or for which there was no growth of pneumococci between vaccinees and placebo recipients. The incidence of “clinical pneumonia” associated with VT colonization was also reduced overall in vaccinees (VE 35%; 95%C.I. 14-50), as well as in HIV infected children (VE: 44%; P=0.004) and HIV uninfected children (VE: 23%; P=0.19). The incidence of bronchiolitis associated with colonization of VT, NVT or “no-growth” did not differ by vaccination status.

Conclusion: This probe-study suggests that protection against colonization with VT conferred through vaccination with PCV may be a marker of the ability of the vaccine to prevent VT pneumonia in children.

Association between carriage of Streptococcus pneumoniae and Staphylococcus aureus in a Dutch birth cohort

Labout, JAM1,2, Duijts, L1,2, Bloemmaart, M1, Jaddoe, VWV1, Hofman, A1, De Groot, R1, Verbrugh, HA1, Moll, HA1,2, Hermans, PWM1,2
1The Generation R Study Group, Erasmus MC, Rotterdam, The Netherlands
2Erasmus MC, Rotterdam, The Netherlands
3Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

Background: The natural balance of microbial species in the nasopharynx may be altered by vaccination. Two recently published papers have shown an inverse association of carriage between Streptococcus pneumoniae, in particular vaccine-type strains, and Staphylococcus aureus in children.1,2 We examined the prevalence and risk factors of carriage of S pneumoniae and S aureus in the pre-vaccination era in Dutch children between 6 weeks and 14 months of age.

Methods: Our study is embedded within The Generation R Study, a prospective population-based cohort study from early fetal life until young adulthood in Rotterdam, The Netherlands. Nasal and nasopharyngeal swabs were obtained at the age of 6 weeks, 6 months and 14 months in a subgroup of 1200 Dutch children. Carriage rates of S pneumoniae and S aureus were determined. Data on risk factors associated with carriage were collected by questionnaires.

Results: At time of analysis, all swabs were obtained from the 6 weeks old children, 95% of the 6 months old children and 75% of the 14 months old children. Carriage of S pneumoniae increased from 7% at 6 weeks to 27% at 6 months to 37% at 14 months. In contrast, the carriage of S aureus decreased from 46% to 20% to 11% in time. Statistical analysis revealed a significant inverse association between these two bacterial species. Risk factor analysis for S pneumoniae and S aureus carriage is currently undertaken and will be demonstrated.

Conclusions: S pneumoniae carriage is negatively associated with S aureus carriage in young children between 6 weeks and 14 months of age. Future risk factor analysis is expected to reveal determinants for bacterial carriage.

Exposure to other children enhances the antibody response to a pneumococcal conjugate vaccine in 1 year old infants

Salt PM1, Banner, C1, Oh, S1, Yu, L1, Griffiths, DT1, Pan D1, Lewis S1, Ferry, BL1, Pollard, A2
1Oxford Vaccine Group, Department of Paediatrics, Churchill Hospital, University of Oxford, Oxfordshire, UK
2Centre for Statistics in Medicine, Wolfson College Annexe, University of Oxford, Oxfordshire, UK

Background: Nasopharyngeal carriage of S. pneumoniae and exposure to cross-reacting antigens is responsible for the development of natural immunity against this important childhood pathogen. Nasopharyngeal carriage of pneumococci stimulates an antibody response to the serotype antigen in adults and, at least for some polysaccharides, may also be an immunising event in infancy. Children with siblings and/or who attend day-care have higher rates of carriage of pneumococci than single children who stay at home and the former may therefore be immunologically primed by carriage.

Methods: One hundred and sixty children aged 1 year were immunised with a 7-valent conjugate pneumococcal vaccine. Pneumococcal carriage rate, antibody concentration and risk factors for carriage (siblings and day-care attendance) were analysed.

Results: After vaccination with a heptavalent pneumococcal conjugate vaccine, children with risk factors for pneumococcal carriage had higher antibody concentrations than children with low risk of pneumococcal carriage for serotypes 4, 9V, 14 and 23F. There was also a trend for enhanced serotype 19F responses in this age group. Serotype 6B was less immunogenic, as found in previous studies, and not affected by mixing with other children in the home or in day-care.

Conclusions: Carriage of S. pneumoniae in the first year of life appears to enhance immune responses against some of the Pnc7 vaccine serotype polysaccharides even though polysaccharides are conventionally believed to be poorly immunogenic in infancy.

This study was funded by a grant from Wyeth Vaccines

Intra-familial transmission of Streptococcus pneumoniae and nonencapsulated Haemophilus influenzae

McKinnon M3, Smith-Vaughan H4, Perez F, Shelby-James T, Mayo M2, Leach AJ1,2
1Menzies School of Health Research, Darwin, Northern Territory, Australia
2Charles Darwin University, Institute of Advanced Studies, Darwin, Northern Territory, Australia

Background: Aboriginal infants in remote communities become colonised with S. pneumoniae and nonencapsulated H. influenzae (NCHi) soon after birth, and consequently experience very early onset of otitis media. For infants over three months of age, their young siblings, and mothers, the prevalence of S. pneumoniae and H. influenzae (predominantly NCHi) carriage was approximately 70% and 80%, 83% and 93% and 40% and 36%, respectively.

Methods: Nasopharyngeal swabs positive for S. pneumoniae and NCHi from infants and their mothers and siblings were selected. Multiple S. pneumoniae isolates from 38 infant and mother paired swabs, and 35 infant and sibling paired swabs were serotyped. Multiple NCHi isolates from 35 infant and mother paired swabs, and 44 infant and sibling paired swabs were PCR-ribotyped.

Results: The hierarchy of pneumococcal serotypes was similar in infants and siblings, and markedly different in the mothers. For NCHi, the majority of PCR-ribotypes detected in the infants were also found in the siblings and mothers. However, four distinct maternal types were not detected in the children. Infant and sibling pneumococcal serotypes were concordant more often than infant-mother paired swabs (Table), and infants and siblings more often carried the common serotypes. However, infants and their mothers had a higher likelihood greater than chance of carrying the same type of S. pneumoniae or NCHi, compared with infants and siblings (Table). Sharing of types between family members was not associated with age or density of bacteria detected in swabs.

Table: Observed concordance of types in paired swabs and likelihood greater than chance of an infant and family member carrying the same S. pneumoniae serotype or NCHi PCR-ribotype.

<table>
<thead>
<tr>
<th>S. pneumoniae</th>
<th>Observed concordance of types in paired swabs</th>
<th>Likelihood greater than chance (observed/expected) of type concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants and mothers (38 pairs)</td>
<td>24%</td>
<td>4.44</td>
</tr>
<tr>
<td>Infants and siblings (35 pairs)</td>
<td>46%</td>
<td>3.47</td>
</tr>
<tr>
<td>NCHi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infants and mothers (35 pairs)</td>
<td>26%</td>
<td>8.13</td>
</tr>
<tr>
<td>Infants and siblings (44 pairs)</td>
<td>39%</td>
<td>5.24</td>
</tr>
</tbody>
</table>

Conclusion: The exceedingly high carriage rates of S. pneumoniae and NCHi in Aboriginal infants in remote communities is related to high carriage rates of these pathogens in some family members, particularly siblings, who also frequently carry the same strain as the infant. The higher likelihood ratios for mother/infant concordance were related to carriage of less common types. Reduction of respiratory pathogen carriage in mothers and siblings and measures to prevent transmission may delay initial acquisition of these pathogens and the associated onset of persistent ear disease in infants.
Multiseroype nasopharyngeal carriage of *Streptococcus pneumoniae* in infants in Fiji

P07.14

**Ranjith Batuwantudawe**

1Fiji School of Medicine, Suva, Fiji

2Centre for International Child Health, Department of Paediatrics, University of Melbourne, Melbourne, Australia

3Ranjith Batuwantudawe

4Research Center for Tropical Infectious Diseases, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan

**Objective**

To identify and describe nasopharyngeal carriage of bacterial respiratory pathogens in post Asian Tsunami temporary shelter camps in Sri Lanka

**Methodology**

Batticaloe district of the Eastern Province, one of the Asian Tsunami affected districts in Sri Lanka was selected for the present study because this district reported the highest number of Respiratory Tract Infections in the Post Tsunami disease surveillance system.

**Results**

From 324 consenting participants nasopharyngeal swabs were collected. At least one respiratory symptom (sore throat, snivel and cough) was reported by 70% of the participants. From nasopharyngeal swab culture 25 *Streptococcus pneumoniae* and 21 *Haemophilus influenzae* isolates were yielded. Seventeen pneumococci were susceptible, five showed intermediate resistance and three were fully resistant to Penicillin G. Molecular typing by PFGE showed 16 strains among the 25 pneumococcal isolates. The serotypes identified were 3, 6A, 6B, 9A, 10A, 10F, 11A, 15B, 17A, 19A, 22A, 22F and 23F.

**Conclusions**

*Haemophilus influenzae* and *Streptococcus pneumoniae* were two of the leading bacterial respiratory pathogens identified in post Asian Tsunami displaced population living in camps. The prevalence of resistance organisms in the circulation of highly congested camps highlights the need for prompt actions to increase the ventilation and hygienic conditions in these camps. Colonization with these resistant strains can become a risk factor in the spread of respiratory infections in any congested living conditions. Since the study focussed on bacterial respiratory pathogens, viral and other causes cannot be excluded.

**Acknowledgments:** Financial support: This study was funded by Grant-in-Aid for Special Purposes (no.16800056), the Ministry of Education, Culture, Sports, Science and Technology, Japan.

**Key words:** Asian Tsunami, respiratory infections, *Streptococcus pneumoniae*, Sri Lanka

---

Nasopharyngeal carriage of *Streptococcus pneumoniae* in a displaced Asian Tsunami population in a camp setting in Eastern Sri Lanka

P07.15

**Ranjith Batuwantudawe**

1Fiji School of Medicine, Suva, Fiji

2Centre for International Child Health, Department of Paediatrics, University of Melbourne, Melbourne, Australia

3Epidemiology Unit, Ministry of Health, Colombo, Sri Lanka

4Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan

**Objective**

To identify and describe nasopharyngeal carriage of bacterial respiratory pathogens in post Asian Tsunami temporary shelter camps in Sri Lanka

**Methodology**

Batticaloe district of the Eastern Province, one of the Asian Tsunami affected districts in Sri Lanka was selected for the present study because this district reported the highest number of Respiratory Tract Infections in the Post Tsunami disease surveillance system. Nasopharyngeal swabs were collected in three randomly selected camps in March 18th to 20th, 2005. Written informed consent was obtained from inhabitants who agreed to participate in the study. Bacterial culture of nasopharyngeal swabs were carried out in Peradeniya University, Kandy, Sri Lanka. *Streptococcus pneumoniae* and *Haemophilus influenzae* isolates were shipped to Nagasaki University Japan for further investigations.

**Results**

There were 133 NP swabs processed. The results are still being analysed and will be presented at the conference. The results will be presented in terms of the prevalence of multi-serotype carriage and the proportion of non-vaccine and vaccine type carriage. The sensitivity of using the proposed methods will also be calculated to detect multiple serotype carriage (singly and combined), and recommend the best method(s) to use in developing countries like Fiji.
The transition in nasopharyngeal microflora accompanying the onset of otitis media in high-risk infants

PO7.16

Smith-Vaughan HC², Byun R¹, Halpin S², Kennedy M², Nadkarni M¹, Jacques NA¹, Hunter N¹, Morris PM¹,², Leach AJ².
¹Menzies School of Health Research, Darwin, Northern Territory, Australia
²Charles Darwin University, Institute of Advanced Studies, Darwin, Northern Territory, Australia
³Institute of Dental Research, Westmead Millennium Institute and Westmead Centre for Oral Health, Sydney, Australia
⁴Flinders University, Adelaide, South Australia, Australia

Background: In Aboriginal children in remote communities of the Northern Territory, Australia, the onset of otitis media (OM) commences within weeks of birth, and is preceded by nasopharyngeal carriage of three principal bacterial respiratory pathogens, Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis. The transition in colonisation pattern shifts from one characterised by a low density of commensal species to increasing density and dominance by pathogens at an average rate of 5% per day with multiple bacterial respiratory pathogens.

Aim: To describe the transition in bacterial colonisation pattern from birth to first episode of otitis media. To identify the window period in which the transition to a dominance by pathogenic bacteria occurs, and to develop methods for use in larger clinical trials of interventions that may prolong non-pathogenic species in the nasopharynx.

Methods: Nasopharyngeal swabs from ten randomly selected Aboriginal infants were analysed. S. pneumoniae, H. influenzae, M. catarrhalis, Staphylococcus aureus, and total load were estimated by quantitative real-time PCR.

Results: Respiratory bacterial density was low in the first three weeks of life, comprising a small proportion of the total bacterial load. In the 3-6 weeks group, density of respiratory bacteria and total bacteria increased significantly (p<0.03 to p<0.001), and all swabs contained multiple respiratory bacteria. This was associated with the transition to OM. No significant difference existed between the 3-6-week and 6-13 week groups.

For S. aureus load, there was no significant difference between any groups. In some children, the dynamics of S. aureus and respiratory pathogen colonisation suggested a protective role for OM. S. aureus may also have a pathogenic role, suggested by cases of transition to OM with a high S. aureus load (up to 75% of total load) and respiratory pathogen load less than 1% of total bacterial load. Despite culture for OM bacteria and viruses, few other OM pathogens were detected in these ten children.

<table>
<thead>
<tr>
<th>Age</th>
<th>Sp</th>
<th>Hi</th>
<th>Mc</th>
<th>S. aureus</th>
<th>Total bacteria</th>
<th>% Sp+Hi+Mc (range)</th>
<th>% S. aureus (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 3 weeks</td>
<td>8</td>
<td>47</td>
<td>28</td>
<td>86</td>
<td>4x10⁴</td>
<td>0.06 (0-14%)</td>
<td>0 (0—100%)</td>
</tr>
<tr>
<td>3 - 6 weeks</td>
<td>4x10⁴</td>
<td>1x10⁴</td>
<td>5x10⁴</td>
<td>434</td>
<td>8x10⁵</td>
<td>35 (0.1-72%)</td>
<td>0.04 (0-65%)</td>
</tr>
<tr>
<td>(all but 1 with OM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 - 13 weeks</td>
<td>2x10⁴</td>
<td>3x10⁴</td>
<td>5x10⁴</td>
<td>459</td>
<td>2x10⁵</td>
<td>44 (0-79%)</td>
<td>0.1 (0-75%)</td>
</tr>
<tr>
<td>(all OM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sp, S. pneumoniae; Hi, H. influenzae; Mc, M. catarrhalis

Conclusions: For Aboriginal children in remote communities, the very early onset of OM is accompanied by a significant increase in multiple bacterial respiratory pathogen load and total bacterial load between 3 and 6 weeks of age. This may be a critical period during which interventions that promote non-pathogenic species or inhibit pathogens in the nasopharynx could be implemented (e.g. probiotics or passive protection such as maternal immunisation).

Pneumococcal carriage study in a semi-closed population of children in north-east Tanzania

PO7.17

Oriyo, N¹,²; Sam, N¹; Gillespie, SIF¹; & Charalambous, BM¹.
¹Tumaini University, Moshi, Tanzania
²Royal Free & University College Medical School, UCL, London, U.K.

Background: Streptococcus pneumoniae is a constituent of the human nasopharyngeal microbiota, but alteration of its commensal relationship with its host can cause disease. Pre-school children constitute the largest reservoir of pneumococci and an important vector for their transmission. Although carriage data exists from developing countries there is little data from Tanzania.

Aim: To undertake a detailed pneumococcal carriage study in healthy children.

Methods: Pneumococcal carriage was studied over 4 months in 24 Tanzanian children living on a sugar plantation. Healthy children <6 years old carrying pneumococci were recruited. Nasopharyngeal and oropharyngeal swabs were taken monthly. Up to 10 presumptive pneumococcal isolates were picked from primary cultures. The serotype or group (STG) and antibiotic sensitivities were determined.

Results: The carriage rate of the pneumococcal carriers decreased to the community rate (32.5%) after 2 to 3 months. All children were colonised at some time during the observation period. Eighteen different STGs were identified with 27% non-typable. Prevalent STGs were 9>19>6>17. 64% of samples had multiple STGs with up to 5 different STGs. All except one STG showed non-susceptibility to antibiotics with 92% resistance to penicillin or cotrimoxazole. Multiple resistance to up to five different antibiotics was observed. Up to 7 different antibiograms were observed in a single sample. No alteration in the carrier state or level of resistance was seen in response to antibiotics.

Discussion: The 2-3 month duration of carriage in this study is similar to other studies. In agreement with the seminal studies of Gundal & Okura (1933) simultaneous colonisation with more than one STG was common with up to 5 different STGs occurring together. This heterogeneity was compounded by simultaneous colonisation of pneumococci with different antibiotic sensitivities. Only 36% of the STG found are covered by the 7-valent conjugate vaccine. Our carriage data indicate that there is a highly heterogeneous nasopharyngeal reservoir of pneumococci in Tanzanian children from which antibiotic and vaccine resistance can develop and spread.

Future work: Non-typable isolates will be analysed by PCR-RFLP of the entire capsulation locus (Batt et al, 2005) and genotyped. Genotyping of the same STGs occurring in simultaneous or consecutive colonisations will also be performed. Host immune responses to pneumococcal antigens will be investigated. Invasive isolates will be captured for comparison with carriage isolates.
Longitudinal study of antibiotic sensitivity of pneumococcal isolates colonizing the nasopharynx of Tanzanian children

Nyiro J, Abdullahi O, Scott JAG
Wellcome Trust/KEMRI Centre for Geographic Medicine Research – Coast, Kilifi, Kenya

Aim: To study antibiotic sensitivity of pneumococcal colonizing the nasopharynx of healthy Tanzanian children over 12 months.

Methods: We randomly collected nasopharyngeal and oropharyngeal swabs from children aged 1-3 years from each survey and re-cultured them. Serotyping was determined using pooled antisera from Statens Serum Institute. Antibiotic susceptibility was determined by disc diffusion and the MIC determined by E-test for the non-susceptible isolates.

Results: Baseline carriage rate was 32.5% with multiple resistances seen in 91.7% of the isolates. Simultaneous carriage of the same serotype (e.g. 6) with different antibiograms was observed. Carriage of serotypes with similar antibiograms were seen over a three month period and thereafter a new strain with different antibiograms emerged. One child carried a single strain of serotype 9 with a penicillin MIC of 0.125-0.19µg/ml and a co-trimoxazole MIC of 4-8µg/ml at the start of the study, lost carriage, and then regained the same ST and antibiotic phenotype after 6 months. In another child non-typable isolates were observed with similar antibiotic susceptibilities. No discernable pattern of acquisition or loss of antibiotic non-susceptibility was observed, and there was no association between carriage of non-susceptible isolates and antibiotic use.

Discussion: Antibiotic susceptibility patterns are highly variable. Simultaneous carriage of multiple isolates with different antibiotic susceptibilities were observed, even between the same serotype/group. Irrespective of the antibiotics taken children were as likely to carry antibiotic susceptible as non-susceptible strains.

Further work: To genotype (MLST) isolates with the same serotype/group and different antibiograms that were simultaneously carried. This will establish the possibility that a pneumococcal strain can acquire antibiotic resistance during nasopharyngeal colonisation. To better define the non-typable isolates with the same or different antibiotic sensitivities their capsulation locus will be characterised by PCR-RFLP that has been shown to produce a unique fingerprint for each serotype.

Quantitative description of the carriage of multiple serotypes of *S. pneumoniae* in the nasopharynges of children in Kilifi District

Nyiro J, Abdullahi O, Scott JAG
Wellcome Trust/KEMRI Centre for Geographic Medicine Research – Coast, Kilifi, Kenya

Introduction: A hazard of 7-valent conjugate pneumococcal vaccine is disease caused by non-vaccine serotypes. The pool of alternative serotypes is described by nasopharyngeal swab (NPS) studies, usually typing 1-4 colonies from each swab culture. This method is relatively insensitive to colonisation with multiple serotypes. In this study we describe the ecology of *S. pneumoniae* in the nasopharynx by serotyping forty-eight colonies per NPS culture and assess the limitations of examining only four colonies.

Methods: The study was nested in a nasopharyngeal carriage study in children in Kilifi District, Kenya during both dry and rainy seasons. Dacron-tipped flexible-wire swabs were passed to the posterior nasopharynx and transported in STGG. They were cultured on horse-blood agar with 2.5mg/l gentamicin and four morphologically distinct pneumococcal colonies were selected, sub-cultured and serotyped. STGG samples were stored frozen at -80°C. We selected 32 positive samples from children aged 1-3 years from each survey and re-cultured them, this time sub-culturing and serotyping 48 different colonies per swab. All serotyping was by Quellung Reaction using polyclonal rabbit antisera (Statens Seruminstitut, Denmark).

Results: Of 64 positive swabs evaluated by serotyping four colonies dual-serotype infections were found in 3 (5%). When re-evaluated by serotyping forty-eight colonies 16 (25%) had >1 different serotype (McNemar Chi: p=0.0003); 3 samples (5%) yielded three different serotypes. The 4-colony method detected 67 (82%) of the 82 isolates detected by the 48-colony method. Season did not affect the results of the 48-colony method but with the 4-colony method no swab yielded >1 serotype during the dry season. We further analysed 13 swabs with two serotypes identified by the 48-colony method; the dominant serotype comprised 90-98% of colonies per swab during the dry season but only 71-90% during the rainy season. The serotype coverage of the 7-valent pneumococcal conjugate vaccine was 54% (7/13) among the dominant isolates and 7% (1/13) among the non-dominant isolates (p=0.03).

Conclusion: Serotyping four colonies per NPS culture revealed only 82% of the serotypes that may be identified by more thorough analyses with forty-eight colonies. In mixed cultures the relative proportions of different serotypes are more even during the rainy season making it easier to detect second serotypes. Pneumococci that are missed by standard NPS laboratory protocols are much more likely to be non-vaccine serotypes than those that are detected.
Dynamic models of pneumococcal carriage and disease, and the impact of the 7-valent pneumococcal conjugate vaccination in the UK

Melegaro A, Choi YH, Gay N, Edmunds J
Centre for Infections, Health Protection Agency, London, UK

The aim of this study was to evaluate the impact of routine vaccination of infants with a 7-valent pneumococcal conjugate vaccine (PCV7) with or without an additional booster in the second year of life or catch-up campaign for pre-school children in the UK. A dynamic, age-structured, compartmental transmission model of vaccine-type and non-vaccine serotypes was developed that incorporated competition between these groups as well as age-dependent mixing patterns. The model was parameterised using a number of datasets including a longitudinal carriage study in the UK and pre-vaccination enhanced pneumococcal surveillance data, and post vaccination surveillance data from the USA. The model was then used to predict the effects of vaccination in the UK. A range of vaccination strategies were implemented, and their impacts on invasive and non-invasive pneumococcal disease were compared. Sensitivity analyses of model parameters were also conducted. The impact of infant PCV7 on pneumococcal disease was sensitive to the degree of competition between vaccine and non-vaccine types and thus the potential for replacement of disease caused by vaccine types with non-vaccine types. Including additional booster or catch-up campaigns leads to more rapid reduction in disease, but no benefit of long term.

The descriptive epidemiology of nasopharyngeal carriage of Streptococcus pneumoniae in Kilifi District, Kenya

Abdullahi O1, Lewa P1, Nyiro J1, Scott JAG1,2
1Wellcome Trust/KEMRI Centre for Geographic Medicine Research - Coast, Kilifi, Kenya
2University of Oxford, John Radcliffe Hospital, Oxford, UK

Background: Acute respiratory infections cause 25-30% of all deaths in children in developing countries and Streptococcus pneumoniae is the commonest identifiable cause of death among these patients. As pneumococcal pneumonia is preceded by nasopharyngeal colonisation describing this intermediate step may aid our understanding of both transmission and disease risk.

Methods: We performed two cross-sectional studies in the same individuals, one during the dry season (March 2004) the second during the rainy season (June 2004). Subjects were selected randomly from a DSS population register in two rural and two urban locations and eight age strata. Dacron-tipped flexible-wire swabs were passed to the posterior nasopharynx, transported in STGG and cultured on horse-blood agar. Cultured pneumococci were serotyped by Quellung reaction and assayed by Etest for antimicrobial resistance. Carriage risk factors were assessed by questionnaire and analysed by logistic regression.

Results: Of 450 subjects sampled in the dry season survey, 414 were resampled in the rains; 279 pneumococci were cultured from 269 positive swabs. Carriage prevalences were 60%, 46% and 6% among 228 subjects aged 0-2 years, 230 aged 3-9 years and 406 aged 10-90 years respectively. Carriage prevalence did not vary by sex or location (rural/urban) but was significantly associated with the rains (conditional OR 1.70, 95% CI 1.15-2.54). Taking account of age and season other significant associations with colonisation were runny nose (OR 2.3), recent use of ampicillin (OR 0.15) and concomitant infection with H. influenzae type a (OR 5.9) and type b (OR 30). The commonest serotypes isolated were 19F (n=34), 6B (21), 6A (20), 23F (20) and 23B (11) among 207 isolates from children <5 years and 15A (6), 19F (5), 19B (5), 6A (4) and 23B (4) among 72 isolates from older subjects. The proportion of pneumococci that were of serotypes included in the 7-valent conjugate vaccine (including also serotype 6A) at ages 6-29 and 30-59 months were 59% (58/98) and 52% (46/89) respectively. The proportions resistant were: amoxicillin 0%, benzylpenicillin 50%, cefotaxime 1%, chloramphenicol <1%, cotrimoxazole 84% and erythromycin 2.8%.

Conclusions: NP carriage of S. pneumoniae was common in Kilifi District among children <10 years old and was higher in the rains than in the dry season. Among colonising pneumococci, antibiotic resistance to drugs used for inpatient (benzylpenicillin) and outpatient pneumonia (cotrimoxazole) was also high.
Can isolates of *Streptococcus pneumoniae* carried by healthy children or children with mild or severe pneumonia predict serotypes and resistance patterns of invasive isolates?  

Abdulahi OF, Lewa P1, Nyiro J, Scott JAG2,2
1Wellcome Trust/KEMRI Centre for Geographic Medicine Research - Coast, Kilifi, Kenya  
2University of Oxford, John Radcliffe Hospital, Oxford, UK

**Background:** Acute respiratory infections cause 25-30% of all deaths in children in developing countries and *Streptococcus pneumoniae* is the commonest identifiable cause of death among these patients. Methods to culture bacteria from normally-sterile sites are not practicable in many developing countries and isolation rates are low. WHO recommends validation of nasopharyngeal isolates as pragmatic alternatives to invasive isolate phenotypes. We aimed to validate nasopharyngeal swab studies of different convenience samples of children for the determination of vaccine serotype coverage and antibiotic resistance prevalence against a large collection of invasive disease isolates collected at Kilifi District Hospital (KDH) over 11 years.

**Methods:** We performed three cross-sectional studies among children aged <5 years, one (March-June 2004) among randomly-selected healthy children in the community, the second (March-October 2005) among cases of mild pneumonia at the outpatient department of KDH and the third (April-December 2005) among children admitted to KDH with severe or very severe pneumonia. Dacron-tipped flexible-wire swabs were passed to the posterior nasopharynx, transported in STGG and cultured on horse-blood agar. Cultured pneumococci were serotyped by Quellung reaction and assayed by Etest for antimicrobial resistance. The results were compared with invasive clinical isolates of *S. pneumoniae* among children presenting to KDH in 1994-2004.

**Results:** In the community, outpatient and inpatient surveys 349, 140 and 87 children were sampled. Carriage prevalence was 57%, 83% and 75% in the three surveys, respectively. The serotype coverage of the 7-valent vaccine among nasopharyngeal isolates was 43% in the community, 47% among outpatients and 45% among inpatient. These did not differ significantly from the vaccine serotype coverage of invasive clinical isolates (40%). The proportions of nasopharyngeal isolates resistant to cefotaxime, chloramphenicol and co-trimoxazole were not significantly different from the proportion among invasive isolates. Benzylpenicillin resistance was observed more commonly among isolates from healthy children (OR 1.4, 95% CI 1.1-1.9), those from outpatients with pneumonia (OR 2.1, 95% CI 1.5-3.0) and those from inpatients with pneumonia (OR 2.0, 95% CI 1.3-3.6) than among invasive isolates. All but one of the benzylpenicillin resistant isolates exhibited intermediate resistance.

**Conclusion:** Nasopharyngeal isolates of *S. pneumoniae* from children in Kilifi District accurately predict the serotype coverage of the 7-valent vaccine and the prevalence of antimicrobial resistance to most antibiotics except benzylpenicillin where carried isolates overestimate resistance.
Background: Previous studies in Papua New Guinea documented universal upper respiratory tract (URT) pneumococcal carriage by age 3 months and high morbidity and mortality from invasive pneumococcal disease in early infancy. To address this, a neonatal 7-valent pneumococcal conjugate vaccine (PCV) trial began in May 2005 in the Asaro Valley, Eastern Highlands Province. We present preliminary results on pneumococcal (Pnc) carriage in infants who received PCV at ages 1, 2 and 3 months, and a control group. Enrolment of neonates has just begun.

Methods: Since May 2005, pernasal swabs (PNS) collected from 39 children (19 PCV, 20 control) at ages 1, 2, 3, 4 weeks and 3 months have been cultured using selective media. Four colonies of Pnc/plate were subcultured and serogrouped by the Quellung reaction. Serotyping is underway.

Results: 177 PNS have been cultured (79 in PCV and 98 in control group). Overall Pnc isolation rate was 43% and 35% in control and PCV groups, respectively (p=0.3). Average age of Pnc colonisation among children who carried Pnc was 17.7 days in PCV and 15.7 days in control groups; 6 babies carried Pnc at age one week. To date we have identified 20 and 34 different serogroups from 12 and 20 Pnc-positive swabs in PCV and control groups, respectively; non-typable Pnc were found more often in PCV (n=8, 53%) than in control swabs (n=2, 10%) (p=0.01). Overall, the most common serogroups were 6, 4, 19 and 14. Common serogroups in the 15 PCV swabs were 6 (20%), 9 (13%), 11 (13%), 16 (13%) and 19 (13%) compared with 4 (25%), 14 (20%), 6 (15%) and 19 (15%) in 20 control swabs. Serogroups 4, 16, 21, 3, and 7 were commonly seen in the first month of life, while 6, 9 and 14 were found mainly after age one month. PCV serogroups were found in 40% and 55% of swabs analysed to date from the PCV and control groups, respectively. Multiple Pnc serogroups were found in 15/20 (75%) Pnc-positive swabs in the control group compared with 7/15 (47%) in the vaccine group (p=0.2).

Conclusion: A highly diverse set of Pnc serogroups are carried by very young PNG children. Differences in isolation rate, serotype distribution and multiple populations between neonatal PCV, infant PCV and control group will be followed closely.
An evaluation of *Streptococcus pneumoniae* carriage rate in the nasopharynx of Filipino children attending well baby clinic in hospital and primary care center

Capeding MRZ1, Tan R2, Calimon NC3, Alpon MM4, Sepulveda JF5, Zeta AB5, Sombrero LT6, for the Department of Microbiology
1Research Institute for Tropical Medicine, Muninlupa City, Philippines
2Bayanan Health Center, Muninlupa City, Philippines
3Weyth Philippines, Inc., Makati City, Philippines

**Aims:** To evaluate the carriage rate and distribution of *Streptococcus pneumoniae* serotypes in the nasopharynx of healthy children and to estimate the prevalence rate of antibiotic resistant *S. pneumoniae*.

**Methods:** Three hundred healthy children 2 months to 5 years old fulfilling the inclusion and exclusion criteria were prospectively enrolled from October to November 2005. Collection of nasopharyngeal secretions using calcium alginate swab with a flexible aluminum shaft was done after the informed consent had been signed by the subject’s parents or legal guardian. Subjects were observed for 15-30 minutes to determine for any adverse effects following nasopharyngeal swabbing. *S. pneumoniae* were identified at the Microbiology Department of the Research Institute for Tropical Medicine, the research center for infectious diseases of the Department of Health. The identity of the isolates was confirmed by colony morphology, Gram’s Stain and susceptibility to optochin. Serotyping was done using the Quellung reaction using specific antisera from the Statens Seruminstitut (Copenhagen). Antimicrobial susceptibility testing was done using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar enriched with 5% sheep blood.

**Results:** Presently a total of 370 healthy children have been enrolled. There were 192 (52%) males and 178 (48%) were females. One hundred thirty six (136) subjects had *S. pneumoniae* isolated in their nasopharynx, belonged to 23 serotypes. Three serotypes 6, 23, 19 were responsible for 48% of all serotypes. Serotypes included in the 7-valent pneumococcal conjugate vaccine accounted for 38% of all isolates, P=0.001. Multivariate analysis found that time since the start of universal PCV7 vaccination, regardless of child age (and vaccination status), predicts lower PCV7 colonization and higher non-PCV7&R colonization, indicating both a strong direct and herd effect that is still evolving. PCV7 has had no effect on colonization with 6B (nor 6A) and 9V. There are just 3 predominant emerging non-PCV7&R serotypes.
Neonatal acquisition of nasopharyngeal carriage of *Streptococcus pneumoniae*.

**Glass, NE**1,2, Mudoga E1, Abdullahi, O1, Nyiro, J1, Schuchat, A2, Scott, JAG1,3

1Kenya Medical Research Institute, Centre for Geographic Medicine Research – Coast, Kilifi, Kenya
2Respiratory Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA
3Nuffield Department of Clinical Medicine, Oxford University, John Radcliffe Hospital, Oxford, UK

**Background:** In transmission modelling acquisition rates are an important parameter for which there is little empiric data. Studies of children in Papua New Guinea estimate a rate of 4.6% per day. Estimating incidence of acquisition in children is inefficient because they have high colonisation prevalence. Therefore we studied entirely naïve individuals - newborns.

**Methods:** For 110 infants born at Kilifi District Hospital we did nasopharyngeal swabs at birth, twice-weekly for two weeks, then once every two weeks for eight weeks. Sampling ceased with the first culture positive swab. We took swabs from mothers at delivery and one month later and recorded data on risk factors for transmission (household structure, socioeconomic status and upper respiratory tract symptoms in family members). Swabs were transported to the laboratory the same day in STGG and cultured on gentamicin horse blood agar. Pneumococci were serotyped by Quellung reaction. The data were analysed by Kaplan-Meier survival function and Poisson regression.

**Results:** Five infants had only one swab and were dropped from the analysis. We analysed 847 swabs, a mean 8.1 per child, of which 52 were positive. The earliest infection was observed on day 3. Among mothers 104 were swabbed once, 31 twice and 1 thrice. Ten of 136 maternal swabs were positive. Survival time of infants without colonisation had the following function. Median time to infection was 50 days. Incidence of acquisition was 1.34 per 100 days observation. There was a significant linear increase in incidence with age (p=0.012). In the regression model, stratified on age, pre-existing maternal colonisation (Rate Ratio=4.37, 95% CI 1.75-11.0) and number of additional children living in the family home (Rate Ratio=1.21 per child, 95% CI 1.00-1.46) were significant risk factors. Among 10 infants of colonised mothers, 9 became infected, 6 with the homologous serotype. Twenty-five different serotypes were detected among infants, the commonest being 23F and 6A (n=5 each), followed by 19F, 23B, 34 and 6B (n=4 each).

**Conclusion:** Young infants in Kilifi had a risk of acquiring nasopharyngeal colonisation by *S. pneumoniae* in the range 1-2% per day rising with age. The study only sampled babies whose families were wealthy enough to pay for hospital delivery; acquisition rates are likely to be higher for the majority of children who are delivered at home.

![Kaplan-Meier survival function without NP infection](image-url)
Nasopharyngeal (NP) carriage of *Streptococcus pneumoniae* (S.pn) in a cohort of healthy, Chilean new born infants (NBI) followed from age 0 to 24 months (mos).

Lagos B1,2, Moñoz A1, San Martín O1, Seasme M1, Maldonado A1, Hovmazáibal JC1, Loyola H2, Levine MM3
1Centro para Vacunas en Desarrollo-Chile (CVD-Chile); Santiago, Chile
2Hospital de Niños Roberto del Río; Santiago, Chile.
3Instituto de Salud Pública de Chile (ISP); Santiago, Chile.
4Center for vaccine Development, University of Maryland; Baltimore, USA

**Aims:** To investigate nasopharyngeal (NP) carriage of *S.pn* in a sample of children residing in the Metropolitan Region (MR) of Chile, and to examine the distribution of capsular serotypes among NP *S.pn* isolates at ages 2, 6, 12, 18 and 24 mos.

**Methods.** Between 09/2001 and 09/2002, 524 NBI representative of the population (ppn) served by the government health care system were recruited in 18 primary care clinics of the MR to participate in a 24-months long follow up primarily aimed at assessing the burden of acute respiratory illnesses (ARI). The study plan included 5 NP swabs (at ages 2, 6, 12, 18 and 24 mos) for investigation of *S.pn* carriage endpoints.

**Results:** The overall attainment and yield of the scheduled NP sampling is shown in the table below. *S.pn* was identified in 870 of 2120 (41%) NP cultures obtained from 424 subjects who provided the 5 scheduled specimens. *S.pn* of capsular serotypes represented in a developmental 10-valent conjugate vaccine increased steadily from age 2 (24.0%) to 18 mos (44.6%), and then fell at age 24 mos (31.7%).

**Conclusions:** Preparatory work for an efficacy trial with a pneumococcal conjugate vaccine has been ongoing in the MR of Chile since mid 1998; yet, this is the first systematic assessment of NP carriage of *S.pn* in infants and children conducted in this study site. Results from this study provide background information for designing a vaccine efficacy trial with *S.pn* carriage endpoints.

**Attainment of the scheduled NP sampling and yield of *Streptococcus pneumoniae* of various serotype categories among 524 participants recruited in the cohort study.**

<table>
<thead>
<tr>
<th>Sampling time (target age)</th>
<th>1 (2 mos)</th>
<th>2 (6 mos)</th>
<th>3 (12 mos)</th>
<th>4 (18 mos)</th>
<th>5 (24 mos)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target Number</td>
<td>524</td>
<td>524</td>
<td>524</td>
<td>524</td>
<td>524</td>
</tr>
<tr>
<td>N and (%) of NP cultures collected</td>
<td>501 (95.6)</td>
<td>476 (90.8)</td>
<td>462 (88.2)</td>
<td>443 (84.5)</td>
<td>434 (82.8)</td>
</tr>
<tr>
<td>Mean age (months) at NP sampling time-points (95% CI)</td>
<td>2.1 (2.1; 2.2)</td>
<td>6.2 (6.1; 6.2)</td>
<td>12.1 (12.1; 12.2)</td>
<td>18.1 (18.0; 18.1)</td>
<td>24.0 (23.9; 24.1)</td>
</tr>
<tr>
<td>N and (%) of <em>S.pn</em> positive cultures</td>
<td>142 28.3</td>
<td>227 47.7</td>
<td>223 48.3</td>
<td>184 41.5</td>
<td>173 39.9</td>
</tr>
<tr>
<td>Mean age (months) at sampling time-points, in 424 subjects who provided all 5 NP specimens (95% CI)</td>
<td>2.1 (2.1; 2.2)</td>
<td>6.2 (6.1; 6.2)</td>
<td>12.1 (12.1; 12.2)</td>
<td>18.1 (18.1; 18.2)</td>
<td>24.0 (23.9; 24.2)</td>
</tr>
<tr>
<td>N and (%) of <em>S.pn</em> positive cultures</td>
<td>121 28.5</td>
<td>202 47.6</td>
<td>203 47.9</td>
<td>177 41.7</td>
<td>167 39.4</td>
</tr>
<tr>
<td>Serotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0.5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>6B</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>7F</td>
<td>1</td>
<td>0</td>
<td>8</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>9V</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>7</td>
<td>5</td>
<td>8</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>18C</td>
<td>1</td>
<td>0</td>
<td>8</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>19F</td>
<td>7</td>
<td>5</td>
<td>8</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>23F</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total, vaccine serotypes</strong></td>
<td>29</td>
<td>24.0</td>
<td>65</td>
<td>32.2</td>
<td>80</td>
</tr>
<tr>
<td>Other vaccine related serotypes / serogroups</td>
<td>21</td>
<td>17.4</td>
<td>43</td>
<td>21.3</td>
<td>48</td>
</tr>
<tr>
<td>Non-vaccine related serotypes / serogroups</td>
<td>40</td>
<td>33.1</td>
<td>52</td>
<td>25.7</td>
<td>52</td>
</tr>
<tr>
<td>Non-Typable <em>Streptococcus pneumoniae</em></td>
<td>31</td>
<td>25.6</td>
<td>42</td>
<td>20.8</td>
<td>23</td>
</tr>
</tbody>
</table>
Clinical characteristics of children with complicated pneumococcal pneumonia caused by serotypes 1 and 3

Tan TQ and the US Pediatric Multicenter Pneumococcal Surveillance Study Group (USPMPSG)
1Feinberg School of Medicine, Northwestern University and Children’s Memorial Hospital, Chicago, IL, USA

The incidence of complicated pneumococcal pneumonia (CPP) in US children has been increasing over the last decade. Surveillance data are showing that pneumococcal serotypes 1 (PS1) and 3 (PS3) are becoming more prevalent as causes of CPP in the pediatric population; these serotypes are not contained in the heptavalent pneumococcal conjugate vaccine (PCV7). The pneumococcal serotypes of hospitalized children with CPP, defined by either loculated pleural fluid (PF) on chest radiograph (CXR) or computed tomography (CT) and chest tube placement or thoracotomy/decortication (TD) or both were determined. Patients (pts) were identified retrospectively, from 9/93-12/03, based on CXR findings and (+) blood and/or PF culture, from pts with infections enrolled in the USPMPSG study (8 children’s hospitals nationwide). The clinical characteristics of pts with CPP caused by PS1 and PS3 were compared to those caused by other pneumococcal serotypes (OPS). Of 201 pts with CPP and serotypes identified, 45 (22.4%) were PS1 and 21 (10.5%) were PS3. Pts with CPP PS1 disease compared to pts with CPP caused by PS3 or OPS, were significantly older (median age 104 mos vs. 24 mos and 34 mos; p < 0.001), more likely to be of Caucasian race (89% vs 62% vs 50%, p < 0.001), and have chest pain on presentation (51% vs. 14% vs. 15.6%, p < 0.001), but were less likely to have multilobar disease (56% vs. 86% vs. 73%, p=0.004), respectively. Pts with PS3 disease had longer chest tube duration (9d vs. 6d vs 5 d, p=0.008) and longer hospital stay (17d vs. 10d vs. 13 d, p < 0.001) compared to pts with CPP due to PS1 or OPS, respectively. Other measures of disease severity did not differ between the groups. Antibiotic resistance was not found in any PS1 and PS3 isolates. Additional surveillance studies are necessary to further characterize PS1 and PS3 as causes of CPP in the pediatric population. Given the increasing frequency and severity of pneumonia caused by PS1 and PS3, these serotypes should be included in future preparations of PCV.

The role of bacterial biofilm in the aetiology of chronic and recurrent ear infections in children

Thornton R1,2, Rigby P1, Thomas W2, Prosser K2,3, Langlands J1, Filion P1, Keil T3, Richmond P1,2,3 & Coates H1,2,3
1University of Western Australia, Perth, WA, Australia
2Telethon Institute for Child Health Research, Perth, WA, Australia
3Princess Margaret Hospital for Children, Perth, WA, Australia

Background: Chronic and recurrent otitis media (OM) is the most common reason children undergo surgery and responds poorly to antibiotic treatment. Bacterial biofilm has been implicated in more than 65% of all human infections and may play a role in OM.

Aims: To demonstrate bacterial biofilm in children suffering with recurrent acute OM (rAOM), OM with effusion (OME) and with chronic suppurative OM (CSOM), and describe the relationship to pathogen specific immune responses.

Methods: To date we have recruited 116 children undergoing surgery for rAOM, OME and CSOM and collected ~2mm biopsies from the middle ear mucosa. Middle ear effusion fluid (MEF) was collected for culture and pneumococcal pneumolysin PCR. Blood was collected for isolation of peripheral blood mononuclear cells and serum. Initially biopsies were analysed for the presence of bacterial biofilm through scanning and transmission electron microscopy (EM).

Preliminary Findings: In the first 16 children with OME, we observed invasive coccal bacteria in middle ear epithelial cells of 4 children (predominantly within mucous containing vacuoles) which correlated with culture negative pneumolysin positive PCR MEF in 3 cases. We were unable to demonstrate associated pneumococcal biofilm which may relate to sample dehydration during preparation for EM. We have now adapted our techniques to use confocal scanning laser microscopy combined with fluorescent in situ hybridization to specifically label the samples for Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis. Using these techniques, we have demonstrated biofilm in tonsils of children with chronic tonsillitis and these techniques are being optimised for middle ear mucosal samples.

Potential Significance: Confirmation of the role of bacterial biofilm in chronic and recurrent ENT infections in children will alter the treatment and prevention strategies for this important problem.
Pneumococcal co-infection with human metapneumovirus

Madhi SA1, Ludewick H1, Kuwenda L1, van Niekerk N1, Cutland C1, Little T1, Klugman KP1,2

1Respiratory and Meningeal Pathogens Research Unit, University of the Witwatersrand; Jhb, Gauteng, South Africa.
2Rollins School of Public Health and Division of Infectious Diseases, School of Medicine, Emory University, Atlanta, GA.

Background: The newly discovered human metapneumovirus (hMPV) may lead to hospitalization of children with lower respiratory tract infection (LRTI), although the pathogenesis thereof remains to be elucidated.

Methods: This hypothesis generating study involved a cohort of children randomized to receive 9 valent conjugate pneumococcal vaccine (PCV) or placebo, and who were investigated for hMPV when hospitalized for lower respiratory tract infections (LRTI). Using a nested reverse-transcription polymerase chain reaction assay (RT-PCR) targeted at amplifying a fragment of the hMPV fusion (F) protein gene, 202 such infections were identified among 2715 episodes of LRTI in children.

Results: Overall, hMPV-LRTI was reduced by 42% (95%CI: 22-56, P=0.0002) among children who had received PCV. Similarly there was a 51% (95%CI:28-67) reduction in hMPV associated clinical pneumonia and a 58% (95%CI: 21, 77) reduction in hMPV associated radiographically confirmed pneumonia. In HIV uninfected vaccinees, hMPV associated LRTI was reduced by 38% (95% CI 14% –56%, P = 0.004) and clinical pneumonia was reduced by 48% (7% - 67%, P = 0.005). Similarly, in HIV infected vaccinees, hMPV associated LRTI was reduced by 47% (1% - 72%, P=0.04) and clinical pneumonia by 56% (12% - 78%; P=0.02). Overall, there was a trend for hMPV associated LRTI being less likely to be reduced in children <6 months of age (14%, 95%C.I. -59 to 53; P=0.63) than in older children (45%, 95%C.I. 25-60; P=0.0002).

Conclusions: Infection by hMPV predisposes children to developing pneumococcal pneumonia, and a significant proportion of LRTI hospitalizations associated with hMPV infection in children older than 6 months of age may be prevented by pneumococcal conjugate vaccine. The molecular mechanisms which enhance the human host’s susceptibility to pneumococcal infection following infection by hMPV remains to be elucidated.

Haemolytic uraemic syndrome associated with invasive pneumococcal disease

Slack M1, George R1, Pitchon B1, Wickens H1, Martin S1, Tullas K2, van’t Hoff W2, Reid C2, Taylor M2, Sharland M1

1Respiratory & Systemic Infection Laboratory, Health Protection Agency Centre for Infections, London, UK
2Great Ormond Street Hospital for Children, London, UK
3Guy’s Hospital, London, UK
4Birmingham Children’s Hospital, Birmingham, UK
5St George’s Hospital, London, UK

Haemolytic uraemic syndrome (HUS), one of the most common causes of acute renal failure in children is generally associated with Escherichia coli O157:H7 infection. HUS has also been described following Streptococcus pneumoniae infection. S. pneumoniae produces a circulating neuraminidase, which cleaves N-acetylneuraminic acid from surface glycoproteins on erythrocytes and endothelial cells, exposing the T-antigen. It is postulated that this may lead to endothelial damage, which in some cases results in thrombotic microangiopathy, which is characteristic of HUS.

An increase in HUS associated with invasive pneumococcal disease (IPD) has recently been observed in the paediatric renal units serving the population of the South-East and Midland Regions of England. 27 cases of pneumococcal-related HUS have been identified since 1998. The median age of onset was 14m. The children affected had severe haematological and nephrological symptoms, with a high proportion requiring dialysis (21/27, 78%) and a high case fatality rate (29%). However, the renal outcome in the survivors appears to be favourable, at least in the short term. In 25% cases the IPD was associated with the development of empyema and /or subdural abscesses. T-antigen activation was demonstrated in 21/27 cases.

11/27 strains of Streptococcus pneumoniae were available for laboratory testing. The strains comprised the following: serotype 19A (n=5); serotype 14 (n=2); serotype 3 (n=2); serotype 6A (n=1); and serotype 12F (n=1). Multi-locus sequence analysis of the strains is being carried out. To date 7/11 strains have been typed by MLST. The strains are of the following serotype/sequence types: serotype 19A/ST 199 (n=3); serotype 19A/ST1201 (n=1); serotype 3/ST 180 (n=2) and serotype 6A/ST 65 (n=1). All of the strains available for testing are fully susceptible to penicillin and cephalosporins.

The neuraminidase activity of the pneumococcal isolates is being determined. It is disappointing that less than half of the pneumococcal isolates were submitted to a reference laboratory for typing. The diagnosis of HUS is usually made at a specialist renal unit, which is often not in the hospital where the child was initially admitted with IPD. However, there does appear to be a genuine increase in the number of cases of pneumococcal-associated HUS. Paediatric nephrologists intend to follow up cases of pneumococcal-associated HUS prospectively. Microbiologists should be encouraged to submit all paediatric invasive pneumococcal isolates for serotyping and genetic analysis.
**Simple method to evaluate biofilm formation of *Streptococcus pneumoniae***

*Tapiainen T, Saukkoriipi A, Kaijalainen T, Leinonen M, Uhari M*

1Department of Pediatrics, University of Oulu, Finland

2National Public Health Institute (KTL), Oulu, Finland

**Background and aims:** Methods to evaluate the biofilm formation of *Streptococcus pneumoniae* have been laborious. As the biofilm formation of oral streptococci has been successful using crystal violet stain on 96-well polystyrene plates we tested this method for pneumococci, too.

**Methods:** Seven clinical isolates were used. Three strains were isolated from nasopharynx (serotypes 6A, 19F, 11) and four from patients with invasive pneumococcal disease (serotypes 6B, 19F, 3). Bacteria were grown in BHI until mid-logarithmic phase and diluted 1:100 in BHI or in BHI supplemented with 0.5%-1.0% glucose, fructose, or sucrose. Two hundred µl of diluted bacterial suspension was incubated (in triplicate) on a polystyrene or polyvinyl plate for 18 h at 37ºC with 5% CO₂. After removal of supernatant and crystal violet stain (0.4% 50 µl 15 min) wells were washed three times with PBS to remove unattached cells. OD was measured at 540 nm after dissolving biofilm in 50 µl of DMSO. Scanning electron microscopy (EM) was used to visualize the structure of biofilms on bottoms of polyvinyl plates.

**Results:** Biofilm formation was poor in BHI (OD < 0.3). Anaerobic conditions did not enhance biofilm formation. In the presence of 0.5% fructose or glucose biofilm formation was enhanced and in two nasopharyngeal isolates (serotypes 19F, 11) good biofilm formation was observed (OD >1.0 and 1.0-1.8 x 10⁸ genomes per well). Biofilm formation was confirmed by scanning EM. The majority of bacteria were viable at 18 hours based on LiveDead kit fluorescent staining. All biofilms were culture negative at 18 hours after washing. Supernatants were culture negative in the presence of 0.5% glucose and 0.5% fructose. However, after 24 h incubation of nonculturable biofilms in fresh BHI, supernatants turned culture-positive.

**Conclusions:** The studied pneumococcal strains formed biofilms in nutrition-rich environment. Crystal violet staining on polystyrene wells appeared to be a simple method to study early events of the biofilm formation of pneumococci.

**Streptococcus Pneumoniae Pili – Purification and Initial Characterization**

*Hilleringmann, M*1; Ferlenghi, I1; Giusti, F2; Mercati, D2; Barocchi M1; Pacchiani, N1; Rappuoli, R1; Covacci, A1

1Chiron Vaccines IRIS, Siena, Tuscany, Italy
2University of Siena, Siena, Tuscany, Italy

*both authors have equally contributed*

*Streptococcus pneumoniae* (pneumococcus), a Gram-positive pathogen that causes pneumonia, bacteremia, meningitis and otitis media, is estimated to be a major cause of morbidity and mortality worldwide. Recently, pneumococcus was found to carry pilius fibers on the cell surface. Similar structures had been identified in other Gram-positive pathogens such as *Corynebacterium diphtheriae*, *Actinomyces spp*, group A and group B streptococci. Pneumococcal pili, detectable not in all clinical isolates, are encoded by an *rrlA* islet, containing 3 sortases and 3 genes coding for LPXTG motifs containing proteins (*rrgA, rrgB* and *rrgC*). Pili are considered to be involved in initiating invasive pneumococcal disease via adhesion to epithelial host cells in the upper respiratory tract. Pililated strains of *S. pneumoniae* show higher virulence when compared to non-piliated isolates. *S. pneumoniae* TIGR4, a clinical, capsular serotype 4 isolate, was used to purify pilus filaments and to perform initial studies on pili structure and their biochemical characteristics. Pili were isolated by enzymatic digestion and purified by sucrose gradient centrifugation followed by column chromatography. The preparation was judged to be homogeneous based on electron microscopy (EM) and sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis. Purified pili showed molecular masses ranging from 2 x 10⁶ to 3 x 10⁶ Da. Heat treatment of pili in the presence of SDS resulted in the dissociation into smaller molecules, yielding a ladder of lower-molecular-weight bands in the gel. Amino acid sequence analysis of pilus tryptic peptide sequences identified a fragment of pneumococcus TIGR4 RrgB protein. EM investigation has been performed on negative stained (1% PTA), immunogold labelled purified pili preparations. Elongated tubular filaments up to 1 µm long and about 10 nm in diameter similar to those detected on whole bacteria were found. Besides single pilus filaments, bundles of strictly packed individual structures were observed. Antiserum against His-tag purified RrgB and RrgC reacted with isolated pili under immunogold EM and in western analysis. The gold labelling pattern indicates a uniform distribution of both, anti-RrgB and anti-RrgC antibodies along the whole filament with a higher relative amount of anti-RrgB. Ongoing structural analysis are performed using quick-freeze deep-etch and cryo-EM.
The influence of allergic airways disease on the immune response to respiratory Streptococcus pneumoniae infection

Preston, J1,Horvat, J C3, Wade, M1, Beagley, K W3, Foster, P S1,4, Gibson, P G3, Hansbro, P M1
1 University of Newcastle, Newcastle, NSW, Australia
2 Respiratory and Sleep Medicine, John Hunter Hospital, NSW, Australia
3 Hunter Medical Research Institute (HMRI), Newcastle, NSW, Australia
4 Australian National University, ACT, Australia

Streptococcus pneumoniae (Spn) infections are a major global cause of morbidity and mortality. Spn infections are most common amongst children under 5, the elderly, immunocompromised and Indigenous Australians. There is conflicting evidence concerning the types of immune responses elicited by an Spn infection. We developed a non-fatal (recovery) model of Spn bronchopneumonia in mice and showed that mild infections elicit a primarily neutrophilic immune response with Th1 biased cytokine production that effectively clears the bacteria and facilitates recovery. Spn vaccination is recommended for high-risk groups, including those with pre-existing allergic airways diseases (AAD) such as asthma. However, the influence of AAD on the severity of Spn disease has not been investigated.

Aim: To determine the effect of AAD on the pathogenesis of a respiratory Spn infection.

Methods: Spn infection was induced in 6-8week old female BALB/c mice by intratracheal inoculation of a non-lethal dose of live bacteria. Respiratory or systemically induced AAD was superimposed on the Spn infection by intranasal or intraperitoneal sensitisation respectively, followed by intranasal challenge with the Th2-inducing antigen ovalbumin (OVA). Immune responses to the Spn infection were assessed.

Results: Spn infection leads to neutrophilic inflammation in the lungs that correlates with impaired lung function compared to naïve controls. Infected animals with pre-existing AAD exhibited an increase in neutrophilic influx into the lungs compared to non-allergic infected controls. However, AAD had no additional effect on the impaired lung function of these mice. Increases in Th1 (Interferon-γ (IFN-γ)) cytokine production in the lung and mediastinal lung draining lymph nodes restimulated with heat-killed Spn were also observed. When Spn infection and allergen sensitisation were induced concurrently, an increase in neutrophilic respiratory inflammation was again observed, and lung function was not significantly different between infected allergic mice and non-allergic infected controls. However, a significant increase in the Th2 (Interleukin-4 (IL-4)), but not IFN-γ, cytokine levels in the lung tissue and lymph node cultures as detected.

Conclusion: Th2-mediated AAD increases the neutrophilic inflammatory responses to Spn infection, however it has no effect on lung function. The effect of AAD on T-cell mediated cytokine responses to Spn infection depends on the timing of allergen sensitisation relative to infection. However, the effect of recently described neutrophilic asthma on Spn infection remains unknown.

Serum amyloid P component protects against Streptococcus pneumoniae in a mouse model of infection

Yuste J, Botto, M, Brown JS
1 Centre for Respiratory Research, University College London, London, United Kingdom
2 Rheumatology Section, Faculty of Medicine, Imperial College London, London, United Kingdom

The pentraxin serum amyloid P (SAP) is a component of the acute phase response that is able to bind to the surface of some pathogens, interact with several complement components and possibly affect innate immunity. Using strains of mice with genetic deficiencies of SAP (SAP−/−) we have investigated the effect of SAP on innate immunity to Streptococcus pneumoniae. Using flow cytometry assays we assessed C3b deposition on S. pneumoniae and phagocytosis by the neutrophil cell line HL-60 in serum from SAP−/− and wild-type mice. Complement C3b factor deposition on S. pneumoniae D39 strain in serum from SAP−/− mice was reduced compared to the results for serum from wild-type mice. In addition, there was a decrease in phagocytosis of S. pneumoniae by neutrophils when the bacteria was incubated in SAP−/− serum compared to wild type serum, suggesting that the decreased C3b deposition on the S. pneumoniae surface reduces phagocytosis of the bacteria. To characterise in more detail the functional consequences of these interaction of SAP with S. pneumoniae, we used mouse models of infection. Wild type and SAP−/− mice were challenged with D39 via intraperitoneal (IP), intranasal (IN) and intravenous (IV) routes. The IP route showed a similar mortality in both groups, although the colony counts of bacteria recovered from spleens and blood were higher in SAP−/− mice. However, after IN inoculation the survival of SAP−/− mice was decreased and the bacteria levels in BAL, lung, spleens and blood were much higher then in the wild-type mice. After IV inoculation, SAP−/− mice had impaired clearance of S. pneumoniae compatible with the reduced phagocytosis seen with the in vitro assay. In addition, during S. pneumoniae infection, SAP−/− mice showed impaired activation of lung / splenic T cells. However the concentrations of pro-inflammatory cytokines (TNF-α, IFN-γ, IL-6, IL-12, and MCP-1) in SAP−/− mice were higher, both in BAL and serum, probably due to the larger number of bacteria present in SAP−/− mice compared to wild-type mice. Our results suggest that SAP plays an important role in innate immunity to S. pneumoniae, possibly by assisting complement activation by the bacterium, so leading to improved phagocytosis and cellular immune response.
Isolation and characterization of *Streptococcus pneumoniae* strains from animal models

Al-Lahham, A¹, Nicklas, W², and Reinert, R²

¹National Reference Center for Streptococci and Institute of Medical Microbiology, Aachen, Germany
²German Cancer Research Centre, Heidelberg, Germany

**Background and Aim:** *Streptococcus pneumoniae* is the major causative pathogen of many childhood community-acquired respiratory tract infections (RTIs), including community-acquired pneumonia, acute otitis media and acute maxillary sinusitis. In the present study *S. pneumoniae* strains isolated from mastomys, guinea pigs and rats were characterized.

**Methods:** Species diagnosis was performed by optochin testing, bile solubility and the serotyping by the Neufeld Quellung reaction. MICs to antibiotics were determined by the microdilution method according to the CLSI recommendations. Multilocus Sequence Typing (MLST) was performed according to standard methods.

**Results:** During the period 1986 to 2005 the number of strains isolated from animals were 29 including mastomys (n=20), guinea pigs (n=3) and Rats (n=6). 20 of these animal models were bred at the German Cancer Research Centre, 3 from the university of Heidelberg in Germany and 6 were brought from a commercial breeding company from Sulzfeld in Germany. Swab samples were taken from the animal models before they were included in any experiment. *S. pneumoniae* were isolated from nose (n=21), lung (n=3), trachea (n=3), abdomen (n=1) and one isolate from eye. Serotypes detected were: 14 (n=23), 19F (n=2), rough (n=1), 7C (n=1), 19A (n=1) and one was non typable. MIC₉₀ (mg/L) of penicillin, clarithromycin, telithromycin and levofloxacin was 0.016, 0.125, 0.016 and 1, respectively. One isolate was clarithromycin resistant. The guinea pigs involved in this study were C4-immune deficient and the pneumococcal isolates derived from them were of serotypes 19A and 19F. 19 isolates were MLST 15 (all of serotype 14) and 10 isolates were new MLS-types.

**Conclusions:** To our knowledge, 10 pneumococcal strains isolated from animal models in this study were not described before in human and the majority of these strains were of serotype 14.
A surveillance system for pneumococcal disease following the successful trial of the 9-valent pneumococcal vaccine in the Gambia

Hill, PC1, Sambo, S1, Lloyd-Evans, N2, Manneh, K3, Greenwood, BM1, Jallow, MF, and Adegbola, RA1
1Bacterial Diseases Programme, MRC Laboratories, Banjul, The Gambia
2Department of State for Health, Government of The Gambia
3Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom

Following success of the pneumococcal vaccine trial in The Gambia it was decided to give one dose of the only commercially available conjugate vaccine, the 7-valent vaccine Prevenar®1, to all study participants and the vaccine may soon be introduced into the EPI programme. There are 90 known pneumococcal serotypes but only a few cause the majority of invasive disease. Virulence and transmissibility vary with serotype. Introduction of the 7-valent vaccine without serotypes 1 and 5 that commonly cause disease in The Gambia, should be accompanied by a surveillance system. We have designed a surveillance system for The Gambia with two primary objectives: (1) to identify trends in the proportion of pneumococcal isolates as a percentage of all positive cultures from blood, CSF and other normally sterile fluids; (2) to identify trends in the proportion of vaccine serotypes among all pneumococcal isolates from blood, CSF, and other normally sterile fluids.

The surveillance system is a collaboration between the Government of The Gambia and the Medical Research Council. It will be centralised and coordinated by government after 2 years. A project steering group comprising three project leaders and key advisors has been established. There will be five key study sites: (1) Basse (Government health centre, MRC Laboratory); (2) Bansang (Government hospital and laboratory); (3) MRC Fajara (hospital and laboratory); (4) Farafenni (Government hospital and laboratory); (5) Royal Victoria Teaching hospital (RVTH; Government hospital and Laboratory).

The key health events under surveillance will be pneumonia and meningitis. A surveillance manual will clearly define indications for investigation of patients with these clinical entities, or with suspected bacteraemia, and standard operating procedures for specimen taking, transportation, laboratory processing and information systems. The MRC laboratory and the RVTH national laboratory will serve as reference laboratories for the surveillance system, receiving isolates from all the other laboratories for serotyping and antibiotic resistance patterns. Quality control will be conducted by collaboration between the two laboratories. We will engage independent auditing on an annual basis for the surveillance system as a whole. Full data collection will commence in early 2006 and will be for five years in the first instance.

Impact on respiratory tract infections of heptavalent pneumococcal conjugate vaccine (Pcv-7) administered at 3, 5, and 11 months of age

Esposito, S1, Lizioli, A1, Lustrico, A1, Faelli, N1, Rognoni, A1, Gualtieri, L1, Cesati, L1, Carreri, V2, Principi, N1
1Institute of Pediatrics, University of Milan, Fondazione IRCCS “Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena”, Milan, Italy
2Department of Health Sciences, Regione Lombardia, Milan, Italy

It has recently been demonstrated that a simplified administration schedule based on two Pcv-7 doses at 3 and 5 months of age, and a booster dose at 11-12 months, can be as immunogenic as the traditional four-dose schedule (three doses at 2, 4 and 6 months, with a booster at 12-15 months). However, as it is important to demonstrate its effectiveness in clinical practice, this study was designed to evaluate the impact of Pcv-7 administered at 3, 5 and 11 months on respiratory tract infections in very young children. In this multicentre, prospective, single-blind trial, 1,571 healthy infants (910 males) aged 75-105 days (median 82 days) were enrolled to receive a hexavalent vaccine and Pcv-7 (n=819) or the hexavalent vaccine alone (n=752) at 3, 5 and 11 months of age. All the children received their first vaccine doses between 1 September and 31 December 2002; morbidity of the study population was recorded for the 24 months after the second dose.

A total of 1,555 subjects (98.9%) completed the study. At least one episode of AOM was diagnosed in 478 children in the Pcv-7 group (58.9%) and 499 in the control group (67.1%), for a mean number of episodes/100 child-years of respectively 637 and 698 (RR: 0.83; 95% CI: 0.61-1.02; p=0.02). There were statistically significant between-group differences in the incidence of AOM in each half-year period of follow-up except the first. Recurrent AOM (defined as ≥3 episodes in six months or ≥4 in one year) was reported in 29 children receiving Pcv-7 (3.5%) and in 43 controls (5.8%) (RR: 0.62; 95% CI: 0.38-0.99; p=0.044). CAP was significantly less frequent in the Pcv-7 group (RR: 0.35; 95% CI: 0.22-0.55; p=0.0001) during the follow-up as a whole, and during the last period of follow-up. The total number of prescribed antibiotic courses was 2,020 in the Pcv-7 group and 2,079 in the control group, for a mean number of courses/100 child-years of respectively 124 and 139 (RR: 0.89; 95% CI: 0.83-0.94; p=0.0001). Pharmacoeconomic analysis showed that Pcv-7 led to an economic saving of 15 euros for each vaccinated subject.

These results show the significant clinical impact of Pcv-7 administered using the simplified schedule of only three doses at 3, 5 and 11 months in terms of non-invasive morbidity, leading to relevant economic advantages.
Immunogenicity of pneumococcal 23-valent polysaccharide and 7-valent conjugate vaccines used in combination in adults 55-70 years old

Miernyk K1, Bulkow L2, Butler J3, Singleton R4, Zanis C5, Dentinger C6, Peters H7, Hennessy T8, Hickel J9, Knutsen B10, Parkinson A10.

1Alaska Native Tribal Health Consortium, 2Arctic Investigations Program/Centers for Disease Control and Prevention, 3Southcentral Foundation, Anchorage, AK, USA.

Background: The value of using pneumococcal conjugate vaccines in adults has not been defined. One proposed use is to prime the immune system with conjugate vaccine and later boost with a dose of polysaccharide vaccine. We studied this strategy and here present data on the immunogenicity of using these two vaccines in combination.

Methods: We randomized immunocompetent adults 55-70 years old who had not previously received any pneumococcal vaccination into 3 groups: Group 1 (n=28) received 0.5mL pneumococcal polysaccharide vaccine (PPV23; Pnu-Immune-23®; Wyeth-Lederle Vaccines); Group 2 (n=29), 0.5mL 7-valent CRM197 pneumococcal conjugate vaccine (PCV7; Prevnar™; Wyeth-Lederle Vaccines) followed two months later by 0.5mL PPV23, and Group 3 (n=29), 0.5mL PCV7 followed six months later by 0.5mL PPV23. We collected sera at enrollment and 2 weeks and 2 months after each vaccination. Sero type-specific IgG and antibody avidity for pneumococcal serotypes (ST) 1, 4, 6B, 14, and 19F were determined by ELISA with pre-absorption with cell wall polysaccharide and ST22F. Sera from the first 10 persons recruited into each group were further analyzed for opsonophagocytic activity (OPA).

Results: Geometric mean IgG concentrations (GMC), median OPA titers, and geometric mean fold increases from baseline (GMFI) 2 months after PPV23 vaccination are in the table. GMC, median OPA titers, and GMFI do not differ between the 3 study groups after PPV23 vaccination (p>.095 for all) with the exception of the serotype-specific IgG GMFI to ST19F (p=.029, 3 way comparison).

<table>
<thead>
<tr>
<th>Serotype-Specific IgG</th>
<th>GMC (ug/mL)</th>
<th>GMFI</th>
<th>Median</th>
<th>GMFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>2.0</td>
<td>5.5</td>
<td>16</td>
<td>2.5</td>
</tr>
<tr>
<td>Group 2</td>
<td>1.7</td>
<td>3.9</td>
<td>12</td>
<td>4.9</td>
</tr>
<tr>
<td>Group 3</td>
<td>1.2</td>
<td>3.3</td>
<td>16</td>
<td>4.0</td>
</tr>
<tr>
<td>ST4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>0.8</td>
<td>5.5</td>
<td>96</td>
<td>9.9</td>
</tr>
<tr>
<td>Group 2</td>
<td>1.2</td>
<td>4.9</td>
<td>128</td>
<td>11.4</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.8</td>
<td>5.1</td>
<td>32</td>
<td>2.6</td>
</tr>
<tr>
<td>ST6B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>4.1</td>
<td>2.6</td>
<td>192</td>
<td>11.4</td>
</tr>
<tr>
<td>Group 2</td>
<td>4.2</td>
<td>2.5</td>
<td>320</td>
<td>34.5</td>
</tr>
<tr>
<td>Group 3</td>
<td>5.1</td>
<td>3.2</td>
<td>256</td>
<td>18.9</td>
</tr>
<tr>
<td>ST14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>6.6</td>
<td>2.5</td>
<td>384</td>
<td>16.0</td>
</tr>
<tr>
<td>Group 2</td>
<td>6.8</td>
<td>2.9</td>
<td>512</td>
<td>16.0</td>
</tr>
<tr>
<td>Group 3</td>
<td>11.1</td>
<td>4.9</td>
<td>128</td>
<td>4.5</td>
</tr>
<tr>
<td>ST19F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>5.1</td>
<td>2.1</td>
<td>512</td>
<td>9.9</td>
</tr>
<tr>
<td>Group 2</td>
<td>6.6</td>
<td>2.8</td>
<td>512</td>
<td>39.3</td>
</tr>
<tr>
<td>Group 3</td>
<td>8.1</td>
<td>4.6</td>
<td>384</td>
<td>11.4</td>
</tr>
</tbody>
</table>

Antibody avidity did not change significantly after the majority of the vaccinations.

Conclusion: A priming dose of PCV7 given 2-6 months earlier did not improve the immune response to PPV23 among immunocompetent adults 55-70 years old.
Immunogenicity following one, two, or three doses of the 7-valent pneumococcal conjugate vaccine and booster response of the 23-valent pneumococcal polysaccharide vaccine at 12 months of age


1Centre for International Child Health, Department of Paediatrics, University of Melbourne, Victoria, Australia
2Murdoch Children’s Research Institute, Royal Children’s Hospital, Melbourne, Victoria, Australia
3Fiji Pneumococcal Project, Ministry of Health, Fiji
4Fiji School of Medicine, Suva, Fiji
5University of Melbourne
6London School of Health and Tropical Medicine, London, United Kingdom

Background: The aim of this study is to find a safe and epidemiologically appropriate pneumococcal vaccination strategy for resource poor countries. A single, blind open-label randomized Phase II study is underway in Fiji documenting the safety, immunogenicity and impact on pneumococcal carriage of various pneumococcal vaccination regimens combining 1, 2, or 3 doses of 7-valent pneumococcal conjugate vaccine (PCV) in infancy followed by a single booster dose of 23-valent pneumococcal polysaccharide vaccine (PPS). In the original study infants were to receive the PPS at 6 or 9 months of age. However, due to concerns about safety the timing was changed and half the infants are now randomized to receive PPS at 12 months of age. Two control groups, one administered PPS at 12 months and the other no PCV or PPS at 12 months are included. All infants will receive a small dose of PPS at 18 months old to mimic a natural challenge and assess immunological responsiveness. The objective is to demonstrate non-inferiority at 19 months of age, in those groups receiving PPS at 12 months and those who do not with respect to the proportion of children in each group, with a satisfactory immune response. We will present preliminary results of the first 228 infants comparing the geometric mean antibody concentrations (GMC) by ELISA at 12½ months of age following a booster of PPS at 12 months of age following 1, 2, or 3 dose primary series of PCV.

Methods: Fijian infants presenting at 6 weeks of age for their first DTP immunization were recruited in Suva, Fiji and were stratified by ethnicity at randomization. They received 0, 1, 2, or 3 doses of PCV (Prevnar®, Wyeth Vaccines) at 6, 10, and 14 weeks of age. At 12 months of age, half the infants received a single dose of PPS (Pneumovax®, Merck and Co., 25µg/serotype). Blood tests were taken at 18 weeks and pre/post PPS.

Results: Data analysis is currently underway and results will be presented at the meeting. Results will be presented in terms of the proportion of infants with serotype specific IgG titres >0.35 µg/mL at 18 weeks of age, persistence of titres at 12 months of age, response to the PPS booster dose, and the effect of prior exposure to PCV on the booster response.

Inferior humoral response in elderly versus young adults to the 23-valent polysaccharide vaccine


1GlaxoSmithKline Biologicals, Rixensart, Belgium
2Centre for Vaccinology, Ghent University Hospital, Ghent, Belgium
3Unité de Pharmacologie Clinique, St Luc Hospital, Brussels, Belgium

Background: Streptococcus pneumoniae remains a major cause of morbidity and mortality, especially in the elderly population. Contradictory results were reported in the literature on the immunological response to pneumococcal vaccination in elderly versus adults: the humoral response recorded in elderly was either lower or comparable to that in adults. Clarification is needed.

Objective: Compare the immunological responses after 23-valent pneumococcal polysaccharide vaccination (23vPS) (Pneumovax®) in young adults versus elderly using two highly standardized assays.

Methods: Two parallel studies were conducted in Belgium, one in young adults (18-45 years) and one in elderly (>65 years) (N=25/group) who were not previously vaccinated against S. pneumoniae. All subjects were administered a single dose of the 23vPS. Blood samples were collected at 1-month post-vaccination. Serum anti-pneumococcal IgG concentrations for 11 serotypes (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F) among the 23 included in the vaccine were measured by a ELISA method that included a pre-adsortion step with serotype 22F polysaccharide in order to increase the specificity of the assay (cut-off 0.05µg/ml). Opsonophagocytic activity (OPA) against the same 11 serotypes was measured by an assay based on the killing of pneumococci by opsonophagocytosis. The results are expressed as a titre (dilution) of serum able to kill 50% viable pneumococci [cut-off is an opsonic titer of eight (1:8)].

Results: ELISA results showed that the antibody levels were significantly lower (Wilcoxon test) in the elderly group for 4 of 11 serotypes measured (1, 3, 4, 14; p-value <0.05), when compared to the young-adult group. Differences in functional activity of the antibodies were even more pronounced. OPA geometric mean titres (GMTs) were significantly lower for 7 of 11 serotypes analysed (1, 4, 6B, 7F, 9V, 14, 18C; p-value <0.05) for the elderly group compared to the young-adult group. For three serotypes (1, 4, 9V), OPA GMTs were found to be approximately 10-fold lower in the elderly versus young adults.

Conclusion: These results, particularly the OPA responses, indicate that upon 23vPS vaccination both the quantity and quality of anti-polysaccharide antibodies are inferior in the elderly as compared to young adults. New vaccine development approaches should aim at improving the immunological profile in elderly, especially by restoring the functionality of the antibodies elicited upon vaccination.
Specificities of immune responses against a serotype 3 pneumococcal conjugate

**Schuerman, L**, Prymula, R, Poolman, J

1GlaxoSmithKline Biologicals, Rixensart, Belgium
2Department of Epidemiology, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

Results from the double blind randomized controlled efficacy trial (POET) conducted with a novel 11-valent protein D pneumococcal conjugate vaccine (11Pn-PD) and hepatitis A vaccine as placebo in 4968 infants in the Czech Republic and Slovakia, demonstrated significant protective efficacy against acute otitis media (AOM) episodes regardless of their aetiology (vaccine efficacy; VE: 33.6% [95%CI: 20.8, 44.3]), as well as against AOM episodes caused by vaccine pneumococcal serotypes (VE: 57.6% [95%CI: 41.4, 69.3]) and non-typeable *Haemophilus influenzae* (NTHi) (VE: 35.3% [95%CI: 1.8, 57.4]). Surprisingly, no protection could be demonstrated against AOM episodes due to serotype 3 pneumococci, despite a sufficient number of episodes (20 episodes in the 11Pn-PD group versus 17 episodes in the control group, VE: -17.1% [95%CI: -126.5, 39.5]). Before the 11Pn-PD booster dose, 6 subjects recorded a serotype 3 AOM episode versus 8 in the control group (VE: 25.1% [95%CI: -116.2, 74.0]), whereas 13 subjects recorded a first serotype 3 AOM episode after the 11Pn-PD booster dose versus 9 subjects in the control group (VE: -43.3% [95%CI: -235.8, 38.9]). Despite the relatively high post-primary anti-polyaccharide ELISA antibody levels against serotype 3, opsonophagocytic activity appeared low and difficult to measure. Unlike the other pneumococcal vaccine serotypes, antibody concentrations measured in POET against serotype 3 polysaccharide following a 4th consecutive 11Pn-PD dose at 12-15 months of age (2.84 µg/ml) remained below the post-primary antibody levels (3.78 µg/ml). Similar observations were previously reported by Finnish investigators (Nurkka et al, PIDJ 2004;23(11):1008-14), who in addition observed that serotype 3 antibody responses elicited by a first 11Pn-PD vaccine dose given in the second year of life exceeded those following a 4th consecutive dose given at the same age to children previously primed with three 11Pn-PD doses in the first year of life. It is intriguing that the lack of protective efficacy against serotype 3, that seems even more emphasized after the booster dose, coincides with the reduced boostability for the same serotype. Although plain polysaccharide boostability for serotype 3 seemed not impaired following primary and booster vaccination with the 11Pn-PD vaccine, these results have led GSK to remove serotype 3 from its final paediatric pneumococcal conjugate vaccine formulation.

The immunogenicity of a nine-valent pneumococcal conjugate vaccine in Gambian children under one year of age


1Pneumococcal Vaccine Trial (PVT), MRC Laboratories, Banjul, The Gambia
2The London School of Hygiene and Tropical Medicine, United Kingdom
3University College London, United Kingdom
4Wyeth Lederle Vaccines, PA, USA

**Background:** *Streptococcus pneumoniae* causes pneumonia, septicaemia, meningitis and death, particularly in developing countries. We showed in a double blind, placebo-controlled Pneumococcal Vaccine Trial (PVT) that a 9-valent pneumococcal conjugate vaccine (PCV, Wyeth Lederle Vaccines PA, USA) containing serotypes 1, 4, 5, 6B, 9V, 14, 19F and 23F had high efficacy against pneumococcal infections and also reduced all-cause admissions and mortality, in Gambian children.

**Aims:** In this sub-study, we evaluated vaccine immunogenicity and assessed the suitability of the WHO recommended ELISA technique for use in pneumococcal serology in Africa.

**Methods:** In 2002, some 212 children living near Basse were recruited after obtaining informed consent. A venous blood sample was obtained one month after the third dose of PCV or placebo had been given for estimation of type specific, capsular polysaccharide anti-pneumococcal IgG antibodies in sera, using a standard ELISA technique. Training and quality control support for the ELISA analysis was provided by Prof. Goldblatt’s WHO pneumococcal serology reference laboratory at University College Hospital, London.

**Results:** Geometric mean IgG antibody concentrations for all nine serotypes were substantially increased in the vaccine group compared to the placebo group. In the placebo group, GMCs were below 0.1 mcg/ml for each serotype. The proportion of children attaining levels over 0.35 mcg/ml was over 92% for each serotype, but geometric mean antibody concentrations varied between serotypes, being highest for types 14, 19F and 6B polysaccharides. Responses were similar in boys and girls with little variation by ethnic group. Functional antibody responses to selected serotypes will be compared in future with results of this ELISA.

**Conclusions:** The standardised ELISA is useful for measuring the response to pneumococcal conjugate vaccine in Africa. The vaccine was highly immunogenic for all serotypes in the study population. Variation in protection against different serotypes is being studied further.
Functionality of antibodies against serotypes 6A and 19A induced by three different pneumococcal conjugate vaccines (PCV) in infants

National Public Health Institute, Helsinki, Finland

Aim: To describe functionality of antibodies to serotypes 6A and 19A induced by three different PCVs containing serotype 6B and 19F conjugates.

Methods: We determined opsonophagocytic activity (OPA) of antibodies to serotype 6A, 6B, 19A and 19F strains after vaccination with PncCRM (Wyeth; N=29-30), PncOMPC (MerckSharp&Dohme; N=29-30) or 11PnPd (GSK; N=25-26). PCVs were given to Finnish infants at 2, 4, 6 and 12-15 months in consecutive vaccine trials. Ratios of the number of samples taken after three and four doses of PCVs were 2:1, except for serogroup 6 in PncOMPC group 1:9. Samples were selected to represent similar antibody concentrations in vaccine groups for 6B or 19F as measured by EIA without 22F. In OPA assay routinely used 6A, 6B, 19A and 19F strains and mutant RX1 strains transformed to express either 6A, 6B, 19A or 19F capsule (J. Paton, Adelaide, Australia) were used. IgG concentrations were reanalysed by 22F EIA. All assays were done in parallel.

Results: Geometric mean IgG concentrations in the PncCRM, PncOMPC and 11PnPd group were 4.09, 3.47 and 4.66 µg/ml for 6B, 0.60, 0.44 and 0.79 µg/ml for 6A, 9.26, 9.32 and 11.87 µg/ml for 19F and 0.66, 0.45 and 0.42 µg/ml for 19A, respectively. The geometric mean OPA titers (the reciprocal of the serum dilution with 50% killing) and percentages of sera with a titer $\geq 8$ were:

<table>
<thead>
<tr>
<th>PCV</th>
<th>6B</th>
<th>6A</th>
<th>19F</th>
<th>19A</th>
</tr>
</thead>
<tbody>
<tr>
<td>PncCRM</td>
<td>DS2212-94</td>
<td>RX1-6B</td>
<td>RHI4211</td>
<td>RX1-6A</td>
</tr>
<tr>
<td></td>
<td>121 (100)</td>
<td>126 (100)</td>
<td>10 (43)</td>
<td>12 (53)</td>
</tr>
<tr>
<td>PncOMPC</td>
<td>93 (100)</td>
<td>76 (97)</td>
<td>7 (30)</td>
<td>6 (23)</td>
</tr>
<tr>
<td>11-PnPd</td>
<td>204 (100)</td>
<td>258 (100)</td>
<td>18 (63)</td>
<td>19 (63)</td>
</tr>
</tbody>
</table>

There were no clear differences in the ability of 6B conjugates to induce anti-6A antibodies capable to mediate killing of 6A strains. The functional activity against 19A strains was highest in the 11PnPd group. From the sera with <10 µg/ml of anti-19F, 2/23, 2/22 and 8/11 had an OPA titer $\geq 8$ against 19A strain DB18, and 3/23, 1/22 and 7/11 for RX1-19A strain in the PncCRM, PncOMPC and 11PnPd group, respectively.

Conclusions: This preliminary analysis suggests that there can be differences in the ability of different PVCs to induce functional cross reactive antibodies.

Impact of 7vPCV and 23vPPV booster in eligible children in the Northern Territory of Australia: Impressive, but not the total answer

Krause, VL, Cook, H, Selvey, CE
1 Centre for Disease Control, Northern Territory Department of Health and Community Services, Darwin, Northern Territory, Australia

Introduction
Exceedingly high rates of invasive pneumococcal disease (IPD) in Northern Territory (NT) Indigenous children and very high rates in Central Australian non-Indigenous children led to the introduction of 7-valent pneumococcal conjugate vaccine (7vPCV) at 2, 4 and 6 months for these groups in June 2001. Indigenous children received a 23-valent polysaccharide pneumococcal vaccine (23vPPV) booster at 18 months.

Aim
To document the impact on IPD of the childhood pneumococcal vaccination program in the NT.

Methods
Enhanced data was prospectively collected on all NT IPD cases since 1994 including demographics, clinical presentation, risk factors, outcome, vaccine status, serotypes and susceptibility testing of isolates. Impact was evaluated with 1994-2000 as pre-vaccine (preV) and 2002-2005 as post-vaccine (postV) years.

Results
IPD in vaccine eligible (VE) children under 2 years fell from 598 per 100,000 preV to 219 postV; a reduction of 63% (p<.001). IPD rates preV and postV due to 7vPCV serotypes (7VT) decreased from 383 to 36 per 100,000 (p<.001) while a slight decrease seen in non-7VT IPD rates was not significant (215 to 182 per 100,000) with no increase in severe or unusual IPD presentations. IPD due to 7vPCV related serotypes e.g., 6A, 19A, in VE children under 2 showed no significant reduction. The predominant serotypes preV were 14, 6B and 23F and postV were 12F and 16F. Overall the IPD rate in those 50 years and over did not fall significantly, however the rate of IPD due to 7VT fell from 28 to 5 per 100,000 (p=.008) in this age group. IPD isolates with reduced penicillin susceptibility from VE children under 2 years declined from an average of 6 cases per year preV to less than 1 postV. Other age groups showed no significant reduction in reduced penicillin susceptibility.

Conclusions
The targeted childhood pneumococcal vaccine program has contributed to a 63% reduction in IPD in VE children under 2 years. Additionally there has been a significant reduction in 7VT IPD in those 50 years and over. IPD with reduced penicillin susceptibility has vanished for the present time in < 2 year olds. IPD due to non-7VT remains quite high. The possibility of serotype replacement demands continued surveillance with attention to drug susceptibility profiles and severity of clinical presentation.
Elevated and persistent functional antibody responses in adults ≥65 years of age after a second dose of a 23-valent pneumococcal polysaccharide vaccine

Manoff, S1, Liss, C1, Gaulfield, M2, Marchese, R1, Silber, J1, Boslego, J1, Romero-Steiner, S1 Rajam, G1, Glass, N1, Whitney, CG2, Carlone, GM2 and the Pneumococcal Revaccination Study Group

1Merck Research Labs, West Point, PA, USA
2Center for Disease Control and Prevention, Atlanta, GA, USA

Background: Streptococcus pneumoniae is the major cause of pneumonia in older adults. Vaccination with a 23-valent pneumococcal polysaccharide vaccine (PPV23) is recommended among persons ≥65 years old. We questioned if a second dose of PPV23 would be immunogenic in this population.

Methods: 1008 subjects in two age groups (50-64 years, ≥65 years) were enrolled who either: (1) received their first vaccination with PNEUMOVAX® 23 during the study (primary vaccination group); or (2) had a documented PPV23 vaccination (3-5 years previously) and were revaccinated with PNEUMOVAX® 23 as part of this study (revaccination group). Antibody responses at day 0 (baseline), and at day 30 and year 5 (postvaccination) were measured in a subset of 120 primary vaccination and revaccination subjects aged ≥65 years by using two assays: (1) an EIA that detects IgG antibody to pneumococcal serotypes 3, 4, 14, and 23F and (2) an opsonophagocytic killing (OPK) assay to measure functional antibody to pneumococcal (Pnc) serotypes 4, 14, and 23F.

Results: Serotype-specific anti-pneumococcal IgG responses were the same for the primary vaccination and revaccination groups for serotypes 3 and 23F (ratio \(\approx 1.0\)) and lower in the revaccination group for serotypes 4 and 14 (ratio \(\approx 0.7\)). OPK geometric mean titers (GMTs) for serotypes 4, 14, and 23F following revaccination were lower but not significantly different than values for primary vaccination. Postvaccination (Day 30) and year 5 EIA and OPK responses in both study groups were higher than those observed in unvaccinated subjects.

Serotype-specific antibody responses at day 30 after primary vaccination or revaccination with PPV23

<table>
<thead>
<tr>
<th>Serotype</th>
<th>EIA (µg/mL)</th>
<th>OPK (GMT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary Vaccination</td>
<td>Revaccination</td>
</tr>
<tr>
<td>3</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>4</td>
<td>3.9</td>
<td>2.6</td>
</tr>
<tr>
<td>14</td>
<td>14.7</td>
<td>10.2</td>
</tr>
<tr>
<td>23F</td>
<td>4.6</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Conclusions: A second dose of PPV23 is immunogenic in older adults. Revaccination leads to increased antibody levels that remain elevated relative to baseline for 5 years postvaccination.
Previous pneumococcal polysaccharide vaccine impacts immune response to subsequent pneumococcal conjugate vaccine in the elderly  

*de Roux, A,1 Schmöle-Thoma, B1, Ahlers, N1, Siber, G1, Gruber, W1, Lockhart, S1, Fernsten, P1, Baker, S1, Welte, T1, Lode, H1*

1Dept. of Chest and Infectious Diseases; HELIOS Klinikum Emil von Behring, Berlin, Germany  
2Wyeth Vaccines Research, Wyeth Pharma GmbH, Münster, Germany  
3Wyeth Vaccines Research, Wyeth Pharmaceuticals, Pearl River, USA

**Background:** The pneumococcal conjugate vaccine (7vPnC) is known to induce a superior immune response compared to the polysaccharide vaccine (23vPS) in adults. This study examined the immunogenicity of the conjugate vaccine administered before or after the polysaccharide.

**Methods:** In an open label study 217 elderly aged ≥70 years without prior pneumococcal immunization were equally randomized to receive either 7vPnC (Prevenar® 2µg saccharide / dose, except 4µg for 6B, Wyeth), or 23vPS (Pneumovax®23, 25µg saccharide/dose, Aventis Pasteur MSD). After one year 23vPS recipients received 7vPnC and 7vPnC recipients received 23vPS. Blood samples were obtained prior to and one month post vaccination. Antibody responses (GMCs) were measured by ELISA.

**Results:** In subjects vaccinated with 7vPnC, GMCs of all serotypes were superior to 23vPS except 19F (non inferior). After one year 7vPnC/23vPS recipients had higher GMCs (point estimates) compared to 23vPS alone. 23vPS/7vPnC recipients had lower GMCs compared to 7vPnC alone.

<table>
<thead>
<tr>
<th>Serotypes</th>
<th>N</th>
<th>4</th>
<th>6B</th>
<th>9V</th>
<th>14</th>
<th>18C</th>
<th>19F</th>
<th>23F</th>
</tr>
</thead>
</table>
| One month after dose 1  
7vPnC | 110 | 3.1 (2.2-4.3) | 8.0 (6.0-10.8) | 9.8 (7.5-12.8) | 17.1 (12.3-24.0) | 13.0 (10.1-16.7) | 5.5 (4.1-7.4) | 12.4 (9.0-17.0) |
| 23vPS | 107 | 1.4 (1.1-2.0) | 4.4 (3.4-5.8) | 3.6 (2.8-4.6) | 8.5 (6.0-12.1) | 6.8 (5.2-8.9) | 4.4 (3.4-5.8) | 3.8 (2.9-5.0) |
| One month after dose 2  
7vPnC/23vPS | 36 | 2.0 (1.2-3.5) | 5.4 (3.3-9.0) | 5.7 | 14.5 | 7.6 | 8.4 | 7.4 |
| 23vPS/7vPnC | 78 | 0.9 (0.6-1.3) | 2.2 (1.5-3.2) | 3.0 (2.2-4.0) | 6.7 (4.5-9.9) | 5.1 (3.7-6.8) | 2.1 (1.5-3.0) | 3.0 (1.9-4.8) |

**Conclusions:** In vaccine naïve subjects ≥70 years 7vPnC induces a superior immune response to 23vPS for six of seven 7vPnC serotypes after one year and increases antibody response to subsequent 23vPS. However, initial 23vPS leads to decreased antibody responses to subsequent 7vPnC.
**Functional activity of antibodies against serotype 19F evoked by pneumococcal conjugate vaccines**

**Soininen, A, Lehtonen, H, Lahdenkari, M, Käyhty, H**  
National Public Health Institute, Helsinki, Finland

**Background:** The pneumococcal conjugate vaccines (PCVs) used in the FinOM Vaccine Trial had modest efficacy against AOM caused by serotype 19F in spite of excellent immunogenicity as measured by antibody concentration. It is possible that the inconsistent efficacy is due to deficiency in the function of the anti-19F antibodies. We describe here whether the opsonophagocytic activity (OPA) against 19F evoked by PCVs with different conjugation chemistries and carrier proteins varies, or whether OPA is generally poor against 19F.

**Methods:** Finnish infants had received either 7-valent PncCRM (Wyeth), 7-valent PncOMPC (Merck), 11-valent PncT/D (Sanofi Pasteur), or 11-valent PncPD (GSK Biologicals) at 2, 4, 6, and 12-15 months of age in separate studies. The PncT/D vaccine was given either with (PncT/D alum) or without (PncT/D) aluminium hydroxide adjuvant. Concomitantly with the Pnc vaccines, the infants received routine childhood vaccines as follows; the PncT/D groups received DTwP-IPV/PRP-T at 2, 4, 6, and 12 mo, the PncPD group received DTaP-IPV/PRP-T at 2, 4, and 6 mo, and the PncCRM and PncOMPC groups received DTwP-HbOC at 2, 4, 6, and 24 mo, and IPV separately at 7 and 12 mo. Serum samples obtained at 7 and at 13-16 months of age were analysed in parallel and randomly for IgG antibody concentration (µg/ml) to 19F by 22F EIA and for OPA against 19F pneumococci by killing assay.

**Results:**

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>7mo</th>
<th>13mo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>GMC</td>
</tr>
<tr>
<td>PncCRM</td>
<td>46</td>
<td>3.67  (2.79-4.82)</td>
</tr>
<tr>
<td>PncOMPC</td>
<td>46</td>
<td>4.60  (3.39-6.24)</td>
</tr>
<tr>
<td>PncT/D</td>
<td>60</td>
<td>5.81  (4.30-7.85)</td>
</tr>
<tr>
<td>PncT/D alum</td>
<td>60</td>
<td>5.96  (4.30-8.26)</td>
</tr>
<tr>
<td>PncPD</td>
<td>48</td>
<td>5.18  (4.04-6.64)</td>
</tr>
</tbody>
</table>

**Conclusion:** After three doses, but not after the fourth dose, the EIA/OPA ratio reflecting the functionality of the antibodies differed between the vaccines. Almost all sera had positive OPA, but the titers remained low irrespective of high concentrations. Antibodies to 19F have in general low functional capacity, with slight differences between the vaccines.
A case-control study to investigate opsonophagocytic activity (OPA) of antipneumococcal antibodies as a serological correlate of protection against acute otitis media (AOM)

Ekstrom N, Lehtonen H, Käyhty H, Kilpi T, Jokinen J, and The FinOM Study Group
National Public Health Institute, Helsinki, Finland

**Background:** For licensing of new pneumococcal vaccines it is important to predict their protective efficacy on the basis of immunogenicity. Based on animal models, both concentration and OPA of antibodies relate with protection against pneumococcal diseases. The antibody concentration EIA is considered the golden standard; it is better standardised, more sensitive and easier to perform than OPA. The aim of this study was to investigate whether OPA measured from sera of children vaccinated with pneumococcal conjugate vaccine (PCV) provides valuable additional information relating to vaccine efficacy against AOM.

**Methods:** This nested case-control study was part of the FinOM Vaccine Trial that evaluated the efficacy of two 7-valent PCVs (PncCRM and PncOMPC). 1666 children were immunized with PCV at 2, 4, 6 and 12 months of age. Serum samples were obtained at 7 or 13 months of age for determination of IgG concentration by EIA and OPA by the standardised killing assay. Events of AOM were identified, and middle ear fluid was cultured for pneumococci. The cases were children with type-specific AOM during 7-12 or 13-24 months of age. The controls were sampled as nested within the cohort; for each AOM-case 5 controls were selected from children who were at risk at that moment, which allows the estimation of relative rate (RR) using stratified Cox regression. Controls were also matched with the cases according to their gender and vaccination status. The precision of this study is 1.1 times poorer compared to the full cohort study. Because of the low number of cases for other serotypes only serotypes 19F and 23F were included in the analysis.

**Results:** An association between OPA and risk of AOM was found, excluding anti-19F induced by PncCRM. OPA correlated with antibody concentration. The effect of a 10-fold increase in antibody concentration or OPA titer at 7 or 13 months of age on the risk of serotype-specific AOM for immunized children (RR, 95% CI):

<table>
<thead>
<tr>
<th></th>
<th>PCV (AOM cases, N)</th>
<th>PncCRM (15)</th>
<th>PncOMPC (13)</th>
<th>PncCRM (11)</th>
<th>PncOMPC (14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>0.81 (0.23-2.81)</td>
<td>0.26 (0.08-0.82)</td>
<td>0.31 (0.10-0.98)</td>
<td>0.42 (0.18-1.00)</td>
<td></td>
</tr>
<tr>
<td>OPA</td>
<td>1.03 (0.38-2.83)</td>
<td>0.19 (0.05-0.69)</td>
<td>0.56 (0.27-1.18)</td>
<td>0.40 (0.15-1.07)</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions:** In agreement with strong correlation between antibody concentration and OPA, the effect of 10-fold increase in antibody concentration or OPA titer to the risk of AOM was very similar. The results confirm that antibody concentration can be regarded as the primary correlate of protection against AOM. However, OPA is useful when evaluating serotype specific differences or antibody responses in different populations e.g HIV-infected.
Pneumococcal vaccination and nasopharyngeal bacterial carriage in Australian Aboriginal infants

Mackenzie GA1,2,3, Carapetis JR1, Leach AJ4, Morris PS1,2,4, Wigger C, Tipakalippa P, Hare K, Kennedy M1, Beissbarth J, Stubbs E2
1Menzies School of Health Research, Darwin, Northern Territory, Australia
2Flinders University, Adelaide, South Australia, Australia
3Centre for International Child Health, Department of Paediatrics, University of Melbourne & Murdoch Children’s Research Institute, Royal Children’s Hospital, Melbourne, Victoria, Australia
4Charles Darwin University, Institute of Advanced Studies, Darwin, Northern Territory, Australia

Background Australian Aboriginal infants acquire respiratory carriage of bacterial pathogens in the first weeks of life with over 80% carriage in infants and young children. Tympanic membrane perforations affect 20% of young children living in remote communities.

Aim: To assess how pneumococcal vaccination might affect carriage of S. pneumoniae and other pathogens and to describe the clinical implications?

Methods Children from 3 communities in the Northern Territory of Australia had monthly clinical assessments and nasopharyngeal swabs from birth to 2 years of age. Comparisons were made between those receiving 7-valent pneumococcal conjugate (2, 4, 6 months) and booster 23-valent polysaccharide vaccine (18 months) (2001-2004) and an historical unvaccinated cohort (1996-2000, randomised trial amoxycillin versus placebo for middle ear effusion). Comparison group data were included if infants were not receiving randomised amoxycillin therapy. Study procedures were the same for both groups with similar examination schedules.

Findings There were 103 comparison subjects, 51 randomised to placebo, and 99 vaccinees. Pneumococcal vaccination was associated with delayed acquisition of S. pneumoniae and reduced carriage of S. pneumoniae at 1-3 and 7-10 months of age. Vaccination was associated with delayed acquisition of vaccine-type (VT). Reduced VT carriage was greatest at 7-10 months, and less at 12-17 months. Acquisition of non-vaccine types (NVT) was earlier among vaccinees and the difference evident at 12-17 months, before administration of 23PPV, was not evident at 18-24 months. Carriage of serotypes 6B and 23F was reduced in vaccinees, carriage of 16F, 19A, and non-typeable isolates was increased. Carriage of H. influenzae and M. catarrhalis was unaffected. Proportions of the two groups experiencing severe otitis were similar. Vaccination was associated with reduced risk of multiple perforations, isolation of VT and serotype 6B, and increased isolation of VR from new perforation discharge.

<table>
<thead>
<tr>
<th>Comparison</th>
<th></th>
<th>Vaccines</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (days) pneumococcal acquisition (75th percentile)</td>
<td>59 (53, ∞)</td>
<td>90 (75, 97)</td>
<td>n/a</td>
</tr>
<tr>
<td>Pneumococcal carriage at 1-3 months</td>
<td>71% (47/66)</td>
<td>47% (40/86)</td>
<td>0.35 (0.17, 0.76)</td>
</tr>
<tr>
<td>Pneumococcal carriage at 7-10 months</td>
<td>80% (44/55)</td>
<td>65% (53/82)</td>
<td>0.38 (0.16, 0.93)</td>
</tr>
<tr>
<td>Age (days) vaccine-type acquisition (50th percentile)</td>
<td>72 (54, 124)</td>
<td>281 (165, ∞)</td>
<td>n/a</td>
</tr>
<tr>
<td>Vaccine-type carriage at 7-10 months</td>
<td>47% (26/55)</td>
<td>9% (7/82)</td>
<td>0.08 (0.03, 0.27)</td>
</tr>
<tr>
<td>Vaccine-type carriage at 12-17 months</td>
<td>56% (30/54)</td>
<td>25% (13/53)</td>
<td>0.19 (0.06, 0.62)</td>
</tr>
<tr>
<td>Age (days) non-vaccine type acquisition (25th percentile)</td>
<td>75 (45, 117)</td>
<td>50 (36, 67)</td>
<td>n/a</td>
</tr>
<tr>
<td>Non-vaccine type carriage at 12-17 months</td>
<td>11% (6/54)</td>
<td>45% (24/53)</td>
<td>5.43 (1.53, 19.3)</td>
</tr>
<tr>
<td>Non-vaccine type carriage at 18-24 months</td>
<td>26% (11/42)</td>
<td>33% (15/46)</td>
<td>0.86 (0.20, 3.70)</td>
</tr>
<tr>
<td>Proportion experiencing perforation</td>
<td>47% (24/51)</td>
<td>38% (37/97)</td>
<td>0.69 (0.33, 1.46)</td>
</tr>
<tr>
<td>Risk of multiple perforation</td>
<td>39% (20/51)</td>
<td>18% (17/97)</td>
<td>0.38 (0.17, 0.84)</td>
</tr>
<tr>
<td>Vaccine-type culture positive new perforation discharge</td>
<td>32% (8/25)</td>
<td>16% (5/32)</td>
<td>0.39 (0.09, 1.66)</td>
</tr>
<tr>
<td>6B culture positive new perforation discharge</td>
<td>20% (5/25)</td>
<td>6% (2/32)</td>
<td>0.27 (0.02, 1.87)</td>
</tr>
<tr>
<td>Vaccine-related type culture positive new perforation dis</td>
<td>8% (2/25)</td>
<td>19% (6/32)</td>
<td>2.65 (0.41, 28.9)</td>
</tr>
</tbody>
</table>

Interpretation Universal pneumococcal vaccination was associated with significant changes in VT and NVT carriage and small effects on the prevalence and incidence of severe otitis media. Indirect effects were reduced pneumococcal transmission to infants. Carriage of other pathogens was unaltered.
Implementation of a 7–valent pneumococcal vaccination program in Germany based on an at risk strategy

Arenz, S1, Kalies, H1, Toschke, AM1, Al-Lahham, A3, Siedler, A2, Reinert, RR1, von Kries, R1
1Ludwig-Maximilians-University of Munich, Institute for Social Pediatrics, Munich, Germany
2Robert Koch-Institute, Berlin, Germany
3National Reference Center for Streptococci, Institute of Medical Microbiology, RWTH-Aachen, Germany

A general recommendation for 7-valent pneumococcal vaccination (PCV7) in the US has prompted a dramatic decrease in the rate of invasive pneumococcal disease (IPD). Germany has opted for an ‘at risk’ only strategy defining risk by prematurity and a broad range of underlying diseases including immune defects and chronic diseases. Aim. To document the impact of vaccination on incidence of IPD in the target population. Methods: The incidence estimates for IPD and the assessment of risk factors were based on prospective active surveillance in laboratories and/or hospitals in the German population <16 years of age. The incidence estimates for the periods 7/2000 to 6/2001 were compared to those for 7/2001 to 6/2003. Adjustment for underreporting was made with the capture-recapture method. Premature birth accounted for most of the indications for PCV7 vaccination in the first six months of life, whereas most other indications emerged later in life. For children born after the recommendation, the proportion vaccinated at least once at 24 months was 8.3% (95% CI 6.4;10.6). The cumulative incidence of children with IPD estimated with the capture-recapture method was constant over the study period 7/2000 to 6/2003. The proportion of preterm infants among children with IPD decreased (not statistically significant). No changes over time for other risk factors for IPD were observed. Conclusion: The implementation of a 7–valent pneumococcal vaccination program based on an at risk strategy that targets only a minority of the children did not significantly impact the overall burden of IPD in Germany.

Long term effects of a 9-valent pneumococcal conjugate vaccine (PCV) on nasopharyngeal colonization with pneumococcal serotypes included in the vaccine

Adrian, PV1, van Niekerk, N1, Jones, S1, Cutland, CL1, von Gottberg, A1, de Gouveia, L1, Klugman, KP3, Madhi, SA1
1RMPRU, Chris Han Baragwanath Hospital, Johannesburg, South Africa.
2RMPRU, NICD, Johannesburg, South Africa.
3Emory University, Atlanta, GA, USA.

Background and aims: PCV has also been shown to reduce the overall incidence of nasopharyngeal colonization with pneumococci, and in particular, by serotypes included in the PCV. An association between reduction in nasopharyngeal carriage of vaccine serotypes and invasive pneumococcal disease has been demonstrated in children. Recent data suggest a loss of effect of PCV on vaccine type nasopharyngeal carriage in children over the age of two.

Methods: Children of mean age, 5.6 years (range 3.9-7.2) who had previously participated in a phase III PCV trial in Soweto, South Africa, and who had received three doses of PCV or placebo respectively, were offered a free booster dose from the study sponsor. A nasopharyngeal swab was collected prior to vaccination. Pneumococci that were identified from nasopharyngeal swabs were serotyped by the Quellung reaction.

Results: Pneumococci were isolated from 48% and 52% of the HIV uninfected children in the vaccinated (n=112) and placebo (n=139) groups respectively. Colonization with vaccine serotypes occurred in 8.9%, and 18.7% of children in the vaccinated and placebo groups respectively (p<0.05, two-sided Fishers exact test). Pneumococci were isolated from 71.9% and 74.0% of the HIV infected children in the vaccinated (n=32) and unvaccinated (n=50) groups respectively. In HIV infected children, there was no significant effect on colonization with vaccine serotypes. Vaccine serotypes occurred in 50% and 42% of the vaccinated and unvaccinated HIV infected groups respectively. HIV infected children had a significantly higher prevalence of colonization with vaccine serotypes than HIV uninfected children (p<0.0001) independent of PCV vaccination status.

Conclusions: PCV is effective in reducing the prevalence of colonization with vaccine serotypes, 5 years after vaccination in healthy children who have not received a booster dose. PCV has no effect on the prevalence of vaccine serotype carriage in HIV infected children.
Response to a conjugate pneumococcal vaccine administered at five years of age in the presence and absence of a primary series of three-doses of vaccine given during early infancy

Madhi, SA1, Adrian, P2, Jassat, W1, Kohler, M1, Jones, S1, Cutland, C1, Kawanda, L1, Klagman, KP1,2
1Respiratory and Meningeal Pathogens Research Unit, University of the Witswatersrand, Jhb, Gauteng, South Africa
2Rollins School of Public Health and Division of Infectious Diseases, School of Medicine, Emory University, Atlanta, GA

Background: The suitability of the recommendation of a single dose of conjugate pneumococcal vaccine (PCV) in unvaccinated children between 5-9 years of age if not previously vaccinated with PCV needs to be validated in HIV infected (HIV+) children. Objective: Determine the immune response at 5 years of age in HIV+ and HIV–uninfected (HIV-) children vaccinated with a single dose of PCV (i.e. newly vaccinated; [NV]), compared to children who were previously vaccinated (RV) with three doses of PCV at 6, 10 and 14 weeks of age.

Methods: A nested study was performed in HIV+ (N=83; 32 RV and 51 NV) and HIV- children (N=286; N=130 RV and 156 NV) participating in a PCV efficacy trial. Immune responses to the 7 pneumococcal serotypes included in Prevenar® were measured pre-immunization and one-month following vaccination using the 22F adsorbed EIA.

Results: The mean time (S.D.) of vaccination following the primary series of PCV was 5.4 (S.D.0.8) years. Pre-vaccination, the geometric mean antibody concentrations (GMC) was greater for all seven serotypes (P<0.001) in RV compared to NV HIV- children; but only for serotypes 6B, 14 and 23F (P<0.05) in RV HIV+ children. Among RV children, the GMC pre-vaccination was greater for all 7 serotypes in HIV- compared to HIV+ children (P=0.05); as well as for serotypes 9V, 18C and 23F in NV HIV- compared to HIV+ children.

Post-vaccination: Among HIV- children, GMCs were greater for all serotypes (P<0.0001), except serotype 19F [P=0.11]) in RV compared to NV children; and to only 2 serotypes (6B and 14) in RV compared to NV HIV+ children. HIV- children had higher GMCs (P<0.001 for all serotypes) than HIV+ children among the RV and NV groups. Among NV children, the proportion of children with antibody concentrations of ≥0.35 ug/ml post-vaccination was less frequent (P<0.001 for all) among HIV+ (range: 32-58%; except 76% for 19F) compared to HIV- children (range: 92-98% for all serotypes, except 6B [78%]). Similarly, among RV children, a lower proportion of HIV+ children had antibody concentrations of ≥0.35 ug/ml to all of the serotypes (range 50-75% for individual serotypes) than HIV- children (range: 99-100%).

Conclusion: HIV+ children vaccinated with PCV during infancy may require more frequent booster doses of PCV. Furthermore, although a single dose of PCV may be protective in older previously unvaccinated HIV- children, HIV+ children probably require additional doses of PCV.

Nation-wide study of IPD in the pediatric population of Germany (1997-2004):

Incidence, epidemiology of resistance and coverage of PCV7

Reinert, RR1, Al-Lahham, A1, Siedler, A1, van der Linden, M1, Toschke, AM1 and von Kries, R2
1National Reference Center for Streptococci and Institute of Medical Microbiology, RWTH-Aachen, Germany
2Robert-Koch Institute, Berlin, Germany

Background and Aim: A population based nationwide study was initiated in 1997 to monitor the epidemiology of invasive pneumococcal disease (IPD) in the pediatric population of Germany (up to 15 years).

Methods: Invasive pneumococcal disease (IPD) in children was reported from all microbiological laboratories and until June 2003 independently from paediatric hospitals. The isolates were sent to the NRCS for confirmation of species diagnosis by optochin testing, bile solubility and for serotyping by the Neufeld Quellung reaction. Determination of the incidence rate was based on capture-recapture method. MICs to antibiotics were determined by the microdilution method according to the CLSI recommendations.

Results: During the period January 1997 to December 2004 the number of confirmed cases reported was 3041. The incidence of IPD among children <2 years of age increased from 18.2 in 1997 (95% confidence interval 16.6-19.8) to 21.4 in 2002 (95% confidence interval 20.0-22.8) per 100,000 children. And the incidence of pneumococcal meningitis in the same age group (<2 years) was 7.5 per 100,000 children in 2002. Between 1997 and 2004 the percentage of cases reported from children <5 years of age varied from 79% to 84.5% of all cases. Predominant serotypes were: 14 (26.5%), 23F (7.2%), 6B (6.8%), 1 (6.6%), 19F (6.5%) 18C (6.2%) and 7F (6.1%). Coverage rate in the age group <5 years (all cases; meningitis cases) by the PCV7 (serotypes 4, 6B, 9V, 14, 18C, 19F and 23F) was 70.1%; 71.8%, and when including serotypes 1, 5, 3, 7F, and 6A the coverage rate was 82.4%; 83%. Resistance rate to penicillin G (intermediate and resistant) increased from 1.4% in 1997 to 8.6% in 2004 and for macrolides has increased from 12.9% in 1997 to 26.8% in 2004.

Conclusions: Universal vaccination with PCV7 could prevent more than 70% of IPD in children below 5 years of age in Germany.
Pneumococcal Population Biology of Vaccine Effectiveness and Serotype Replacement in S. pneumoniae (SP) Isolated During the American Indian Clinical Trial of the 7-Valent Pneumococcal Conjugate Vaccine (PCV)

Lipsitch, M1, O’Neill, K2, Cordy, D3, Bugalter, B4, Trzcinski, K5, Thompson, C6, Goldstein, R7, Pelton, S8, Huot, H9, Bouchet, V10, Bronsdon, M11, Parkinson, A12, Reid, R13, Santosh, M14, O’Brien, KL15
1Harvard University, Boston, MA, USA
2Broad Institute, Cambridge, MA, USA
3Boston University, Boston, MA, USA
4Johns Hopkins University, Baltimore, MD, USA
5Centers for Disease Control & Prevention, Anchorage, AK, USA

Background: A cluster-randomized clinical trial of PCV vs. meningococcal conjugate vaccine (MCV) was performed among American Indians in the southwestern US between 1997 and 2000. We sought to characterize the effect of PCV on the SP population by characterizing 375 SP isolates from nasopharyngeal carriage (NP) in trial participants during the last 18 months of the trial, as well as 89 otitis media (OM) and 126 invasive disease (INV) isolates collected throughout the trial.

Methods and Scientific Questions: Multilocus sequence types (MLST) were obtained. To assess influence of pneumococcal strain background on the serotype (ST)-specific effectiveness of the vaccine in preventing carriage of vaccine-serotype (VT) SP, MLST frequency was compared among SP isolates between PCV and MCV communities, within ST strata for all VT. The same analysis was performed for NVT to determine SP strain background effects on replacement. To detect possible vaccine-induced capsular switching, we sought MLST that were represented in PCV, but not MCV, samples with an NVT capsule, but were also present with at least one VT capsule. To determine whether serotype replacement consisted mainly of expansion of existing clones or appearance of novel ones, we calculated the frequency of MLST that were associated with NVT and were present only in SP isolates from PCV communities.

Results: 1) PCV had differential effectiveness against different VT ST, but no statistical differences in effectiveness were found between MLST within a ST, either for NP, OM, or INV isolates. 2) Only one putative vaccine-induced capsule-switched isolate was found, a ST35F isolate of MLST 124, usually associated with ST 14. 3) 76% of NVT MLST in PCV communities were also present in our sample from MCV communities; thus, at most 24% of replacing strains were due to appearance of new strains. 4) Little heterogeneity was found in the isolate of MLST 124, usually associated with ST 14. 3) 76% of NVT MLST in PCV communities were also present in our sample from MCV communities.

Conclusions: Serotype, rather than SP strain background or capsular switching, appears to be the main determinant of the PCV’s ability to inhibit VT and to cause replacement by NVT. Serotype appears also to be the main determinant of carriage vs. disease potential. These findings represent a setting of early stage PCV use and further studies are required to determine their durability.

Post-PCV7 expansion and emergence of sequence types among serotype 19A and serogroup 15 Streptococcus pneumoniae (SP) in Massachusetts children 1999-2004

Hanage, W1, Huot, H2, Huang, S3, Goldstein, R4, Bishop, C5, Lipsitch, M6, Pelton, S7, Finkelstein, J8
1Imperial College, London, UK
2Boston University Medical Center, Boston, MA, USA
3Harvard Medical School and Harvard Pilgrim Health Care, Boston, MA, USA
4Harvard School of Public Health, Boston, MA, USA

Following introduction of PCV7, serotype 19A and serogroup 15 have emerged as frequent replacement types among invasive and carriage isolates in Massachusetts (MA). We performed MLST on isolates of 19A and 15 obtained from MA children between 1999 and 2005 to define the expansion and emergence of individual sequence types (ST) within these serotypes.

During 1999, carriage SP isolates were collected from children < 7yrs, during well and sick visits in two MA cities (N=90), and subsequently in 2001 and 2004 from 16 cities or towns (N=126 and 222 respectively). MLST was performed on all carriage isolates. Invasive isolates were collected in MA from 2001-2004 in collaboration with MA Dept of Public Health—MLST of these isolates is underway.

85 carriage isolates of 19A (n=37) and 15 (n=48) were identified. Prevalence of 19A changed little between the first two years of the study (8% and 6% of samples respectively) but increased to 11% in 2004. Serogroup 15 strains expanded from 6% and 7% in 1999 and 2001 to 15% in 2004. eBURST analysis of MLST data showed that both serotypes/groups were predominated by STs of the same clonal complex: (CC) 199, suggesting a history of capsular switching. Of interest, the 19A/199 strains show reduced susceptibility to penicillin. In MA, serotype 19A is also the most frequent serotype causing invasive disease (57 of 350 strains). While expansion of CC199 is an explanation for increased prevalence, additional unrelated CCs of 19A strains have emerged in the study population, in marked contrast with serogroup 15 (for which all but one isolate fall into only two CCs). Three STs previously limited to vaccine serotypes have been identified within type 19A, supporting CDC surveillance data reporting 19A strains associated with capsule switching (Pai, JID, 2005). We are currently in the process of analyzing invasive isolates in order to compare 19A and 15 isolates in parallel carriage and invasive populations.
Safety and immunogenicity of the 13-valent pneumococcal conjugate vaccine in healthy adults

Dana, A, Wilson, E, Goss, MA, Olivero, K, Benson, J
Merck & Co., Inc. West Point, PA., USA
Background: The Advisory Committee on Immunization Practices (USA) currently recommends vaccination with PPV at age 65 years. The implementation of a vaccination program starting at age 50 years would mean that patients could receive more than the recommended 1 or 2 doses. While the safety of 1 or 2 doses of PPV has been established, there is limited data on the safety of 3 doses.

Methods: The database contained 47 reports involving 3 doses of PPV overall and were consistent with the product label. Where recovery status was available, recovery was complete in most cases.

Results: In 27 years from initial licensure of Pneumovax®23 through March 31, 2005, approximately 85 million doses of Pneumovax®23 have been distributed worldwide. This review revealed that AEs in patients receiving ≥3 doses were comparable in type and seriousness to those reported for PPV overall and were consistent with the product label. Where recovery status was available, recovery was complete in most cases.
Salivary and serum responses to 7-valent pneumococcal conjugate vaccine (PCV) in HIV-infected adults

French N1, Kayhty H2, Haikala. R2, Mwalukomoo T1, Nurkka A2, Gordon S3.
1Malawi-Liverpool/Wellcome Trust Laboratories & Dept of Medicine, College of Medicine, Box 30096, Blantyre, Malawi. 2National Public Health Institute, Mannerheimintie 166, Helsinki, Finland. 3Liverpool School of Tropical Medicine, L3 5QA, Liverpool, United Kingdom

Objective: To determine the immunogenicity of a 7-valent PCV in HIV-infected Malawian adults.

Background: Invasive pneumococcal disease (IPD) is a common life threatening complication of HIV-infection particularly in Africa. Pneumococcal polysaccharide vaccine has proven ineffective for the control of IPD in this setting. PCVs offer an alternative approach, although reports to date from the US have measured sub-optimal serum responses in HIV-infected adults when compared to uninfected controls. However as PCVs are capable of producing mucosal responses and protection of carriage, these responses may be a relevant indicator of possible vaccine efficacy.

Methods: 20 HIV-infected and 22 HIV-uninfected adults were randomly assigned to receive 2 doses of PCV or placebo 1 month apart. Saliva and serum were collected at baseline, 1, 2, and 6 months after enrolment. Serotype-specific IgG (serum, saliva) and IgA (saliva) anti-capsular antibodies (anti-PS) to serotypes 6B, 14, 19F & 23F were measured using a standard EIA method incorporating a cell wall and 22F polysaccharide adsorption step.

Findings: None of the 22 placebo recipients (10 HIV +ve, 12 HIV –ve) showed measurable responses in serum or saliva to vaccination. Amongst the HIV-infected PCV recipients there were significant increases in anti-PS IgG to all serotypes in sera and to all serotypes except 19F in saliva (1 month post the first dose of PCV geometric mean concentration, GMC, anti-PS IgG in saliva – 6B: 3.7, 14: 13.0, 19F: 9.8, 23F: 9.2 ng/ml). Salivary anti-PS IgA concentrations increased to serotypes 6B and 23F (1 month post the 1st PCV GMCs - 6B: 39.6, 14: 123.8, 19F: 56.7, 23F: 66.4 ng/ml). This pattern was also found in the HIV-uninfected PCV recipients (1 month post 1st PCV GMCs of salivary IgA anti-PS - 6B: 45.7, 14: 118.9, 19F: 49.4, 23F: 29.8 ng/ml). No difference in anti-PS concentration in serum or saliva was discernible at any time point by HIV status. No increase in anti-PS was noted after a second dose of vaccine in either vaccine group. Serum functional data will also be presented.

Interpretation: PCV induces an immunological response systemically and mucosally in HIV-infected Africans and this is similar to response in the HIV-uninfected. A second dose of PCV after one month confers no additional increase in anti-PS. These findings support the evaluation of PCV in this population in a clinical efficacy trial.

Serotype replacement in invasive Streptococcus pneumoniae (ISP) infections after introduction of 7-valent pneumococcal conjugate vaccine (PCV7) in Canada: Implications for an expanded 13-valent conjugate vaccine

Kellner JD 1, Church DL 1, MacDonald J 1, Scheifele D 2, Tyrrell GJ 1, for Calgary Area S. pneumoniae Epidemiology Research (CASPER)
1University of Calgary, Calgary, Alberta, Canada
2University of British Columbia, Vancouver, BC, Canada
3National Centre for Streptococcus, Edmonton, Alberta, Canada

Background. Post-licensure surveillance of PCV7 use in children has demonstrated significant direct and indirect effects to prevent ISP in the USA (Whitney et al. NEJM 2003 and others) and Canada (Kellner et al. CMAJ 2005:173:1149). As ISP infections caused by PCV7 serotypes decline, it is uncertain what will be both the relative and absolute change in non-vaccine serotype infections.

Methods. Population-based surveillance for ISP (sterile site cultures) began in 1998 in Calgary (pop. ~1,000,000). All cases are identified through active laboratory surveillance. Clinical data is obtained and isolates are serotyped. Routine 4-dose PCV7 vaccination for children was introduced in Alberta in Sept 2002. We compared the pre-PCV7 period (1998-2001) with the first 2 full years of PCV7 use (2003-2004) to determine proportion of ISP serotypes that were PCV7 serotypes, PCV13 (PCV7 serotypes and 1, 3, 5, 6A, 7F, 19A, see others) and Canada (ISP) infections after PCV7 introduction.

Results. There were 714 cases of ISP in Calgary from 1998 to 2004 and all were serotyped.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV7</td>
<td>62%</td>
<td>45%</td>
<td>39%</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>PCV13 (less PCV7)</td>
<td>21%</td>
<td>20%</td>
<td>20%</td>
<td>P=0.44</td>
</tr>
<tr>
<td>Other (non-PCV13)</td>
<td>17%</td>
<td>40%</td>
<td>40%</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>&lt;16 yrs</td>
<td>N=111</td>
<td>N=31</td>
<td>N=13</td>
<td></td>
</tr>
<tr>
<td>PCV7</td>
<td>81%</td>
<td>74%</td>
<td>62%</td>
<td>P=0.14</td>
</tr>
<tr>
<td>PCV13 (less PCV7)</td>
<td>11%</td>
<td>16%</td>
<td>23%</td>
<td>P=0.19</td>
</tr>
<tr>
<td>Other (non-PCV13)</td>
<td>8%</td>
<td>10%</td>
<td>15%</td>
<td>P=0.32</td>
</tr>
<tr>
<td>16 yrs and above</td>
<td>N=306</td>
<td>N=164</td>
<td>N=76</td>
<td></td>
</tr>
<tr>
<td>PCV7</td>
<td>55%</td>
<td>39%</td>
<td>36%</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>PCV13 (less PCV7)</td>
<td>19%</td>
<td>21%</td>
<td>20%</td>
<td>P=0.87</td>
</tr>
<tr>
<td>Other (non-PCV13)</td>
<td>26%</td>
<td>40%</td>
<td>45%</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

Conclusions. PCV7 serotypes now comprise a much smaller, and still declining, proportion of ISP infections in the overall population but the proportion caused by other serotypes in an expanded PCV13 has not yet changed. ISP serotype epidemiology determined before the introduction of PCV7 can no longer be used to anticipate the coverage of PCV13 at any age.
Efficacy estimates for a 7-valent pneumococcal conjugate vaccine: Impact of case definition used for acute otitis media

**Palmu, AAI, Jokinen, J, Mäkelä, H, Kilpi, TM.**
National Public Health Institute, Helsinki, Finland.

**Background.** An 11-valent pneumococcal conjugate vaccine with protein D of *Haemophilus influenzae* as a carrier (11-PnPD) was recently reported to have higher vaccine efficacy (VE, 34%) against clinical acute otitis media (AOM) than the currently available 7-valent pneumococcal conjugate vaccine (7-PncCRM) had in the FinOM Vaccine Trial (~6%). 11-PnPD also reduced AOM due to *Haemophilus influenzae* (Hi) by 35%, but the incidence of AOM was considerably lower (appr. 0.1/pyr) in this study than in the FinOM study (1.24/pyr).

We re-analysed our VE results for 7-PncCRM using a similar definition as used in the 11-PnPD study and a definition giving a similar incidence for AOM in the control group as that obtained in the 11-PnPD study.

**Methods.** 1662 children were recruited into the FinOM Trial between 1995 to 1999 to evaluate the efficacy of 7-PnCRM against AOM compared to control vaccine (Hepatitis B). Vaccinations were given at 2, 4, 6 and 12 months of age. The study children were followed from 2 to 24 months age for occurrence of AOM. The etiology of AOM attacks were determined by bacterial culture and pneumococcal serotyping of the middle ear fluid samples. The methods used in the 11-PnPD study were quite comparable.

Results. When the definition used in the 11-PnPD study (abnormal eardrum associated with at least two symptoms, instead of one) was applied, there were 993 episodes (0.9/pyr) of clinical AOM in the 7-PncCRM group compared to 1097 (0.99/pyr) in the control group during the per protocol follow-up from 6.5 to 24 months of age. Thus, VE was 9% (95%CI -3, 19). Severe AOM (bulging tympanic membrane or spontaneous perforation accompanied by fever of at least 38.0°C) was found in an incidence similar to that of any AOM in the 11-PnPD study, 0.14/pyr in the control group, VE estimate being 9% (-16, 28). VE estimates for AOM due to 7-PnCRM serotypes were similar regardless of the case definition used: 57, 53, and 46% for the original FinOM, 11-PnPD study and FinOM severe, respectively.

**Conclusions.** The differences between the efficacy estimates of 11-PnPD and 7-PncCRM cannot be explained by the different case definitions used for AOM. Differences in selection of AOM cases between the two studies and efficacy of the 11-PnPD vaccine for Hi AOM are likely to account for the difference.

Pneumococcal conjugate vaccines in preventing vaccine-type invasive pneumococcal disease and pneumonia with consolidation on x-ray in children under two years of age

**Lucero MG 1, Dulalia VE 1, Parreño RAN 1, Lim-Quianzon DM 1, Nohynek HM 1, Makela H 2, Williams G 3**
1Research Institute for Tropical Medicine, Muntinlupa City, Philippines
2National Institute of Public Health, Helsinki, Finland
3University of Queensland, Brisbane, Australia

Pneumonia, most commonly caused by *Streptococcus pneumoniae* (Pnc), is a major cause of morbidity and mortality among young children, particularly in developing countries. The global trend of increasing prevalence of antibiotic-resistant Pnc prompts evaluation of effectiveness of preventive strategies against pneumonia such as the pneumococcal conjugate vaccine (PCV).

This review determines the effectiveness of PCV in terms of vaccine efficacy (VE) in reducing the incidence of invasive pneumococcal disease (IPD) from vaccine serotypes (VT) and x-ray confirmed pneumonia in children given PCV before 12 months of age.

We searched The Cochrane Library (Issue 2, 2005), MEDLINE (1990 to August 2005) and EMBASE (1990 to August 2005), reference list of articles and books of abstracts of relevant symposia, and corresponded with researchers via email.

We selected randomized controlled trials on children below age two in which PCV was compared with placebo or another vaccine, with IPD and clinical/radiographic pneumonia as outcomes.

Two reviewers independently identified eligible studies, assessed trial quality, and extracted data. Differences were resolved by discussion.

The inverse variance method was used to pool effect sizes.

We identified seven randomized double-blind controlled trials that showed PCV was highly effective in preventing IPD among children who received PCV before 12 months of age. The pooled VE for vaccine serotype IPD was 88%, 66% for all serotype-IPD, and 22% for x-ray confirmed pneumonia.

The results of the Gambian trial published in June 2005 showed 77% VE against IPD and 37% VE against x-ray confirmed pneumonia. This study incorporates new information from the Gambian trial that strengthens even more the evidence on the effectiveness of PCV against IPD and x-ray confirmed pneumonia.
Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia

Catts FT1, Zaman SMA1, Enwere G1, Jaffar S1, Levine OS1,2, Biney EO1, Pierce NP1, Greenwood BM1, Adegbola RA1
1Medical Research Council Laboratories, Fajara, The Gambia
2London School of Hygiene and Tropical Medicine, London, England
3National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA
4Johns Hopkins Bloomberg School of Public Health, USA

Background: Pneumonia is estimated to cause almost 2 million deaths each year in children. *Streptococcus pneumoniae* is the most important cause of severe pneumonia. We conducted a randomised, placebo-controlled double-blind trial of a nine-valent pneumococcal conjugate vaccine in eastern Gambia, with the primary objective of evaluating efficacy against a first episode of radiological pneumonia. Results were published in 2005; here we present more detail on invasive disease.

Methods: We randomised children aged 6-51 weeks to receive 3 doses of pneumococcal conjugate vaccine or placebo with intervals of at least 4 weeks between doses. We conducted surveillance for radiological pneumonia, invasive disease and adverse events at a major health centre and hospital. We monitored mortality by recording outcome of admissions and conducting three-monthly home visits to each child.

Results: Results of per-protocol and intent to treat analyses were similar. In per-protocol analyses, pneumococcal vaccine efficacy was 37% (95% CI: 27, 45) against radiological pneumonia; 77% (95% CI:51, 90) against vaccine-type invasive pneumococcal disease (IPD); 50% (95% CI:21, 69) against all IPD; 15% (95% CI:7, 21) against all-cause admissions, and 16% (95% CI:3, 28) against mortality. Of all children with IPD, 65% had radiological pneumonia and only 5% had meningitis (no confirmed pneumococcal meningitis cases occurred in per-protocol vaccinated children). Only one child had more than one episode of invasive disease; serotype 33F was obtained in the first episode and serotype 22F in the second, 182 days later. In per-protocol analyses we found significant efficacy of 88%-100% against individual serotypes 5, 14 and 23F but no evidence of efficacy against serotype 1 (4 cases in vaccinated children and 2 in controls) or 9V (2 cases in each group). There was a relative reduction in IPD associated with vaccine-related serotypes and increase in IPD associated with non-vaccine serotypes among PCV recipients, though neither was statistically significant.

Conclusion: In this rural African setting, pneumococcal conjugate vaccine has high efficacy against pneumonia and invasive pneumococcal disease, and can significantly reduce hospital admissions and improve child survival.

Opsonophagocytic activity of antibodies against Type 6B and 19F *Streptococcus pneumoniae* after vaccination of HIV-infected Malawian adults with 7-valent pneumococcal conjugate vaccine (PCV)

Käyhty H1, Haikala, R1, Gordon S2,3, Mwaluko T,3, Mthuntha N1,3, French N2,3
1National Public Health Institute, Mannerheimintie 166, Helsinki, Finland,
2Malawi-Liverpool-Wellcome Trust Laboratories & Dept of Medicine, College of Medicine, Box 30096, Blantyre, Malawi.
3Liverpool School of Tropical Medicine, L3 5QA, Liverpool, United Kingdom.

Objective: To determine the functional activity of antibodies after vaccination of HIV-infected and HIV-uninfected Malawian adults with 7-valent PCV.

Background: Invasive pneumococcal disease is a common life threatening complication of HIV-infection particularly in Africa. PCVs seem to evoke a satisfactory antibody response in HIV-infected individuals. However, there are only few reports on the opsonophagocytic activity (OPA) of antibodies after vaccination of HIV-infected adults.

Methods: 20 HIV-infected (HIV+) and 22 HIV-uninfected (HIV-) adults were randomly assigned to receive 2 doses of PCV or placebo 1 month apart. OPA against type 6B (6BPnc) and 19F (19FPnc) *Streptococcus pneumoniae* was determined by standard killing assay in serum samples collected at 1, 2 and 6 months after the first dose of the vaccines. The results are given as reciprocals of serum dilution with 50% killing. IgG anti-capsular antibodies (anti-PS) were measured using a standard 22F- EIA method (see French et al.).

Findings: Four/11 and 3/12 HIV-, and 2/10 and 2/10 HIV+ placebo recipients had OPA against 6BPnc and 19FPnc, respectively. Geometric means titers (GMT) remained low in both groups (20-25 and 7.5-10.1 for HIV- and 4.7-5.7 and 4.6-5.3 for HIV+ group, respectively). After vaccination there was a wide variation in anti-PS concentrations and OPA titers among both HIV- and HIV+ PCV recipients. The geometric mean antibody concentrations of the HIV- and HIV+ PCV recipients did not differ significantly (respectively 6.9 and 0.92, depending on the time after vaccination and the serotype). The number of OPA positive samples and geometric means titers (GMT) seemed to be greater, and anti-19F needed for killing seemed to be lower, in the HIV- PCV recipients, but the differences were not significant:

<table>
<thead>
<tr>
<th>Time, mo</th>
<th>Type 6B OPA</th>
<th>Type 19F OPA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV-</td>
<td>HIV-</td>
</tr>
<tr>
<td></td>
<td>N OPA+</td>
<td>GMT(95%CI)</td>
</tr>
<tr>
<td>1</td>
<td>7/9</td>
<td>47(54-4266)</td>
</tr>
<tr>
<td>2</td>
<td>9/10</td>
<td>54(78-3849)</td>
</tr>
<tr>
<td>3</td>
<td>7/9</td>
<td>40(52-3200)</td>
</tr>
</tbody>
</table>

Interpretation: This preliminary study with a small sample size suggests that PCV induces functionally active antibodies in HIV-infected recipients. These findings support the evaluation of PCV in this population in a clinical efficacy trial.
Global review of pneumococcal serotype distribution in children under 5 years of age in the era of conjugate pneumococcal vaccination

Rodgers GL, Schranz J
Global Medical Affairs, Vaccines, Wyeth, Collegeville, PA

Background. Globally, infections caused by Streptococcus pneumoniae lead to much morbidity and mortality among infants and children. In addition to burden of disease information, knowledge of the serotype distribution of pneumococcal isolates causing disease is essential in evaluating the potential effect of conjugate pneumococcal vaccination.

Methods. We undertook a systematic review of published literature of pneumococcal serotype distribution for invasive pneumococcal disease (IPD) and acute otitis media (AOM). We accessed English and non-English literature using computerized databases and other sources for the period 1966 through 2005. Serotype distribution of IPD and AOM isolates were described geographically by country and region, and by age group and disease. Serotype coverage for the currently licensed 7-valent (7V) pneumococcal conjugate vaccine (Prevenar) and for experimental 10- and 13-valent vaccines were calculated.

Results. Pneumococcal serotype distribution was assessed for North America (NA), Europe, Asia, Latin America (LA) and Africa. Information varied greatly among regions and within regions. The majority of available information was from US, Canada, Western Europe and Australia. Africa had the least amount of information. Comparisons of serotype distributions prior to and post-Prevenar introduction were available for NA and Australia. The majority of studies were laboratory-based surveillance from hospitalized patients with pneumonia and meningitis; AOM information was limited.

Differences from previously published reviews in coverage of the 7V vaccine were demonstrated in LA and Asia. Many countries in LA have 7V vaccine coverage rates between 60 and 70% for children under the age of 2 years. 7V vaccine coverage rates for Asia showed wide variation with 75 to 90% of isolates from some countries (Hong Kong, Korea, Japan, Singapore, Taiwan), with little added increment for the 10- and 13-valent vaccines. Other countries in Asia (India, Pakistan, Bangladesh) demonstrated lower 7V vaccine coverage rates and potential additional benefits from vaccines that include serotypes 1 and 5. Serotype changes have been documented in countries with national immunization programs (US, Canada and Australia), demonstrating small but statistically significant increases in nonvaccine serotypes, especially 19A in the US.

Conclusions. Distribution of pneumococcal serotypes causing disease varies worldwide. Studies should be based on active surveillance with standard case definitions and serotyping, not serogrouping should be performed and reported for all isolates. Knowledge of serotype distribution is essential to assess potential impact of current and future pneumococcal conjugate vaccines.

Antimicrobial resistance among Streptococcus pneumoniae isolates in the era of pneumococcal conjugate vaccine: A review of the literature

Center KJ, Schranz J
Global Medical Affairs, Wyeth Pharmaceuticals, Collegeville, PA, USA

Introduction. S. pneumoniae is a major cause of invasive disease in all age groups, and is associated with significant mortality, morbidity, and costs. Furthermore, antimicrobial resistance among S. pneumoniae continues to complicate treatment of disease. To reduce the burden of illness, pneumococcal polysaccharide vaccines have been recommended for certain populations, although their use is not universal. Pneumococcal conjugate vaccine (PCV7) was first licensed in 2000 for use in infants and young children. In regions where PCV uptake is widespread, incidence of invasive pneumococcal disease has declined dramatically, among both immunized and unimmunized populations. The purpose of the current review was to investigate whether pneumococcal conjugate vaccines have also influenced trends in antimicrobial resistance among colonizing and disease-causing isolates of S. pneumoniae since their introduction into clinical practice.

Methods: Surveillance and prevalence studies were identified by electronic searches of MEDLINE and EMBASE (January 1998 to December 2005). In addition, national databases including the Active Bacterial Core surveillance, United States (1998 to 2003) and European Antibiotic Surveillance System (1999 to 2005) interactive database were searched to identify trends in pneumococcal resistance. Data was then analyzed using Stata9 (Timberlake Consultants) to explore the trends in antibiotic susceptibility over time.

Results: 1,244 citations were retrieved, of which 91 reviewed were considered valid for inclusion in the review. The review showed that despite wide geographical variations, a leveling or decline in rates of resistance was reported in a number of regions. While some regions reported dramatic declines in antibiotic resistance among pneumococci causing invasive disease since widespread use of PCV7, these findings were not consistently seen.

Conclusions: Declines in pneumococcal antimicrobial resistance have been reported since licensure of PCV7, especially in regions where it is widely used. However, these trends may be attributable to a number of initiatives, and this finding has not, to date, been a consistent one. Further studies are needed to investigate the long-term impact of PCV7 use on pneumococcal antimicrobial resistance. It is likely that control of pneumococcal antimicrobial resistance will require a combined strategy of conjugate vaccination and reduction of inappropriate antimicrobial use.
Evidence of non-PCV vaccine serotypes replacement invasive pneumococcal disease in 5 counties in Tennessee in children ≤2 years of age

Halasa, NH, Talbot, TR\(^1\), Arbogast, PG\(^2\), Schaffner, W\(^3\), Griffin, MR\(^1\), and Craig, AS\(^2\)

\(^1\)Vanderbilt University, Nashville, TN, USA  
\(^2\)Tennessee Department of Health, Nashville, TN, USA

**Background:** We previously reported a decrease in the incidence of invasive pneumococcal disease (IPD) in Tennessee, which began in the year 2000 coincident with the introduction of pneumococcal conjugate vaccine (PCV).

**Objective:** To evaluate the incidence of IPD with two additional study years, 2003 and 2004.

**Methods:** Active laboratory-based surveillance from the Active Bacterial Core Surveillance program identified persons with IPD from 1995-2004, who resided in 5 Tennessee counties. Trained nurses collected clinical data. Incidence of IPD was calculated as the number of IPD cases in county residents divided by the county population as determined from US census data, and expressed per 100,000. Rates were calculated by age (<2, ≥2 years) and by whether the isolate was serotype included in PCV.

**Results:** The rate of IPD in persons ≥2 years continued to decline after the introduction of PCV (p<0.01, test for trend) (Figure 1). The rate of IPD in children <2 years declined markedly after vaccine introduction (p <0.01, test for trend) (Figure 2). However, no further decline in IPD rates was seen in children younger than 2 years since 2002 (p-value=0.194) There was an increase in disease due to non-PCV serotypes (p-value 0.006), particularly with 19 A identified in 17/34 non-PCV isolates in 2004 (Table 1).

**Conclusion:** In the 5 TN surveillance counties, IPD in persons ≥2 years of age has continued to decrease through 2004. The rate of IPD in children <2 years has declined remarkably since 2000 but an increase non-PCV vaccine serotypes was seen. Serotype replacement of circulating strains is suggested by the isolation of serotype 19A in 50% of instances of IPD due to non-PCV vaccine serotypes in 2004.
Infections in children with sickle cell disease in the pneumococcal conjugate vaccine era

Adamkiewicz, TA, Brown, K, Silk, B, Strayhorn, G, Farley, MM

Morehouse School of Medicine, East Point, GA, USA
Emory University, Atlanta, GA, USA
Atlanta VAMC, and Georgia Emerging Infections Program, Atlanta, GA, USA

Introduction: The incidence of and mortality from invasive pneumococcal infections are significantly higher in children with Sickle Cell Disease (SCD) than in children in the general population. Following licensure of a 7-valent pneumococcal conjugate vaccine (PCV) in February 2000, invasive pneumococcal disease declined dramatically in the general population, and in children with SCD. In this study, we compared the incidence of invasive S. pneumoniae, N. meningitidis and H. influenzae infections before and after the introduction of PCV.

Methods: An electronic registry of children with SCD in Metropolitan Atlanta, Georgia born after 1989 was compared with a database of invasive respiratory bacterial infections occurring between 1/1/95 and 12/31/02 in the Georgia Active Bacterial Core Surveillance (ABCs)/Emerging Infections Program, a laboratory-based, surveillance network. Person-years were counted from the start of the study period for all patients seen once or more in a hematology service prior to that date. For patients who were seen for the first time after the start of the study period, the first observation time point was the date of the first hematology care visit. Last observation was the last recorded medical visit of any type, or the end of the observation period, whichever came first.

Results: The four reported invasive H. influenzae infections in children 0-5 years of age with hemoglobin SS all occurred after PCV licensure. No N. meningitidis infections were reported during the entire study period in this group of patients. All H. influenzae isolates were capsule serotype f.

<table>
<thead>
<tr>
<th>Years</th>
<th>Patients</th>
<th>S. pneumoniae</th>
<th>H. influenzae</th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td>95-99</td>
<td>369 24</td>
<td>2.6</td>
<td>0</td>
<td>24 2.6</td>
</tr>
<tr>
<td>00-02</td>
<td>388 9</td>
<td>1.5</td>
<td>4</td>
<td>0,7* 13 2.1</td>
</tr>
</tbody>
</table>

*Rate differs from zero (p=0.02).

Conclusion: As invasive pneumococcal infections covered by PCV decreased in children with SCD after PCV licensure, a small but significant increase in invasive H. influenzae serotype f infections was noted. There were no reported cases of N. meningitidis infections in either time period. Future population-based studies are necessary to determine the risk of infections with invasive respiratory bacteria not covered by licensed conjugate vaccines.

S. pneumoniae (Pnc) surface exposed glutamyl tRNA synthetase (GtS), a putative adhesin, is able to induce protective immune response in mice


Soroka University Medical Center, Ben Gurion University of the Negev, Beer Sheva, Israel

Background: Proteins with age-dependent immunogenicity in children were found to induce protective immune response to Pnc in the mouse model system (Ling et al, CEI 138:290; 2004). One of these proteins, GtS, was tested for its ability to induce protective immune response in mice and to interfere in Pnc adhesion to cells.

Methods: GtS (NP_346492, molecular weight 55.9kDa) was cloned, expressed and purified. 7 week-old BALB/c mice (n=22) were immunized intraperitoneally with 25µg protein at 0 and 21 days in the presence of Alum. Control mice (n=20) were immunized with Alum only. Mice were challenged with a lethal dose of Pnc serotype 3 strain WU2. Western blot with cell-wall proteins obtained from Pnc serotypes 2, 3, 6B, 9V and 14 were probed with serum obtained from mice immunized with GtS. The ability of GtS protein and anti-GtS serum to interfere with adhesion to A549 human lung adenocarcinoma cell line was tested with 3 pairs of capsulated and unencapsulated respective Pnc strains (serotype 3: WU2 and 3.8, serotype 2 D39 and R6 and serotype 14 strain 14DW and 14.8).

Results: The serum obtained from immunized mice cross-reacted with a 55.9kDa GtS protein in the cell wall protein-fraction obtained from the genetically and capsularly variable Pnc strains tested. The surface localization of GtS was confirmed using FACS analysis. 39% of the immunized mice survived intranasal challenge, given 7 days following last immunization, while 100% of adjuvant immunized mice succumbed (p<0.05). Incubation of three genetically- and capsularly-unrelated pairs of capsulated and unencapsulated strains of Pnc with GtS, inhibited their adhesion to the A549 cells in a concentration-dependent manner (r=-0.748 to r=-0.876 p<0.001). Antibodies to GtS inhibited 90% of Pnc adhesion to A549 cells in a concentration dependent manner (r=-0.791; p<0.001).

Conclusions: GtS, an age dependent Pnc immunogen is a surface-located putative adhesin capable of inducing protective immune response against Pnc in mice.
DNA vaccines based on genetically detoxified derivatives of pneumolysin fail to protect mice against challenge with Streptococcus pneumoniae

Ferreira, DM, Arêas, APM, Darrieux, M, Leite, LCC, Miyaji, EN
Centro de Biotecnologia, Instituto Butantan, São Paulo, SP, Brazil

The currently administered 7-valent polysaccharide conjugate vaccine against Streptococcus pneumoniae has been shown to be highly effective in high risk groups, but its use in developing countries will probably not be possible due to elevated costs. The use of conserved protein antigens using the genetic vaccination strategy is an interesting alternative for the development of a cost-effective vaccine. We have analyzed the potential of DNA vaccines expressing genetically detoxified derivatives of pneumolysin (pneumolysoids) against pneumococcal infections and compared with immunization using recombinant protein. The purified recombinant pneumolysoid with the highest residual cytolytic activity was able to confer partial protection against a lethal intraperitoneal challenge, with the induction of high antibody levels. Immunization with DNA vaccines expressing pneumolysoids, on the other hand, induced a significantly lower antibody response and no protection was observed. This work was supported by FAPESP and Fundação Butantan.

Solubility and carrier potential of three non-hemolytic pneumolysin proteins

Dilts, DA1, Liu, DF1, Douglas, M1, Núñez, L1, Koster, M1, Kirkham, LA2 and Mitchell, T2
1Wyeth Vaccines, Pearl River, NY, USA
2University of Glasgow, Glasgow, Scotland

Chemically toxoided pneumolysin (Ply) has been shown to function as a carrier for bacterial polysaccharides, but the wild type recombinant toxin (WTrPly) has some characteristics that are unfavorable for further development in vaccines. Although WTrPly is expressed well in E. coli, the majority of the protein exists as insoluble aggregates that require a more extensive purification process than the protein in the soluble fraction. After purification, the WT protein cannot be concentrated above 2 mg/ml without spontaneous aggregation. WTrPly is also highly toxic and presents significant biosafety concerns during fermentation and purification. To this end, genetically detoxified Ply proteins have been described in the literature. Most of these previously derived toxoids have retained some degree of cytotoxicity and their solubility in aqueous buffers is unknown. The aim of our research was to identify a genetically detoxified Ply that was highly soluble, both in the E. coli cytoplasm and after purification. Three non-hemolytic mutants of Ply have been recently identified: rPlyΔ6, rPlyΔ7 and rPlyΔ8. The three mutant rPly proteins varied in their proportion of soluble protein when expressed in the same host/vector system; rPlyΔ6 was the most soluble and rPlyΔ8 was the least soluble. This suggested that slight differences in the protein conformation affected the proportion of cytosolic rPly that aggregated. rPlyΔ6, was found almost exclusively in the soluble fraction of induced cells. The soluble:insoluble ratio of rPlyΔ6 in E. coli was investigated using three expression plasmids to direct gene expression. Vectors using the trc promoter produced more protein in the soluble fraction than the vector that used the arabinose promoter. Two E. coli strains of different lineage were compared as hosts, and it was determined that strain background did not significantly affect cytoplasmic soluble:insoluble ratios. Despite differences in cytoplasmic solubility, all three purified mutant proteins were stable at concentrations exceeding 3.5 mg/ml. Immunogenicity of the mutant rPly proteins was also investigated in mice. All three mutant proteins were as immunogenic as WTrPly. The mutants were conjugated to S. pneumoniae type 14 polysaccharide (Pn14) and compared to WTrPly and CRM197 in their ability to perform as carriers for the polysaccharide. Conjugates using the mutant rPly proteins elicited antisera that had equivalent anti-Pn14 titers as the WTrPly and CRM197.
Vaccination with non-toxic pneumolysin conjugated to capsule polysaccharide protects against IPD

Kirkham, LS, Douce, GR, Dilts, DA, Koster, M and Mitchell, TJ
1Division of Infection and Immunity, Institute of Biology and Life Sciences, University of Glasgow, Glasgow, UK.
2Vaccines Discovery Research, Wyeth, New York, USA

Our group has recently described the construction of a pneumolysin mutant (Δ6 Ply) that was demonstrated to be as protective as native pneumolysin against pneumococcal infection without the cytotoxic effects. To further investigate the potential use of Δ6 Ply as a vaccine candidate, the protein was covalently conjugated to capsule polysaccharide (CPS) from serotype 4 S. pneumoniae. BALB/c mice were subcutaneously vaccinated with Δ6 Ply, CPS, Δ6 Ply conjugated to type 4 CPS, Prevnar and Prevnar plus Δ6 Ply. Serum was taken before vaccination and before each boost and the anti-Ply IgG titres were measured by ELISA. Groups immunised with free and conjugated Δ6 Ply had high and comparable anti-pneumolysin IgG titres in their serum. Vaccinated mice were then challenged intraperitoneally with an infectious dose of serotype 4 (TIGR4) S. pneumoniae and monitored for survival. Mice vaccinated with Δ6 Ply-type 4 CPS or Prevnar were completely protected from challenge with TIGR4 S. pneumoniae whereas mice given free Δ6 Ply or CPS alone were not fully protected. As mice were completely protected from TIGR4 challenge when vaccinated with type 4 CPS conjugated to Δ6 Ply or Prevnar, Δ6 Ply can be considered to be as efficacious as CRM197 in acting as a carrier protein for pneumococcal polysaccharide. This has also been confirmed in vaccination experiments with conjugation of Δ6 Ply to type 14 CPS. The use of a pneumococcal carrier protein, such as Ply, prevents the possibility of carrier suppression occurring, which has been shown to arise with tetanus toxoid. Whether Δ6 Ply confers additional protection against IPD caused by heterologous serotypes remains to be determined.

Differential Regulation of memory and primary B cell responses to pneumococcal protein antigens in children by TLR-2 against bacterial lipopeptide

Zhang Q, Bernatoniene J, Bagrade L, Clarke E, Paton J, Mitchell T, Finn A
1University of Bristol, Bristol, UK,
2University of Adelaide, Adelaide, Australia
3University of Glasgow, Glasgow, UK

Background: Pneumococcal protein antigens are currently under study as potential new generation of vaccines. Synthetic bacterial lipopeptide (BLP) which is known as a TLR2 agonist, has been shown to be a potent immunological adjuvant to some antigens in animal models. The aim of this study is to investigate whether and how BLP regulates the mucosal B cell antibody responses to pneumococcal protein antigens CbpA and pneumolysin (Ply), PsaA and PspA in adenoidal mononuclear cells (MNC) from children undergoing adenoidectomy.

Methods: Nasopharyngeal swabs were cultured for pneumococcus. Adenoidal MNC or MNC depleted of memory T cell (CD45RO+) and/or memory B cell (CD27+) were cultured with pneumococcal culture supernatant (CCS) which contains secreted CbpA, Ply, PsaA and PspA. Antigen-specific antibody responses were measured by immunoassay.

Results: Co-stimulation with BLP significantly enhanced the CCS-induced primary type IgG antibody response in memory T and/or B cell depleted adenoidal MNC (p<0.01), regardless the carriage status of the patients from whom cells were isolated. However, BLP significantly reduced the memory type IgG response induced by CCS in adenoidal MNC isolated from patients cultured positive for pneumococcus (p<0.01), although in those who were culture negative for pneumococcus, BLP enhanced the IgG response (p<0.05).

Conclusion: TLR2 agonist BLP could differentially modulate the primary and memory B cell responses to pneumococcal protein antigens in human NALT in children with and without pneumococcal carriage. These results may have important implications in the understanding of mucosal immune responses to pneumococcus and vaccination strategies.
Cellular responses to a candidate pneumococcal whole cell vaccine in human nasopharyngeal tonsils

Bagrade L1, Zhang Q1, Malley R2, Finn A1
1University of Bristol, Bristol, UK
2Children’s Hospital, Boston, MA, USA

Background: A candidate ethanol-killed whole cell pneumococcal vaccine (WCV) has been shown to be protective against pneumococcal infection in mouse models. Data related to the effects of WCV in humans are limited. The aim of this study was to investigate whether WCV induces significant T cell responses in human adenotonsillar cells.

Methods: Human adenotonsil mononuclear cells (MNC) were cultured in the presence of two types of ethanol-killed autolysin negative unencapsulated WCVs – one expressing pneumolysin and one pneumolysin negative mutant. Cellular depletion of CD45RO+ cells was performed by negative selection using MACS microbeads and magnetic sorting. T cell proliferation after stimulation was analysed using a FACS-based CFSE assay.

Results: Significant CD4+ T cell proliferation was observed after stimulation with both types of WCVs. The pneumolysin positive vaccine showed significantly higher CD4+ T-cell proliferation than the pneumolysin-negative mutant. No significant proliferation of naïve CD4 T cells (CD45RA+) was observed after stimulation with the pneumolysin-negative mutant, in contrast to significant proliferation induced by the pneumolysin positive WCV (p<0.01).

Conclusion: Stimulation with pneumolysin positive WCV induces significant CD4 T cell proliferation, including both memory and naïve phenotype in a dose dependent fashion in human adenotonsillar cells. Further studies are in progress to elucidate the nature and regulatory mechanisms of interactions between WCV and human mucosal immune cells.

Efficiency of polysaccharide pneumococcal vaccine and alternative means for preventing pneumonia in recruits in Russia

Jogolev, KD1, Zhogolev, SD1, Ogarkov, PI1, Dobritsa VP2, Petrov LN2
1Epidemiology Department of Military Medical Academy, St Petersburg, Russia
2State Scientific Center Research Institute of Highly Pure Biopreparations, St Petersburg, Russia

Some groups of recruits aged 18-20 (more than hundred of persons in each group) were given various prophylactic means against influenza, acute respiratory disease and pneumonia. They included vaccines Grippol (against H1N1 and H3N2 influenzae) and Pneumo 23 (against main serotypes of Streptococcus pneumoniae) (parenteral administration during first days after the unit arrival), IRS-19 – bacteria lyases of 6 serotypes of S.pneumoniae and 13 species of other bacteria (as aerosol, one dose in each nasal meatus, once a day for first 10 days), Thymogen – synthetic analog of thymus gland extract (as aerosol, the same scheme), Dibazol (0.02 g) in combination with ascorbic acid (0.3 g) twice a day for 10 days, probiotic Vitaflor from two strains of Lactobaccillus acidophilus as a milk-acid product (a cup t.d.s. for three days before vaccination by Grippol and Pneumo 23), Imudon – a composite of bacteria lyases of 4 species of lacto bacteria, Staphylococcus aureus, Klebsiella pneumoniae and other bacteria (one tablet three times a day for 10 days).

The prophylactic drugs significantly improved immunologic indices in recruits, decreased a number of agent carriers compared to control groups. Vitaflor increased immunogenicity of vaccines. The prophylactic drugs significantly improved immunologic indices in recruits, decreased a number of agent carriers compared to control groups. Vitaflor increased immunogenicity of vaccines.

General incidence of influenza, other ARD, acute bronchitis and pneumonia in recruits who received the drugs was 2.2–3.1 times lower than in control groups. The most effective anti-pneumonia drug appeared to be pneumococcal vaccine combined with influenza vaccine and probiotic. The most cost effective means were Dibazol in combination with ascorbic acid. The course of pneumonia was slighter and significantly shorter if it was happened among those who had received prophylactic drugs.
DNA vaccines expressing PspA (Pneumococcal surface protein A) elicit protection levels comparable to recombinant protein

Ferreira DM, Miyaji EN, Oliveira MLS, Darrieux M, Arêas APM, Ho PL and Leite LCC
Centro de Biotecnologia, Instituto Butantan, São Paulo, SP, Brazil

Pneumococcal surface protein A (PspA) is a promising candidate for the development of cost-effective vaccines against Streptococcus pneumoniae. In the present work BALB/c mice were immunized with DNA vaccine vectors expressing the N-terminal region of PspA. Animals immunized with a vector expressing secreted PspA developed higher levels of antibody than mice immunized with the vector expressing the antigen in the cytosol. However, both immunogens elicited similar levels of protection against intraperitoneal challenge. Furthermore, immunization with exactly the same fragment as a recombinant protein using alum as adjuvant elicited even higher antibody levels, but this increased humoral response did not correlate with enhanced protection. These results thus show that DNA vaccines expressing PspA are able to elicit protection levels comparable to recombinant protein, even though total anti-PspA IgG response is considerably lower.

Supported by: Fapesp, Fundação Butantan, CNPq

Expression of pneumococcal conserved protein antigens in S. typhi live vectors

Barry, EM, Santiago, AE, Davis, T, Herrera, A, Singh S, Levine MM
University of Maryland School of Medicine, Baltimore, Maryland

Streptococcus pneumoniae continues to be an important cause of life-threatening invasive disease throughout the world. While polysaccharide conjugate vaccines are proving to be efficacious in disease prevention, the use of conserved pneumococcal protein antigens in vaccine formulations offers an alternative vaccination strategy. Our approach involves the use of live, oral, attenuated S. Typhi vaccine strains as carriers of heterologous pneumococcal antigens. Three protein antigens, which are conserved among S. pneumoniae serotypes, include pneumococcal surface adhesin A (PsaA), pneumolysin (Ply), and pneumococcal surface protein A (PspA). Each of these purified proteins has shown promise as a protective antigen in animal models. We have engineered stabilized plasmids directing high-level expression of these antigens with the use of signal peptides for periplasmic localization or fusion to ClyA for secretion. A series of constructions were compared for protein expression in the S. Typhi live vector as well as immunogenicity in the mouse model. Antibody responses were elicited against the live vector as well as each pneumococcal antigen following vaccination. In general, expression levels correlated with immunogenicity. Challenge studies for the evaluation of protective responses are underway. Additional plasmids were engineered to direct expression of pairs of antigens using optimized promoter sequences. Antibody responses were elicited against each antigen following immunization in the mouse model. These data support a model for a multivalent Salmonella-pneumococcal vaccine candidate.
Comparative immunogenicity of different protein D-polysaccharide conjugated vaccine formulations in rat and mouse animal models

Kyd, JM1, Cripps, AW2, Denoël, P3, Godfroid, F4, Poolman, J
1Central Queensland University, Rockhampton, QLD, Australia
2Griffith University, Gold Coast, QLD, Australia
3GlaxoSmithKline Biologicals, Rixensart, Belgium

This study aimed to compare the induced antibody responses for different formulations of an 11-valent polysaccharide-protein D conjugated vaccine (Pn-PD) following immunisations in rat and mouse animal models. PD is a nontypeable Haemophilus influenzae protein used as the protein carrier for the polysaccharide serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F. This study reports the affect of the vaccine formulation on antigen-specific antibody responses following a combination parenteral and mucosal immunisation regime in BALB/c mice, DA and Sprague Dawley rats. IgG, IgG1, IgG2a and IgA levels specific to PD, Ps14, Ps19F, Ps23F and Ps6B were measured in sera and either lung (BAL) or middle ear (MEL) lavage fluids collected on day 21 after immunisations on days 0 and 14. The Pn-PD formulations were prepared in a saline carrier or adjuvanted with either MPL or AS7. The results show that: (1) the adjuvant and formulation affected the levels of specific antibody titre; (2) the species of animal also resulted in differences in antibody responses; (3) significant antibody titres to PD were measured in sera and lavage fluids for some formulations across all animal species; (4) detectable anti-Ps titres were more frequently measured in the sera rather than in the lavage fluids; (4) both IgG1 and IgG2a-specific Ps responses were measured in sera, with IgG1 responses being more frequently detected for specific Ps; and (5) in general for these formulations, Ps6B was the least immunogenic polysaccharide. In conclusion, these results demonstrate that the animal model used and the formulation of the vaccine both affect the isotype and titre of antibody specific to the protein carrier (PD) and the polysaccharide antigen.


Intranasal immunization with Cholera toxin B-Pneumococcal surface antigen A fusion protein induces protection against colonization with Streptococcus pneumoniae and has negligible impact on the nasopharyngeal and oral microbiota of mice

Arêas, AP1, Pimenta, FC2, Pimenta, EN2, Oliveira, MLS1, Ho, PL1, Leite, LCC1
1Centro de Biotecnologia, Instituto Butantan, São Paulo, Brazil
2Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, Brazil

Streptococcus pneumoniae is the most important causative agent of bacterial pneumonia and it is also responsible for other mucosal as well as systemic infections such as otitis media, sinusitis, meningitis and sepsis. Recent efforts for the development of new vaccines against infections caused by Streptococcus pneumoniae have focused on proteins that may be conserved throughout the different pneumococcal serotypes. One of the candidate proteins is PsaA (pneumococcal surface antigen A), a protein involved in the transport of manganese and zinc in the bacterium, which was shown to have a significant role in protection against pneumococcal carriage, being proposed as component for a mucosal vaccine. Vaccines targeting mucosal immunity may raise concerns on possible alterations in the normal microbiota, specially in the case of PsaA, which was shown to have homologs with elevated sequence identity in other viridans streptococci normally isolated from human clinical specimens. Our group has previously shown an increase in both systemic and mucosal antibodies in saliva, nasal and bronchial wash samples after intranasal immunization of mice with a Cholera toxin B subunit-PsaA (CTB-PsaA) fusion protein. In this work we demonstrate that intranasal immunization with CTB-PsaA is able to protect mice against colonization with S. pneumoniae, but does not significantly alter natural oral or nasopharyngeal microbiota in mice. We have isolated streptococcal species in the natural microbiota of mice that have proteins that cross-react with anti-PsaA antibodies and immunization with CTB-PsaA did not alter total amounts of streptococci nor did it eliminate any streptococal species showing reaction with anti-PsaA antiserum.

This work was supported by FAPESP, CNPq and Fundação Butantan
**Pneumococcal surface protein A (PspA) diversity among Indian clinical isolates**

*Verma, RK, Kumar R, Das BK*

All India Institute of Medical Sciences, New Delhi, India

Streptococcus pneumoniae is a major cause of meningitis and pneumonia in children and elderly worldwide. Presently available polysaccharide and conjugates vaccines are not enough to deal with this pathogen. We are working on Pneumococcal surface protein A (PspA), which can be a potent future vaccine candidate. This protein exhibits large amounts of diversity. Therefore we are interested in describing the extent of diversity in PspA by PCR and sequencing in Indian clinical isolates.

We have collected 106 non-invasive and 19 invasive pneumococcal isolates. All isolates were confirmed as pneumococcus by gram staining and optochin sensitivity. We used 3 primer sets to type PspA into families. Most of the isolates belong either family 1 or family 2. Out of 106 non-invasive isolates, 49% family 1, 46% family 2, and only 2 isolates (1.8%) were typed as family 3. Six isolates (5.2%) were positive for both the families (family 1 and family 2). While, out of 19 invasive isolates, 12 were positive for PspA family 1 and 7 isolates were positive for family 2. We used LSM-13 and SKH-2 primer set to sequence PspA alpha helix. Preliminary data shows considerable variation between Indian sequences and sequences from other parts of the world.

**Middle ear fluid (MEF), nasopharyngeal (NP) and oropharyngeal (OP) Streptococcus pneumoniae (Spn) serotype distribution in Costa Rican (CR) children with otitis media (OM)**

*Arguedas A, Soley C, Porat N, Brilla E, Porras W, Dagan R.*

Instituto de Atención Pediátrica and Universidad Autónoma de Ciencias Médicas San José, Costa Rica, Ben-Gurion University, Beer-Sheva, Israel

**Background:** *Spn* is a key pathogen in otitis media worldwide. It is important to establish its serotype distribution in each geographical region in order to evaluate the impact of current and future pneumococcal vaccines.

**Objective:** To establish the most common *Spn* serotypes present in the MEF, NP/OP of CR children with OM and to assess the potential protection provided by the licensed, conjugate heptavalent *Spn* vaccine (PCV-7) against OM.

**Methods:** From year 2002 to 2005, MEF, NP and OP samples were obtained from CR children with OM who participated in various antimicrobial clinical trials. *Spn* was identified according to standard procedures. Strains were Serotyped by the Quellung reaction and antimicrobial susceptibility to penicillin, TMP/SMX, macrolides, cephalosporins and quinolones was determined by Kirby Bauer or E-test.

**Results:** A total of 110 MEF and 207 NP/OP isolates were obtained at the time of OM diagnosis. Children median age was 15 months (range: 3 – 47) and 65% (72 patients) were < 24 months of age. The most common MEF *Spn* serotypes were 14 (16%), 3 (16%), 19F (15%) 6B (12%) and 23F (10%). The most common NP/OP *Spn* serotypes were 19F (23%), 6B (14%), 14 (19%), 3 (9%) and 23F (8%). Of MEF strains, PCV-7 vaccine type (VT) constituted 90%, 69%, 87%, 100% and 100% of penicillin-resistant, TMP/SMX-resistant, macrolide-resistant, cephalosporin-resistant and multidrug-resistant strains respectively. Coverage with PCV-7 and the investigational 10-valent and 13-valent vaccines against MEF strains was 62%, 64% and 80%, respectively and against NP/OP strains 68%, 69% and 78%, respectively.

**Conclusions:** *Spn* vaccine coverage with PCV-7 in CR children with otitis media is similar to that observed in developed countries and may be enhanced with the investigational 13-valent vaccine currently undergoing clinical trials. Vaccine protection includes most of the drug-resistant strains.
Antibodies to PspA families 1 and 2 in saliva but not in serum of children were associated with a lower risk of pneumococcal AOM


1National Public Health Institute, Helsinki, Finland
2University of Alabama, Birmingham, AL, USA

Background: Pneumococcal surface protein A (PspA) is a highly variable, yet cross-reacting virulence factor divided into two major families. In the present study, we assessed the role of natural human serum and salivary anti-PspA antibodies in defense against pneumococcal acute otitis media (pncAOM). Our previous studies with a family 1 PspA antigen suggested that young children develop only low levels of serum and salivary anti-PspA antibodies. The present study was extended to include antigens from both PspA family 1 (PspA1) and PspA family 2 (PspA2).

Methods: Enzymeimmunoassay (EIA) was used to measure the serum IgG and salivary IgA to PspA1 and PspA2 in serum and saliva samples of children from the Finnish Otitis Media (FinOM) Cohort Study at the ages of 12 months (N=287 and N=161, respectively) and 18 months (N=258 and N=133, respectively). Cross-tabulation was used to preliminarily analyze the association of the serum and salivary anti-PspA1 and anti-PspA2 with pncAOM. Cox proportional hazard model was used to evaluate the relative risk (RR) of subsequent pncAOM during the following 6 months in relation to the presence of serum or salivary anti-PspA at the ages of 12 and 18 months.

Results: The percentages of anti-PspA positive serum and saliva samples were markedly higher than those observed in our previous studies with a single PspA antigen. The geometric mean concentrations of serum anti-PspA1 and anti-PspA2 IgG were similar at 12 and 18 months in children with and in those without pncAOM during the following 6 months. The children having detectable anti-PspA IgA in saliva at 12 months seemed to have pncAOM as often as children with undetectable salivary anti-PspA. However, at 18 months the children with salivary anti-PspA had a significantly lower frequency of pncAOM during the following 6 months than the children with no salivary anti-PspA (p < 0.05). Serum anti-PspA IgG seemed to have no association with protection against pncAOM at 12 and 18 months. In contrast, at 18 months, the presence of salivary anti-PspA (anti-PspA1 and/or anti-PspA2) IgA was associated with a decreased risk of pncAOM during the following 6 months [RR 0.3 (95% CI 0.11-0.69)].

Conclusion: The decreased risk of pncAOM associated with the presence of salivary anti-PspA at 18 months of age suggests that salivary anti-PspA antibodies may have a role in prevention of pncAOM.
Comparison of *S. pneumoniae* (sp) carriage and penicillin resistance between vaccinated and non-vaccinated young children with acute otitis media (AOM)

Cohen, R1, Levy, C2, de La Rocque, F2, Bonnet, E3, Fritzell, B1, Telentoum, R1, Boucherat, M3, Varon, E4

1CHI Créteil, France
2ACTIV, France
3Wyeth, France
4HEGP Paris, France

**Background:** In order to follow the Sp serotypes and antibiotic resistance trends following the implementation of the 7-Valent pneumococcal conjugate vaccine (PCV7), a 5-year French national surveillance network was started in 2001.

**Methods:** 95 pediatricians collected nasopharyngeal swabs from children 6 to 24 months of age with AOM who had not received antibiotics within 7 days. Sp isolation, serotyping and antibiotic susceptibility were performed using standard methodology by the French National Reference Center.

**Results:** Over the first 4 years, 2532 patients were enrolled. 1096 received at least one shot of PCV7: 1 dose (11.6%) 2 doses (28.8%) and ≥3 doses (59.6%). Vaccinated and non vaccinated children were comparable regarding sex ratio, number of siblings, clinical signs and symptoms, proportion of children who received antibiotics within 3 months prior enrollment. Non vaccinated children were more frequently home cared (37.5% vs 30.2%, p=0.0001) and were older (14.1 months ± 5.5 vs 13.4 months ± 5, p=0.0005) than vaccinated.

<table>
<thead>
<tr>
<th>Carriage according to vaccination status (2526 samples available):</th>
<th>Non vaccinated n=1432 (%) IC 95%</th>
<th>Vaccinated ≥1 dose n=1094 (%) IC 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non Sp carriers</strong></td>
<td>424 (29) [27;32]</td>
<td>467 (43) [40;46]</td>
</tr>
<tr>
<td>Vaccine serotypes without 19F</td>
<td>440 (31) [28;33]</td>
<td>126 (11) [10;13]</td>
</tr>
<tr>
<td>19F</td>
<td>189 (13) [11;15]</td>
<td>104 (9) [8;11]</td>
</tr>
<tr>
<td>19A</td>
<td>97 (7) [5;8]</td>
<td>100 (9) [7;11]</td>
</tr>
<tr>
<td>6A</td>
<td>84 (6) [5;7]</td>
<td>57 (5) [4;7]</td>
</tr>
<tr>
<td>Other vaccine related serotypes</td>
<td>27 (2) [1;3]</td>
<td>17 (1) [0.9;2]</td>
</tr>
<tr>
<td>Non vaccine serogroups</td>
<td>166 (11) [10;13]</td>
<td>206 (19) [16;21]</td>
</tr>
<tr>
<td>Non Typable</td>
<td>5 (0.3) [0.1;0.9]</td>
<td>17 (1) [0.9;2]</td>
</tr>
</tbody>
</table>

The risk for a child to carry a highly penicillin resistant strain was 4% for immunized children who had not received antibiotics within 3 months, 8% for those vaccinated and who had received antibiotics, 10% for children not immunized and who had not received antibiotics and 15% for children not vaccinated who had received antibiotics.

**Conclusion:** This study shows synergy between vaccination and prudent use of antibiotics to reduce the carriage of resistant pneumococci.
Microbiology of acute otitis media with perforation (AOMwiP) in Aboriginal children living in remote communities – monitoring the impact of 7-valent pneumococcal conjugate vaccine (7vPCV)

Leach A1,2, Beissbarth J2,3, Halpin S2,3, Hare K1,2, Kennedy G1,2, Mackenzie G1,2, Mellon C1,2, Wigger C1,2, Wilson C1,2, Smith-Vaughan H1,2, Stubbins E2,3, Tipakalippa P1,2 & Morris P1,2,3

1Menzies School of Health Research, Darwin, Northern Territory, Australia
2Charles Darwin University, Institute of Advanced Studies, Darwin, Northern Territory, Australia
3Flinders University, Adelaide, South Australia, Australia

Background
Otitis media (OM), particularly tympanic membrane perforation (TMP), remains a significant health problem for Aboriginal children living in the Northern Territory of Australia. Pneumococcal conjugate vaccine (7vPCV), which was introduced into the Australian infant immunisation schedule in 2001, has the potential to reduce rates of TMP.

Aim
To describe the microbiology of acute otitis media with perforation (AOMwiP) in young Aboriginal children pre- and post-introduction of 7vPCV vaccination.

Methods
Data from studies conducted between 1996 and 2004 were combined and cases of AOMwiP were included if the child was less than 18 months of age, was not enrolled in an antibiotic arm of a randomized controlled trial, had either their first AOMwiP episode, or a new AOMwiP episode (occurring 4 weeks after a previous AOMwiP episode, or following a diagnosis of an intact non-bulging tympanic membrane) and a swab of ear discharge had been cultured. The control group consisted of children enrolled in studies prior to 7vPCV availability in July 2001 and the vaccine group consisted of children enrolled in studies conducted after July 2001. Standard microbiological methods were used.

Results
Fifty three cases of AOMwiP were identified in 21 children in the control group and 83 cases in 45 children in the vaccine group. Mean age was identical (267 days) and there were more males in the control group (55% versus 45%). 7vPCV coverage (>2 doses) was 87% in the vaccine group.

H. influenzae was the most prevalent OM pathogen and S. pneumoniae the second most prevalent, in both groups (Table 1). There was a marked reduction in AOMwiP associated with 7vPCV type pneumococci in vaccinees compared to controls (RD -12% [-23, 1]). The third most common pathogen detected was S. pyogenes (6% overall). Staphylococcal spp. were very common, but rarely as a sole species, and these were considered likely contaminants.

Table 1. Otitis media pathogens isolated from episodes of acute otitis media with perforation in 7vPCV-vaccinated and non-vaccinated Aboriginal children less than 18 months of age.

<table>
<thead>
<tr>
<th>Organism (alone or in mixed culture)</th>
<th>Overall (N=136)</th>
<th>CONTROL (N=53)</th>
<th>VACCINE (N=83)</th>
<th>*Risk Difference [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. influenzae</td>
<td>57%</td>
<td>55%</td>
<td>58%</td>
<td>+3% [-14, 20]</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>34%</td>
<td>38%</td>
<td>31%</td>
<td>-6% [-23, 10]</td>
</tr>
<tr>
<td>VT**</td>
<td>10%</td>
<td>17%</td>
<td>5%</td>
<td>-12% [-23, 1]</td>
</tr>
<tr>
<td>VRT</td>
<td>9%</td>
<td>4%</td>
<td>12%</td>
<td>+8% [-0.4, 17]</td>
</tr>
<tr>
<td>Non-VT</td>
<td>14%</td>
<td>17%</td>
<td>12%</td>
<td>-5% [-17, 7]</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>6%</td>
<td>9%</td>
<td>6%</td>
<td>-3% [-13, 6]</td>
</tr>
<tr>
<td>M. catarrhalis</td>
<td>4%</td>
<td>8%</td>
<td>1%</td>
<td>-6% [-14, 1]</td>
</tr>
<tr>
<td>Negative for spp. above</td>
<td>34%</td>
<td>34%</td>
<td>34%</td>
<td>+0.2% [-16, 17]</td>
</tr>
<tr>
<td>Staphylococcal spp.</td>
<td>62%</td>
<td>57%</td>
<td>66%</td>
<td>+10% [-7, 26]</td>
</tr>
</tbody>
</table>

*Adjusted for repeat measures in the same child. **VT = 7vPCV type, VRT = not 7vPCV but related to 7vPCV, Non-VT = not 7vPCV not related to 7vPCV.

Conclusion
This analysis of acute perforations shows a marked reduction in AOMwiP associated with VT, and substantial replacement by VRT, in vaccinees compared to controls. H. influenzae was the dominant respiratory pathogen (57% overall), and GAS was more prevalent than previously appreciated (6% overall). In this analysis there was no significant replacement of VT pneumococcal AOMwiP by non-pneumococcal species. AOM with perforation remains a significant health problem needing urgent attention.
Effect of pneumococcal vaccination in otitis-prone children during influenza epidemics

Hak, E1, Veenhoven, RH2, Uiterwaal, C3, Schilder, A4, Sanders, EAM5
1Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, the Netherlands
2Spaarne Hospital, Hoofddorp, the Netherlands
3Wilhelmina Children’s Hospital, University Medical Center Utrecht, the Netherlands

Background. Primary viral infections induce mucosal alterations that allow for adherence of bacteria to the respiratory epithelia and increased bacterial colonization in the upper airways. In a cohort study among children with acute otitis media (AOM), *S. pneumoniae* was isolated more frequently in middle-ear fluid containing influenza virus (100%) than in those ears containing other respiratory viruses. We hypothesized that pneumococcal vaccination reduces AOM occurrence during influenza epidemics in otitis-prone children.

Aims. We assessed the effects of pneumococcal vaccination on the occurrence of AOM during influenza epidemics, per-influenza seasons and summer seasons.

Methods. A secondary analysis of a randomized controlled trial among 383 children with recurrent AOM aged 1-7 years with two or more AOM episodes before inclusion was conducted. Children were randomly allocated to receive either 7-valent pneumococcal conjugate vaccine (PCV-7) followed by 23-valent pneumococcal polysaccharide vaccine (PPSV-23), or hepatitis A or B control vaccines. AOM was the primary outcome. We subdivided 24 months of follow-up for each participant into person-periods during influenza epidemics, per-influenza and summer seasons in the period 1998-2001. In an intention-to-treat analysis, proportions of person-periods with AOM were compared between the pneumococcal vaccine and control groups and presented as 1-relative risks*100 percent with corresponding 95% confidence intervals [95% CI].

Results. Prevalence of males and mean age were similar in the comparison groups in all seasons. During influenza epidemics, pneumococcal vaccination prevented 35% of AOM episodes (95% CI, -3%-60%, *P*=.07) and was statistically significant more effective in the PCV-7/PPSV-23 group (effectiveness, 64%; 95% CI, 4%-87%, *P*=.03) than the PCV-7 group (16%; 95% CI, -42%-51%, *P*=.50, see table). Pneumococcal vaccination did not prevent AOM in the non-influenza seasons.

Conclusions. This study among children with recurrent AOM showed substantial reduction in the rates of AOM by pneumococcal vaccination during an influenza epidemic. Studies are urgently warranted to determine benefits of combinations of influenza and pneumococcal vaccines during influenza epidemics.

Pneumococcal infection in preschool children: incidence of acute otitis media and pneumonia related to influenza and respiratory syncytial virus circulation

Jansen, AGSC1, Sanders, EAM2, Hoes, AW2, van Loon, TM3, Rovers, MM1, Hak, E1
1Julius Center for Health Sciences and Primary Care
2Department of Pediatric Immunology
3Department of Virology, UMC Utrecht, the Netherlands

Background: *Streptococcus pneumoniae* is a major bacterial pathogen involved in acute otitis media (AOM) and pneumonia. Primary viral infections induce mucosal alterations that allow for adherence of bacteria to the respiratory epithelia, and predispose to bacterial infections. Evaluation of the occurrence of AOM and pneumonia during influenza and respiratory syncytial virus (RSV) season may predict the potential impact of pneumococcal vaccinations combined with influenza and/or RSV vaccination.

Objective: To determine the incidence of AOM and pneumonia in preschool children in primary care in relation to the circulation of influenza viruses and RSV.

Study design and setting: We performed a retrospective cohort study including all children aged 0-5 years registered in the computerised research database of the Utrecht general practitioner (GP) network. During the period 1998-2002 (18,801 child years), participating GPs recorded episodes of AOM and pneumonia according to the International Classification of Primary Care. Viral surveillance data were obtained from the Weekly Sentinel System of the Dutch Working Group on Clinical Virology. Peak viral seasons were defined as the weeks with more than 5% of the yearly number of isolates of influenza or RSV. The baseline period was defined as the weeks with less than 1% of the yearly number of isolates of both influenza and RSV.

Results: On average 177 and 19 episodes of AOM and pneumonia were recorded per 1,000 child years. Children below two years of age had higher incidence rates of AOM than older children (196 and 123 per 1,000 child-years; relative risk (RR) 1.60, 95%CI: 1.48-1.72). During peak influenza season notably more consults occurred for AOM (RR 1.86, 95%CI: 1.66-2.08) and pneumonia (RR 2.08, 95%CI:1.48-2.94). During peak RSV season consultation rates even appeared even higher for AOM (RR 2.24, 95%CI:2.01-2.50) and pneumonia (RR 3.67, 95%CI:2.72-4.95). The excess consultation rates for AOM and pneumonia during peak influenza and RSV seasons appeared highest in children below two years of age.

Conclusion: AOM and pneumonia cause substantial burden on primary care during influenza and RSV season. Pneumococcal vaccination combined with vaccination against influenza and RSV may substantially reduce the burden of AOM and pneumonia, notably in children under two years of age.
Use of ototopical antibiotic treatment on chronic suppurative otitis media: A randomised controlled trial and feasibility study

Gadil, E1,2, Morris, PS1,2, Beissbarth, J3, Chang, A1, Gibson, P3, Hare, K1,2, Halpin, S3, Hansbro, P1, Kennedy, M1,2, Masters, B5, Valery, P3, Timmins, NT, Torzillo, P3, Wigger, C2, Leach, AJ1,2

1Menzies School of Health Research, Darwin, NT, Australia
2Charles Darwin University, Darwin, NT, Australia
3Royal Children’s Hospital, Brisbane, Queensland, Australia
4John Hunter Hospital, Newcastle, NSW, Australia
5Royal Newcastle Hospital, Newcastle, NSW, Australia
6Queensland Institute of Medical Research, Brisbane, Queensland, Australia
7Nganampa Health Council, NT, Australia

Background: Chronic suppurative otitis media in Australian Indigenous communities is a major health problem that is extremely difficult to treat. Use of ototopical antibiotics is currently the recommended practice. While bacterial eradication is an important step in promoting cure, little is known about the role of local immunity and persistent inflammation in CSOM and how this is affected by antibiotic therapy.

Aims
- To evaluate the short-term clinical effects of ototopical antibiotics combined with dry-mopping versus dry-mopping alone in Aboriginal school children with CSOM.
- To conduct a pilot study on the feasibility of using ear and nasal discharge samples for immunologic studies in CSOM.

Study Design: An assessor-blinded randomised controlled trial was conducted involving Aboriginal school children aged between 4 and 13 years, diagnosed with CSOM, with perforation size at least 5%, and moderate to profuse discharge. They were randomly assigned to 4 days of either the “dry mopping alone” (BD) or the “antibiotic group” (dry mopping plus either ciprofloxacin or Sofradex® [4 drops BD]). Specimens of ear discharge were collected for immunological (IL8) and bacteriological studies. Clinical improvement was defined as moderate to profuse discharge becoming scant or dry after intervention. Discharge was clinically assessed by a direct swab into the middle ear space. The primary outcome was the proportion of children with no clinical improvement.

Results: Thirty two of 78 consented children were eligible for randomisation. After 4 days, 5 of the 20 in the “antibiotic group” and 9 of the 12 in the “dry mopping alone group” did not have clinical improvement. One from the “antibiotic group” had dry ears. All ear discharge specimens were positive for micro-organisms at baseline and forty percent had Pseudomonas. At the end of intervention, none of 11 in the “dry mopping alone group” and seven out of 18 from the “antibiotic group” showed bacterial clearance. The concentration of IL-8 in ear discharge was low (~300 pg/mg of ear discharge) and not significantly different between intervention groups.

<table>
<thead>
<tr>
<th>Day 4</th>
<th>Dry-mopping alone</th>
<th>Antibiotic</th>
<th>Relative Risk [95%CI]</th>
<th>Risk Difference [95%CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Failure - no improvement in discharge</td>
<td>9/12 (75%)</td>
<td>5/20 (25%)</td>
<td>0.33 [0.14, 0.76]</td>
<td>-50% [-81, -1]</td>
</tr>
<tr>
<td>Failure - no bacterial clearance</td>
<td>11/11 (100%)</td>
<td>11/18 (61%)</td>
<td>0.61 [0.42, 0.88]</td>
<td>-39% [-61, -16]</td>
</tr>
</tbody>
</table>

Conclusion: Topical antibiotics were significantly better at clearance of ear discharge and bacteria than dry-mopping alone. A significant reduction in ear discharge may be a good indication of early clinical response. Our study documented low levels of IL-8 in the ear discharge of children with CSOM consistent with chronicity of ear infection.
Otitis media research transfer. The Aboriginal Ear And Hearing Health Web Resource and National Network

Hopkins, S1, Brands, J1, Thompson, N2, Eikelboom, RH1, Nelson, S4, Dearing, S1, Morris, P6,7
1Cooperative Research Centre for Aboriginal Health, Darwin, NT Australia
2Indigenous Health InfoNet, Edith Cowan University, Perth, WA, Australia
3Lions Ear and Hearing Institute, Perth, WA, Australia
4Danila Dilba Health Service, Darwin, NT, Australia
5Department of Employment, Education and Training, Darwin, NT, Australia
6Menzies School of Health Research, Darwin, NT, Australia
7Flinders University, Adelaide, SA, Australia

Background
The World Health Organisation states that rates of chronic suppurative otitis media greater than 4% represent a serious public health problem. In some remote Aboriginal communities, rates of ear drum perforation exceed 60% and up to 50% of school children are eligible for hearing aids. There is an urgent need to improve access of otitis media and to disseminate useful information about the effectiveness of treatment and prevention interventions for ear and hearing health.

Aim
The primary aim of this project is increasing the availability and sharing of quality information and educational resources through an internet-based integrated knowledge resource. It is hoped that this will improve ear and hearing health and the associated social and educational disadvantage. We aim to evaluate the impact of this resource, as part of a comprehensive research transfer plan, on the uptake of evidence based policy and practice.

Methods
An internet-based integrated knowledge resource will be developed using a systematic approach to the provision of evidence-based information, developed by the Indigenous Health InfoNet (www.healthinfonet.ecu.edu.au). Components of the resource would include; reviews and other synthesised knowledge, prevention and management guidelines/protocols, ear and hearing health resources and a bibliography. It would also include information about research activity and funding, organisations, agencies and individuals working in the field, news and events and training programs. Lastly, an ear and hearing health ‘community of practice’ or network would be developed.

Quality assurance will be ensured through expert peer review throughout the development and implementation of this project.

Results
An email database of approximately 50 people including nurses, doctors, Aboriginal Health Workers, policy officers, audiologists, teachers, researchers and other scientists has been established. It includes people from Queensland, New South Wales, Western Australia and the Northern Territory. They were consulted in the development of the project proposal and have expressed their support. The Cooperative Research Centre for Aboriginal Health has funded the initial development phase of this project and collaboration with The Menzies School of Health Research and the Indigenous Health InfoNet has been established.

Conclusion
This web resource represents a novel approach to addressing the problem of presenting complex information. The support received during the development of the proposal from people working in the field indicates there is a need for improved access to information and educational resources.

Middle ear fluid Streptococcus pneumoniae serotype distribution and antimicrobial susceptibility patterns in Mexican children with acute otitis media

Lopez-Enriquez, C1, Espinosa Monteros LE2, Rojas L1, Gomez-Barreto D2
1Hospital Español, Mexico City, Mexico
2Hospital General Manuel Gea Gonzalez, Mexico City, Mexico
3Hospital Infantil de Mexico, Federico Gomez, Mexico City, Mexico
4Hospital Infantil de Mexico, Federico Gomez, Mexico City, Mexico

Background: The acute otitis media (AOM) is the most common microbial respiratory tract infection in early childhood. Streptococcus pneumoniae is 1 of 2 most common pathogens isolated from patients with AOM worldwide. The American Academy of Pediatrics (AAP) advises immunization with a 7-valent pneumococcal conjugate vaccine in children with recurrent AOM. The pneumococcal conjugate vaccine could be preventing the AMO in Mexican children.

Objectives: To establish the most common S. pneumoniae serotypes present in the middle ear fluid (MEF) of Mexican children with otitis media (OM) and to analyze serotype distribution and antimicrobial susceptibility patterns and assess the potential protection provided by the new conjugated S. pneumoniae vaccines.

Methods: During 2002 and 2003, 69 S. pneumoniae isolates were obtained from the MEF of Mexican, ages 3-109 months, with AOM. Serotyping was performed by the quellung reaction with antisera from Statens Serum Institute, Copenhagen, Denmark.

Results: The most common S. pneumoniae serotypes isolated were 6B, 15.5%; 14, 15.5%; 19F, 15.5%; 23F, 15.5%; and 6A, 9.86%.
Serotype distribution was similar among children younger than 24 months of age or 24 months of age or older and by disease distribution. The OM was most frequent among children between 2-24 months, 43.38%.

Overall rates of resistance (defined as the rate of intermediate resistance plus the rate of resistance) were as follows: penicillin, 68.42% (intermediate and resistant categories were 31.58% and 36.84%, respectively); amoxicillin, 6.3%; cefuroxime, 35.92%; cefotaxime, 7.1%; erythromycin, 26.32%; and clindamycin, 21.05%.
The most common resistant serotypes isolated were 19F and 23 F.

Conclusions: The serotype distribution of S. pneumoniae causing pediatric OM in Mexico, City is similar to those reported from developed countries. The current heptavalent pneumococcal conjugate vaccine covers 71.97% of OM episodes in Mexican children.
Acute otitis media in Denmark; symptoms, treatment and control

Lous, J1
1University of Southern Denmark, Odense, Denmark

The aim of this multicenter study in primary care in Denmark was to describe the symptoms, the treatment and control of acute otitis media (AOM) in children up to 10 years of age. 58 general practitioners (GP) participated in the 2-year observational study. A total of 331 children with AOM were included in the study. AOM was defined as fever and pain combined with either redness and protrusion of the tympanic membrane or with whiteness and decreased mobility of the tympanic membrane or simply as acute purulent secretion from the middle ear. When the GP had diagnosed an AOM he took a nasopharyngeal swab, which was sent for culture and susceptibility testing. The symptoms were recorded during the consultation by the doctor. All children were encouraged to return for a 4-week control.

Results: The mean age was 2½ years. More than 90% of the children reported they suffered from earache, half of them for 12 hours or less, and 10% for more than 48 hours. About 85% of the children had symptoms of common cold, and about 60% had fever. For about 15% this acute otitis media was their first episode. About half the children had received antibiotics within the last 6 months, one sixth had never had antibiotics. More than half of the children had bilateral AOM. About 15% were classified by the GP as light, 60% as medium and 25% as severe AOM. Eighteen percent was culture negative by nasopharyngeal swab, 48% had pneumococci, 33% Haemophilus influenzae or Moraxella catarrhalis, and 26% Streptococcus pneumoniae were isolated.

No relationship was found between bacterial species and symptoms. Thirty percent were initially treated without antibiotics. When antibiotic was given, penicillin V was the drug of choice in 60%, amoxicillin in 33%, and other antibiotics in 7% of cases. At the 4-week control more than 80% of the children had recovered, 2-5% still had symptoms and signs of AOM, and about ¼ had signs of middle ear effusion. (secretory otitis media).

Conclusion: Some children with AOM are treated without antibiotics. Penicillin V was the drug of choice in most treated children. No serious problems were seen after 4 weeks.
The impact of pneumococcal conjugate vaccine on otitis media in the outpatient setting

**Background**
Routine childhood immunization with the 7-valent pneumococcal conjugate vaccine (PCV7) has substantially reduced invasive pneumococcal disease (IPD) in children aged < 2 years in the U.S. Otitis media (OM) is much more common than IPD and is associated with $3 billion in direct and indirect costs annually. Since the impact of PCV7 on OM is not well documented, we evaluated the effect of PCV7 on OM outpatient visits in two communities.

**Methods**
We reviewed outpatient charts of children attending family medicine and pediatric practices in Monroe County, NY and Davidson County, TN (92 of 117 [79%] practices participated). We compared the risk of developing OM between 6 and 24 months of age in two cohorts of children born before and after PCV7 introduction. We used Cox regression to evaluate time to first and third OM episode, and time to frequent OM (defined as ≥ 3 episodes in 6 mos or ≥ 4 episodes in 12 mos). Episodes are defined as visits for OM occurring after 6 mos of age and at least 21 days after the previous visit for OM.

**Results**
There was no significant difference in the timing of first OM between children in the two cohorts (hazard ratio [HR], 0.94; 95% CI 0.84-1.04; p=0.233). However, the HR (0.86, 95% CI 0.73-1.00) for time to third OM was significant (p=0.046) with an associated 10% reduction in the occurrence of a third OM episode by 2 years. Children in the post-PCV7 cohort showed a 14% decline in the cumulative proportion with frequent OM by 2 years and the associated HR of 0.82 (95% CI 0.67-1.01) was marginally significant (p=0.055).

**Conclusions**
We observed a modest reduction in the risk of frequent OM attributable to PCV7 in the clinical outpatient setting. However, PCV7 shortages that reduced the number of children in the post-PCV7 cohort vaccinated according to the recommended schedule and catch-up vaccinations that resulted in a number of children in the pre-PCV7 cohort receiving PCV7 during their second year of life may have reduced potential differences between the cohorts. These findings suggest that physicians are unlikely to observe notable decreases in OM visits in their practices in the short term, but the population-wide long-term reduction in morbidity and costs from frequent OM may be substantial.

---

Telithromycin resistance in clinical isolates of pneumococci from Europe: Resistant mechanisms and time-kill kinetics

**Background**
Telithromycin is a novel ketolide antibiotic with significant in-vitro activity against *S. pneumoniae*. The aim of this study project is to characterize the possible resistance mechanisms of clinical isolates of *S. pneumoniae* with reduced susceptibility to telithromycin (>1 mg/l) and their time-kill kinetics with telithromycin.

**Methods**
Determination of MICs was performed by the microbroth dilution method according to the CLSI (formerly called as NCCLS) and the serotyping by the Neufeld Quellung reaction. Multilocus sequence typing, sequencing of the 23S rRNA, sequencing of genes encoding ribosomal proteins (L4 and L22), ermB and the time-kill kinetics were performed according to standard methods.

**Results**
In two nation-wide studies and one European surveillance study (n= 6604) performed at the National Reference Center for Streptococci in Germany, reduced susceptibility to telithromycin (>1 mg/l) was detected in 17 isolates (0.3%). MICerm/MICerm (mg/l) of the strains to other antibiotics were as follows: Penicillin G 2/2, cefuroxim 8/8, erythromycin A >32/>32, clindamycin >32/>32, tetracycline 32/32, telithromycin 2/8, and gatifloxacin 0.25/0.25. Two major serotypes were detected, serotype 14 (58.8%) and serotype 19A (29.4%). Four isolates were selected for molecular characterization and time-kill, two of which with a telithromycin MIC 2 mg/l and two strains with a telithromycin MIC of 8 mg/l. All isolates possess the cMLS B phenotype (ermB positive). The isolates showed a wide range of combinations of resistance determinants including multiple alterations in the 23S rRNA (A138G, C150T, A260G, A1745T, and C2216T.), a S20N alteration in the ribosomal protein L4 (n = 9), and a N100S alteration in the erm(B) gene (n=14). The predominant clone was serotype 14 sequence type 143 (8 of 17 isolates), which was seen in France (n = 7) and Germany (n = 1). Telithromycin-resistance has also spread to the Spain23F-1 clone (ST 81; n = 1) and its serotype 19A variant. In-vitro time-kill showed a minimal kill from 0-8 hours and then regrowth. Bactericidal activity was achieved with 8 times the MIC in all strains.

**Conclusions**
Although the incidence of telithromycin resistance remains rare worldwide, the spread of telithromycin resistance to multi-drug resistance clones with world-wide distribution is worrisome.
A novel oral carbapenem, Tebipenem Pivoxil (ME1211), showed high bacterial and clinical effectiveness against penicillin-resistant *S. pneumoniae* on a phase II clinical trial in otolaryngological infections

Yamana, N1,2, Fujihara, K1, Hotomi, M1, Furakawa, M1, Furuya, N1, Suzuki, K2, Totsuka, K1, Ubakatu, K1, Baba, S1

1Wakayama Medical University, Wakayama, Japan
2ME1211 Otolaryngology Study Committee, Tokyo, Japan

**Background:** ME1211 is a novel oral carbapenem antibiotic having potent activity against *S. pneumoniae* on which increased resistance has become a serious problem worldwide.

**Methods:** A double-blind comparative study was conducted to establish the recommended dose for adult otolaryngological infections in 3 groups at 150 mg tid(150T), 250 mg bid(250B), or 300 mg tid(300T) for 7 days, which were estimated from non-clinical PK/PD data and Phase I. Clinical and bacteriological efficacy and safety were investigated mainly in acute otitis media (AOM) and acute sinusitis (AS). For the clinical study, the severity of disease was assessed by using the clinical scoring system (Eur J Pediatr, 2005,164:3-8). Bacteriological assays were performed by conventional culture methods and PCR methods to identify molecularly the genes associated with the resistance to β-lactam antibiotics in *S. pneumoniae*. In addition, PK/PD analysis was performed to obtain the clinical target values of PK/PD parameters.

**Results:** Of 212 patients enrolled, 112 patients diagnosed to be infected with common causative pathogens were subjected for clinical efficacy analysis. Clinical efficacy as the primary endpoint was obtained in 72.1% (31/43, 95% CI 56.3-84.7) in 150T, 88.6% (31/35, 95% CI 73.3-96.8) in 250B and 85.3% (29/34, 95% CI 68.9-95.0) in 300T, showing high efficacy in 250B and 300T. The clinical efficacy rate in AOM was 100% in any groups (6/6, 9/9, 8/8), and that in AS was 68.0% (17/25, 95% CI 46.5-85.1) in 150T, 93.8% (15/16, 95% CI 69.8-99.8) in 250B and 84.2% (16/19, 95% CI 60.4-96.6) in 300T. The bacteriological efficacy was 90.7% in 150T, 91.4% in 250B and 91.2% in 300T, showing high efficacy in 250B and 300T. The clinical efficacy rate in AOM was 100% in any groups (6/6, 9/9, 8/8), and that in AS was 68.0% (17/25, 95% CI 46.5-85.1) in 150T, 93.8% (15/16, 95% CI 69.8-99.8) in 250B and 84.2% (16/19, 95% CI 60.4-96.6) in 300T. The bacteriological efficacy was 90.7% in 150T, 91.4% in 250B and 91.2% in 300T, and all strains of *S. pneumoniae* including penicillin-resistant strains (PRSP) were eradicated on day 3 (MIC of ME1211 distributed below 0.063μg/ml). A major adverse drug reactions (ADRs) was mild diarrhea and no serious ADR was observed Based on the results of PK/PD analysis, the target values of AUC/MIC and Cmaxf/MIC were calculated as 10 and 4, respectively.

**Conclusion:** The recommended clinical dosage of ME1211 for the treatment of otolaryngological infections was considered to be 250 mg bid, showing marked bacteriological effects of ME1211 against common causative pathogens including PRSP.

---

**Molecular characterization of tetracycline-resistant isolates of *Streptococcus pneumoniae* in Poland**

Izdebski, R., Sadowy, E., Hryniewicz, W.
National Institute of Public Health, Warsaw, Poland

**Aims:** To study molecular mechanisms of tetracycline resistance and clonal diversity of clinical tetracycline-resistant *Streptococcus pneumoniae* strains isolated in Poland and to investigate the relationship between the presence of tetracycline resistance determinants and the MICs of tigecycline, doxycycline, minocycline and as well as other clinically important agents.

**Methods:** Tetracycline susceptibility of 866 clinical isolates of *S. pneumoniae* from respiratory tract infections, collected in Poland between 1998 and 2003 was investigated by the disk diffusion method as recommended by the CLSI. Serotyping of resistant isolates was performed by capsule swelling reaction or PCR. Clonality of the resistant isolates was studied by PFGE of Smal-digested bacterial DNA. Selected isolates representing main PFGE types were analysed by the MLST. Resistance determinants (tetM, tetO) were detected by PCR. MICs were determined using the CLSI broth microdilution method.

**Results:** In total, 242 (27.9%) isolates non-susceptible to tetracycline represented 27 serotypes. The majority of isolates (77.3%) belonged to serotypes 19F, 23F and 14 (69, 59 and 16 isolates, respectively), and to serogroup 6 (43 isolates). Among serotype 19F isolates, 13 PFGE types were present. The main type in this group (56 isolates) had 9 subtypes, with the most numerous subtype (45 isolates – 18.6% of all resistant isolates) corresponding to ST423. Isolates of serotype 23F (21 PFGE types) belonged to STs: 81 (16 isolates), 602 (15) and 173/272 (5). In the group of serotype 14, STs 143 (7 isolates) and 124 (4) were identified. Among isolates of serogroup 6 (16 PFGE types), STs 315 (13 isolates) and 490 (6) were found. All tetracycline-nonsusceptible isolates harbored the tetM gene. Among studied isolates, MIC for tetracycline ranged from 8-128 μg/ml, for doxycycline and minocycline 0.5-16 μg/ml. Twenty six percent of isolates were penicillin-nonsusceptible. Resistance rates to erythromycin, trimethoprim-sulphamethoxazole and chloramphenicol were 26.4%, 38.8% and 39.2% respectively.

**Conclusions:** The observed high level of resistance to tetracycline among *S. pneumoniae* isolated in Poland appears to result from spread of relatively few epidemic clones, harboring the tetM gene as resistance determinant. Tigecycline showed an excellent activity against tetracycline-resistant *S. pneumoniae*.
**Epidemiology of Streptococcus pneumoniae resistance to antimicrobial agents in Taiwan**

Shieh, GJ\(^1\), Hwang, B\(^2\), Tang, RB\(^2\)

\(^1\)National Taiwan University, Taiwan
\(^2\)Taipei Veterans General Hospital, Taipei, Taiwan

**Introduction:** During the past several decades, the spread and emergence of penicillin-nonsusceptible and multidrug-resistant *Streptococcus pneumoniae* has become a global problem. In Taiwan, penicillin-nonsusceptible *S. pneumoniae* (PNSSP) was first reported in 1986. Since then, antibiotic resistant strains of *S. pneumoniae* have been isolated throughout the whole island. Due to inappropriate prescription and use of antibiotics, such as antibiotic overprescription in primary care units and multiple-antibiotic prescription contributes to increasing antibiotic resistance in Taiwan. Close monitoring of antimicrobial resistance and proper antibiotic usage are major ways to control of pneumococcal resistance.

**Objective:** Our objective was to review the resistance of *S. pneumoniae* strains isolated in Taiwan over time.

**Methods:** A systematic review of published literature and unpublished data on pneumococcal susceptibility in Taiwan from 1995 to 2004 was performed.

**Results:** In Taiwan, the prevalence of clinical isolates of PNSSP increased over time; rates were as follows: 12%-22% in 1989-1995, 56%-61% in 1996-1997, 56%-76% in 1998-1999, 60%-80% in 1999-2000, 72% in 2000-2001, and 70% in 2002. Susceptibility testing of other β-lactams from 1996 to 2002 demonstrated the following resistance rates: 14%-43% to cefotaxime, 13%-56% to ceftriaxone, 46%-49% to cefepime, 43%-67% to cefuroxime, 14%-21% to imipenem, and 4%-39% to meropenem. Pneumococcal resistance to erythromycin and azithromycin in clinical isolates has increased markedly in Taiwan: 9% in 1984, 56% in 1989-1992, 74%-82% in 1996-1997, 76%-94% in 1998-1999, and 94%-100% in 2000-2001. *S. pneumoniae* resistance to tetracycline has increased from 69% in 1989-1992 to 71%-74% in 1990-1993, and to 83% in 1996-1997. *S. pneumoniae* resistance to trimethoprim-sulfamethoxazole (TMP-SMZ) was 65%-98% in 1996-2002. Resistance to quinupristin-dalfopristin in 1996-2001 was 8%-42%. Fluoroquinolone resistance remains at low levels (0%-7%) in Taiwan. Almost all *S. pneumoniae* isolates remain susceptible to rifampicin (93%), vancomycin (100%), and linezolid (100%). More than 90% of PNSSP isolates in 1996-2002 were also resistant to multiple antibiotics. Among the PNSSP isolates, 63%-100% were resistant to cefotaxime, ceftriaxone, and carbapenems; 66% were resistant to cefepime; 59%-97% were resistant to erythromycin; and 85%-100% were resistant to TMP-SMZ.

**Conclusions:** Pneumococcal resistance to penicillin, cephalosporins, macrolides, tetracyclines, and TMP-SMZ in Taiwan has increased markedly over time. Rates of multidrug resistance are also extremely high. Judicious antibiotic usage and pneumococcal vaccination may help in controlling of pneumococcal resistance.
Antibiotic susceptibility of pneumococci from acute otitis media in Denmark

Lous, J, Hansen, BL, Hansen, KG, Molstad, S, Nielsen HU, Konradsen HB, Frimodt-Møller, N
1University of Southern Denmark, Odense, Denmark
2General Practice, Aalborg, Denmark
3Unit of Research and Development in Primary Care, Mjölby, Sweden
4Statens Serum Institute, National Center for Antimicrobials and Infection Control, Copenhagen, Denmark

The aim of this multicenter study in primary care in Denmark was to describe the bacteria in nasopharynx in children with acute otitis media (AOM) with special focus on the antibiotic susceptibility of S. pneumoniae.

A total of 331 children (0-10 years old) with AOM were included in this observational study in 1997-1998. AOM was defined as fever and pain combined with either redness and protrusion of the tympanic membrane or with whiteness and decreased mobility of the tympanic membrane or simply as acute purulent secretion from the middle ear. When the GP diagnosed AOM he took a nasopharyngeal swab for culture and susceptibility testing.

Results: The mean age of the children was 2½ years. Of the 331 children 74 (22%) had no pathogenic bacteria in their nasopharynx, 157 (47%) had Streptococcus pneumoniae, 108 (33%) had Haemophilus influenzae, 85 (26%) had Moraxella catarrhalis, and 13 (4%) had Streptococcus pyogenes. That means, that in 78% of the children we found one or more of these pathogenic bacteria. In 161 children (63%) of culture positive only one pathogen was found.

About half the children had received antibiotics within the last 6 months, one sixth had never had antibiotics. Penicillin V was the antibiotic most often used, closely followed by amoxicillin or ampicillin.

Ninety-nine of the S. pneumoniae were tested for susceptibility towards selected antibiotics. We found one (1%) multi-resistant S. pneumoniae and 3 (3%) with intermediate susceptibility towards Penicillin V.

Discussion: Due to the relatively low use of antibiotics in Denmark, and of penicillin as first drug of choice we still have relatively few problems with antibiotic resistance in children with acute otitis seen in general practice.

Hellenic study on the susceptibility of Streptococcus pneumoniae isolated from the nasopharynx of healthy children in 2004

14th Dept of Internal Medicine, ATTIKON University General Hospital, Athens Greece
2The Hellenic Study Group on the Susceptibility of Streptococcus pneumoniae

Objectives. To present data from the first nation-wide surveillance study on the susceptibility of Streptococcus pneumoniae strains from nasopharyngeal colonization in 2004.

Methods. Nasopharyngeal swabs from healthy children (0,5-6 years) attending day-centers were collected from February to May 2004. Identification was performed with optochin sensitivity, bile solubility and slide agglutination assays. E-test susceptibility testing was performed for penicillin(PG), cefuroxime(CXM), ceftriaxone(CXT), erythromycin(ERY), tetracycline(TET), cotrimoxazole(COT), levofloxacin(LEV) and moxifloxacin(MOX), and agar dilution for telithromycin(TEL) in ERY-R strains. NCCLS 2004 breakpoints for intermediate resistance (IR) and resistance (R) were used. CIP nonsusceptibility breakpoint was >2mg/L. Macrolide resistance phenotype was identified with the double disk diffusion test. Serogrouping/serotyping for strains R at least to 1 antibiotic class was performed with the Quellung reaction with group/type-specific antisera (Statens Serum Institut). Multiresistance was defined as R to ≥3 different antibiotic classes.

Results. A total of 2536 healthy children were swabbed and 746 definite pneumococcal strains were isolated. Results for IR/R respectively per antibiotic (%) were: PG(20,6/14), CXM(1,7/23,9), CXT(1,2/0,1), ERY(0,8/32,7), TET(1,5/24,8), COT(1,8/25,1), and CIP(3,2). No resistance to LEV/MOX was observed. Twenty-six PG-R strains (3,4% of total) had an MIC≥4mg/L (range 4-6). Distribution per resistance phenotype was: M 53,6%, cMLSB 39,1% and iMLSB 7,2%. Three strains were TEL-R (all cMLSB). Per phenotype, TEL MIC50/MIC90 were: M 0,25/0,5 - cML 0,06/0,25. Full susceptibility to all classes was 43,9%. PG and ERY R co-existed in 10,5%. Multiresistance to 3, 4 or >4 classes accounted for 3,8/2,0/1,9mg/L. For TEL, values were 0,06/0,25 in the multiR population. Heptavalent conjugate vaccine (PCV7) would protect against 64,5% and 79% of serotypes without or with intra-serogroup protection, respectively. For multiR strains most prevalent serotypes were 19F and 23F.

Conclusions. Despite existing rates of resistance for PG, the low number of strains beyond ultra-high resistance cut-offs (MIC≥4 mg/L) and the extremely low CXT-R rates render penicillin at appropriate dosing and ceftriaxone still effective against pneumococcal respiratory tract infections. Increased resistance to macrolides may prohibit monotherapy, while LEV/MOX and TEL display potency as alternative agents for resistant strains. PCV7 could afford protection against resistant strains. For the post-vaccination era in Greece (after 2005), periodic surveillance is required for the assessment of pneumococcal resistance and serotype distribution.
**PO13.09**

**Antibiotic consumption and the recovery of nonsusceptible pneumococci from the nasopharynx of healthy children in Greece**

Poulakou, G1, Katsarolis, I1, Matthiopoulos, F1, Tsiodras, S1, Kafetzis, D1, Daikos, GL2, Kavaliotis, F1, Pneumatikos, F, Samonis, G2, Giamarellou, H1

14th Dept of Internal Medicine, ATTIKON University General Hospital, Athens Greece
2the Hellenic Study Group on the Susceptibility of Streptococcus pneumoniae

**Objectives.** To correlate epidemiological data regarding healthy children with the nasopharyngeal (NP) carriage of non-susceptible pneumococci and investigate possible risk factors on an individual level.

**Methods.** A nation-wide NP carriage survey among healthy children 0.5-6ys attending day-care centres took place from Feb-May 2004 in Greece. Questionnaires with antibiotic usage and health info were filled by parents prior to sampling. Non-susceptibility (NS) to penicillin (PG), erythromycin (ERY), tetracycline (TET), cotrimoxazole (COT) and ciprofloxacin (CIP) was determined by E-test according to NCCLS 2004 breakpoints. OR with 95%CI were calculated for each individual variable and X2-test with Yates correction was used for statistical analysis.

**Results.** NP culture with matched questionnaire was obtained from 2536 children (1246 boys) and 746 pneumococcal strains were isolated (mean carriage rate 29%). Results for NS(%) were: PG (34,7), ERY (33,5), TET (26,3), COT (43,6), and CIP (3,2). Carriage rates and susceptibility patterns varied among different geographic departments and differed significantly between sampling periods. No sex difference was observed in carriage and susceptibility rates. No difference was observed for number of siblings, hospitalization history, steroid use or other underlying morbidity. Antibiotic consumption was similar in total sample and carriers for the month (30,8% vs. 27,5%, respectively), but marginally higher for the trimester prior to sampling (31,9% vs. 27,7% respectively, OR = 1,22, p = 0,03). Otitis media history did not differ between carriers and total sample. Among carriers, those treated with antibiotics within the previous month were at greater risk of harboring NS strain. OR for previous month antibiotic consumption per antibiotic NS were: PG 1,7<1,2-2,4> p=0,000, ERY 1,83<1,2-2,6> p=0,004, COT 1,43<1,09-2,15> p=0,01, TET 2,32<1,64-3,41> p=0,000. Respective values for antibiotic use in the last trimester did not reach statistical significance. CIP-NS was not associated with antibiotic use. Although antibiotics were prescribed by a physician in 90% of cases, "viral" symptoms were reported in 42% of children. Amoxicillin/clavulanate (32%) and cephalosporins (29,3%) were the most frequently prescribed antibiotics in the month prior to sampling. No difference in carriage was observed with any particular class. Cephalosporin use in the last month was associated with ERY-NS (OR 5,85<3,28-10,45> p=0,000).

**Conclusions.** Prior antibiotic consumption, especially within the last month, is the major risk factor for the recovery of NS pneumococci in the nasopharynx of healthy children in Greece. Prudent use of antimicrobials should be an essential component of the national antibiotic policy, as the nasopharynx of healthy children could serve as a niche of resistance.

**PO13.10**

**Serotype distribution & antibiotic resistance patterns among invasive Streptococcus pneumonia isolates in Saudi Arabia**

Atef M Shibl

King Saud University, Riyadh, Saudi Arabia

A study was initiated to determine the vaccine coverage of prevalent invasive pneumococci from children aged less than 10 years in Saudi Arabia. A total number of 350 invasive isolates were serotype & their antibiotic susceptibilities were determined. The strains were predominantly isolated from blood (82%) & from CSF (18%). All invasive isolates include non-duplicate pneumococci isolated in the hospital laboratories of 18 major & regional hospitals distributed throughout Saudi Arabia. All isolates were identified according to the standard methods & serotype by quelling reaction with sera of various reactivities from the Staten’s serum institute (Copenhagen, Denmark).

E-test strips of Penicillin; Erythromycin & Cefotaxime were used to determine the MIC’s following the manufacturer’s instructions (AB Biodisk, Solna, Sweden). Results were interpreted according to published breakpoints of the NCCLS, 2003. Quality control strains were included with each run. Multi-drug resistance was defined as having resistance to the 3 antibiotics. The most common serotypes were 23F, 19F, 14, 18C & 6B. Serogroups I & 5 were absent in this study.

Overall serotypes in the 7-valent conjugate pneumococcal vaccine accounted for approximately 62% of the total incidence of invasive disease. If the cross-reacting serotypes 6A, 19A, 9N, 18F & 9A are added to this, the coverage increases to approximately 79%. Approximately 6% of the isolates are non-typable.

The 7-valent vaccine comprised 88% of the isolates from children aged < 2 years & 74% from children aged 2 – 6 years. There were some differences in the frequencies in the rank order of the most common serotypes, but no significant differences in the frequencies of the individual serotypes were found between the same 2 age groups.

Approximately 46% of the isolates were sensitive to Penicillin, 42% were intermediate & 12% were resistant. The rates of resistance to Erythromycin & Cefotaxime were 26% & 6% respectively. Approximately 18% of the Penicillin resistant isolates were also resistant to Erythromycin. Almost 60% of the isolates were resistant to at least one of the three antibiotics tested. The vaccine comprised 82% of invasive isolates with resistance to Penicillin, Erythromycin & Cefotaxime.

The finding that the 7-valent vaccine included serotypes that account for the majority of the penicillin non-susceptible as well as the multi-drug resistant pneumococci is encouraging. The use of this vaccine in young children is expected to have a substantial impact on the burden of pneumococcal resistance & invasive disease.
Role of efflux pumps in fluoroquinolone-resistant *Streptococcus pneumoniae* and failure of current CLSI breakpoints in identifying fluoroquinolone-susceptible strains with *parC* mutations

Kim, YS, Park, SJ, Jun, JB, Choi, SH, Jeong, JY
Division of Infectious Diseases, Asan Medical Center, and Center for Antimicrobial Resistance and Microbial Genetics, University of Ulsan, Seoul, Korea

Concurrent with increased use of Fluoroquinolone (FQ) for the treatment of pneumococcal infections, increased percentage of isolates demonstrates resistance. The aims of this study were (1) to measure the prevalence of FQ resistance in *Streptococcus pneumoniae* (SP) from Korea, (2) to assess the contribution of efflux pump (EP) to FQ resistance, (3) to determine whether levofloxacin (LEV) CLSI breakpoints can identify isolates that harbour recognized resistance mechanisms. SP (n=881) collected across Korea over a period of 7 years (1997-2004) were susceptibility tested against six FQ by agar dilution method. Mutations in the QRDR were characterized by PCR and sequencing. MIC were tested in broth before and after adding reserpine (10 µg/ml), an EP inhibitor, to check any contribution of EP to FQ resistance. Nineteen (2.1%) among 881 strains showed LEV MIC ≥ 4 µg/ml. Thirty-three (3.7%) had CIP MIC ≥ 4 µg/ml; 18 (2.2%) gatifloxacin MIC ≥ 2 µg/ml; 21 (2.4%) sparfloxacin MIC ≥ 2 µg/ml; and 6 (0.7%) gemifloxacin MIC ≥ 1 µg/ml. Among 19 LEV-nonsusceptible strains, 15 (79%) had amino acid (aa) substitutions for Ser79 or Asp93, 3 had aa substitutions only for Lys137, 1 had no substitution, and 10 (53%) showed decrease of their MIC, 2 or more fold, after adding reserpine. Among randomly selected 10 strains with LEV MIC=2 µg/ml, 8 (80%) showed aa substitutions for Ser79 or Asp93, 2 had aa substitutions only for Lys137. Among randomly selected 10 strains with LEV MIC=1 µg/ml, 3 showed aa substitutions only for Lys137,7 had no substitution. FQ resistance rate in SP from Korea is still not so high despite widespread use of FQ. EP play an important role in FQ-resistant SP. LEV susceptibility testing that uses current CLSI clinical breakpoints does not identify most SP isolates with *parC* mutations.

Serotype-specific real-time PCR for identification of *Streptococcus pneumoniae* PO13.11

Hu, A, Colella, M, Li, F, Zhao, P, Tam, J, Rappaport, R, and Cheng, S-M
Wyeth Vaccines Research, Pearl River, NY, USA

*Streptococcus pneumoniae* (*S. pneumoniae*) is a leading cause of community-acquired pneumonia (CAP) among children and adults worldwide. The introduction of Prevnar, a vaccine directed against 7 of the most commonly isolated pneumococcal serotypes, has led to a reduction in incidence of disease. Meanwhile, monitoring changes in the distribution of the circulating pneumococcal serotypes in the community are needed. In order to conduct surveillance of *S. pneumoniae* in ongoing clinical studies of Prevnar and other pneumococcal vaccines, a rapid diagnostic assay was developed. Based on the published sequences of capsular polysaccharide synthesis (*cps*) gene clusters, real-time PCR assays were designed for the detection, quantification and serotyping of all 7 serotypes present in Prevnar, namely 4, 6B, 9V, 14, 18C, 19F and 23 F. Individual serotype-specific real-time PCR assays were established, and subsequently combined into a multiplex format. Assay sensitivity was determined by the amplification of 10-fold serial dilutions of purified *S. pneumoniae* DNA of each serotype. Results showed that the 7 serotype-specific real-time PCR assays detected 5-100 copies of the respective *S. pneumoniae* genomes in both singleplex and multiplex formats. To assess assay specificity, each of the 7 serotype-specific assays was tested against a panel of 68 unique *S. pneumoniae* serotypes. The results showed that the primers/probe pair used in the 7 serotype-specific assays identified only the corresponding serotype. The sensitivity and specificity of these serotype-specific PCR assays allows the direct detection, quantification and serotyping of the 7 Prevnar-related serotypes in clinical specimens.
**Rapid differentiation of *Streptococcus pneumoniae* serotypes 6a and 6b using multiplex real-time PCR**

**Podt.03**

Hu, A, Li, F, Zhao, P, Tam, J, Rappaport, R, and Cheng, S-M

Wyeth Vaccines Research, Pearl River, NY, USA

Timely differentiation of two closely related *Streptococcus pneumoniae* (*S. pneumoniae*) serotypes 6A and 6B is needed for monitoring the serotype-specific epidemiology of pneumococci after introduction of Prevnar because the 7-valent vaccine contains serotype 6B, but not 6A. To this end, we employed real-time PCR as a sensitive and specific alternative to classical methods for detection and differentiation of these two pathogens that share extremely high sequence homologies, particularly in their capsular polysaccharide synthesis (*cps*) gene clusters. However, rare polymorphic *cps* sites at nucleotides, 2750-2764 of 6A and 8770-8785 of 6B were identified and used to design the real-time PCR probes for each serotype. The assays were first established as a mono-specific reaction, and subsequently advanced to a multiplex format. We also designed a serogroup 6 real-time PCR assay for confirmation purposes. Assay sensitivity was determined by amplification of a series of 10-fold dilutions of purified 6A or 6B pneumococcal DNA. The results showed that all the assays detected 5-100 copies of the respective genomes, regardless of whether the assay was performed as a single or duplex reaction. To assess assay specificity, all the assays were tested individually against a panel of 80 *S. pneumoniae* specific strains including two 6A and eight 6B isolates. The results demonstrated that the serogroup 6 assay detected all 6A and 6B isolates, but none of the other *S. pneumoniae* serotypes. The 6A specific assay detected two 6A isolates but not other *S. pneumoniae* serotypes including all 6B isolates; parallel results were observed for the 6B specific assay. In summary, we describe a rapid, quantitative and cost-effective real-time PCR-based assay to identify *S. pneumoniae* serotype 6 as well as a duplex assay to further discriminate between serotypes 6A and 6B.

---

**Rapid diagnosis of invasive pneumococcal infections using Binax® NOW™ immunochromatographic test**

**Podt.04**

Lalitha MK¹, Aaronjeyaseelan S¹, Jesudason MV², Thomas K³, and Steinhoff MC³

¹Department of Clinical Microbiology, ²Department of Medicine, Christian Medical College and Hospital, Vellore, India. ³Department of International Health, Johns Hopkins University Baltimore USA

Background: Infections caused by *Streptococcus pneumoniae* are the major cause of morbidity and mortality worldwide. The isolation of organism in culture is affected by prior antimicrobial therapy and available antigen detection methods require the presence of ≥10⁴ cells per ml. for optimal sensitivity of the test. Therefore it is essential to evaluate an alternate, rapid and less cumbersome detection method for diagnosis of invasive pneumococcal infections. We have evaluated Binax® NOW™ immunochromatographic test, which detects C polysaccharide antigen of *Streptococcus pneumoniae*.

Material and Methods: Prospectively cerebrospinal fluid samples (*n* = 20) from clinically suspected and pathologically suggestive of bacterial meningitis were included. The test was also done on blood culture broths (*n* = 38) giving growth positive signal in a BactT-Alert system, showing Gram-positive cocci in smear examination. In addition retrospectively the kit was tested with fluids from normally sterile sites (*n* = 8) of patients with known pneumococcal infection. The results were compared with existing detection method viz, Latex agglutination, culture as well as Polymerase Chain Reaction assay. The analytical sensitivity was determined by testing with *S. pneumoniae* dilutions. The cross reactivity was determined by testing with pathogens commonly isolated from cases with pyogenic meningitis and bacteremia.

Results: The analytical sensitivity was determined as 7X10³ cells/ml. There was no cross reactivity with other common pathogens tested. Twelve out of 20 CSF samples were positive (Sensitivity 100%, Specificity 67%, PPV 67%, NPV100%, kappa 0.615, Chamberlain’s Positive Present Agreement 67%). There were four additional pickup as compared to culture. Ten out of 38 blood culture samples were positive (Sensitivity 100%, Specificity 94%, PPV 80%, NPV 100%, kappa 0.86, Chamberlain’s Positive Present Agreement 80%). All fluid samples (pleural fluid n=6 & ascitic fluid n=2) were also positive. The test was positive with one pleural fluid when culture was negative.

Conclusion: The results of Binax® NOW™ test correlated well with smear, Latex agglutination and PCR. The sensitivity and the specificity are found to be high. In two instances it could detect *S. pneumoniae* in CSF when culture and LA tests failed. The test gives positive result even with mixed infection. The results are available within 15 minutes as compared to LA (30 minutes) as well as PCR (~4 Hours). Therefore we concluded that Binax® NOW™ test is a useful tool for the rapid diagnosis of pneumococcal disease.
Comparison of citrated sheep and human blood with defibrinated horse and sheep blood as culture media supplements for the isolation and antibiotic susceptibility testing of *Streptococcus pneumoniae*

Russell, EM1, Biribo, S2, Selvaraj, G3, Oppedissano, F4, Warren, S5, Seduadua, A6, Mulholland, EK7, Carapetis, JR8
1Centre for International Child Health, Department of Paediatrics, University of Melbourne, Victoria, Australia
2Fiji School of Medicine, Suva, Fiji
3Murdoch Children’s Research Institute, Royal Children’s Hospital, Melbourne, Victoria, Australia
4New Children’s Hospital, Sydney, New South Wales, Australia
5Fiji Pneumococcal Project, Ministry of Health, Suva, Fiji
6London School of Health and Tropical Medicine, London, United Kingdom

**Background:** *Streptococcus pneumoniae* requires a source of blood as a culture media supplement for its isolation and susceptibility testing. Defibrinated sheep, horse, pig or goat blood is recommended. In developing countries, unused expired human blood is often the most convenient source of blood used. Apart from the occupational health and safety risk to laboratory personnel, human blood agar (HuBA) is not recommended due to poor isolation rates of bacteria. Citrated sheep blood is easy to obtain and a reliable source of blood, however the effect of this additive has not been previously documented on the isolation rate and susceptibility pattern of *S. pneumoniae*. In this study a comparison was made between HuBA, citrated sheep blood agar (SCBA) and the gold standard defibrinated horse and sheep blood agars (HBA, SDBA) on the isolation and antibiotic susceptibility patterns of reference and clinical strains of *S. pneumoniae* in cerebrospinal fluid (CSF) and saliva.

**Methods:** Various dilutions of reference and clinical strains of *S. pneumoniae* were cultured on HBA, SDBA, SCBA and citrated HuBA agar plates. Clinical isolates in CSF were identical for all blood agars except alpha-hemolysis was not obvious for any strain on citrated HuBA. Antibiotic susceptibility results for *S. pneumoniae* were identical for the two different sheep blood agars. However citrated HuBA demonstrated larger zone sizes. The *S. pneumoniae* susceptibility reference strain failed quality control on the citrated HuBA.

**Results:** For all strains, growth of the reference and clinical strains were identical on all blood agars at all dilutions including the strains in the saliva and CSF. Morphological appearances were similar for all blood agars except alpha-hemolysis was not obvious for any strain on citrated HuBA. Antibiotic susceptibility results for *S. pneumoniae* were identical for the two different sheep blood agars. However citrated HuBA demonstrated larger zone sizes. The *S. pneumoniae* susceptibility reference strain failed quality control on the citrated HuBA.

**Conclusions:** HuBA supports the growth of *S. pneumoniae* from sterile sites. However, the characteristic morphological appearance is not evident. SCBA is recommended along with HBA and SDBA for the susceptibility testing of *S. pneumoniae*. HuBA is not recommended for susceptibility testing as the quality control for the *S. pneumoniae* susceptibility reference strain fails on media using human blood.

---

Purification and structure characterization of the active component in the pneumococcal 22F polysaccharide capsule used for absorption in pneumococcal enzyme-linked immunosorbent assays

Skovsted, I1, Kerrn, MB2, Sonne-Hansen, J3, Sauer, LE3, Nileson, AK4, Konradsen, HB4, Petersen, BO5, Nyberg, N4, Daus J3
1Statens Serum Institut, Copenhagen, Denmark 2 Carlsberg Laboratory

Protection against pneumococcal disease is thought to be mediated primarily by antibodies that are opsonic. Pneumococcal capsular polysaccharide (CPS) is immunogenic and induces type-specific protective immunity. For convenience, the protective capacity of serum antibodies is often evaluated by the measurement of antibody titers in an ELISA test. The pneumococcal capsular polysaccharide (CPS) used in ELISA contains several impurities; these include about 5% by weight of teichoic acid (CWPS). All individuals have antibodies to CWPS possible as a result of early exposure to pneumococci, *Streptococcus mitis* and *Streptococcus oralis*. The concentration of the CWPS antibodies in non immunized individuals often exceeds the concentration of the serotype specific pneumococcal antibodies. Therefore, the pneumococcal ELISA requires an absorption step to remove the unprotective CWPS antibodies. Recently a new pneumococcal CPS ELISA was recommended with an extra serum absorption step with 22F CPS to improve the results. To characterize and purify the active component in the 22F capsule, a non capsulated pneumococci was prepared from a 22F capsulated pneumococci. The cell wall polysaccharide (CWPS2) purified from this pneumococci was compared to 22F CPS in the ELISA. The result showed that CWPS2 and 22F CPS have the same absorption potential indicating CWPS2 being the active component in 22F CPS.

CWPS has been shown to exist in different forms depending on there phosphorylcholin amount in the tetrasaccharide subunit, therefore a PCR analysis of the LicD2 gene (responsible for the attachment of the second phosphorylcholin in the tetrasaccharide subunit) and an extensive NMR analysis was conducted. The result showed that CWPS contains one phosphorylcholin whereas CWPS2 contains two showing an immunological difference between the two CWPS variants. In conclusion pneumococcal ELISAs absorption could be performed with a combination of CWPS + 22F CPS or CWPS + CWPS2. Since the concentration of CWPS2 varies in different 22F CPS preparations it would be useful and timesaving to implement the purified polysaccharide CWPS2 in the commercial CWPS product.
Ability of brain heart infusion broth as a holding medium to support the growth of Streptococcus pneumoniae and Haemophilus influenzae

Lalitha MK1, Aaronjeyaseelan S1, Thomas K2, and Steinhoff MC3
1Department of Clinical Microbiology, 2Department of Medicine, Christian Medical College and Hospital, Vellore, India. 3Department of International Health, Johns Hopkins University Baltimore USA

Background: Streptococcus pneumoniae and Haemophilus influenzae are the two major pathogens causing life-threatening invasive infections in the developing world. Isolation of these fastidious pathogens is affected by a delay in transportation and culturing of clinical material. Though automation is available for performing blood culture, most laboratories perform manual methods using Brain Heart Infusion (BHI) broth. Therefore we evaluated a commercially available BHI broth in India at suitable temperature when holding CSF, Blood and other body fluids before inoculating into appropriate media.

Material and Methods: Suspensions of S. pneumoniae ATCC 49619 and H. influenzae ATCC 49247 equal to 0.5 McFarland’s were made and decimally diluted seven times to achieve approximately 10³ cells/ml. The dilutions with one, ten and hundred cells were inoculated separately into two sets of BHI broth (Delta Biologicals, Bangalore), for incubation at 37°C and room temperature (28°C) respectively. At 12, 18, 24, 48, 72 hours interval, 100µl of media were plated onto Trypticase Soy Blood Agar (TSBA) for S. pneumoniae and Modified Chocolate Agar (MCA) media for H. influenzae. The plates were incubated at 37°C with CO₂ for 24 hours and the colony forming units (cfu) determined.

Results: At 37°C both S. pneumoniae and H. influenzae were able to produce >300 cfu with all three dilutions from 12th hour. However, at RT the yield was poor. From Dilution 1 (one cell), <30, 32, 72 and from dilution 2 (ten cells), 128, 202, 284 cfu of S. pneumoniae were produced at 12, 18, 24 hours respectively. Only from 48 hours there were >300 cfu produced. From Dilution 3 (Hundred cells), more than 300 cfu of S. pneumoniae were produced from 12 hours. Only <50 cfu of H. influenzae were produced from dilution 1. From Dilution 2, <30, 286 H. influenzae cfu were observed at 12, 18 hours. More than 300 cfu were produced from 24 hours. From Dilution 3, 123 cfu were formed from 12 hours and >300 cfu were produced from 18 hours but invariably from 24 hours the growth of H. influenzae declined and there was no growth at 72 hours.

Conclusion: Therefore we concluded that the inoculated BHI broths should be transported immediately to the laboratory and incubated at 37°C and it is ideal to do the plating at 24 hours after inoculation for the optimal recovery of these pathogens.

Development of a four-serotype, multiplexed opsonophagocytic killing assay for detecting opsonic antibodies against Streptococcus pneumoniae

Burton, RL and Nahm, MH
University of Alabama at Birmingham, Birmingham, Alabama, USA

Aim: Measuring opsonic capacity of antibodies induced with pneumococcal vaccines is important in pneumococcal vaccine evaluations. However, opsonophagocytic killing assay (OPA) has been a labor-intensive assay. To reduce efforts, we have previously developed a two-serotype multiplexed OPA (Kim et al. Clin Diag Lab Immn 10:616, 2003). We have now extended the assay principle to a 4-serotype multiplexed OPA (MOPA-4).

Methods: S. pneumoniae expressing serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F were obtained. Each strain was made resistant to one of the following antibiotics: optochin, streptomycin, spectinomycin, or trimethoprim. MOPA-4 was performed as before with the above bacteria and the following modifications. Briefly, bacteria stocks were washed, and 10 µl containing ~500 cfu of MOPA-4 (or ~1000 cfu for single serotype assay) was added to each well containing 20 µl test serum. After incubation for 30 minutes at room temperature, 10 µl complement and 40 µl differentiated HL60 cells (4 x 10⁵ cells) were added. After incubation for 45 minutes at 37°C in a humidified 5% CO₂ atmosphere, reaction mixture was spotted onto agar plates (5 µl for single serotype, 10 µl for MOPA-4). After addition of appropriate antibiotic plates were incubated overnight at 37°C, and colonies were counted. Opsonization unit is defined as the reciprocal of the dilution that kills 50% of bacteria.

Results: Sera from twenty healthy adults (5 pre-vaccination and 15 post-vaccination with 23-valent polysaccharide (PS) vaccine) were tested in the MOPA-4 and the single-serotype assays. The opsonization units obtained with both assays agreed well, with high R² values (>0.95) and slopes of best fit lines ranging from 0.91 to 1.07. Fifteen of the 240 results (~6%) obtained differed by more than two-fold, and none differed by more than three-fold. The assay was specific: pre-absorbing the test sera with homologous PS (5 µg/ml) of each had little effect. Cross-reactive PS also inhibited, but less efficiently than the homologous PS.

Conclusions: The MOPA-4 is a specific assay that yields results similar to those obtained with the single serotype assay. Since antibodies to four serotypes can be measured simultaneously, a more comprehensive evaluation of pneumococcal vaccine efficacy can be performed with a limited volume of serum.
The impact of bacterial strains, bacteria/PMNLs ratio, complement source, and complement concentration on opsonophagocytic activity for pneumococcal serotype 19F

Yu, XH, Hu, BT, Belanger, K, Fernsten, P
Wyeth Vaccine Research, Pearl River, NY

Host protection against *Streptococcus pneumoniae* is primarily believed to be mediated by opsonin-dependent phagocytosis. Therefore, it is crucial to accurately evaluate opsonophagocytic activities of antibodies after vaccination. Evaluation of opsonophagocytic assay (OPA) data from pneumococcal polysaccharide vaccine trials has shown that functional antibody rise to most vaccinated serotypes is significantly higher than that for serotype 19F when measured by current OPA procedures. Others have also reported that higher concentration of anti-19F antibodies were required to yield similar OPA titers compared to anti-6B antibodies. We have evaluated four assay parameters in the PnOPA to determine whether the lower Pn19F OPA titers were the result of sub-optimal assay conditions. Factors considered in our evaluation were (1) source of Pn19F bacterial strains, (2) source of exogenous complement, (3) complement concentration, and (4) the ratio of polymorphonuclear lymphocytes (PMNs) to bacteria (effector to target (E/T) ratio). Standard assay conditions utilize 12.5% baby rabbit complement and an E/T ratio of 400:1.

Forty-nine sera from infants immunized with a 7-valent pneumococcal conjugate vaccine were used to evaluate four different strains of Pn19F target cells in the PnOPA. Resulting OPA titers covered a low-medium-high titer range and demonstrated less than 2-fold difference in GMT when the different target strains of Pn19F were compared. The use of baby rabbit complement resulted in similar to slightly higher Pn19F OPA titers than did precolostral bovine complement. No significant changes in Pn19F OPA titers were observed when complement concentration was increased step-wise to a maximum concentration of 31.25%. E/T ratios of 400:1 yielded approximately four-fold higher Pn19F OPA titers than did E/T ratios of 100:1 when precolostral bovine complement was used. There was not a significant difference between E/T ratios of 400:1 and 100:1 when baby rabbit complement was used. However, E/T ratios higher than 400:1 did not yield higher OPA titers, regardless of complement source. In summary, none of four evaluated assay parameters were found to have a major influence on OPA titers for pneumococcal serotype 19F.

---

Parameters that influence the performance of HL-60 cells for *S. pneumoniae* opsonophagocytic assay

Yu, XH, Hu, BT, Belanger, K, Fernsten, P
Wyeth Vaccines Research, Pearl River, NY

**Background:** *S. pneumoniae* Opsonophagocytic Assay (PnOPA) measures the functional antibodies against *Streptococcus pneumoniae* (*Pn*) *in vitro*. Many laboratories routinely use this method to evaluate the effectiveness of current and novel pneumococcal vaccines. Following differentiation with dimethylformamide (DMF) HL60 cells acquire the morphological, cell surface, and functional characteristics of mature human polymorphonuclear leukocytes (PMNs). However, proper maintenance, differentiation of HL-60 cells and closely monitoring of cell surface marker expression are crucial to achieve accurate and consistent measurement of vaccine-induced immune protection.

**Methods:** In our study, we have evaluated the impact of HL-60 cells generation numbers, supplementation with fresh culture media (feeding), and apoptosis on HL-60 function in PnOPA. Cell surface markers expression of CD35 (CR1), CD11b (CR3), CD32 (FcRII), CD16 (FcRIII), and CD71 at different passage numbers and growth media conditions was determined by flow cytometric analysis. Cell function was assessed by PnOPA for pneumococcal serotypes 6B, 14, and 19F.

**Results:** The generation number of HL60 cells was calculated based on cell population doublings, and generation one was assigned when the cells were first received from ATCC. The use of differentiated HL-60 cells in PnOPA at generation numbers greater than 210 resulted in a change in bacterial killing kinetics for all serotypes tested when compared with HL-60 generation number 105. Poor OPA performance was observed when there was low expression of CD71 on undifferentiated HL-60 cells and low expression of CD35 on differentiated HL-60 cells. Expression of CD16 was lower in differentiated HL-60 cells when compared with PMNs, and expression of CD16 on differentiated HL60 cells decreased at generation numbers greater than 210. The decreased expression of CD16 at higher HL-60 generations may be associated with the change in bacterial killing kinetics that was observed when these cells were used in the PnOPA. When separated, the non-apoptotic portion of differentiated HL-60 cells functioning in the PnOPA equivalently to unseparated cells and freshly isolated human PMNs. Daily supplementation with fresh culture media did not affect the function of differentiated HL-60 cells. Conclusion: The use of HL-60 cells that are well maintained and differentiated and at the appropriate passage number will result in a more accurate and consistent PnOPA to measure functional antibodies.
Effect of addition of heterologous pneumococcal polysaccharide 22F to the Wyeth/WHO pneumococcal polysaccharide ELISA on IgG assignments for infant sera

Baker, S, Hu, BT, Hackell, J, Razmpour, A, Scott, DA, Tam, JS, Fernsten, P
Wyeth Vaccines Research, Pearl River, NY, USA

The specificity of single absorbent anti-pneumococcal polysaccharide ELISA (anti-PnPs ELISA) responses were evaluated using heterologous pneumococcal polysaccharide (PnPs) competitors, including PnPs 22F, in the presence of pneumococcal absorbent containing C polysaccharide (PnA). The addition of a second absorbent, PnPs 22F, has been recommended to improve assay specificity when testing sera from an adult population. However, the effect of the second absorbent on infant serum pneumococcal IgG assignments was not demonstrated. For the current study, the effect of pre-absorption with PnPs 22F on anti-PnPs ELISA assignments was evaluated using sera from the US Northern California Kaiser Permanente Prevnar efficacy study. Serum specimens from 368 subjects that received either 7-valent pneumococcal conjugate vaccine (7vPnC), or meningococcal group C conjugate vaccine (MnCC) were re-assayed contemporaneously for antibody to the 7 serotypes (4, 6B, 9V, 14, 18C, 19F, 23F) contained in the 7vPnC vaccine, using either a single absorbent (PnA without PnPs 22F) or a double absorbent (PnA plus PnPs 22F) method.

Within the 7vPnC group, 5 of the 7 serotypes (4, 6B, 9V, 14 and 18C) showed geometric mean concentration (GMC) ratios for the two assays with 95% confidence intervals that included 1, suggesting no statistically significant differences between the 2 assay methods. The remaining 2 serotypes, 19F and 23F, had GMC ratios close to 0.90 and an upper limit of the confidence interval less than 0.95, suggesting that the difference in GMC is statistically, but not clinically, significant. Pre-absorption with 22F slightly reduced the resulting assignments and suggests that there may be non-specific antibody responses with these 2 serotypes.

The impact of 22F absorption was greater in the MnCC control treatment group than in the 7vPnC group. Six of the 7 serotypes had GMC ratios below 0.60 and an upper limit for the confidence interval below 0.65; serotype 14 has a ratio of 0.85 and an upper limit of 0.95. These results were not unexpected and suggest that much of the anti-pneumococcal antibody detected in sera from 7-month old infants not immunized with 7vPnC is not specific for capsular polysaccharide. The results from the comparison of these 2 assay methods suggest that antibody responses to the serotypes in 7vPnC are serotype-specific and that employment of the second absorbent would have little impact on correlates of protection based on this study.

Equivalence of differentiated HL60 cells and human polymorphonuclear leukocytes as effector cells in pneumococcal opsonophagocytic assay

Hu, BT, Yu, X-H, Harvey, H*, Kirch C**, and Fernsten, P
Wyeth Vaccines Research, Pearl River, NY, USA

Freshly isolated human polymorphonuclear leukocytes (PMNs) have been used as effector cells in an in vitro pneumococcal opsonophagocytic assay (PnOPA) to facilitate the measurement of serotype-specific functional antibodies in killing Streptococcus pneumoniae. However, the demand for freshly isolated human PMNs presents an operational challenge for routine large scale clinical testing. Substituting human PMNs with terminally differentiated cultured cells can solve the supply problem, simplify the process, and improve the PnOPA performance consistency. We evaluated the performance (equivalence) of differentiated HL60 cells as effector cells in replacing freshly isolated human PMNs in PnOPA. Non-vaccinated human adult sera (n = 26-27) and vaccinated human infant sera (n = 25) from post-primary bleeds of US infants immunized with 9-valent PnC vaccines were tested for their opsonophagocytic antibodies using differentiated HL60 cells and human PMNs. The acceptance criteria for equivalence have been defined as 1) a Pearson correlation coefficient (r value) between the sets of data generated using the two different effectors of 0.8 and 2) 70% of the specimens in the panel have less or equal to a 2-fold difference in assigned OPA titer when tested with the two effector cells. Results show that the Pearson correlation coefficients from the vaccinated infant serum panel were 0.94, 0.91, 0.92, 0.93, 0.96, 0.83, 0.97, 0.94, and 0.96 for serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F, and 23F, respectively. Additionally, more than 70% of the specimens from eight of nine test serotypes passed the second acceptance criterion (serotypes 4, 5, 6B, 9V, 14, 18C, 19F, and 23F). Comparable results were observed from a panel of sera from non-vaccinated adults. We conclude that differentiated HL60 effector cells show equivalent performance to freshly isolated PMNs in PnOPA.

*current address: NIAID, National Institutes of Health, Bethesda, MD, USA
**current address: Johnson and Johnson, West Henrietta, NY14586
Improved pneumococcal opsonophagocytic assay for increased throughput and automation

Shivaprakash, SB1, Shah, RN2, Jones, TR1, Nahm, MH1, Tam, JS1, Fernsten, PD1
1Wyeth Vaccines Research, Pearl River, NY, USA.
2University of Alabama at Birmingham, Birmingham, Alabama

The pneumococcal opsonophagocytic assay (OPA), which measures killing of S. pneumoniae cells by phagocytic effector cells in the presence of functional antibody and complement, is the preferred surrogate assay for demonstration of vaccine efficacy. Since the traditional OPA is highly labor-intensive, efforts have been made to improve the assay. We have integrated multiplex OPA, using antibiotic resistant target pneumococci (Kim et al. Clinical and Diagnostic Laboratory Immunology 10: 616-621, 2003), with microcolony readout for quantitation of surviving bacteria (Liu et al. Journal of Immunological Methods 292: 187-193, 2004). These assay improvements were assessed for equivalence to traditional OPA, higher throughput rate, and for potential automation of microcolony quantitation. The duplex OPA involving pneumococcal serotypes 18C or 19F (optochin resistant) and 23F (streptomycin resistant) was compared with corresponding monoplex OPA following quantitation of antibiotic resistant microcolonies on filter plates. Antibody titers of four control sera for the homologous pneumococcal serotypes as measured by the duplex OPA were generally within one serum dilution of those measured by the monoplex OPA. The duplex OPA with microcolony readout led to at least a 2-fold improvement in efficiency as compared to the traditional OPA. Additional multiplexing and automation of sample preparation and processing can further enhance this efficiency improvement. The data suggest that integration of these improvements yields results equivalent to those obtained by traditional OPA and will significantly reduce cost, time, reagents (including patient’s sera), and labor involved in the clinical evaluation of multivalent pneumococcal vaccines.

Apoptosis of differentiated HL60 cells and effects on Streptococcus pneumoniae opsonophagocytic assays

Hu, BT, and Harris, SG*
Wyeth Vaccines Research, Pearl River, NY, USA

HL60 cells are immortalized promyelocytic leukemia cells and can be induced to differentiate into more mature neutrophil-like cells by N,N-dimethylformamide (DMF). As early as 3 days post-DMF treatment, two populations with different cellular morphologies emerge. One population, defined as the upper population, has both increased size and granularity as characterized by flow cytometry and decreased density when compared to the other population. The emergence of the upper population of differentiated HL60 cells coupled with the decrease in cell viability over time led us to test the hypothesis that the upper population of cells were undergoing apoptosis, although ≥ 90% cells were still viable, as measured by trypan blue dye exclusion method. The two populations of cells were separated by Percoll gradient density centrifugation and further characterized by Annexin-V and PI staining. The upper population of differentiated cells, found in the less dense fractions of the Percoll gradient, showed early indication of apoptosis. The lower, denser, population of differentiated cells did not show signs of apoptosis. The complement receptor CD35 and CD11b of the non-apoptotic cells were expressed at higher levels than those of the apoptotic cells. However, the transferrin receptor CD71 was expressed at a higher level in the apoptotic than in the non-apoptotic cell population. The functionality of these two populations of day 5 post-DMF-differentiated HL60 cells in opsonophagocytic assay for Streptococcus pneumoniae (PnOPA) was evaluated with multiple human serum specimens. The non-apoptotic differentiated cells function as well in the PnOPA as freshly isolated human polymorphonuclear leukocytes (PMNs). However, the viable but apoptotic population of cells appears non-functional in PnOPAs. This suggests that to assure maximum robustness of the assay, the optimal time window for harvesting differentiated HL60 cells for use in PnOPA is from day 3 to day 5 post-DMF induction.

* current address: Biogen IDEC Inc., San Diego, CA, USA
Evaluating the cross-reactive immune responses to pneumococcal polysaccharide conjugates of serotypes 6A, 6B, 19A & 19F by ELISA and opsonophagocytic assay

**PODT.15**

**Hu, B T, Yu, X-H, Baker, S, Fernsten, P**  
Wyeth Vaccines Research, Pearl River, NY, USA

*Streptococcus pneumoniae* is a gram-positive diplococcus that is a major causative agent of pneumonia, otitis media, bacteremia, and meningitis. Pneumococcal serotypes 6 and 19 are among the most common serogroups causing invasive pneumococcal disease (IPD) worldwide for young children. The currently licensed 7-valent pneumococcal conjugate (PnC) vaccine covers ~85% of the disease burden in North America, including serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F; but does not include serotypes 6A and 19A. However, serotype 19A IPD cases, in particular, have risen from 3% of total cases for all age groups in the US in 1998-1999 to 9% of total cases in 2003. High degrees of structural homology in pneumococcal polysaccharides (PnPs) are found within serogroups 6 and 19, thus cross-reactive immune responses are anticipated. The question arises: what degree of cross-reactivity is expected within serogroup, and is the cross-reactive response functional? We have evaluated the immune responses in a subset of post-primary and post-booster bleeds of US infants immunized with 7-valent or 9-valent PnC vaccine, which does not contain 6A and 19A PnC immunogens. Results showed that considerable amounts of detectable antibodies to 6A and 19A were found in both post-primary and post-booster bleeds by ELISA. Significant amounts of cross-reactive opsonophagocytic antibodies to 6A were generated in response to 6B PnC immunogen and were correlated with the magnitude of the 6B response. However, significantly less cross-reactive functional antibody to 19A was elicited by 19F PnC immunogen at both post-primary and post-booster bleeds, even when significant amounts of 19F opsonophagocytic antibodies were measured. It therefore appears that the pneumococcal ELISA may detect immune responses to both shared and non-shared epitopes within serogroups 6 and 19. However, functional antibodies may not be shared within a serogroup but may be serotype specific, particularly within serogroup 19. GPA may therefore be a key assay for distinguishing the functional cross-reactive immune responses elicited within serogroups.

Thermodynamics and stoichiometry of binding of a panel of antibodies to pneumococcal polysaccharides

**PODT.16**

**Harris, S.L. and Fernsten, P.**  
Wyeth Vaccines Research, Pearl River, NY, USA

The thermodynamics of the interaction between anti-polysaccharide (PS) antibodies and their antigens have been investigated via isothermal titration microcalorimetry (ITC). One ITC experiment directly determines the thermodynamic binding constant (K), the enthalpy of binding (ΔH) and the stoichiometry of binding (N). The free energy of binding (ΔG) can be calculated from K (ΔG=−RTlnK) and the entropy of binding (ΔS) can be calculated from ΔG=ΔH-TΔS. Earlier work in the field, investigated the binding of monoclonal antibodies (mAbs) with oligosaccharide portions of PS. We investigated the binding of six different anti-pneumococcal PS mAbs and three Fabs (monovalent antibody fragments) with their respective PS. The K values for each of the mAbs and Fabs to PS were in the 10^5 to 10^7 M^-1 range, one to two orders of magnitude greater than K values reported for other antibody-carbohydrate interactions. We believe the increased affinity may be the result of using glycoconjugates to generate these mAbs. As described for other antibody-carbohydrate interactions, binding for both the mAbs and Fabs is enthalpically driven. Generally, the ΔS for mAb binding is small and favourable. For two of the three Fabs, the ΔS was unfavourable. The N provides information regarding how many mAbs (or Fabs) can bind simultaneously to one PS chain. The N was determined in terms of number of oligosaccharide repeat units for each PS and ranged from approximately 1 to 4 depending upon the PS. Multiplying the N by the number of monosaccharides in a repeat unit provides the number of monosaccharides found, on average, between bound mAb or Fab. For the six mAbs, the N values, in terms of monosaccharides, were very similar, with an average of twelve monosaccharides per bound mAb. The N for each Fab was smaller than for the corresponding mAb. These results suggest that steric interactions between antibody molecules are the major influence on stoichiometry of binding.
Molecular identification and typing of *Streptococcus pneumoniae* direct from nasopharyngeal aspirates using mPCR/RLB

Brown, M1, Fanrong, K1, Wang, Y2, Gilbert, L1
1Centre for Infectious Diseases and Microbiology, Westmead Hospital, Westmead, NSW, Australia
2Beijing Children’s Hospital, Beijing, P.R. China

Serotyping *Streptococcus pneumoniae* isolates is useful in epidemiological studies of pneumococcal disease and evaluation of vaccine efficacy. It is important to have a clear understanding of the serotype distribution of colonising pneumococci prior to the widespread use of pneumococcal vaccines in Australia, so that changes in serotype distribution as a result of this can be understood. Studying colonising strains can also prove useful in identifying virulence factors involved with invasive pneumococcal diseases.

Staff at the CIDM have developed a molecular method of serotyping *Streptococcus pneumoniae* using a multiplex PCR based Reverse Line Blot (RLB) assay, which can identify 40 different pneumococcal serotypes. (Kong et al 2005 submitted) This method has been evaluated for use on cultures against the traditional gold standard for serotyping pneumococci, the Quellung reaction. The next step is to validate the use of this assay directly on clinical samples thus removing the culture step, saving time, money, and hopefully increasing sensitivity (in colonisation studies low organism numbers which are still significant may be missed by culture, but hopefully can be identified by these molecular methods.)

Samples used in the study consist of 498 nasopharyngeal aspirates (NPAs) collected from children presenting with respiratory viral symptoms. 185/498 NPAs were culture positive for *Streptococcus pneumoniae* and these isolates have been serotyped both by the conventional Quellung reaction method, and by the mPCR/RLB. These results will be used to validate the mPCR/RLB methods use directly on clinical samples.

Validation of a multiplex pneumococcal serotyping assay (multibead typing) with clinical samples

Lin, J1, Kaltoft, M2, Brandao, A3,4, Echaniz-Aviles, G1, Brandileone, MC1, Hollingshead, S1, Benjamin, W1, and Nahm, MN1
1University of Alabama at Birmingham, Alabama, USA, 2Statens Serum Institut, Copenhagen, Denmark, 3Adolfo Lutz Institute, Sao Paulo, Brazil, 4IOC/FIOCRUZ, RJ, Brazil, 5National Institute of Public Health, Cuernavaca, Mexico

We have recently developed a rapid pneumococcal serotyping method called “multibead assay” (Yu et al., J. Clin Micro. 43:156, 2005) based on a multiplexed inhibition-type immunoassay for capsular polysaccharide in lysates of pneumococcal cultures. A mixture of fluorescent capsule-specific antibodies is allowed to bind to a mixture of latex beads in the presence of pneumococcal lysates and fluorescence of each latex bead is measured with a flow cytometer. Anti-capsule antibodies are either monoclonal (to 14 common serotypes) or polyclonal. Fourteen flow-cytometry-distinguishable latex beads are used and each bead type is coated with one capsule polysaccharide. The multibead assay has been developed to identify 36 serotypes (1, 2, 3, 4, 5, 6A, 6B, 7A/7F, 8, 9L/9N, 9V, 10A/10B/39/33C, 11A/11D/11F, 12A/12B/12F, 14, 15B/5C, 17F, 18C, 19A, 19F, 20, 22A/22F, 23F, and 33A/33F). More than 90 % of the US isolates have been reported to express one of these serotypes. To validate the new assay, we examined 495 clinical isolates of pneumococci obtained in Brazil, Denmark, and Mexico. Pneumococci were serotyped by the Neufeld’s test in the countries of origin and lysates of each strain were coded and mailed to the USA for the multibead assay at ambient temperature without any thermal protection. After breaking the code, 54 discrepancies (11 % of samples) were noted but 46 were due to non-reproducible technical problems or insufficient growth of the pneumococci. All of the isolates grew well for a second test and therefore the culture medium used for the multibead assay is adequate. The discrepancies persisted for 8 isolates involving 6A, 11A, and 18C serotypes. Additional studies of the 8 isolates showed that the discrepancies were due to differences in the reagents used in the multibead or Neufeld’s tests for these 3 serotypes. For instance, 5 isolates were typed as 6A with the Neufeld’s test but as non-typeable by the multibead assay. Selection of another new monoclonal antibody for the multibead assay resulted in all 5 discrepant isolates typing as 6A. This finding indicates validity of the multibead assay and emphasizes the need to validate any new pneumococcal serotyping assay with a large number of clinical isolates from different locations.
A multiplexed assay for simultaneously detecting multiple pneumococcal capsular polysaccharides in urine

Yu J, Nahm MH
University of Alabama at Birmingham, Birmingham, Alabama, USA

Aim: Urine samples from pneumococcal pneumonia patients often contain more than 1 ng/ml of capsular polysaccharides (PS) (Scott et al., Clin. Inf. Dis. 28:764, 1999). Detection of the PS in urine samples may help us identify the serotypes of pneumococcal pneumonia and determine the efficacy of pneumococcal vaccines against pneumonia. We have, therefore, developed a sensitive multiplexed assay for pneumococcal capsular PS by modifying a multiplex assay originally developed for serotyping pneumococcal isolates (Yu et al., J. Clin Micro. 43:156, 2005).

Method: To detect capsular PS of the seven serotypes (4, 6B, 9V, 14, 19F, and 23F) in Prevnar vaccine, monoclonal antibodies (mAbs) specific for each of the seven PSs were attached to seven different types of latex beads coated with goat-anti-mouse Ig antibody. Each bead type was coated with one mAb. A mixture of beads was incubated with urine samples and washed. The PS bound to the beads was visualized using rabbit anti-capsular PS antisera, fluorescein-conjugated anti-rabbit Ig antibody, and a flow cytometer.

Results: The assay was sensitive and specific. One ng/ml of PS added to normal urine samples produced fluorescence signals 2 fold over the background for serotypes 6B, 14, 19F, and 18C. 10 ng/ml of PS produced similar signals for serotypes 4, 9V, and 23F. The sensitivity of the assay could be increased further by precipitating urine PS in 70% ethanol and concentrating it 5-10 fold. The presence of unrelated or two related (6A and 19A) PS at 0.1 ug/ml did not produce detectable signals. Since the assay is largely automated, it could be used to analyze many samples (>100 per day). To begin assessing the performance of our assay and the effects of antigenuria following recent vaccination, we analyzed urine samples from two adults obtained 0, 1, 3, 6, or 9 days after vaccination with Pneumovax. All the samples had undetectable amounts (<1ng/ml) of capsular PS. To confirm our results, we tested the urine samples using the same mAbs with a classical sandwich-type ELISA method that could detect and 0.1-2 ng/ml of capsular PS. The ELISA also did not detect any PS.

Future work: We are improving our assay to detect additional serotypes. We plan to test our assay with urine samples from patients with pneumococcal pneumonia or individuals who carry pneumococci.

Enzyme-linked immunosorbent assay for quantitative determination of capsular polysaccharide production in Streptococcus pneumoniae Cuban clinical isolates

Cruz-Leal, Y1, Menéndez, T1, Coizex, C4, Espinosa, R1, Canaan, L1, Blanco, F1, Carmenate, T2, Chang, F1, Quiñones, D3, Tamargo, P1, Cremata, J1, Verez-Bencomo, V2 and Guillén, G1
1Centro de Ingeniería Genética y Biotecnología (CIGB), La Habana, Cuba.
2Centro de Estudio de Antígenos Sintéticos, Facultad de Química, Universidad de la Habana, La Habana, Cuba.
3Instituto de Medicina Tropical “Pedro Kourí” (IPK), La Habana, Cuba.

A simple, specific, sensitive, and reproducible enzyme-linked immunosorbent assay (ELISA), has been developed to quantify the level of capsular polysaccharide (CPS) production by Streptococcus pneumoniae Cuban clinical isolates. Strains belonging to serotype 23F are one of the most frequent clinical isolates in Cuba. The quantitation method is based on two type 23F serotype-specific polyclonal antibodies: immunoglobulin G (IgG), purified from sera of mice immunized with a pneumococcal type 23F CPS conjugate, used in the coating step and a serotype-specific rabbit serum as the second antibody. Solutions of purified type 23F CPS were used as standards. Relationship between absorbance at 492 and type 23F CPS concentration was linear over the range of 1 to 310 ng/ml (R=0.989); with 1ng/mL as lower limit of sensitivity. The specificity of ELISA was assessed because purified type 19F CPS and cell-wall polysaccharide samples were not detected after they evaluation by the ELISA described in this study. Repeatability and intermediate precision of assay were good, being the CV 3 % and 10 %, respectively. This ELISA allowed characterized of 23F pneumococcal clinical isolate and selection of appropriate vaccine strain, for a natural polysaccharide vaccine, also constituted a valuable analytical tool for S. pneumoniae fermentation and CPS purification follow up. This system was adapted to quantify CPS from strains belonging to other pneumococcal serotypes. Experiments are conducted at present to evaluate whether the ELISA system described here could be applied for pneumococcal cell typing or diagnosis.
Comparison of antimicrobial sensitivity results on citrated sheep blood Mueller Hinton and citrated human blood Mueller Hinton for invasive Streptococcus pneumoniae clinical isolates

Sedudau, AN1, Mulholland, EK1, Carapetis, JR1, Baadromo E1, Russell, FM2
1Fiji Pneumococcal Project, Ministry of Health, Suva, Fiji
2Centre for International Child Health, Department of Paediatrics, University of Melbourne, Victoria, Australia
3London School of Health and Tropical Medicine, London, United Kingdom
4Murdoch Children’s Research Institute, Royal Children’s Hospital, Melbourne, Victoria, Australia
5Ministry of Health, Fiji

Background: Sheep blood Mueller Hinton (SBMH) agar is the standard media for performing antimicrobial sensitivity tests (AST) for Streptococcus pneumoniae. In developing countries such as Fiji with poor availability of a regular supply of sheep blood, expired human blood from blood donors is used as convenient source of blood for media preparation. The aim of this study is to compare the AST results obtained from the SBMH and citrated human blood Mueller Hinton (HBMH) using clinical pneumococcal isolates from normally sterile sites.

Methods: Both media were made according to the manufacturer’s instructions and were kept between 2 to 8°C until required. All S. pneumoniae isolates from normally sterile sites were collected from the Colonial War Memorial Hospital laboratory from July 2004. The isolates were immediately placed into brain heart infusion broth and made up to a 0.5 Macfarland Standard, then plated onto both the 5% SBMH agar and 5% HBMH agar. The plates were incubated in CO2 at 35°C for 18 to 24 hours. AST was initially determined by the Bauer and Kirby disk diffusion method. The strains were tested for susceptibility using 1 µg oxacillin, 15 µg erythromycin, 30 µg chloramphenicol, and 0.5-2.5 µg trimethoprim/sulfamethoxazole disks. The zone diameters were measured and recorded on the two media by two different scientists. For those isolates that were nonsusceptible, the level of resistance was confirmed by minimum inhibitory concentrations (MIC), using E tests.

Results: Twenty-two invasive isolates have been compared so far. 2/22 were nonsusceptible to oxacillin on both medias. E test results showed that both isolates had intermediate sensitivity to penicillin. 13/33 isolates on HBMH agar showed smaller zone sizes than citrated SBMH agar (median 6mm, range 1-12mm). 7/22 on HBMH agar showed larger zone sizes than citrated SBMH agar (median 11mm, range 4-20mm). 1/22 had the same zone size on both agars. 21/22 isolates showed susceptibility to all the other three antibiotics with only one isolate showing resistance to trimethoprim/sulfamethoxazole.

Conclusion: There is an inconsistency between zone sizes between the two different blood agars. There have been only 2 penicillin resistant isolates so far so the interpretation of these results requires ongoing surveillance to determine whether the AST results obtained from the 2 different medias have any clinical relevance.

Blood cultures in pneumococcal bacteraemia: The effect of blood and broth volume

Rele, M1, Buttery, JP2, Daley AJ3
1Department of Microbiology
2Infectious Diseases Unit and NHMRC CCRE in Child and Adolescent Immunisation
3Murdoch Childrens Research Institute, Royal Children’s Hospital, Melbourne, Australia

Introduction: Between 1% and 11% of neonatal sepsis is caused by Streptococcus pneumoniae with a mortality rate of 14% for invasive disease. Blood culture (BCs) remain the gold standard for detection of bacterial and fungal sepsis. Despite highly advanced and sensitive continuous monitoring systems, 50% of patients admitted to Paediatric and Neonatal Intensive Care Units with well defined criteria for disease.

Between 1% and 11% of neonatal sepsis is caused by Streptococcus pneumoniae with a mortality rate of 14% for invasive disease. Blood culture (BCs) remain the gold standard for detection of bacterial and fungal sepsis. Despite highly advanced and sensitive continuous monitoring systems, 50% of patients admitted to Paediatric and Neonatal Intensive Care Units with well defined criteria for disease.

Objectives: To evaluate in both an automated paediatric BC system and a 2mL microculture system: (1) the minimum volume of blood required to obtain a positive BC; (2) to determine the optimum blood: broth ratio, and (3) to estimate the minimum required number of organisms for detection.

Methods: Systems were injected with varying volumes of adult blood (0.2mL, 0.5mL, 1.0mL, 2mL and 4mL for BC bottles; 0.1mL and 0.2mL for microculture systems) containing S. pneumoniae in concentrations of 1, 5, 10, 50, 100 cfu/mL (colony forming units/milliliter). Results: At high colony counts (≥50cfu/mL) both systems reliably detected bacteraemia, with the microculture tubes yielding positive cultures consistently earlier than the BC bottles (mean 6 hours vs 15 hours). At low colony counts (<10cfu/mL) and blood volumes < 2mL, the BC bottles system failed to detect bacteraemia in 91% of episodes. Autolysis was more common in bottles with lower blood volumes (0.5 and 2mL) giving false negative blood culture results.

Conclusions: Both blood volume and broth ratio has an impact on the time to positivity of a blood culture. Less than 2mL blood volume is insufficient for detection of low organism density (<10cfu/mL) in conventional automated BC systems. Our data reflects the potential benefit of a newer technology with small volume of blood culture broth media to facilitate more frequent and rapid identification of pneumococcal bacteraemia.
Improving the sensitivity of blood culture for pneumococcus


1Department of Microbiology, Dhaka Shishu Hospital, Dhaka, Bangladesh; 2Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA; 3Saving Newborn Lives Initiative (SNL), Save the Children-US, Washington, DC, USA; 4Centre for Population and Health Research (ICDDR,B), Bangladesh; 5Center for International Health and Development, Boston University School of Public Health, Boston, MA, USA.

Background

Streptococcus pneumoniae is frequently undetected because blood cultures are insensitive. Production of autolysin by S. pneumoniae and contamination with fast-growing organism(s) further reduces the sensitivity of blood cultures. We attempted to improve the detection of S. pneumoniae from blood culture bottles by use of an immunochromatographic test for the C-polysaccharide molecule of S. pneumoniae (ICT, BinaxNow®) and sub-culturing on a selective medium.

Methods

We obtained blood from febrile patients at risk for invasive pneumococcal disease. Blood culture bottles were incubated at 37°C and sub-cultured on days 2, 3 and 7. The ICT was performed if bottles appeared to be hemolyzed, but without growth. In addition, the decision to perform ICT was compared for two microbiologists in blinded, independent fashion on a subset of culture bottles. Contaminated bottles were cultured on gentamicin (5µg/ml) blood agar (GBA) plates, selective for S. pneumoniae. All culture-negative and ICT-positive broths were further tested for pneumococcal genome (ply) by PCR.

Results

From 2,739 blood cultures from pneumonia and meningitis cases, we isolated 48 S. pneumoniae strains. Eight pneumococcal disease cases were detected by ICT of culture-negative broths (N=2,231). All ICT-positive cases were also positive for the ply genotype. In addition, S. pneumoniae was isolated from 3 of 249 contaminated blood culture bottles by sub-culturing on GBA; thus, an additional 11 cases of invasive pneumococcal disease were detected by use of these techniques. Out of 150 blood culture bottles subjected to observational screening, 142 were recommended for not doing ICT by both (N=134) or just one (N=8) of the observers, and 140 (99%) of them were negative. Both the observers voted in favor of doing ICT on the same 3 bottles, and 2 of them were positive.

Conclusion

Using ICT and further culturing of contaminated specimens increased the detection of S. pneumoniae by 23%. Bacteremic pneumococcal cases can be missed because the organism may be lysed by its own toxin, so it is important to perform subculture earlier than commonly practiced. Any contaminated bottle from suspected pneumococcal cases should be plated on GBA before discarding. ICT represents an alternative method for detecting false-negative pneumococcal cases by blood culture, but it is impractical for all cases due to increased cost and labor. However, a preliminary visual screening can rationalize use of this test with about 99% accuracy.

Characterization of differentiated cell lines and implications of use in the pneumococcal opsonophagocytic killing assay

Care RS, Gillet M, Feavers J, Fleck RA

National Institute of Biological Standards and Control, South Mimms, Herts, UK

The opsonophagocytic killing assay (OPKA) is the “gold standard” for the evaluation of serological responses to pneumococcal vaccines. It is used to quantify functional antibodies through the use of human cells to phagocytose and kill serum opsonised bacteria. However, there remains reluctance to use the OPKA because of its complexity, as well as concerns over its reliability and reproducibility. The effector cell is an important variable in the OPKA but chemically differentiated continuous human cell lines offer a standard source of phagocytic cells. The accuracy and reproducibility of the assay is critically dependent upon the optimization of cell differentiation when a cultured cell line is employed. This study confirmed that both HL-60 and NB-4 cells have the potential to be used as effector cells in the OPKA, although differentiation induced by dimethylformamide (DMF). Receptors required for phagocytosis in vivo were present on the cell surface of both HL-60 and NB-4, although the level of expression varied. Expression levels of receptors specific for the Fe portions of IgG antibodies, FcγII (CD21), FcγII (CD32) and FcγIII (CD16), did not significantly increase upon differentiation. In addition HL-60 cells were homozygous for the low affinity receptor allotype, FcγIIa R131, which is prevalent among patients suffering from pneumococcal infection. In contrast, NB-4 is heterozygous containing both high and low affinity H131/R131 allotypes, although the level of expression of FcγIIa was low. Expression of the Fc receptor (CD89), which binds IgA, increased upon differentiation with both ATRA and DMF indicating that the OPKA could also measure IgA levels. Complement dependant opsonophagocytic pathway remains central to the clearance of S. pneumoniae in vivo and receptors bind activated complement component C3 on the bacterial surface. Expression of complement receptors CR1 (CD35), CR3 (CD11b) and CR4 (CD11c) were all increased on differentiated cells. However ATRA differentiation induces higher levels which could provide greater sensitivity of the OPKA. Choice of cell line and differentiation method used has important ramifications for the OPKA and permit the assay to be adapted for the analysis of different elements of the immune response to pneumococcal disease or vaccination.
Effect of swab composition and comparison of the use of swab and swab-containing STGG media on the culture and PCR detection of pneumococci from simulated nasopharyngeal specimens

Rubin, LG, Dayan, NE, Rizvi, A
Schneider Children’s Hospital of the North Shore-Long Island Jewish Health System, New Hyde Park, NY, USA

Background: Nasopharyngeal Streptococcus pneumoniae colonization is detected using a calcium alginate (CA) or Dacron polyester (D) swab placed in STGG pneumococcal transport media and cultured. However, for PCR-based detection there are reports of inhibition of PCR assays for detection of Bordetella pertussis DNA using CA NP swabs. In addition, it is unclear if the STGG media or the swab is the optimal material for culture or PCR-based pneumococcal detection.

Methods and Results: D, Rayon (R), or CA-tipped swabs (or no swab control) were inserted in duplicate STGG tubes containing ~10^3 cfu of exponential phase S. pneumoniae serotype 1 or 19F. After 30 min incubation at ambient temperature or 24 h at 4°C and vortexing of tubes, culture of serial dilutions of STGG yielded similar number of colonies. After overnight storage at 4°C, 0.2 ml STGG was removed, DNA was extracted using High Pure PCR Template Preparation Kit (Roche), and serial ten-fold dilutions of extracted DNA were subjected to PCR to amplify a 178 bp or 208 bp sequence specific for serotype 1 or 19F, respectively. Following PCR with DNA from ~10 cfu of type 1, product was detected by PCR in all 24 tubes with swabs and 7/8 control tubes. Using a 1:10 dilution, PCR was positive in 6/8, 5/8, and 8/8 tubes containing D, R, CA swabs, respectively, and 6/8 control tubes and in 4/8, 2/8, 2/8, and 1/8 tubes after a 100-fold dilution. Similar results were obtained using the 19F strain. In a separate experiment CA swabs were inoculated with ~10^3 cfu and serial dilutions of types 1 or 19F pneumococci, placed in STGG broth, and vortexed. DNA was separately extracted from the swab and 0.2 ml STGG using NucliSens (bioMerieux), serially diluted, and amplified with PCR. Detection of product was ~100-fold more sensitive using the swab than using STGG. Comparison of subculture of STGG containing inoculated swabs or inoculated directly with an equivalent number of pneumococci showed a 10-20-fold higher number of cfu from the tubes inoculated directly.

Conclusions: D, R, and CA NP swabs do not inhibit PCR amplification of pneumococcal DNA (using the DNA extraction method employed) or recovery of pneumococci by culture. Despite vortexing in STGG, CA swabs retain >90% of organisms. PCR detection of pneumococci is more sensitive using swabs than STGG broth.

Use of the Binax NOW® immunochromatographic test for the rapid detection of pneumococcal meningitis

Plouffe, JF, Graham, AK, Murdoch, DR
Ohio State University, Columbus, OH, USA
Canterbury Health Laboratories, Christchurch, New Zealand
Christchurch School of Medicine & Health Sciences, Christchurch, New Zealand

Streptococcus pneumoniae is the most common cause of bacterial meningitis in children and adults. The rapid diagnosis of pneumococcal meningitis is hindered by the relative insensitivity of existing tests, which is decreased further if antibiotics are commenced prior to sample collection. The NOW S. pneumoniae antigen test (Binax, Scarborough, ME USA) is easy to perform, provides a result within 15 minutes, and has been approved by the FDA for testing CSF samples from patients with suspected pneumococcal meningitis. However, to date this application of the test has only been evaluated in a limited number of published studies. We identified 238 patients with suspected meningitis from 2 databases who had CSF samples tested by the NOW test, and combined this data with that from 973 cases reported in the published literature. Of the total of 191 CSF samples obtained from patients with pneumococcal meningitis by culture or PCR, 190 (99.4%) had positive results with the NOW test. The results of the NOW test were not affected by prior antibiotic therapy, unlike for culture and Gram stain. Conversely, 1020 assays of CSF in patients not shown to have pneumococcal meningitis were negative using the NOW test.

The one exception was a patient with a cross reacting Streptococcus oralis (a rare cause of meningitis). The recent IDSA guidelines for the management of bacterial meningitis emphasize the use of empiric broad-spectrum antibiotics because of the increased prevalence of multi-drug resistant S. pneumoniae. Empiric therapy included adjunctive steroid therapy, vancomycin and rifampin in addition to the standard 3rd generation cephalosporin until suspicion of S. pneumoniae as the etiology of meningitis was excluded. The performance of the NOW test on CSF samples is a promising tool that may facilitate the initiation of appropriate therapy designed to treat or not to treat pneumococcal meningitis. In areas of the world where it is difficult to perform PCR, the NOW test for pneumococcal antigen in CSF will allow studies to be performed to measure the burden of S. pneumoniae disease. The effectiveness of vaccines in the prevention of pneumococcal meningitis will be more accurately measured. Therapeutic decisions based on the NOW test may be cost saving, may reduce unnecessary antibiotic use, and lead to improved patient care.
The rapid spontaneous death of *S. pneumoniae* (SP) is caused by H$_2$O$_2$ and can be inhibited by catalase. 

Regev-Yochay G$^1$, Thompson C$^1$, Trzinski K$^1$, Malley R$^2$ and Lipsitch M$^1$

$^1$Department of Epidemiology, Harvard School of Public Health, $^2$Children’s Hospital, Harvard Medical School, Boston, MA, USA

The rapid spontaneous death of SP during the stationary phase in broth culture, has long been believed to be caused by LytA amidase. This study demonstrates a major role of H$_2$O$_2$ in this process of SP. **Methods:** Laboratory strain Rx1 and clinical isolates 603 and Pn20-14 (serotype 6B and 14 respectively), were examined. LytA-negative variants (lytA$^-$) were constructed by replacing lytA with a kanamycin-resistance cassette. H$_2$O$_2$ production of the strains and their variants was measured by a colorimetric assay. Growth of lytA$^-$ variant strains versus their respective parents was assessed in microaerophilic conditions (5% CO$_2$) or anaerobic conditions, with or without supplementing the media with bovine liver catalase in excess (1000U/ml). Growth curves were measured both by optical density (OD) and colony forming units (CFU). **Results:** Each lytA$^-$ variant secreted similar amounts of H$_2$O$_2$ as its parent strain. Rx1 and its lytA$^-$ variant secreted significantly less H$_2$O$_2$ than all other isolates. 603, Pn20-14 and their variants died within 15-24 hours in microaerophilic conditions, whereas Rx1 and its variant survived in culture for at least 32h-48h. Survival of 603, Pn20-14 and their variants could be prolonged to >48h by adding catalase to the media, or by incubating in anaerobic conditions. Growth of lytA$^-$ variants differed markedly from their respective parents by optical density, since autolysis of lytA$^-$ variants was reduced. However, the CFU did not differ between lytA$^-$ variants and their parents in any of the strains examined. **Conclusion:** The rapid spontaneous death of SP is related to H$_2$O$_2$ production. SP Survival is prolonged by supplementing the media with catalase (thereby implicating H$_2$O$_2$ in this process), or by culturing in anaerobic conditions (in which H$_2$O$_2$ is not produced by the organism). Differences between lytA$^-$ and wildtype strains in growth curves measured by OD reflect differences in autolysis rates, rather than in viable counts. The survival of different strains is correlated with the amount of H$_2$O$_2$ produced by the strain, and not with a functional LytA amidase.

Characterization of pneumococcal polysaccharides and serotyping of clinical isolates from *Streptococcus pneumoniae* by Fourier-transform infrared spectroscopy

Menéndez, T$^1$, Bosch, A.$^2$, Rodríguez, ME$^2$, Cruz-Leal, Y.$^1$, Sierra, D.$^1$, Prieto, C.$^2$, Cañada, L.$^1$, Chang, J.$^1$, Guillén, G.$^1$ and Yantorno, O.$^2$

$^1$Centro de Ingeniería Genética y Biotecnología (CIGB), La Habana, Cuba.
$^2$Centro de Investigación y Desarrollo en Fermentaciones Industriales (CINDEFI), Facultad de Ciencias Exactas, UNLP, La Plata, Argentina.

Infection control and epidemiological studies primarily require rapid and simple means of identifying and typing clinical isolates. Rapid and accurate identification of *Streptococcus pneumoniae* serotypes is an essential task in clinical microbiology since this organism is one of the leading causes of acute respiratory infections worldwide. Fourier transform (FT)- infrared (IR) spectroscopy could provide potential alternatives to conventional typing methods, because it is fast, easy to perform, and economical. This vibrational spectroscopic technique could also be useful in the characterization of capsular polysaccharides purified from cells of *S. pneumoniae*, intended to be used in the formulation of plain or conjugated polysaccharide-based anti-pneumococcal vaccine. In the present work we present an analysis of capsular polysaccharides purified from strains of *S. pneumoniae* belonging to several serotypes. Contents of protein, lipopolysaccharide, DNA and cell-wall polysaccharide were assessed using FT-IR. Capsular polysaccharides belonging to several serotypes were well discriminated showing the potential usefulness of this techniques in the differentiation of pneumococci. Furthermore clinical isolates, belonging to several serotypes, were also discriminated by FT-IR. These results demonstrated the potential of FT-IR technique as routine methods for *Streptococcus pneumoniae* microbiological studies.
Interlaboratory comparison of the specific IgG response to serotypes in Prevenar

Balloch A1, Mininni TL2, Nurkka A3, Mackenzie G4, Leach AJ5, Kayhty H6, Fernsten PD7, Tang MLK1
1Murdoch Children’s Research Institute, Melbourne, Victoria, Australia
2Wyeth Vaccines Research, Pearl River, NY, USA
3Vaccine Immunology Laboratory, National Public Health Institute (KTL), Helsinki, Finland
4Menzies School of Medical Research, Darwin, Northern Territory, Australia,

Background: In 2004 the Pneumococcal Laboratory at the Murdoch Children’s Research Institute was established to service the FiPP (Fiji Pneumococcal Project) immunization trial in Fiji. Assays for specific IgG to the 7 serotypes in Prevenar were developed based upon the WHO guidelines, www.vaccine.uab.edu and using the method of the Vaccine Immunology Laboratory, KTL, Finland. High, medium and low controls were included in each assay.

There are no QA programs for pneumococcal testing available in Australia or Internationally. A QC serum set available from NIBSC is useful, however direct comparisons are difficult as the absorption and dilution schedules have altered since these samples were characterised.

Method: Wyeth Applied Immunology and Microbiology Laboratory, Pearl River, New York, provided a serum set (14 samples). A comparison was made between the three laboratories: MCRI Pneumococcal laboratory, Wyeth Vaccines Research Laboratory and the Vaccine Immunology Laboratory, KTL. Each laboratory tested each sample three times for specific IgG to the 7 serotypes in Prevenar. We have compared data from the 3 laboratories using both a correlation coefficient and a measuring agreement analysis. As the sample size is small, it is considered, by some, to be inappropriate to use a correlation coefficient. For measuring agreement, a plot of difference in value versus mean value was used. Limits are set as Mean (of the differences) + 2 standard deviations of the differences.

Results: There are some differences in the methods used by the individual laboratories. The MCRI and KTL laboratories use a foetal bovine serum blocking step and absorb the samples overnight with 10 μg/ml C-PS and 30 μg/ml serotype 22F. The Wyeth method does not include a blocking step. The Wyeth and MCRI laboratory use a medium binding plate, whereas KTL use a high binding plate. Despite these minor differences the comparison of values from the 3 laboratories, for the 7 serotypes in Prevenar, demonstrated good agreement.

Conclusion: This study has presented us with an excellent opportunity to compare our method with those of two other internationally recognised laboratories. In the absence of external QAP being available, a similar serum set for use across different laboratories would be helpful for any laboratory wishing to establish testing for specific IgG to Pneumococcal serotypes.

A novel method for serotyping pneumococcal strains using quantitative immunoabsorption as measured by ELISA

Eastham, V1, van Niekerk, N1, Little, T1, von Gottberg A2, de Gouveia L2, Adrian, PV3, Madhi, SA3
1Respiratory and Meningeal Pathogens Research Unit, CHBH, Soweto, South Africa.
2Respiratory and Meningeal Pathogens Research Unit, NICD, South Africa

Background and aims: The pneumococcal conjugate vaccine PCV covers a limited number of pneumococcal serotypes. In order to assess vaccine coverage in different populations the identification of the burden of disease caused by different pneumococcal serotypes needs to be accurately assessed. The gold standard method for pneumococcal serotyping is the Quellung method. This method is time consuming, and requires expensive typing serum, which makes it expensive to perform large clinical and epidemiological studies. Interpretation of capsular swelling is subjective and can lead to discrepant results.

Methods: We have developed a novel method that uses the ability of the organisms to absorb the capsule type specific antibodies from a pool of polyvalent human serum. The absorption of the type specific antibody is quantitatively measured by capsule type specific Enzyme-Linked Immunosorbent Assay (ELISA). The new method was used to identify serotypes 4, 6A, 6B, 9V, 14, 18C, 19A, 19F and 23F in 235 nasopharyngeal isolates of pneumococci. These results were compared with the Quellung method with a checkerboard of pooled sera followed by sub-typing.

Results: Of the pneumococcal isolates, the capsular subtypes 19F (10%), and 23F (8%) were the most prevalent, followed by 6A, 14, and 6B (6%). Strains that are covered by the vaccine and are of low prevalence are 19A (4%), 9V (3%), 18C and 4 (1%). The results of this new method correlated 99% to that of the Quellung method. Of the 1% discrepancy (n=3), one isolate proved to be a mix of two strains, and two isolates were misidentified as 19A and were re-typed as 19F by the Quellung method. Thus, the sensitivity and specificity of the immunoabsorption method for the serotypes tested was 100%.

Conclusions: This method provides a cost effective alternative to identify the sub-set of serotypes that are covered by the current 7 valent PCV. Expansion of this method to include about 25 of the most common serotypes will optimise its cost effectiveness. Identification of rare serotypes should be done by established methods.
Usefulness of dot blot test for the serotyping of Streptococcus pneumoniae

Brandão, AP1,2,3, Gorla, MCO1, Lemos, APS1, Yara, TI1,4, Zanella, RC1, Almeida, SCG1, Brandileone, MCC1
1Instituto Adolfo Lutz (IAL), São Paulo, SP, Brazil
2Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil
3FUNASA, São Paulo, SP, Brazil
4Fundação Butantan, São Paulo, SP, Brazil

Serotype identification of pneumococci is very important as baseline information for control of pneumococcal diseases by vaccination, and to monitor them after vaccination of the population at risk, since there are temporal, geographic and age group differences on pneumococcal serotypes causing infection. As more vaccine efficacy studies and epidemiological carriage surveys are being conducted to improve vaccine coverage and also due to the enhancement of the national pneumococcal invasive disease surveillance programs, there is an increasing need for serotype-specific diagnosis. Because Neufeld (Quellung) test is labour-intensive and expensive, we decided to evaluate a dot blot (DB) assay as a screening test for the serogroups and types most frequently isolated from pneumococcal invasive disease in Brazil. After introducing some changes in the DB assay as described by Fenoll et al. (J.Clin.Microbiol.,1997;35:764-6), mainly related to antigen production and colour development system, we have analysed 1,349 invasive strains received during 2003 by the Brazilian reference centre for S. pneumoniae, IAL, in order to validate the assay. Overnight cultures on blood agar plates were harvested and killed at 56°C/30min and dotted on strips of nitrocellulose membrane. After drying, the strips were incubated with rabbit antiserum (Statens Serum Institut) against 12 serogroups/types 1, 4, 5, 6, 7, 8, 9, 11, 12, 14, 18, and 23. After 1h incubation at room temperature, the strips were washed, incubated with anti-rabbit IgG-peroxidase conjugate for 1h, washed and exposed to 3-amino-9-ethylcarbazole solution. When rose-red colour was obtained for the positive control, the strips were washed and evaluated. The same bacterial culture was used for the DB assay and Quellung test.

Of 944 strains belonging to one of the 12 serogroups assessed by Quellung reaction, 887 were positive by DB assay (94% sensitivity). All the remainder 462 isolates belonging to serogroups/types other than 1, 4, 5, 6, 7, 8, 9, 11, 12, 14, 18, and 23 were Negative by DB assay (100% specificity). The assay has then been routinely performed at IAL, and was shown to be very useful, as it is a cost-effective technique and does not require special equipment. About 70% isolates have been typed or grouped, leaving few strains and lesser steps for further typing by Quellung test to define serotypes within a serogroup.

Development of a multiplex, rapid non-culture bead based assay for serotype identification of pneumococci

Lahe, G1, Balmer P2, Harrison, T2, Borrow R1
1Vaccine Evaluation Unit, Health Protection Agency, Manchester, United Kingdom
2Centre for Infections, Health Protection Agency, Colindale, London, United Kingdom

Diagnostic procedures for the identification of a pneumococcal serotype require a positive isolate. There are non-culture diagnostic protocols available, but these only confirm the presence of pneumococci and do not identify the serotype. The increased use of pneumococcal polysaccharide and conjugate vaccines, especially in high risk groups and the likely increase in the number of serotypes included in future versions of the conjugate vaccines has necessitated the need for improved enhanced surveillance (and diagnosis) in order to assess their impact on public health. Recently, a multiplex bead-based assay was described using serotype specific monoclonal antibodies to identify serotypes which was demonstrated to have good specificity in identifying previously characterised pneumococcal isolates. We have developed, a competitive inhibition assay for the identification of pneumococcal serotypes from clinical samples (blood, CSF or urine) without the restriction of requiring monoclonal antibodies and has the potential for expansion of the repertoire of serotypes currently included in the assay. Pneumococcal capsular polysaccharides were conjugated to microspheres as previously described and mixed with the unknown sample and pneumococcal antiserum (89-SF). The presence of a pneumococcal serotype-specific polysaccharide is identified by an inhibition of fluorescence in comparison to a control (microspheres plus antiserum only). Initially, 13 serotypes were included in the assay and showed good inhibition curves upon titration of known concentrations of pneumococcal serotype-specific polysaccharides and demonstrated between 49 – 138% homologous recovery of polysaccharide in spiked IgG-deficient sera. Analysis of pneumolysin PCR positive CSF samples with the 13-plex assay gave 57% samples positively identified. Of the negative samples, 75% had low bacterial loads with PCR cycle numbers between 30-40. The assay has been expanded to incorporate 23 serotypes and has shown good specificity following the addition of homologous polysaccharide. A multiplex, rapid non-culture assay for pneumococcal serotype identification has been developed which will be useful in assessing the impact of pneumococcal vaccines.


Assessment of anti-6A antibody response induced by Serotype 6B in pneumococcal polysaccharide vaccine in Koreans

Kim, KH1, Seoh, JY1, Nahm, MH2
1Ewha Woman’s University, Seoul, Korea
2University of Alabama at Birmingham, AL, USA

Purpose: *Streptococcus pneumoniae* serotype 6A and 6B are important pathogens in pneumococcal infections in Korea. It is commonly assumed that the 6B vaccines elicit antibodies cross-reacting with 6A serotype and the cross-reactive antibodies may protect against infections of 6A. To examine this assumption, we investigated the antibody titer and opsonophagocytic capacity to serotype 6A and 6B in Koreans who were immunized with pneumococcal polysaccharide (PS) vaccine.

Method: Twenty-seven adults, 9 children and 33 elderly persons were immunized with pneumococcal PS vaccine that contains 6B PS. The antibody titer and capacity to opsonize 6B and 6A pneumococcal serotypes were studied in pre- and postimmune sera with 3rd generation ELISA and opsonophagocytic killing assay (OPKA).

Results: Antibody titers and opsonization titers to 6B (vaccine) serotype were significantly increased after immunization in all age groups. Although antibody titers to 6A (cross-reactive) did not change significantly after immunization in 3 age groups, opsonization titers to 6A showed significant increases in adults and elderly after immunization. Therefore, immune responses to 6A were demonstrated by the OPKA but not by ELISA. Anti-6B antibody levels and the opsonization titer against 6B correlated in children (r=0.35), adults (r=0.45) and elderly (r=0.65) after immunization.

Conclusions: Although 6B PS in PS vaccine elicits functional antibodies cross-reacting with 6A in most of adults and elderly persons, it did not occur in children and in some adults. Opsonization titers by OPKA seemed to be more sensitive than antibody titers by ELISA to assess the formation of cross-reactive antibodies induced by vaccine.
PneumoADIP's Strategic framework to establish the value of pneumococcal vaccination in developing countries

Lee, EH1, Mahbub, FB1, Wonodi, CB1, O'Brien, KL1, Cherian, T2, Moitch, J1, and Knoll, MD1
1Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland, USA
2World Health Organization, Geneva, Switzerland

Historically, 15-20 years passed before children in the world’s poorest countries benefited from new vaccines used in industrialized countries. The Pneumococcal Accelerated Development and Introduction Plan (PneumoADIP), created by Global Alliance for Vaccines and Immunizations (GAVI), aims to shave years off this time lag for pneumococcal conjugate vaccines (PCVs). To establish the value of PCVs in developing countries in a timely manner, PneumoADIP uses a strategic framework to invest in research and surveillance activities. Decision-makers at country, regional, and global levels will require key pieces of information to evaluate the case for PCV introduction. Once PCV safety and efficacy have been established globally, it is essential to determine local and regional disease burden. Local microbiologic surveillance demonstrates presence of invasive pneumococcal disease, and serotype distribution is used to project vaccine impact. Vaccine-probe studies provide regional data on the greater burden of vaccine-preventable clinical disease, which is not captured by invasive disease surveillance. Cost-effectiveness studies allow countries to compare and prioritize PCV introduction to other health programs. Demonstration projects evaluating PCV administered through the Expanded Programme on Immunization provide estimates of the vaccine’s indirect effects, specifically herd immunity and serotype replacement. Immunogenicity studies in representative populations provide data for vaccine licensure and global/regional recommendations.

In <3 years, PneumoADIP has committed ~$6.9 million dollars to surveillance and research activities aimed at improving the evidence-based case for PCV introduction. These activities are underway in 58 countries, with serotype distribution data collected in >25 countries where data previously were absent or severely limited. To date, surveillance has yielded >31,000 blood and cerebrospinal fluid specimens, with 554 pneumococcal and 271 Haemophilus influenzae b (Hib) isolates.

PneumoADIP’s strategy was shaped by lessons learned with Hib vaccine and aims to accelerate evidence-based demand for PCV introduction. These activities are linked with plans for developing an ‘investment case’ for global and country-level decision-makers and for communicating results to key opinion leaders and sponsors. Activities are carefully timed to meet projected demand forecasts, which in turn will be used to assure financing from multiple sources and an affordable, sustainable vaccine supply from manufacturers. Lacking an evidence base in representative populations will unnecessarily delay decisions about introduction of new PCVs for years. Accelerated use of pneumococcal vaccine in developing countries can prevent nearly 3.6 million child deaths by 2025.

PO14.03

PneumoADIP-driven Communications Efforts Lead to a Marked Increase in Media Coverage Around Pneumococcal Disease

Kvist H1, Keeling E2, Griffin C3
1GAVI’s PneumoADIP (Pneumococcal Vaccines Accelerated Development and Introduction Plan), Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
2Ruder Finn, London, UK
3Echo Research, Godalming, Surrey, UK

Context: When PneumoADIP’s communications activities commenced in June 2003, baseline research indicated that only infrequent mentions of pneumococcal disease occurred in the media, mainly relating to the use of vaccines in the developed world. <Google> searches for <pneumococcal disease> brought up zero results. PneumoADIP has subsequently coordinated efforts to increase awareness of the burden of pneumococcal disease and the need for routine immunization for the world’s children. Echo Research was independently commissioned to assess whether pneumococcal disease had moved up the media agenda since PneumoADIP’s inception.

Methodology: News coverage was assessed from April 2003 to September 2005. Articles from Internet searches, automated online news and print media provided the data for the analysis. The search was for English-language coverage – print and online – focusing on key donor countries. Coverage of Hib (Haemophilus influenzae type b) disease in the same time period was the comparator – to show whether any increase in coverage was due to changes in the general media environment for neglected diseases.

Results: Pneumococcal disease consistently attracted more media coverage than Hib throughout the period analysed (158 vs 89 articles). As PneumoADIP stepped-up communications efforts, increased coverage was generated – with a sevenfold increase in the first nine months of 2005 compared with the same period in 2004 (17 vs 122 articles). There were no negative pneumococcal stories – all were assessed as neutral or positive. Almost half (45%) of pneumococcal stories had a developing world focus – PneumoADIP was deemed directly responsible for generating 36% of these. All developing world pneumococcal articles contained at least one PneumoADIP-driven key message. The Gambia pneumococcal vaccine trial outreach generated 13% of all developing world-focused pneumococcal coverage. All Gambia vaccine trial coverage contained the key message that pneumococcal vaccination reduced all-cause mortality by 16%. In addition, the PneumoADIP-driven outreach surrounding the Gambia Hib vaccine study generated 44% of all Hib coverage in 2005 (1 January–30 September 2005).

Discussion: This independent analysis showed that a coordinated communications programme has been successful in helping to increase media coverage around pneumococcal disease and the existence of life-saving vaccines – from an extremely low baseline. When the price of inaction is so high - up to one million child deaths annually - the value of increased disease awareness in influencing policy and donor response is immense.
The Case for Routine Use of Pneumococcal Conjugate Vaccine (PCV) for HIV-infected Children in the Developing World

Bliss, S.1, Levine, O.2,3, O’Brien, KL.4,5
1Johns Hopkins School of Medicine, Baltimore, MD, USA
2Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
3GAVI’s PneumoADIP, Baltimore, MD, USA

Background: PCV use among children in settings of high human immunodeficiency (HIV) seroprevalence may be an effective strategy for preventing pneumococcal disease in these high-risk children. We reviewed the published literature to assess the evidence for a policy to use PCV in HIV-burdened regions.

Methods: We searched PubMed to identify articles on pneumococcal disease epidemiology (i.e. rates of IPD, serotype distribution) and PCV effects (i.e. safety, immunogenicity, efficacy, indirect protection, serotype replacement) among HIV-infected people.

Results: Eight reports documented IPD incidence rates in HIV-infected children between 18.4 and 113 episodes per 1000 child-years, a 13- to 43-fold increased risk compared to HIV-uninfected children. Eleven reports identified incidence in HIV-infected adults ranging from 2.0 to 57 episodes per 1000 person-years, a 6.2- to 60-fold increased risk. Seven studies demonstrated no serotype differences among IPD isolates from HIV-infected versus uninfected children. Among nine studies, HIV-infected adults generally had a greater proportion of vaccine-type pneumococcal isolates than HIV-uninfected adults. Three studies assessed PCV safety in HIV-infected children and found the vaccine was well-tolerated. In six studies among HIV-infected children, PCV was highly immunogenic, but with lower antibody concentrations as compared to uninfected children; in a single study, function of these antibodies was also reduced. One phase III trial among HIV-infected infants showed that 9-valent PCV was 65% efficacious for preventing first episodes of vaccine-type IPD (a reduction of 569 episodes per 100,000 child-years) and reduced clinically-diagnosed lower respiratory tract infection by 2573 cases per 100,000 child-years. In the USA, surveillance shows that IPD rates declined among HIV-infected adults after routine childhood vaccination started, but with some attenuation of benefit due to serotype replacement disease. Most studies reviewed were from the USA and South Africa.

Discussion: The strengths of a case for routine PCV vaccination of HIV infected children are that HIV infection significantly increases the risk of IPD and pneumococcal pneumonia, the serotype distribution of pediatric IPD isolates is unaffected by HIV status and includes a high proportion of vaccine types, and PCV appears safe, immunogenic, and efficacious among HIV-infected children. The main gaps in the data relate to limited geographic diversity of the data, and that the data are based on use of 7-valent and 9-valent vaccines. Data from other countries and with other vaccines would improve an already strong case.

Coordinated communications efforts between 2003 and 2005 lead to a marked increase in media coverage around pneumococcal disease

Kvist H.1, Haylock S.2, Griffin C.3
1GAVI’s PneumoADIP (Pneumococcal Vaccines Accelerated Development and Introduction Plan), Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
2Ruder Finn, London, UK
3Echo Research, London, UK

Context: When PneumoADIP’s communications activities commenced in June 2003, baseline research indicated that only infrequent mentions of pneumococcal disease occurred in the media, mainly relating to the use of vaccines in the developed world. <Google> searches for pneumococcal disease brought up zero results. PneumoADIP has subsequently coordinated efforts to increase awareness of the burden of pneumococcal disease and the need for routine immunization for the world’s children. Echo Research was independently commissioned to assess whether pneumococcal disease had moved up the media agenda since PneumoADIP’s inception.

Methodology: News coverage was assessed from April 2003 to September 2005. Articles from Internet searches, automated online news and print media provided the data for the analysis. The search was for English-language coverage – print and online – focusing on key donor countries. Coverage of Hib (Haemophilus influenzae type b) disease in the same time period was the comparator – to show whether any increase in coverage was due to changes in the general media environment for neglected diseases.

Results: Pneumococcal disease consistently attracted more media coverage than Hib throughout the period analysed (158 vs 89 articles). As PneumoADIP stepped-up communications efforts, increased coverage was generated – with a sevenfold increase in the first nine months of 2005 compared with the same period in 2004 (17 vs 122 articles). There were no negative pneumococcal stories – all were assessed as neutral or positive. Almost half (45%) of pneumococcal stories had a developing world focus – PneumoADIP was deemed directly responsible for generating 36% of these. All developing world pneumococcal articles contained at least one PneumoADIP-driven key message. The Gambia pneumococcal vaccine trial outreach generated 13% of all developing world-focused pneumococcal coverage. All Gambia vaccine trial coverage contained the key message that pneumococcal vaccination reduced all-cause mortality by 16%. In addition, the PneumoADIP-driven outreach surrounding the Gambia Hib vaccine study generated 44% of all Hib coverage in 2005 (1 January–30 September 2005).

Discussion: This independent analysis showed that a coordinated communications programme has been successful in helping to increase media coverage around pneumococcal disease and the existence of life-saving vaccines – from an extremely low baseline. When the price of inaction is so high - up to one million child deaths annually - the value of increased disease awareness in influencing policy and donor response is immense.
Global, regional, and country estimates of Streptococcus pneumoniae disease burden

1World Health Organization, Geneva, Switzerland  
2Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Background: Published research suggests Streptococcus pneumoniae (SP) is a leading cause of vaccine preventable deaths among children <5 years of age globally. However, disease prevention efforts including vaccination and case management are hampered by lack of more precise, comprehensive disease burden estimates at local, regional and global levels. The World Health Organization designed a methodology and process that was transparent, rigorous, and verifiable to estimate the number of SP cases and deaths worldwide.

Methods: We constructed a model for estimating cases and deaths of SP attributable meningitis, pneumonia and non-meningitis/non-pneumonia invasive disease, based on data availability and input from an independent expert panel. To populate model parameters, we searched available literature from nine databases using search terms for SP and associated life-threatening syndromes (i.e. pneumonia, meningitis, or invasive disease) from 1980 through 2005; we also searched unpublished studies known to the authors and additional articles derived from references of abstracted papers. Identified articles were screened for relevance, and articles meeting screening criteria were abstracted by two independent reviewers using a structured form including study quality indicators. Where required, differences in abstracted data were resolved by a third reviewer. Because primary data of adequate quality were not available for all countries, disease burden estimates at the country level required extrapolation. Country groupings based on geographic proximity and child mortality levels were constructed together with decision rules for extrapolation and were reviewed by the independent expert panel.

Results: The literature search identified 11,149 articles. SP meningitis burden was estimated using incidence of SP meningitis, case fatality ratios, and direct and indirect protection from SP vaccination. SP pneumonia burden was estimated from WHO global estimates of pneumonia deaths and cases combined with data from efficacy trials of conjugate pneumococcal vaccines against pneumonia. The non-pneumonia/non-meningitis model relies on the relative proportion of cases attributable to various disease syndromes. When point estimates are presented, they should be presented with ranges reflecting the uncertainty in those estimates. Rigorously derived estimates of SP disease burden are essential for global, regional, and local decision-making on disease prevention and reduction strategies.