

ST 01

Antimicrobial agents resistance of *Streptococcus pneumoniae* at the far East of Russia: results of investigation

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Background: Resistance of main respiratory pathogens to antimicrobial agents has recently become the chief problem in treating infections of lower respiratory tract all over the world. The Russian Far East is not an exception. Our study is the first to determine the susceptibility patterns of various classes of antimicrobial agents against *S. pneumoniae* isolates from Vladivostok. We used such antimicrobial agents as Penicillin, Ceftriaxone, Cefotaxime, Ciprofloxacin, Levofloxacin, Vancomycin, Chloramphenicol, Clindamycin, and Erythromycin.

Methods: We had worked with 134 isolates of *S. pneumoniae* from lab of the Vladivostok Navy Hospital. Accordingly to NCCLS standards we used disco diffusion method (DDM) and method of dilution in broth for the determining of minimal inhibitory concentrations (MICs). The lowest concentration of antibiotic giving no visible growth was determined to be the MIC.

Results: Among all the penicillin resistant isolates (23%), 84% showed intermediate resistance and 16% were highly resistant to Penicillin. Of the penicillin resistant isolates, 8.4% were resistant to Erythromycin, 13.8% were resistant to third generation Cephalosporins, and 10% were resistant to Clindamycin. There was an overall resistance of 15% for Fluoroquinolones.

Conclusions: The high level of Erythromycin and Penicillin resistance reported emphasises the global trend of increasing resistance. The most applied agents in our Region are Erythromycin, Ciprofloxacin and Penicillin. Control of the problem of antimicrobial resistance will require more judicious and appropriate use of antimicrobials, the development of new agents with novel targets of action, and strategies for preventing disease from occurring in the first place. In addition, the pursuit of an understanding of resistance mechanisms and pharmacodynamics as they relate to clinical outcome must be an ongoing effort, and that knowledge must be applied to the development of more effective approaches for the treatment of the disease.

ST 02

Molecular epidemiology of erythromycin-resistant *Streptococcus agalactiae* in southern Taiwan

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Background: Erythromycin resistance is common among streptococci, including group A and B streptococci, and *S. pneumoniae* in Taiwan. Several resistance phenotypes and mechanisms mediating erythromycin resistance among these gram-positive cocci have been recognized. It remains unresolved that the widespread erythromycin resistance among clinical isolates of *S. agalactiae* with different resistance phenotypes is related to the dissemination of certain genetically related clones.

Methods: From 1993 to 2002, clinical isolates of *Streptococcus agalactiae* were consecutively collected in Tainan area. Antimicrobial susceptibility was determined by agar dilution method and the possible molecular mechanism mediating erythromycin resistance was studied by polymerase chain reaction to detecting *erm* (B), *erm* (TR) and *mef* (A/E) genes. Pulse field gel electrophoresis (PFGE) was used to investigate the genetic relatedness of erythromycin-resistant isolates.

Results: 189 (24.3%) of 779 clinical isolates of *Streptococcus agalactiae* consecutively collected in Tainan area were resistant to erythromycin. 145 (76.7%) isolates were categorized as MLSB type (macrolide-lincosamide-streptogramin B, resistant to clindamycin and erythromycin) and 44 (23.3%) isolates, M type (macrolide, resistant to erythromycin but susceptible to clindamycin). Of MLSB phenotypes, the majority (136, 93.8%) had *erm* (B) gene and 3.4% (5 isolates) had *erm* (TR) gene, whereas 36 (81.8%) of 44 isolates with M phenotype had *mef* (A/E) gene. The genetic relationship of 177 isolates was studied by pulse field gel electrophoresis (PFGE). Forty-three (24.3%) isolates were PFGE type 1, accounting for 18.1% (25/138) of MLSB phenotype isolates and 46.2% (18/39) of M phenotype isolates. Of isolates with MLSB phenotype, PFGE type 9 accounted for 13.8% (19/138); type 3, 12.3% and type 7, 10.9%. Of those with M phenotype, 23.1% (9/39) were PFGE type 11.

Conclusions: Our results suggest that erythromycin resistance is not uncommon among clinical isolates of *S. agalactiae*, is mainly related to the presence of genes *erm* (B) and *mef* (A/E), and is, at least, partially related to the spread of certain genetic clones.

ST 03

Dynamic analysis of nasopharyngeal *Streptococcus pneumoniae* strains during azithromycin prophylaxis (DIANA project).

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CAP is a common cause of morbidity (230/1000) in Russian military training camps. Prophylaxis with azithromycin is an effective method of outbreak control. But it's commonly accepted that high level of macrolide consumption correlates with macrolide and, sometimes, clindamycin resistance of *S. pneumoniae*.

To assess carriage of macrolide-resistant pneumococci after azithromycin prophylaxis of CAP outbreak in military we conducted this prospective study.

Two prophylaxis schemes with azithromycin vs control (group 3) were evaluated: 500 mg/w for 8 weeks (group 1), and once-through sanitation with 1500 mg (group 2). Nasopharyngeal carriage of *S. pneumoniae* and its susceptibility to erythromycin, azithromycin, miocamycin, clindamycin, penicillin, amoxicillin, cefotaxime, tetracycline and co-trimoxazol evaluated by broth microdilution

trice: before and after treatment: on weeks 9th and 20th. *S. pneumoniae* carriage rate at Visit 0 was 34-43%, on week 9 in groups 1, 2, and 3 -75, 66 and 50% (<.05); on week 20 -69, 57, and 36% in the same groups (<.05). At visit 0 no macrolide resistant strains were detected in all 40 strains tested. But background level of intermediate penicillin resistance estimated in 0-14% of strains (>.05). Dramatic growth of macrolide resistance detected on 9 week in the group 1-95.7% (44 resistant strains. Azithro+Clinda resistance in 37% of them) and group 2-89.5% (n=34. Azithro+Clinda in 11.9% of them). By week 20 resistance rate in group 1 decreased down to 40% (n=16. Azithro+Clinda in 10%) and 22.6% (n=7. Azithro+Clinda in 5.4%) in group 2. As for penicillin, we didn't find unfavorable shift.

As for this preliminary results, prophylactic courses of azithromycin correlates with carriage of macrolide resistant pneumococci, but penicillin-G therapy failures didn't observe.

ST 04

Four-year trend analysis of antimicrobial resistance to *Streptococcus pneumoniae* in the USA: TRUST surveillance program, 1999-2002

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Background: The emergence of resistance to β -lactams, macrolides, and trimethoprim-sulfame-thoxazole among *Streptococcus pneumoniae* (SP) in the past few years has made the empiric treatment of respiratory infections more complicated. The objective of this study was to analyze the changing levels of pneumococcal resistance over the past four respiratory seasons (1999-2002) in the U.S.A. by comparing the in vitro activity of levofloxacin (LVX) with the activities of penicillin (PEN), azithromycin (AZI), trimethoprim-sulfamethoxazole (TMP-SMX), and ceftriaxone (CRO). The findings are from the surveillance study, TRUST (Tracking Resistance in the U.S. Today) conducted by Focus Technologies laboratory.

Methods: In total, 27,828 SP were collected from up to 240 U.S. hospitals from 50 states over the four consecutive respiratory seasons, 1999-2002 (n= 4296, 9499, 6362, 7671 respectively). All isolates were centrally tested by NCCLS broth microdilution with MICs interpreted using NCCLS breakpoints. Annual resistance (R) rates, correlative patterns between agents, and MIC distribution analyses were performed.

Results: Over the four-year period, 1999-2002, PEN resistance (MIC ≥ 2 μ g/mL) among SP increased by 3.7% to 18.4% and AZI-R increased by 4.8% to 27.5%, while TMP-SMX remained elevated at 26.0%. During the same period CRO-R (nonmeningitis breakpoints) increased by 0.5% to 1.7% and LVX-R increased by 0.3% to 0.9%. Overall PEN-R correlated with co-resistance to TMP-SMX (87.3%), AZI (76.3%), and CRO (9.1%) and did not statistically correlate with LVX (1.3%). In the 2002 respiratory season, the multi-drug R phenotype, PEN-AZI-TMP/SMX was present in 14.5% of isolates, and only 0.2% were concurrently resistant to PEN and LVX. From 1999 to 2002 the MIC₉₀ for AZI increased from 8 to 16 μ g/mL, while the MIC₉₀ for LVX remained unchanged at 1 μ g/mL among SP.

Conclusions: Over four consecutive respiratory seasons (1999-2002) in the U.S.A., PEN and AZI resistance among SP increased to 18.4 and 27.5% respectively, while LVX-R remained rare (<1%). No evidence of levofloxacin MIC "drift" has occurred: the LVX MIC₉₀ value of 1 μ g/mL has not changed while AZI MIC₉₀ has increased from 8 to 16 μ g/mL over the four-year period.

ST 05

Cumulative clinical trial experience with levofloxacin in community-acquired pneumococcal pneumonia due to macrolide-resistant strains

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Background: *Streptococcus pneumoniae* (SP) is an important cause of community-acquired pneumonia in adults and children. The prevalence of antibiotic-resistant pneumococci has increased dramatically in many parts of the world, including the United States. Results from a recent US surveillance study of antimicrobial resistance in respiratory tract pathogens demonstrated resistance rates of 18.4% to penicillin, 27.5% to macrolides, and 0.9% to levofloxacin. Empiric treatment of pneumococcal infections is complicated by the fact that penicillin resistance is frequently associated with multidrug resistance, including resistance to macrolides: susceptibility of SP to levofloxacin is independent of penicillin resistance. Levofloxacin has demonstrated excellent clinical and microbiological efficacy in the treatment of CAP due to SP, including penicillin- and macrolide-resistant strains. Current levofloxacin US labeling includes CAP due to penicillin-resistant SP strains (MIC value for penicillin (≥ 2 mcg/mL)).

Methods: Retrospective review of CAP trials conducted by Johnson & Johnson Pharmaceutical Research & Development/Ortho-McNeil Pharmaceutical to evaluate the clinical and microbiological efficacy of levofloxacin in patients with CAP due to macrolide-resistant (erythromycin MIC value (≥ 1 mcg/mL) SP. All patients were treated with levofloxacin 500mg QD, IV or PO for 7-14 days or levofloxacin 750mg QD, IV or PO for 5 days.

Results: Of the XXX levofloxacin-treated patients enrolled in these trials who had pneumococcal pneumonia, a total of 38 cases were due to erythromycin-resistant strains; 36 cases were microbiologically evaluable. Clinical and microbiological response rates were identical: 35 (97.2%) patients were deemed clinical successes and 35 (97.2%) of the macrolide-resistant SP were eradicated.

Conclusions: These results confirm the effectiveness of levofloxacin 500mg QD for 7-14 days and levofloxacin 750 mg QD for 5 days in the treatment of CAP due to macrolide-resistant strains of SP.

ST 06

Activity of levofloxacin and other orally available antibiotics against bacterial isolates from CAP from four Asian countries: A current perspective from the GLOBAL surveillance 2001-2002

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Background: *S. pneumoniae* (SP), *H. influenzae* (HI), and *M. catarrhalis* (MC) are common pathogens in community-acquired respiratory infections (CAP) and are treated empirically with orally available antibiotics. Previous reports from Asian countries have shown increases in multidrug-resistant (MDR) SP and ampicillin (AMP)-resistant (R) HI. Global surveillance initiatives provide current perspectives on antimicrobial resistance and identify new trends in resistance, especially for new agents such as levofloxacin (LEV).

Methods: During 2001-2002, SP, HI, and MC were collected from patient specimens at hospital laboratories in China, Hong Kong (HK), South Korea (SK), and Thailand and submitted for susceptibility testing. Isolates were tested against penicillin (PEN; SP), AMP (HI, MC), cefuroxime (CFX), azithromycin (AZI), clarithromycin, erythromycin, trimethoprim-sulfamethoxazole (SXT), and LEV.

Results: PEN R among SP was 10.0% in China, 46.4% in Thailand, 60.4% in SK, and 50.0% in HK. CFX R among SP was 10.6% in China, 54.2% in Thailand, 67.0% in SK, and 50.0% in HK. AZI and SXT R were >50% in all countries. LEV R was 1.1% in China, 1.2% in Thailand, 2.1% in SK, and 8.5% in HK. The prevalence of MDR SP ranged from 3.9% in China to 50.9% in SK with >90% of all MDR phenotypes R to PEN, AZI, and SXT. LEV R was rarely associated with MDR. Among HI, beta-lactamase (BL) production (range 12.9 to 58.5%) and SXT R (range 17.1 to 56.7%) showed no discernible relationship. No LEV R HI were collected. BL production among MC ranged from 91.7% in China to 100% in HK and Thailand.

Conclusions: High levels of resistance to common therapies such as PEN, AZI, and SXT were detected in SP. In contrast, most isolates remained susceptible to LEV. In urban environments such as HK, LEV R SP were clonal, suggesting improved infection control may reduce emergence of resistance. Continued surveillance is needed to track changes in resistance and continued clonal spread of SP within Asian countries.

ST 07

Persistence of erythromycin resistance of group A streptococci in Korea from 1998 through 2002

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Background: We have been monitoring group A streptococci obtained from children with pharyngotonsillitis and invasive diseases with T antisera since late 1990s. We had found sudden increased T12 and T4, which mostly resistant to erythromycin, in 1998. We would like to know the trends of incidence of erythromycin resistant group A streptococci before and after introducing of the separation of dispensary from medical practice in mid 1999.

Methods: Five hundred thirty two isolates from children with Group A streptococcal infections from 1998 through 2002 were typed serologically and 330 among 532 isolates which were selected randomly were determined antibiotic susceptibilities by tube dilution methods.

Results: The prevalent T serotypes were 12 (36.1%), 4 (12.8%), 1 (10.9%), 2/28 (8.8%), and nontypable (7.1%). Resistance rates of isolates to erythromycin was 46.2% in 1998, 18.4% in 1999, 15.4% in 2000, 27.1% in 2001, and 36.5% in 2002. Relatively lower rate of erythromycin resistance in 2000 seemed to be caused by sudden increase of T1. The number of isolates of T12 and T4 tends to be decreased in more than 15 years old group compared to less than 6 years old age group. All isolates tested were susceptible to penicillin, vancomycin and ceftriaxone. The rate of tetracycline resistance was similar to that of erythromycin, but clindamycin resistance rate decreased gradually from 41.0% to 13.5%. The nationwide consumption of erythromycin decreased gradually from \$ 11 million in 1988 to \$ 4.3 million in 2002, but the consumption of macrolides and their similar antibiotics increased gradually from \$ 24.3 million in 1998 to \$ 38.8 million in 2002.

Conclusions: There are somewhat decreased resistant strains after initiating separation of dispensary, and increased again according to the increased consumption of macrolides. We have to examine the mechanisms of resistant group A streptococci and their epidemiologic characteristics related to the increased consumption of antibiotics.

ST 08

Susceptibility and mechanisms of erythromycin resistance in clinical isolates of group B streptococcus

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Background: Group B streptococcus (GBS) is one of the pathogens most frequently responsible for peripartum maternal and neonatal infections. However, resistance to erythromycin in GBS strains has emerged and become a severe problem in recent years. In GBS, resistance to macrolides is conferred either by methylases encoded by *erm* genes, that modify the ribosomal target of macrolides or by pumps encoded by *mef* (A) genes that efflux these antibiotics.

Objectives: The aim of this study was to determine the susceptibility to erythromycin of GBS and to characterize the mechanisms of macrolide resistance in erythromycin-resistant strains.

Materials and Methods: A total of 185 clinical strains isolated from vaginal or cervical swabs from pregnant women in Beijing and Guangzhou Obstetrics and Gynecology Hospital from 1994 to 1999, of which, 6 invasive strains of GBS were isolated from infectious babies in Beijing Children's Hospital. The E-test method was used to determine the erythromycin and clindamycin resistance of GBS strains. All erythromycin resistant isolates were screened for erythromycin resistant genes. The *erm* B and *mef* A genes were detected by PCR amplification.

Results: Overall, 45 (24%) of 185 individuals carried GBS resistant to erythromycin, whereas 39 (21%) of 185 carried GBS resistant to clindamycin. All clindamycin resistant isolates were also resistant to erythromycin. There were no erythromycin resistant GBS strains which was from 1994 to 1997, whereas the rate of GBS erythromycin resistant varied from 35% in 1998 to 46% in 1999. The *erm* B and *mef* A genes were harbored by 44% (20/45), 29% (13/45) of these strains, respectively. Twelve (12) isolates did not harbor *erm* B or *mef* A genes.

Conclusions: The level of erythromycin and clindamycin resistance in China GBS isolates is of concern. Among the erythromycin-resistant strains, *erm* B genes were widely distributed and were prevalent; the *mef* A gene is far less frequent in China GBS isolates; The strains without *erm* B or *mef* A might possess other macrolide resistance mechanisms such as mutation in 23S rRNA or one of the ribosomal proteins.

ST 09

Serotype distribution of penicillin-nonsusceptible *Streptococcus pneumoniae* from invasive diseases and nasopharyngeal carriage among children in Taiwan-implication for vaccine strategy

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Penicillin-nonsusceptible *Streptococcus pneumoniae* is an emerging problem worldwide. We have reported an extremely high prevalence (70%) of penicillin-nonsusceptible *Streptococcus pneumoniae* among children in Kaohsiung, Taiwan. Furthermore, 98% of the 594 isolates were nonsusceptible to more than 1 drug tested. Immunization with an effective pneumococcal vaccine is the optimal approach to resolve the issue of increasing resistance. The knowledge of distribution of serotypes among pneumococci within certain geographic area is essential for the preparation of vaccine. Moreover, it is inconclusive whether the serotype distribution among nasopharyngeal (NP) carriage isolates represent the distribution of clinical invasive disease. Thus we conduct this study to elucidate the relationship between serotype and penicillin-nonsusceptibility among both nasopharyngeal carriage and clinical isolates. The serotypes of clinical and NP specimens were tested by Quellung reaction using pool serum and factor serum from Copenhagen Serum Institute. Altogether 589 nasopharyngeal isolates and 70 clinical isolates (isolated from blood, CSF, pleural fluid and ear discharge) were tested. Among the 408 nonsusceptible NP isolates, the predominant serotypes were 23F (31.6%), 19F (29.4%), 6B (22.5%), 14 (6.6%) and 6A (5.4%). Serotype 23F was also the leading serotype among multiple-resistant isolates. Serotype 14 was the predominant isolates among clinical isolates. The clinical isolates were further divided to invasive and noninvasive isolates; serotype 14 was the leading cause of invasive isolates with serotype 19F the leading cause among non-invasive isolates. Although the rank of order differed between NP and clinical isolates, the overall serotype distribution was similar among these 2 groups of isolates; serotype 23F, 19F, 14, 6B and 6A accounted for 80% of both clinical and nasopharyngeal isolates. Surveillance of NP carriage may provide a useful predictor of the pneumococcal serotypes causing clinical diseases and facilitate the adequate formulation of protein-conjugate vaccine in different geographic area.

ST 10

Comparison of *emm* genotyping and erythromycin resistance of group A Streptococci isolated from the elementary school children and acute pharyngitis in Jinju, Korea

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Backgrounds: Group A Streptococci (GAS), the most common cause of bacterial pharyngitis, has become more resistant to erythromycin (EM) or clindamycin (CC), as these antibiotics are widely used. The degree of resistance and its mechanism is not well known for the isolates in Korea.

Methods: Throat cultures were taken from 581 healthy elementary school children and from 246 children of acute pharyngitis at a local clinic in 2002. T typing and *emm* genotyping was performed. Antibiotic sensitivity with disk diffusion, phenotypic distribution and resistance genes of EM resistance were investigated for the isolates.

Results: The isolation rate of GAS was 17% in the carriers and 50% in the acute pharyngitis. Although T12 is most common, 27.6% in the carriers and 37.4% in acute pharyngitis. *Emm*12 (33.7%), *emm*18 (9.2%), *emm*22 (8.2%) and *emm*1 (7.1%) were common in the carriers, while *emm*12 (28%), *emm*75 (18.4%), *emm*22 (12.8%) and *emm* 2 (12%) were frequent in the pharyngitis. Resistance to EM and CC were 50% and 34% in the carriers, compared to 46% and 20% in the acute pharyngitis respectively. Constitutive resistance (CR) and M phenotype were 65% and 33% in the carriers, compared to 42% and 58% in the pharyngitis. Inducible resistance phenotype was rarely seen as 2% in the carriers.

Conclusions: GAS is very common in school children and in acute pharyngitis. *Emm*12 is most common and it has high resistance to EM and CC. The antimicrobial resistance to GAS has become a significant problem in our community, which is higher in the carriers than in the pharyngitis. Precise diagnosis and adequate treatment for acute bacterial pharyngitis is needed.

ST 12

Antibiotic susceptibility of *Streptococcus uberis* from bovine mastitis milk in Korea

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Background: *Streptococcus uberis* (*Str. uberis*) are now the most common cause of environmental mastitis. Environmental mastitis incidences caused by *Str. uberis* have been continuously increased in low somatic cell count (SCC) herds. The purpose of this study was to investigate the antibiotic susceptibility and resistance of environmental *Str. uberis* isolated from bovine mastitis milk.

Methods: Total 16 of *Str. uberis* were isolated and identified by VITEK system (bioMerieux, U.S.A.) from 163 milk samples with SCC above 500,000 cells/mL in 58 farms on a national scale from 1999 to 2002. Antimicrobial susceptibility tests for each isolates were carried out by disc diffusion method and MIC.

Results: The prevalence of *Str. uberis* was 28.6% as 16 strains among 49 isolates of *Streptococcus* spp.. All of isolates showed high susceptibility against ampicillin (93.75%), cephalothin (81.25%), penicillin (81.25%), oxacillin (56.25%), and chloramphenicol (50%). But, they showed low susceptibility to gentamicin (25%), streptomycin (25%), kanamycin (18.75%), neomycin (18.75%), erythromycin (12.5%), tetracycline (12.5%), and amikacin (6.25%), respectively. Also, all of them showed susceptibility against one or more antimicrobial agents.

Conclusions: Almost *Str. uberis* from bovine mastitis milk practically showed low susceptibility against antimicrobial agents. But, there was no visible changes of resistance or susceptibility against antimicrobial agents among isolates from 1999 to 2002. Therefore, continuous monitoring for *Str. uberis* will be necessary to prevent and control clinical or sub-clinical mastitis.

ST 14

Surveillance of antimicrobial susceptibility of *S. pneumoniae* and *H. influenzae* in children among three hospitals of P. R. China from 2000- 2002

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Background: *S. pneumoniae* (Sp) and *H. influenzae* (Hi) remains the leading cause of community-acquired pneumonia, otitis media, and meningitis in children. In this study, we investigate the nasal carriage of antibiotic-resistant *S. pneumoniae* and *H. influenzae* in Chinese children under 5 years old during 2000-2002.

Methods: A total of 947 Sp strains and 940 Hi strains were isolated and tested from patients who visited Children's hospitals in Beijing, Shanghai and Guangzhou with respiratory infections. Minimal inhibition concentrations (MICs) of antibiotics were determined by E-test. Disk diffusion test was used for antimicrobial susceptibility.

Results: The average rates of nasopharyngeal carriage for Sp and Hi in those hospitals were 26.7% and 24.5%, respectively. For *S. pneumoniae* isolates, prevalence of penicillin non-susceptibility in this study was 41.6%. The majority of penicillin non-susceptibility isolates (37.3%) were low level resistance (MIC \geq 0.64 μ g/ml and \leq 1.5 μ g/ml). The penicillin resistant Sp (PRSP) isolates accounted for 4.3%. The most sensitive antimicrobials in terms of percentage of susceptible organisms were amoxicillin-clavulanic acid (97%), and to less extent, ceftriaxone (92%); cefurxime and cefactor were slightly stronger sensitive than penicillin with susceptibility of 80% and 75%. Erythromycin, tetracycline and TMP-SMZ were highly resistant (82.6%, 82.3% and 77% respectively) to Sp. Among erythromycin resistant isolates, 100% were resistant to azithromycin, 98.6% to clarithromycin, 97.2% to roxithromycin and Spiramycin, and 96.6% to clindamycin. 97.2% were shown typical of the macrolides-lincosamides-streptogramons B (MLS_B) resistance phenotype, 2.8% were M phenotype. The group of penicillin non-susceptibility (92.9%) was with significantly higher rates of multi-drug resistance than that of PEN-S group (59.2%). For *H. influenzae* isolates, the resistant rates to ampicillin and chloramphenicol were 10.5% and 11%, respectively. Amoxicillin-clavulanic acid, ceftriaxone, cefurxime and cefactor were active against all Hi isolates. 53% of isolates were resistant to TMP-SMZ.

Conclusions: The resistant Sp and Hi isolates from Chinese children with respiratory tract infection would be a severe problem. Data in this study documented the high rate of penicillin or multi-drug resistance among isolates of pneumococci and the high prevalence of multi-drug resistance in Chinese population may be becoming more serious in this century.

ST 15

Vancomycin-resistant *Streptococcus equines* isolated from food animals

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Background: Glycopeptide antibiotics such as avoparcin was used as growth stimulating agents in animal husbandry. As a result, vancomycin-resistant gram-positive bacteria were found in food animals. We have investigated the rate of vancomycin resistance in food animals.

Methods: Streptococci were isolated directly from streptococcal selective agar plates supplemented with 2 mg of vancomycin per liter. The isolated strains were identified by Vitek system. MIC of several antibiotics was determined and genotyping by PCR analysis were investigated. And the cell wall thicknesses were determined by TEM. PCR-RAPD was done to verify some genetic relatedness between resistant strains.

Results: 7 of vancomycin-resistant *Streptococcus equines*, 2 of vancomycin-resistance *Streptococcus viridians* and 3 of vancomycin-resistant *Enterococcus faecium* were isolated. Among this strains, all of *S. equines* showed high level resistance to vancomycin and teicoplanin (100 mg /ml <). But we could not find out any *van* genes in these strains and could not find out any difference of cell wall thickness between type strains and resistant strains. The PCR-RAPD results showed some common band between type strain and resistant strains.

Conclusions: From food animal, we have isolated vancomycin-resistant *S. equines*. These resistant strains might have new resistant determinant compared with existing mechanisms of resistance.

ST 16

In vitro* development of resistant mutants to DW-286a, a new fluoroquinolone antibiotic, in *Streptococcus pneumoniae

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Background: Topoisomerase IV mutations in *S. pneumoniae* are important for resistance to ciprofloxacin and levofloxacin, which initially target ParC. On the other hand, DNA gyrase and topoisomerase IV are dual targets for clinafloxacin in *S. pneumoniae*. This study was performed to analyze the ability of DW-286a to cause resistance development in *S. pneumoniae* and to establish whether DNA gyrase or topoisomerase IV is primary target.

Methods: We examined the roles of DNA gyrase and topoisomerase IV in determining the susceptibility of *S. pneumoniae* C9211 to DW-286a. DW-286a resistant mutants of *S. pneumoniae* C9211 were generated by stepwise selection at increasing drug concentration. Sequence analysis of PCR products from the mutant strains was used to examine the quinolone resistance-determining regions (QRDR) of *GyrA* and *GyrB* proteins of DNA gyrase and the analogous regions of *ParC* and *ParE* subunits of the DNA topoisomerase IV.

Results: First-step mutants exhibiting low-level resistance had an alteration in *GyrA* at Ser-83, with Ser-83 to Tyr or Phe being observed. Second-step mutants had mutations in *GyrA* at Ser-83 to Tyr and in *ParC* at Ser-79 to Tyr at the same time. Third-step mutants displaying more high-level resistance were found to have additional change in *GyrA* at Glu-87 to Lys. Moreover, fourth-step mutants had additional mutations in *ParC* at Asp-83 to Asn, together with other mutations. No changes in *GyrB*, and *ParE* were observed in these mutants. There were no increases in their susceptibility to DW-286a in the presence of reserpine. This phenotype indicated the absence of efflux mechanism of resistance in these mutant strains.

Conclusions: Complementary genetic and biochemical studies revealed that *GyrA* and *ParC* are dual targets for DW-286a in *S. pneumoniae* and resistance to DW-286a in *S. pneumoniae* occurs *in vitro* at a low frequency.

