ANTIMICROBIAL SUSCEPTIBILITY OF *STREPTOCOCCUS PNEUMONIAE* ISOLATED FROM PATIENTS WITH RESPIRATORY TRACT INFECTIONS IN THAILAND

Somporn Srifuengfung[1], Chanwit Tribuddharat[1], Preecha Champreeda[2], Justin Daniels[3], Kulkanya Chokephaibulkit[4], Nithita Wongwan[1] and Pitimon Polwichai[1]

[1]Department of Microbiology, [4]Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; [2]GlaxoSmithKline Thailand; [3]GlaxoSmithKline Research and Development, United Kingdom

Abstract. A total of 400 clinical *Streptococcus pneumoniae* strains from patients with respiratory diseases were collected from January 2002 to December 2005. In this study, an increased prevalence of penicillin-nonsusceptible *S. pneumoniae* (PNSP) from 63% in 2002-2003 to 69% in 2004-2005 was found. During 2004-2005, 56% were erythromycin-nonsusceptible *S. pneumoniae* (ENSP) and 54% were both PNSP and ENSP. The PNSP, ENSP and PNSP+ENSP groups showed similar trends, *ie*, sensitive to amoxicillin/clavulanate (range 97.2-98.5%), levofloxacin (range 90.7-92.4%), ceftriaxone (range 87.1-89.4%), and ofloxacin (range 64.8-66.1%). Lower levels of susceptibility were detected for azithromycin, clarithromycin, cefdinir, cefprozil, clindamycin, co-trimoxazole, chloramphenicol and tetracycline in penicillin and erythromycin-nonsusceptible strains. Of the macrolide-resistant *S. pneumoniae,* 55% of strains exhibited the M phenotype and 45% the constitutive MLS_B phenotype. No pneumococci with the inducible MLS_B phenotype were detected in Thailand.

INTRODUCTION

A steady increase in drug-resistant pneumococcal infections has been reported in many parts of the world. Within the last decade, *Streptococcus pneumoniae*, a common cause of respiratory tract infections, has exhibited a striking increase in resistance to agents that traditionally cleared this infection easily (Felmingham *et al*, 2000; Reinert *et al*, 2005). The emergence of multiple drug resistance in *S. pneumoniae* has complicated the empirical treatment of respiratory tract infections. Penicillin resistance has become widespread and is a worldwide occurrence. Increasingly, penicillin-resistant organisms are also resistant to other drugs *ie*, macrolides, and a growing number of clinical failures following the use of these agents has been reported in different parts of the world (Kelley *et al*, 2000). It is, therefore, important to monitor changes in drug susceptibilities to provide information for empirical clinical therapy. Beta-lactams inhibit cell-wall synthesis by binding to penicillin-binding proteins (PBPs). Resistance to penicillin and other beta-lactams results from chromosomally mediated mutations in PBPs; the pneumococci do not produce beta-lactamase. Resistance to macrolides results from two main mechanisms: target alteration and active efflux. For the former, expression of a ribosomal methylation encoded by the *ermB* (erythromycin-resistance methylase) gene results in alteration of 16S rRNA subunit target sites. This resistance mechanism is called "MLS_B type" (macrolide-lincosamide-streptogramin B type) which can

Correspondence: Chanwit Tribuddharat, Department of Microbiology, Faculty of Medicine Siriraj Hospital, Bangkok 10700, Thailand.
Tel: 66 (0) 2419-9684 ; Fax: 66 (0) 2411-3106
E-mail: sissf@mahidol.ac.th

be either constitutive MLS_B or inducible MLS_B. The active efflux is encoded by the *mef* (macrolide efflux) gene which is more specific and causes the so called "M type" mechanism of resistance to 14- and 15-membered ring macrolides (Palavecino *et al*, 2002). Large variations in penicillin and erythromycin non-susceptible (intermediate and resistant) strains were observed among countries. Global penicillin non-susceptible *S. pneumoniae* (PNSP) ranged from 3.8% to 73.6% according to a study of 8,882 pneumococcal strains from 26 countries. In European countries, France and Spain have high rates of PNSP (Jacobs *et al*, 2003). Looking only at Asian countries, PNSP rates are very high, ranging from 7.8% to 92% (Song *et al*, 2004). Internationally, erythromycin non-susceptible *S. pneumoniae* (ENSP) rates vary from 6.4% to 91% (Sahm *et al*, 2000; Bruinsma *et al*, 2004). This study provides the surveillance data for Thailand regarding drug susceptibilities of pneumococci isolated from patients with community-acquired respiratory tract infections. We also evaluated the drug susceptibilities in penicillin and erythromycin non-susceptible (intermediate and resistant) strains.

MATERIALS AND METHODS

A total of 400 pneumococcal strains, one per patient, were collected from January 2002 to December 2005 from respiratory tract specimens or blood of patients exhibiting respiratory symptoms from four centers: Siriraj Hospital, Ramathibodi Hospital, Queen Sirikit National Institute of Child Health, and National Institute of Health. Identification of *S. pneumoniae* was assessed by standard microbiological techniques (Ruoff *et al*, 2003). The strains were cultured from sputum (276 strains, 69%); blood (96 strains, 24%); bronchial washings (16 strains, 4%) and bronchoalveolar lavage (12 strains, 3%). Drug susceptibilities were performed on 200 specimens (isolated during 2002-2003) by the disk diffusion method according to standard (CLSI, 2006) recommendations (5 drugs: oxacillin, erythromycin, clindamycin, co-trimoxazole, levofloxacin) and for minimal inhibitory concentrations (MICs) by Etest using the manufacturer's recommendations (9 drugs: penicillin, amoxicillin/clavulanate, cefdinir, cefprozil, cefuroxime, cefotaxime, azithromycin, roxithromycin, and levofloxacin). Drug susceptibility testing of another 200 strains (isolated during 2004-2005) was divided into 2 phases. In Phase 1, all strains were tested by disk diffusion (7 drugs: oxacillin, erythromycin, clindamycin, co-trimoxazole, chloramphenicol, tetracycline, ofloxacin). In Phase 2, penicillin or erythromycin non-susceptible strains (PNSP or ENSP) were evaluated for their MICs against further key therapeutic agents (8 drugs: penicillin, amoxicillin/clavulanate, cefdinir, cefprozil, ceftriaxone, azithromycin, clarithromycin, levofloxacin). Quality control strains *S. pneumoniae* ATCC 49619, *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *Staphylococcus aureus* ATCC 25923 and *S. aureus* ATCC 29213 were used for routine susceptibility testing. The three different macrolide resistance phenotypes were investigated by a previously described method (Palavecino *et al*, 2002) using a 15 μg erythromycin disk placed 15 mm away from a 2 μg clindamycin disk. Blunting of the clindamycin zone of inhibition proximal to the erythromycin disk indicated inducible MLS_B phenotype, and resistance to both erythromycin and clindamycin indicated a constitutive MLS_B phenotype, and susceptibility to clindamycin with no blunting indicated an M phenotype.

RESULTS

In this study, the ratio of male: female patients was 1.86: 1. The age range was 6 months-98 years. The patient age groups were: <2 years (5.5%), 2-<5 years (6.5%),

5-9 years (6.5%), 10-19 years (5.5%), 20-29 years (7.5%), 30-39 years (10%), 40-49 years (8.5%), 50-59 years (17%), and ≥ 60 years (33%); most strains were recovered from adults. The overall results from the disk diffusion method are summarized in Table 1. There were increased prevalences of PNSP and ENSP from 63% and 51% in 2002-2003 to 69% and 56% in 2004-2005, respectively.

For Etest results (Table 2), the MIC_{50} and MIC_{90} ranges and sensitivities for pneumococci to various drugs were compared. This data was from our 2002-2003 study. Our data showed that amoxicillin/clavulanate had excellent (100%) *in vitro* activity, followed by levofloxacin (99%), and cefotaxime (75.5%), but there was poor activity against pneumococci with other drugs tested. The azithromycin and roxithromycin results indicated that the macrolide resistance exceeded penicillin resistance.

Table 3 shows that for 200 pneumococcal strains isolated during 2004-2005, there were 132 (66%) PNSP, 112 (56%) ENSP, and

Table 1
Percentages of drug susceptibilities using the disk diffusion method (2002-2005).

Drugs	Years					
	2002-2003			2004-2005		
	S	I	R	S	I	R
Oxacillin	37	-	63	31	-	69
Erythromycin	49	1.5	49.5	44	0.5	55.5
Clindamycin	76	1	23	68	-	32
Co-trimoxazole	32	9.5	58.5	23.5	3.5	73
Chloramphenicol	NT	NT	NT	70	-	30
Levofloxacin	99	-	1	NT	NT	NT
Ofloxacin	NT	NT	NT	62.5	32	5.5
Tetracycline	NT	NT	NT	21.5	2	76.5

NT=not tested; S=susceptible; I=intermediate; R=resistant.

Table 2
Drug susceptibilities using the Etest method (2002-2003)[a].

Drugs	MIC (μg/ml)			% of strains		
	50%	90%	Range	Sensitive	Intermediate	Resistant
Penicillin	0.5	2	0.008-8	40	29	31
Amoxicillin/clavulanate	0.25	2	0.016-2	100	0	0
Cefdinir	1	8	0.016-16	49	2.5	48.5
Cefprozil	1	8	0.016-16	58.5	22	19.5
Cefuroxime	0.5	4	0.016-16	52.5	9	38.5
Cefotaxime	0.25	2	0.016-16	75.5	21.5	3
Azithromycin	4	>256	0.064->256	50.5	3.5	46
Roxithromycin	8	>256	0.064->256	49	0.5	50.5
Levofloxacin	1	2	0.5->32	99	0	1

[a] All 200 strains were tested.

Table 3
Cross-resistance of penicillin or erythromycin-nonsusceptible *S. pneumoniae* strains to various drugs by Etest (2004-2005)[a].

Drugs	% of strains with the indicated resistance profile[b]								
	PNSP (*n*=132)			ENSP (*n*=112)			PNSP and ENSP (*n*=108)[c]		
	S	I	R	S	I	R	S	I	R
Disk diffusion									
Oxacillin	0	0	100	3.6	0	96.4	0	0	100
Erythromycin	18.2	0.8	81	0	0.9	99.1	0	0	100
Clindamycin	53.8	0	46.2	43.7	0	56.3	43.5	0	56.5
Co-trimoxazole	6.8	0.8	92.4	7.1	0.9	92	7.4	0.9	91.7
Chloramphenicol	59.1	0	40.9	50	0	50	50	0	50
Ofloxacin	65.9	25.8	8.3	66.1	24.1	9.8	64.8	25	10.2
Tetracycline	9.9	1.5	88.6	0	1.8	98.2	0	1.9	98.1
Etest									
Penicillin	0	36.4	63.6	3.6	25	71.4	0	25.9	74.1
Amoxicillin/clavulanate	98.5	1.5	0	97.3	1.8	0.9	97.2	1.9	0.9
Cefdinir	20.5	3	76.5	13.4	0.9	85.7	9.2	1.9	88.9
Cefprozil	31.1	24.2	44.7	24.1	27.7	48.2	21.3	28.7	50
Ceftriaxone	89.4	6.8	3.8	87.5	8	4.5	87.1	8.3	4.6
Azithromycin	18.9	6.1	75	0.9	8	91.1	0.9	7.4	91.7
Clarithromycin	19.7	0	80.3	1.8	0	98.2	1.9	0	98.1
Levofloxacin	92.4	0	7.6	91.1	0	8.9	90.7	0	9.3

[a]Only PNSP/ENSP strains were tested; [b]S=susceptible; I=intermediate; R=resistant; [c]PNSP=penicillin-nonsusceptible *S. pneumoniae*; ENSP=erythromycin-nonsusceptible *S. pneumoniae*.

108 (54%) PNSP that were also nonsusceptible to erythromycin. In our study, the PNSP, ENSP and PNSP+ENSP groups showed similar trends regarding susceptibility to amoxicillin/clavulanate (range 97.2-98.5%), levofloxacin (range 90.7-92.4%), ceftriaxone (range 87.1-89.4%) and ofloxacin (range 64.8-66.1%). Other drugs tested were not effective. In this study, there was an increase in levofloxacin-nonsusceptibility between 2002-2003 and 2004-2005. We found that the prevalence of levofloxacin-nonsusceptible strains among PNSP+ENSP was 9.3%, whereas it was 2.8% for amoxicillin/clavulanate. Although our study did not suggest as high a prevalence of levofloxacin resistance with other drugs, the strains showed a high MIC ≥ 64 μg/ml.

For the macrolide resistant phenotype, 55% of strains exhibited the M phenotype and 45% the constitutive MLS_B phenotype (Fig 1). No pneumococci with the inducible MLS_B phenotype were detected in this study.

DISCUSSION

The results of this study indicate the rates of antimicrobial resistance among pneumococcal strains isolated in Thailand continue to increase. The Asian Network for Surveillance of Resistant Pathogens (ANSORP) reported 53.8% PNSP and 42.3% ENSP for Thai isolates during January 2000 to June 2001 (Song *et al*, 2004). The SENTRY Antimicrobial Sur-

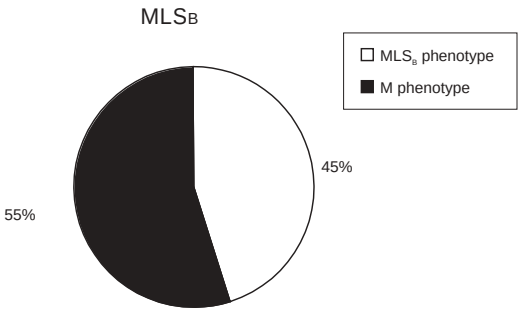

Fig 1–The percentages of macrolide resistance phenotypes.

veillance Program during 1999-2003 reported that PNSP varied from 28.6% in Europe to 33% in North America, and ENSP varied from 13.6% in Latin America to 28.9% in North America (Johnson *et al*, 2006). The PROTEKT (Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin) study during 1999-2003 reported that penicillin non-susceptibility rates were stable over the study period; 21.8% of isolates were penicillin resistant (Schito *et al*, 2005). In this study, the prevalence of resistance of nonsusceptible strains of *S. pneumoniae* to chloramphenicol was high (30%), but less than that of Singapore (40%) (Soh *et al*, 2000), and higher than that in European countries, which ranged from 1.9% to 25.8% (Reinert *et al*, 2005). As shown in Table 2, macrolide resistance in pneumococci *ie*, the resistance to azithromycin and roxithromycin, is a serious problem in many Asian countries, including Thailand (Song *et al*, 2004). The CLSI guidelines state that pneumococci resistant to erythromycin exhibit cross-resistance to other macrolides, such as azithromycin and roxithromycin (CLSI, 2006). Our findings are consistent with these guidelines.

Macrolide resistance is reported to be more prevalent in Asian countries than in the Western world. In this study, the average percentage of ENSP during the 4-year period was 53.5%. This resistance level was less than that of previous reports of 93.7% and 87.8% ENSP in Vietnam and in Taiwan, respectively (Song *et al*, 2004).

The predominant macrolide resistance phenotype was the M phenotype, followed by the constitutive MLS_B phenotype. It is good the inducible MLS_B phenotype was not detected in Thailand although this phenotype is prevalent in some countries, such as Japan (Kimura *et al*, 2003), Spain (Perez-Trallero *et al*, 2001) and Chile (Palavecino *et al*, 2002). The MLS_B antimicrobial agents are a macrolide, lincosamide and streptogramin B type, a group of structurally distinct drugs all of which act on the 50S subunit of 70S ribosomes. According to the literature (Singleton and Sainsbury, 2001), pneumococci that express the M phenotype contain an efflux-based mechanism which confers resistance specifically to macrolides. The mechanism does not affect susceptibility to lincosamide or streptogramin B type. It is important to note that pneumococci which express the inducible MLS_B phenotype have resistance to macrolides, lincosamide and streptogramin B type. Therefore, clindamycin may not be used for treatment of pneumococcal infection with the inducible MLS_B phenotype. The clindamycin disk used for detecting the macrolide resistant phenotype was a 7-chloro-7-deoxy-lincomycin, a semisynthetic derivative of lincomycin (Singleton and Sainsbury, 2001).

In summary, the major clinical implication of this report is that there is definitely an increasing potential for treatment failure with most drugs currently recommended for empirical treatment of community-acquired pneumococcal infection.

ACKNOWLEDGEMENTS

We would like to thank GlaxoSmithKline (Thailand), Vej Dusit Foundation, and the Faculty of Medicine, Siriraj Hospital (graduate research fund) for their kind financial support.

REFERENCES

Bruinsma N, Kristinsson KG, Bronzwaer S, *et al*. Trends of penicillin and erythromycin resistance among invasive *Streptococcus pneumoniae* in Europe. *J Antimicrob Chemother* 2004; 54: 1045-50.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Wayne, Pennsylvania: CLSI, 2006; 26, M100-S16.

Felmingham D, Grunebergand RN, the Alexander project group. The Alexander project 1996-1997: latest susceptibility data from the international study of bacterial pathogens from community-acquired lower respiratory tract infections. *J Antimicrob Chemother* 2000; 45: 191-203.

Jacobs MR, Felmingham D, Appelbaum PC, Gruneberg RN, the Alexander project group. The Alexander project 1998-2000: susceptibility of pathogens isolated from community-acquired respiratory tract infection to commonly used antimicrobial agents. *J Antimicrob Chemother* 2003; 52: 229-46.

Johnson DM, Stilwell MG, Fritsche TR, Jones RN. Emergence of multidrug-resistant *Streptococcus pneumoniae*: report from the SENTRY antimicrobial surveillance program (1999-2003). *Diagn Microbiol Infect Dis* 2006; 56: 69-74.

Kelley MA, Weber DJ, Gilligan P, Cohen MS. Breakthrough pneumococcal bacteremia in patients being treated with azithromycin and clarithromycin. *Clin Infect Dis* 2000: 31: 1008-11.

Kimura T, Horii T, Morita M, Muramatsu H, Kanno T, Maekawa M. Detection of macrolide resistance in *Streptococcus pneumoniae*. *Chemotherapy* 2003; 49: 56-61.

Palavecino EL, Riedel I, Duran C, *et al*. Macrolide resistance phenotypes in *Streptococcus pneumoniae* in Santiago, Chile. *Int J Antimicrob Agents* 2002: 20:108-12.

Perez-Trallero E, Fermandez-Mazarrasa C, Garcia-Rey C, *et al*. Antimicrobial susceptibilities of 1,684 *Streptococcus pneumoniae* and 2,039 *Streptococcus pyogenes* isolates and their ecological relationships: results of a 1-year (1998-1999) multicenter surveillance study in Spain. *Antimicrob Agents Chemother* 2001; 45: 3334-40.

Reinert RR, Reinert S, Linden M, Cil MY, Appelbaum P. Antimicrobial susceptibility of *Streptococcus pneumoniae* in eight European countries from 2001 to 2003. *Antimicrob Agents Chemother* 2005; 49: 2903-13.

Ruoff KL, Whiley RA, Beighton D. *Streptococcus*. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, eds. Manual of clinical microbiology. 8th ed. Washington DC: American Society for Microbiology, 2003: 405-21.

Sahm DF, Jones ME, Hickey ML, Diakun DR, Mani SV, Thornsberry C. Resistance surveillance of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* isolated in Asia and Europe, 1997-1998. *J Antimicrob Chemother* 2000; 45: 457-66.

Schito GC, Felmingham D. Susceptibility of *Streptococcus pneumoniae* to penicillin, azithromycin and telithromycin (PROTEKT 1999-2003). *Int J Antimicrob Agents* 2005: 26: 479-85.

Singleton P, Sainsbury D. Dictionary of microbiology and molecular biology. 3rd ed. New York: John Wiley and Sons, 2001.

Soh SW, Poh CL, Lin RP. Serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* isolated from pediatric patients in Singapore. *Antimicrob Agents Chemother* 2000; 44: 2193-6.

Song JH, Jung SI, Ko KS, *et al*. High prevalence of antimicrobial resistance among clinical *Streptococcus pneumoniae* isolates in Asia (an ANSORP study). *Antimicrob Agents Chemother* 2004; 48: 2101-7.