

**A-1****The Association between the Risk Factors for Nasopharyngeal Colonization with Penicillin Resistant Pneumococci and the Relevance of Infection Control in Children Day Care Centers****Pa Tambyah, MI Thong, D Goh, Yf Chiew**  
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The prevalence of penicillin-resistant *S. pneumoniae* (PRSP) has been increasing among hospitalised patients in Singapore. We undertook a prospective study of children attending 15 different day care centers to better define the prevalence of PRSP in the community. Nasopharyngeal swabs were obtained from 470 children. A total of 114 (24.3%) children were colonized by *S.pneumoniae* (SP), 33 (7%) by PRSP. Only one of the isolates had high-level resistance to penicillin (MIC > 2.0 µg/mL). PRSP were more likely than penicillin-susceptible SP to be resistant to erythromycin (67% vs 25%,  $P < 0.0001$ ) and tetracycline (91% vs 34%,  $P < 0.0001$ ) and intermediately resistant to cefotaxime (68% vs 0%,  $P < 0.0001$ ). In a multivariate logistic regression model, young age ( $P = 0.005$ ) and presence of rhinorrhea ( $P = 0.025$ ) were the only predictors of colonization with SP while rhinorrhea was the only predictor of colonization with PRSP ( $P = 0.04$ ). Gender, ethnicity, household size, number of younger siblings, frequency of colds and recent hospitalization or antibiotic uses were all not associated with carriage of SP. When an analysis was restricted only to children colonized with SP, only recent upper respiratory tract infection (a possible surrogate marker for antibiotic use) was predictive of colonization with PRSP in a multivariate model. We conclude that infection control measures such as wiping runny noses and even encouraging these children to stay at home are necessary to prevent dissemination of PRSP in the community.

**A-2****Recent Antibiotic Use and Penicillin-resistant Pneumococcal Bacteremia****J. Ruhe\*, R. Hasbun, and D. Mushatt**  
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**BACKGROUND** An increase from 10% to 50% in the incidence of penicillin-resistant *Streptococcus pneumoniae* (PRSP) blood isolates has been observed in two tertiary care medical centers in New Orleans between 1995 and 2000.

**METHODS** To determine risk factors for PRSP (intermediate [MIC 0.1-1 µg/mL] and high-level [MIC > 2 µg/mL]), we have performed a retrospective cohort study of 102 patients (pts.) with pneumococcal bacteremia at the Medical Center of Louisiana in New Orleans and at Tulane University Hospital.

**RESULTS** The median age of the cohort was 39 years (range 2 months to 87 years); 64% were male; 74% were black. The majority of pts. were immunocompetent, but up to 40% had a Charlson comorbidity score > 1 and 31% had HIV/AIDS. The site of infection included lung (69%) and otitis (9%). On bivariate analyses, predictors that were associated ( $p < 0.05$ ) with intermediate or high-level PRSP were: recent hospitalization (OR 3.2, 95% C.I. [1.2-9.1]) recent pneumonia [OR 4.3, 95% C.I. (1.5-12.3)], recent sinusitis/otitis [OR 4.3, 95% C.I. (1.4-13.4)], previous antibiotic use in the last 30 days [OR 3.2, 95% C.I. (1.3-8.1)] and previous penicillin use in the past 6 months [OR 3.1, 95% C.I. (1.3-7.3)]. Previous macrolide [OR 3.2, 95% C.I. (0.9-11.8)] and quinolone [OR 0.9, 95% C.I. (0.3-3.0)] use were not associated with PRSP bacteremia.

**CONCLUSION** Recent antibiotic use (especially penicillins) and recent respiratory infections are important risk factors for PRSP bacteremia in New Orleans.

**A-3****Drug Resistant *S. pneumoniae* - Trends in Hong Kong****D.J. Lyon\* and M. IP**  
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Screening for reduced penicillin susceptibility using the oxacillin 1 µg disc method was introduced in 1992 at the Prince of Wales Hospital, Hong Kong. The proportion of *S.pneumoniae* isolates of reduced penicillin susceptibility rose from 10% in 1993 to 50% in 1997 and 63% in 2000. Resistance to erythromycin, cotrimoxazole and tetracycline also rose sharply in the same period, and by 2000 it was 71%, 71%, & 82% respectively. Of 117 isolates submitted for MIC, the penicillin MICs were mostly 1 or 2 µg/mL, with the highest being 4 µg/mL. The highest cefotaxime MIC was 2 µg/mL. The rate of rise of drug resistance was greatest during the period 1993-95. Penicillin nonsusceptible strains belonged to only 5 serotypes - 23F, 19F, 6B, 14 & 9V. *pbp* fingerprint studies and PFGE analysis showed that most penicillin insusceptible isolates were of two main groups; the largest, group A, expressed capsular types 23F, 19F or 14, and the other, group B, expressed capsular type 6B. Within each group, the strains were highly similar to each other and to representatives of the Spanish 23F and 6B clones. Multilocus sequence typing (MLST) showed that the strains within group A and within group B had the same MLST profile. The MLST profile for group A was the same as the Spanish 23F clone, and group B the Spanish 6B clone. Several clones of multidrug resistant *S.pneumoniae*, with the genetic background of the two main Spanish clones, have spread widely in Hong Kong.

**A-4****Antimicrobial Resistance of *Streptococcus pneumoniae*: a Comparison between the Middle of 1980's and 1990's****Li J, Yang YH, et al.**  
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**OBJECTIVE** To study the antimicrobial resistance of *Streptococcus pneumoniae* (Sp) in 1983-1985 and 1995-1997.

**METHODS** The compared the susceptibility of Sp to antibiotics in 1983-1985 (49 isolates recovered from normally sterile body sites) and 1995-1997 (33 isolates recovered from nasopharyngeal swabs, CSF and ear drainage of inpatients with agar dilution).

**RESULTS** Penicillin-resistant Sp increased significantly from 6% in 1983-85 to 21% in 1995-1997. The resistance of Sp to cefuroxime, cefotaxime, imipenem increased from 0 to 15%, 9% and 12%, respectively. Erythromycin-resistant Sp increased significantly from 2% to 79%. The categories of resistance increased in 1995-1997 compared to the period 1983-1985. Some penicillin resistant Sp were resistant to cefuroxime, cefotaxime, and imipenem significantly. The main patterns in 1983-1985 were tetracycline and tetracycline/ chloramphenicol; in 1995-1997 erythromycin, clarithromycin, clindamycin were added to the main patterns.

**CONCLUSIONS** The antimicrobial resistance of Sp to penicillin, cefuroxime, cefotaxime, imipenem, erythromycin, etc. had increased in 1995-1997 as compared to 1983-1985 within the scope of this study. Rational use of antibiotics should be emphasized.

## A-5

A Study of Penicillin Resistance in Clinical Isolates of *Streptococcus pneumoniae*

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To investigate the current status of penicillin resistant *S. pneumoniae* (Sp), we measured the minimal inhibitory concentrations (MICs) of penicillin G, ampicillin and oxacillin in 130 clinical isolates of Sp by using an agar dilution method. The results showed that 20 strains (15.4%) had penicillin G MIC  $\geq 2 \mu\text{g}/\text{mL}$ , 16 strains (12.3%) had ampicillin MIC  $\geq 4 \mu\text{g}/\text{mL}$ , and 32 strains (24.6%) had oxacillin MIC  $\geq 4 \mu\text{g}/\text{mL}$ . The rate of penicillin-resistance (40.7%) in hospitalized patients with pneumonia were much higher than that of outpatients and carriers. The results indicate that strains of Sp resistant to penicillins have become a serious problem in Beijing. Strategies to limit the spread of highly resistant pneumococcal strains should include encouraging judicious use of antibiotics.

## A-6

Characteristics of *Streptococcus pneumoniae* Isolates from Patients in Korea: Capsular Serotypes and Antimicrobial Sensitivity Patterns

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**BACKGROUND** *Streptococcus pneumoniae* is a major infectious agent in humans, and the emergence of penicillin- and multidrug-resistant strains threatens to complicate the management of pneumococcal infections. And the distribution of pneumococcal serotypes differs over geographic area, time period, age group, and clinical source.

**METHOD** One hundred forty-two isolates of *S. pneumoniae* obtained from patients in Seoul, Korea were serotyped by the capsular typing test (quellung reaction) and tested to determine their susceptibility to eight antimicrobial agents using the broth dilution method. Fifty-three strains of different serotypes were performed PFGE to investigate the genetic relatedness of pneumococcal strains.

**RESULTS and CONCLUSIONS** Of all 142 pneumococcal isolates, 37(26%) were isolated from blood and CSF, and 105(74%) from sputum, throat, and other body sites. Four serotypes (19F, 23F, 14, and 9V) were responsible for 85.6% of the cases. There were no significant differences in the relative frequency of serotypes isolated from blood and CSF versus those isolated from other body sites. The 23-valent pneumococcal vaccine types represented 92% of all isolates, 89% of isolates from blood and CSF, and 95% of isolates from other sites. The proportion of serotypes covered by pneumococcal vaccine showed a few difference by the infection sites. Overall, 76.8% of all isolates were not susceptible to penicillin, exhibiting either intermediate resistance (12.0%) or high level resistance (64.8%). The prevalence rates (59.5%) of invasive pneumococcal isolates not susceptible to penicillin were much lower than those (82.8%) of other sites. Multidrug-resistance to three or more of antibiotics tested was observed in 86.6% of the isolates. Fifty-three strains of several serotypes showed 33 distinct PFGE patterns, but isolates of the same serotype always do not show the related PFGE pattern. We confirmed that the prevalence of antimicrobial-resistant pneumococci is very highest in Korea and it is emphasized the need for improved surveillance and continuous control of pathogens that are resistant to antimicrobial agents.

## A-7

Serotype Distribution and Antimicrobial Susceptibility Profiles of Group B *Streptococcus* Strains from Pregnant Women in Beijing

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**OBJECTIVE** To investigate the serotype distribution among group B streptococcus (GBS) strains isolated in Beijing areas from 1994 to 1999 in order to determine the optimal components of future GBS vaccines; also, to assess the antimicrobial susceptibility profile of the strains.

**METHODS** A total of 155 GBS strains were isolated from 1994 to 1999. Serotyping was performed by double diffusion in agarose (Ouchterlony method). Susceptibility to ampicillin, penicillin G, oxacillin, erythromycin, clindamycin, cephalosporin, cefuroxime, cefoperazone, gentamicin and amikacin was assessed by disc diffusion.

**RESULTS** Seven serotypes were identified. Type II (II/C; 36%), type III (III/R; 26%), type Ia (Ia/c; 18%) were the most common. All strains were susceptible or moderately susceptible to ampicillin, penicillin G, oxacillin, cephalosporin, cefuroxime and cefoperazone. A dramatic increase in erythromycin and clindamycin resistance occurred when comparing the period 1994-97 and 1998-99. For serotype III/R, the proportion resistant to macrolides was 22.5%.

**CONCLUSIONS** GBS serotypes Ia, II and III may be important components of a future multivalent GBS vaccine for use in Beijing areas. The susceptibility of the GBS strains to penicillin G and ampicillin supports continued use of either of these antibiotics for prevention of early-onset GBS diseases. The use of erythromycin as a second-line agent can be questioned with regard to the increased bacterial resistance. Continuous surveillance for changes in susceptibility is considerable clinical importance.

## A-8

Macrolide Resistance Determinants in  $\beta$ -Haemolytic Streptococci and *Streptococcus pneumoniae* in Hong Kong

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**OBJECTIVES** The prevalence and distribution of macrolide resistance determinants responsible for macrolide, lincosamide and streptogramin (MLS) group of antibiotics among  $\beta$ -haemolytic streptococci and *Streptococcus pneumoniae* in Hong Kong were investigated.

**METHOD** 206 isolates of Group A (*S. pyogenes*), B (*S. agalactiae*), C and G streptococci and 197 *Streptococcus pneumoniae* strains isolated during 1993 - 1999 were studied. The MICs of penicillin, erythromycin, clarithromycin, clindamycin, tetracycline, chloramphenicol, cefotaxime and ciprofloxacin were determined by the NCCLS agar dilution. The macrolide resistance determinants were detected by PCR using primers specific for the *ermA*, *ermB*, *ermC*, *ermTR*, *mreA* and *mef* genes.

**RESULTS** 24% (50/206) of  $\beta$ -haemolytic streptococci were resistant to erythromycin, but the rates varied from 15% in *S. agalactiae*, to 21% in Group C and G streptococci and 56% in *S. pyogenes*. 58% of the resistant isolates carried the *erm* genes and expressed the MLS<sub>B</sub> phenotype. *mreA* gene was detected in *S. agalactiae*. The remaining strains showed the M phenotype and carried the *mef* gene. Erythromycin resistance rates amongst penicillin-susceptible *S. pneumoniae* were 38% and 92% in penicillin-intermediate or resistant *S. pneumoniae*. 27% (43/158) showed the MLS<sub>B</sub> phenotype and carried the *ermB* gene. 73% (115/158) displayed the M phenotype and possessed the *mef* gene.

**CONCLUSIONS** A high prevalence of erythromycin resistance was seen among  $\beta$ -haemolytic streptococci and *S. pneumoniae*. Various *erm* and *mef* genes were present in varying proportions depending on the species. In *S. pneumoniae*, widespread presence of macrolide resistance is likely due to extensive spread of penicillin-resistant clones in Hong Kong.

**A-9*****Streptococcus pneumoniae* Bacteremia in Adults during 1996-2000 in Korea**

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**OBJECTIVE** A retrospective analysis was performed to measure the incidence of pneumococcal bacteremia, and to identify risk factors for penicillin resistance and prognostic factors for outcome among adult patients.

**METHODS** Medical records of all patients with pneumococcal bacteremia during 1996-2000 was reviewed, and demographic, clinical, and microbiologic data were obtained. Pediatric patients under 15 years of age were excluded from the study.

**RESULTS** A total of 149 cases with pneumococcal bacteremia was identified from 147 adults. 100 (67.1%) cases were men, and 44 (29.5%) were nosocomial. Of the 149 cases, 74 (49.7%) had penicillin-resistant isolates, exhibiting intermediate resistance in 45 (30.2%) and high level resistance in 29 (19.5%). The incidence of penicillin-resistant pneumococci among patients with pneumococcal bacteremia remained stable from 54.5% in 1996 to 49.7% in 2000 (P=0.82). Resistance rates to ceftriaxone, clindamycin, erythromycin, and trimethoprim-sulfamethoxazole were 22.4%, 52.1%, 61.2%, and 44.2% respectively. Multidrug resistance showed in 47%. Penicillin resistance was significantly associated with malignancy, placement of biliary drainage catheter, and previous  $\beta$ -lactam antibiotic use in univariate analysis. However, their association was not significant as independent risk factors for penicillin resistance in multivariate analysis. Mortality due to pneumococcal bacteremia was 23.4%, and it has not changed significantly in adults during 1996-2000 (P = 0.08). The mortality was associated with age, neurologic disease, chemotherapy, placement of indwelling urinary catheter, bedridden state, leukopenia, polymicrobial bacteremia. **DEREBPMENT** of complications in univariate analysis. However,

**CONCLUSIONS** Penicillin resistance, and even high level penicillin resistance, was not a risk factor for mortality and the mortality has not been changed in adults with pneumococcal bacteremia. Considering the highest and still increasing incidence of penicillin and multidrug resistance of pneumococci in Korea, continued surveillance for resistance patterns as well as for data on clinical outcomes is should on a regular basis, and judicious use of antibiotics is mandatory.

**A-10****Chromosomal DNA Analysis of Group B Streptococcal Clinical strains**

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A collection of 45 epidemiologically unrelated *Streptococcus agalactiae* strains (group B streptococcus, GBS), isolated from pregnant women in P.R.China and Russia, were characterized by pulsed field gel electrophoresis (PFGE), hybridization analysis to virulence gene probes and ribotyping to look at the relationship of PFGE and ribotype profiles and presence of 9 genes potentially involved in virulence. 7 of these genes were present in all the strains. However, bac gene and bca gene were discovered in part of the strains. Ribotyping analysis revealed 3 different HindIII, 9 EcoRI and 12 PvuII ribotypes among 45 strains. A strong correlation between the PvuII ribotype and the presence of the bac gene was observed, with 21 of 22 bac+ strains belonging to the same PvuII ribotype P1. PFGE analysis revealed a strict correlation between a specific PFGE profile and presence of the bac gene in the genome. We propose that the acquisition of the bac gene by an ancestral GBS recipient strain provided some biological advantages, resulting in the rapid and effective spreading of this new clone within the human population. Independently of the presence of the bac gene, the molecular size of the entire GBS chromosomes varies from 2027,5 kb to 2293 kb. Virulence genes, located on SmaI restriction fragments, were used to correlate the physical and genetic maps of the strains, revealing the conservatism of the overall genome organization of GBS. Interestingly, potential virulence genes scpB, hylB, lmb were located on a 91 kb fragment that is equal to 4,5% of total genome.

**A-11****Antimicrobial Resistance of 90 Strains of *Streptococcus pneumoniae* in Children**

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The penicillin MIC of 90 strains were detected by K-B diffusion method and by E-test. Results 48/90 strains(51.5%) were possible resistance to penicillin based on disk diffusion. The MICs detected by E-test, however, showed that of these 48 strains, only one strain(2.1%) had the MIC 2  $\mu$ g/mL, and 23/48 strains(47.9%) were penicillin intermediate resistance(MIC 0.12~1.0  $\mu$ g/mL), but 24/48 strains(50%) were actually penicillin susceptible(MIC  $\leq$  0.06  $\mu$ g/mL). The percentage of resistance to erythromycin, chloramphenicol, clindamycin and TMP-SMZ was 75.6%, 35.6%, 53.3% and 85.5% respectively. No resistant strain to vancomycin was detected. Conclusion For *S.pneumoniae*, laboratories should report MICs for penicillin and the results based on disk diffusion are only suitable for screening. *S.pneumoniae* resistance to penicillin is 26.7% in Shanghai Children's Hospital in 1999. It must be extremely concerned that the *S.pneumoniae* resistance to erythromycin, clindamycin and TMP-SMZ is very high.

**A-12****The Effect of Priming on Anti -CPS Immune Responses after Intranasal Immunization with Group B Streptococcus Type III Capsular Polysaccharide-cholera Toxin B Subunit Conjugate**

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The effect of priming with Group B Streptococcus type III capsular polysaccharide (GBS CPS III)-recombinant cholera toxin B subunit (rCTB) conjugate, pure GBS CPS III or rCTB alone on anti-CPS III systemic and mucosal immune responses after intranasal (i.n.) immunization in mouse model were investigated. Priming with pure GBS CPS III alone or with GBS CPS III-rCTB conjugate induced comparable levels of specific IgG and IgA in serum, lungs and vagina. However, the immunization scheme of both priming and boosting with conjugate was superior to priming with CPS and boosting with conjugate or the reverse, on inducing strongest anti-CPS responses. All the immunization schemes, except priming and boosting with CPS, could induce similar high levels of IgG1 antibody responses in serum. The mice primed with CPS III and boosted with CPS III-rCTB conjugate by i.n. route failed to produce significant levels of IgG2a, IgG2b and IgG3 in serum, comparing with the levels in mice primed with the conjugate. Pre-immunization with rCTB did not suppress specific serum IgG response induced by GBS CPS III-rCTB conjugate intranasally, but did inhibit IgA responses. Moreover, a strong inhibitory effect on anti-CPS IgA response in lungs was observed. I.n. immunization with GBS CPS III-rCTB conjugate could not overcome the inhibition caused by pre-immunization with rCTB intraperitoneally and the levels of pre-existing anti-rCTB antibody correlated with the suppression in the anti-CPS specific response. These findings suggest that priming with CPS affects the distribution of IgG subclasses to GBS CPS and that preexisting rCTB immunity has an inhibitory effect on the mucosal immune responses elicit..

