A Description of Antimicrobial Susceptibility of *Streptococcus pneumoniae*-Siriraj Hospital, Thailand: 2008

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Streptococcus pneumoniae was isolated from 170 patient specimens at Siriraj Hospital during January-December 2008. Patients were 66% male and ranged in age from 3 months to 94 years (mean ± SD = 38.2 ± 31.7). The largest proportion (29.4%) of isolates were from patients older than 60 years, followed by patients aged 2-5 years (20%) and from patients less than 2 years (12.4%). Monthly isolation was highest in December (22 isolates in December compared to the average of 13 isolates of the other months). Antimicrobial susceptibility for eight drugs was determined by the disk diffusion method. Overall, susceptibility was generally high to chloramphenicol (71.8%), linezolid (100%), ofloxacin (93.5%) and vancomycin (100%), but less susceptible to erythromycin (35.3%), penicillin (31.1%), tetracycline (28.8%) and trimethoprim/sulfamethoxazole (24.1%). Among the 105 (62%) isolates resistant to three or more drugs, the most common resistance pattern was erythromycin–penicillin–tetracycline–trimethoprim/sulfamethoxazole, accounting for 39% of such isolates, followed by chloramphenicol–erythromycin–penicillin–tetracycline– trimethoprim/sulfamethoxazole (29.5%). The minimal inhibitory concentrations (MIC) of penicillin and cefotaxime were determined by broth microdilution. By 2008 CLSI criteria, 92% and 90% of 51 sterile site isolates were penicillin and cefotaxime susceptible, including one of two meningitis cases. In contrast, of 26 non-sterile site isolates, only 26.9% and 76.9% were susceptible to penicillin and cefotaxime, respectively. The MICs of penicillin were higher for isolates from non-sterile sites than for those from sterile sites.

Keywords: *Streptococcus pneumoniae, Antimicrobial susceptibility, Drug resistance*

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Streptococcus pneumoniae is a leading cause of pneumonia, meningitis, otitis media and bacteremia, resulting in substantial morbidity and mortality worldwide[1]. In 2005, the World Health Organization estimated that 1.6 million people die of pneumococcal disease every year, including the death of 0.7 to 1 million children aged less than 5 years[2]. A recent report estimated 11-18 million episodes of serious pneumococcal diseases occurred in the year 2000, causing about 826,000 deaths in children aged less than 5 years[3]. The emergence of penicillin- and multidrug-resistant pneumococcal isolates has been a growing concern. Treatment of penicillin-resistant *S. pneumoniae* has become a challenge, and reports of treatment failure, especially with invasive multidrug-resistant *S. pneumoniae* infections, have increased[4,5]. The aim of this study was to describe the prevalence and the antimicrobial susceptibility patterns of *S. pneumoniae* isolates obtained from patients at Siriraj Hospital in 2008.

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Material and Method

S. pneumoniae were isolated from 170 clinical specimens from patients at Siriraj Hospital during January-December 2008. Each isolate represents only one patient. Isolates from the same patient were included only once, giving preference to sterile site isolates. *S. pneumoniae* isolation was performed by the Bacteriology Laboratory, Department of Microbiology, Faculty of Medicine at Siriraj Hospital, Mahidol University. All isolates were identified as *S. pneumoniae* based on colony morphology,

susceptibility to optochin and bile solubility. They were kept at -70°C in 5% brain heart infusion broth plus 20% glycerol (V/V). Antimicrobial susceptibility testing was performed by plating isolates on Mueller Hinton agar (Becton Dickinson Ltd., USA) supplemented with 5% sheep blood and a nephelometer, and then incubated for 18-20 hours at 35°C in 5% CO_2 with antimicrobial disks containing chloramphenicol (30 µg), trimethoprim /sulfamethoxazole (1.25/23.75 µg), erythromycin (15 µg), linezolid (10 µg), ofloxacin (5 µg), oxacillin (1 µg), tetracycline (30 µg) and vancomycin (15 µg). Susceptibility to each antimicrobial agent was determined using the interpretation criteria for disk diffusion as described by the 2008 Clinical and Laboratory Standards Institute (CLSI) guidelines[6]. *S. pneumoniae* ATCC 49619 was used for quality control. We defined multi-drug resistance as non-susceptibility to 3 or more classes of antimicrobial agents.

The minimal inhibitory concentrations (MIC) for penicillin and cefotaxime were determined by the broth microdilution method as described by CLSI[6]. Cation-adjusted Mueller Hinton broth (Becton Dickinson Ltd., USA) supplemented with 2-5% lysed horse blood was used. Both drugs were obtained as a standard powder. *S. pneumoniae* ATCC 49619 was used for quality control. Susceptibility results were interpreted using the breakpoints described in the 2008 CLSI guidelines. The comparison between the new[6] and former[7] breakpoints is shown in Table 1. The breakpoints for meningitis were applied only to isolates from cerebrospinal fluid (CSF). For isolates from other normally sterile sites, the breakpoints for "nonmeningitis treated with parenteral penicillin" were used. For isolates from non-sterile sites, breakpoints for "nonmeningitis treated with oral penicillin" were used.

Results

One hundred and seventy isolates of *S. pneumoniae* were collected from clinical specimens of 170 patients (Table 2). Fifty-five isolates were from sterile sites, 46 (83.7%) of which were from blood. Among the 115 isolates from non-sterile sites, 75 (65.2%) were from true sputum of adults with respiratory tract infections. Overall, 66% of isolates were from males; 29.4% were from patients older than 60 years, while 22% were from patients aged less than 2 years and 14.1% were from patients aged 2-5 years (Fig. 1). The highest number of *S. pneumoniae* isolates were obtained in December (Fig. 2). There were 22 isolates in December compared to the average of 13 isolates of the other months.

Based on disk diffusion results (Table 3), all *S. pneumoniae* isolates were susceptible to linezolid and vancomycin; 93.5% were susceptible to ofloxacin while 71.8% were susceptible to chloramphenicol. Less than 50% of isolates were susceptible to erythromycin (35.3%), penicillin (31.1%), tetracycline (28.8%) and trimethoprim/sulfamethoxazole (24.1%). Drug susceptibility of *S. pneumoniae* by specimen sites was shown in Table 4. There is not much difference between sterile and non-sterile sites.

Of 170 isolates, 105 (61.8%) were multi-drug resistant *S. pneumoniae* (Table 5). The most common

Table 1. Comparison of former and new MIC breakpoints for determining *Streptococcus pneumoniae* susceptibility to penicillin, according to CLSI guidelines

Standard	Susceptibility category MIC (µg/ml)		
	Susceptible	Intermediate	Resistant
Former MIC breakpoints[6] from CLSI 2005 (all clinical syndromes, regardless of penicillin administration method)	≤ 0.06	0.12-1	≥ 2
New MIC breakpoints[7] from CLSI 2008 (by clinical syndrome and penicillin route)			
Meningitis, parenteral penicillin	≤ 0.06	-*	≥ 0.12
Nonmeningitis, parenteral penicillin	≤ 2	4	≥ 8
Nonmeningitis, oral penicillin	≤ 0.06	0.12-1	≥ 2

MIC = minimal inhibitory concentration, CLSI = Clinical and Laboratory Standards Institute
*No intermediate category for meningitis under new penicillin breakpoints

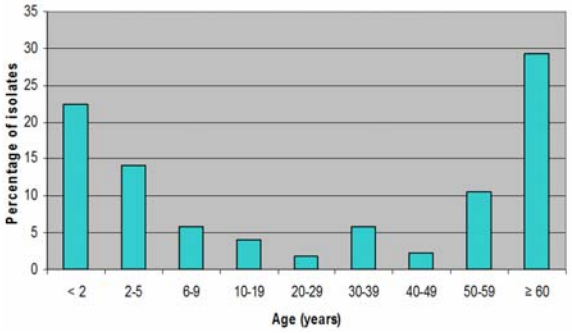

Fig. 1 Distribution of *S. pneumoniae* isolates by age group

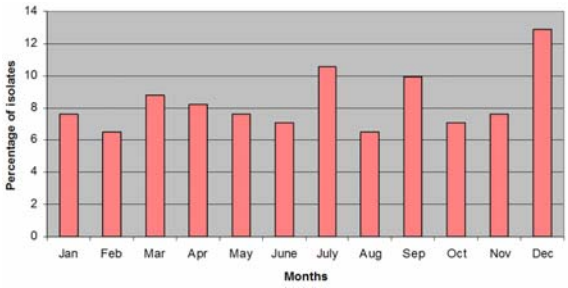

Fig. 2 Distribution of *S. pneumoniae* isolates by month

Table 2. Distribution of *S. pneumoniae* isolates by specimen sites

Clinical specimens	Number of isolates	%
Sterile sites		
Blood	46	86.8
Cerebrospinal fluid	2	3.8
Plural effusion	3	5.6
Others	2	3.8
Total	53	100
Non-sterile sites		
Sputum	75	64.1
Broncho-alveolar lavage	2	1.7
Nose	9	7.7
Adenoid tissue	7	6.0
Ear	7	6.0
Eye	7	6.0
Sinus	4	3.4
Others	6	5.1
Total	117	100

multi-drug resistance pattern was erythromycin-penicillin-tetracycline-trimethoprim/sulfamethoxazole, accounting for 39% of all multi-drug resistant isolates, followed by chloramphenicol-erythromycin-penicillintetracycline-trimethoprim/sulfamethoxazole (29.5%).

Susceptibilities to penicillin and cefotaxime based on MICs are shown in Table 6. Overall, 92.2% of pneumococcal isolates from sterile sites were susceptible to penicillin; one of 2 CSF isolates and 46 (93.9%) of 49 isolates from other sterile sites were penicillin susceptible. Cefotaxime susceptibility was present in 90.2% of sterile site isolates, including both CSF isolates and 89.8% of isolates from other sterile sites. Among the isolates from non-sterile sites, only 26 (22.6%) had MIC determined. The penicillin and cefotaxime susceptibilities were 26.9% and 76.9%. The MIC_{50} and MIC_{90} of penicillin from non-sterile site isolates were higher than that of sterile site isolates.

Discussion

Many clinical studies of pneumococcal disease show a slight male predilection for disease[8]. Although the reasons for this are unclear, our results are consistent with this finding. Children younger than 2 years experience the highest pneumococcal disease burden worldwide. In developed countries, the incidence is highest in those aged 6 months to 1 year. Individuals older than 55-65 years are the second most commonly affected age group worldwide. Immuno compromised persons of any age are at a higher risk for pneumococcal disease[8]. In our study, however, the most common age group from isolates were obtained was > 60 years old. This age distribution of pneumococcal isolation may relate the higher number of elderly compared to pediatric patients seen in our hospital and the fact that sputum, the source of 44% of our isolates, is not routinely collected as part of the evaluation of pediatric patients. However, this study was not necessarily designed to assess differences in disease burden by age, sex, or season, because population denominators are not known and because many of the isolates (non-sterile site) may not have been associated with disease. In addition, we don't get sputum from small children, the number of isolates from adults is certainly going to be higher, regardless of true disease rates. From laboratory culture, as seen in other investigations[8], *S. pneumoniae* was isolated in higher frequency in winter.

Antimicrobial resistance in *S. pneumoniae* began to emerge in some parts of the world during the late 1970s[9]. Since then, it has developed into a major public health problem worldwide and raises concern for treatment failures[10]. In some settings, *S. pneumoniae* has rapidly developed a clinically relevant resistance to essential antimicrobial agents

Table 3. Drug susceptibility of *S. pneumoniae* by disk diffusion method

Drugs	Number (%) of susceptibility		
	Susceptible	Intermediate	Resistant
Chloramphenicol	122 (71.8)	-	48 (28.2)
Erythromycin	60 (35.3)	-	110 (64.7)
Linezolid	170 (100)	-	-
Ofloxacin	159 (93.5)	5 (3.0)	6 (3.5)
Penicillin (using oxacillin disk)	53 (31.1)	-	117 (68.9)
Tetracycline	49 (28.8)	8 (4.7)	113 (66.5)
Trimethoprim/sulfamethoxazole	41 (24.1)	13 (7.7)	116 (68.2)
Vancomycin	170 (100)	-	-

Table 4. Drug susceptibility of *S. pneumoniae* by specimen sites by disk diffusion method

Antimicrobial agents	Percentage of susceptibility[a]					
	Sterile sites (n = 53)			Non-sterile sites (n = 117)		
	S	I	R	S	I	R
Chloramphenicol	73.7	-	27.3	70.4	-	29.6
Erythromycin	36.4	-	63.6	35.7	-	64.3
Linezolid	100	-	-	100	-	-
Ofloxacin	98.2	1.8	-	91.3	3.5	5.2
Penicillin (using oxacillin disk)	29.1	-	70.9	32.2	-	67.8
Tetracycline	32.7	1.8	65.5	26.1	6.1	67.8
SXT[b]	30.9	10.9	58.2	20.9	6.1	73
Vancomycin	100	-	-	100	-	-

[a] S, susceptible; I, intermediate resistant; R, resistant.
[b] Trimethoprim/sulfamethoxazole

(*e.g.*, penicillin, macrolides, third generation cephalosporins, carbapenem drugs and trimetroprim/sulfamethoxazole)[5,11,12]. A 2004 report from the Asian Network for Surveillance of Resistant Pathogens (ANSORP) showed that about half of *S. pneumoniae* isolated from normally sterile body sites were nonsusceptible to penicillin[13]. This report included isolates from Thailand in 2000-2001 which showed a high prevalence (53.8%) of penicillin nonsusceptibility (26.9% fully resistant). In the current study, 68.9% of isolates were nonsusceptible to penicillin based on disk diffusion, and all nonsusceptible isolates were fully resistant (Table 3). This 68.9% was higher than the previous value of 63% reported for isolates in Thailand in 2002-2003, but approximately the same to the value of 69% in 2004-2005[14]. It is important to point out that by using the disk diffusion method, penicillin resistance occurred in isolates from both sterile and non-sterile sites, almost at the same rate (Table 4). The CLSI does not recommend the disk diffusion method, but rather use the MIC method for isolates from sterile sites.

The prevalence of penicillin nonsusceptibility can vary geographically. Compared to results from a European study during 2001-2003 in *S. pneumoniae* from sterile or nonsterile sites in adults $\geq$ 16 to > 70 years, the prevalence of penicillin resistance among *S. pneumoniae* was higher in the current study than it was in Austria (4.4%), Belgium (11.5%), France (47.6%), Germany (6%), Italy (13%), Portugal (19%) and Switzerland (17.3%), whereas it was similar to Spain (61.9%)[15]. Our study found a higher prevalence of erythromycin-resistant *S. pneumoniae* (64.7%) than

what was described in a previous Thailand study, which found 49.5% resistance in 2002-2003 and 55% in 2004-2005[13]. We found that erythromycin-resistant *S. pneumoniae* was also highly resistant to other antimicrobial agents: penicillin (91.8%), trimethoprim/sulfam ethoxazole (83.7%), and tetracycline (89.1%).

Table 5. Multi-drug resistant patterns of *S. pneumoniae* by disk diffusion method

Pattern of drugs	No. of isolates	%
E-P-TET-SXT	41	39
C-E-P-TET-SXT	31	29.5
E-P-TET	12	11.4
C-E-TET-SXT	4	3.8
E-TET-SXT	4	3.8
C-E-OFX-T-TET-SXT	3	2.9
C-E-P-SXT	3	2.9
C-E-OFX-P-SXT	2	1.9
E-P-SXT	2	1.9
C-E-P-TET	1	1
C-OFX-P-SXT	1	1
C-E-TET	1	1
Total	105	100

C, chloramphenicol; E, erythromycin; OFX, ofloxacin; P, penicillin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole

As in other investigations[16], erythromycin-resistant *S. pneumoniae* isolates were often resistant to multiple antimicrobial agents (data not shown). Non-susceptibility of *S. pneumoniae* to other amtimicrobial agents, including tetracycline and trimethoprim/sulfamethoxazole, was also found in this study. Our findings are consistent with previous studies of *S. pneumoniae* isolates in Europe showing that trimethoprim/sulfamethoxazole resistance has become common[17]. Similar to reports from the USA[18] and China[19], our findings demonstrate that ofloxacin resistance is uncommon. However, increasing and inappropriate use of fluoroquinolones may promote emergence of resistance to quinolones and related agents[20].

All *S. pneumoniae* isolates were susceptible to linezolid and vancomycin. Studies suggest that linezolid may be a promising option for the treatment of community-acquired pneumonia due to penicillin-resistant *S. pneumoniae*[21]. Both linezolid and vancomycin are recommend to use only after treatment with other antimicrobial agents has failed[19]. For pneumococcal meningitis, combination therapy with vancomycin and cefotaxime or ceftriaxone is recommended initially until the results of susceptibility testing are available[22].

In this study, resistance to multiple (three or

Table 6. Penicillin and cefotaxime susceptibilities of *S. pneumoniae* isolates from sterile and non-sterile sites by broth microdilution

Drugs	%S[a]	%I[a]	%R[a]	MIC_{50}[b] (µg/ml)	MIC_{90}[b] (µg/ml)	MIC Range (µg/ml)
Sterile sites (n = 51)						
Penicillin						
Overall (n = 51)	92.2	3.9	3.9	0.12	2	0.03-8
CSF (n = 2)	50	-	50	0.06	0.5	0.06-0.5
Other sites (n = 49)	93.9	4.1	2	0.12	2	0.03-8
Cefotaxime						
Overall (n = 51)	90.2	7.8	2	0.25	2	0.03-4
CSF (n = 2)	100	-	-	0.25	0.5	0.25-0.5
Other sites (n = 49)	89.8	8.2	2	0.25	2	0.03-4
Non-sterile sites (n = 26)						
Penicillin	26.9	7.7	65.4	2	4	0.03-16
Cefotaxime	76.9	19.2	3.8	0.5	2	0.03-4

[a] S = susceptible, I = intermediate, R = resistant

[b] MIC_{50} and MIC_{90} are the minimal inhibitory concentration required to inhibit the growth of 50% and 90% of pneumococci, respectively.

The isolates from CSF were determined by meningitis criteria. The isolates from other sterile sites were determined by nonmeningitis with parenteral penicillin criteria. The isolates from nonsterile sites were determined by nonmeningitis with oral penicillin criteria.

more) antimicrobial agents was common among *S. pneumoniae* isolates. In Canada, multiple resistance was observed in 56 of 1,354 invasive pneumococcal isolates (4.1%). The isolates were resistant to beta-lactams (except for four isolates) and to at least two of the following agents: erythromycin, chloramphenicol, trimethoprim/sulfamethoxazole and ofloxacin[23]. These findings highlight the importance of judicious and appropriate antibiotic use as well as the value of effective pneumococcal vaccines, which have been shown to lead to reductions in antimicrobial resistance[24].

Penicillin susceptibility of invasive pneumococcal isolates (*i.e.*, isolated from normally sterile sites) was high based on MICs using new CLSI guidelines. (However, penicillin susceptibility of invasive isolates was very low based on disk diffusion). This is in contrast to our 2005 report[25] which found 88.2% penicillin nonsusceptibility using old CLSI guidelines. The current study was limited by the lack of clinical information to aid the interpretation of laboratory testing. Blood isolates from patients who may have also had meningitis but who did not have CSF isolates, could be wrongly interpreted as susceptible using the non-meningitis criteria. Although this study included only two CSF isolates, findings support current recommendations that penicillin should not be used to treat meningitis and that cefotaxime is still effective, at least based on *in vitro* testing.

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การศึกษาความชุกและความไวของเชื้อ Streptococcus pneumoniae ซึ่งแยกได้จากผู้ป่วยในโรงพยาบาลศิริราชต่อยาต้านจุลชีพในปี พ.ศ. 2551

สมพร ศรีเฟื่องฟุ้ง, กุลกัญญา โชคไพบูลย์กิจ, ชาญวิทย์ ตรีพุทธรัตน์, โสภิตา คำรังษี

การศึกษา เพื่อหาความชุกของเชื้อ Streptococcus pneumoniae ซึ่งแยกได้จากผู้ป่วยในโรงพยาบาลศิริราช ระหว่างเดือนมกราคม-ธันวาคม พ.ศ. 2551 โดยเชื้อแยกได้จากสิ่งส่งตรวจทั้ง sterile และ nonsterile site พบว่ามีเชื้อจำนวน 170 สายพันธุ์ ที่แยกได้จากผู้ป่วยซึ่งมีอายุระหว่าง 3 เดือน ถึง 94 ปี (mean ± SD = 38.2 ± 31.7 ปี) เพศชายมากกว่าเพศหญิงสองเท่า โดยแยกเชื้อได้บ่อยที่สุดจากผู้ป่วยที่มีอายุมากกว่า 60 ปี (29.4%) แยกเชื้อได้รองลงมาคือ ผู้ป่วยที่มีอายุ 2-5 ปี (20%) และที่มีอายุน้อยกว่า 2 ปี (12.4%) เดือนธันวาคมจะสามารถแยกเชื้อได้บ่อยที่สุด (22 ราย) โดยเปรียบเทียบกับค่าเฉลี่ยของเดือนอื่น ๆ (13 ราย) ผลการศึกษาความไวของเชื้อต่อยาต้านจุลชีพ 8 ชนิด ด้วยวิธี disk diffusion พบว่าเชื้อทั้งหมดมีความไวดีต่อยา chloramphenicol (71.8%) , linezolid (100%), ofloxacin (100%) และ vancomycin (100%) แต่ให้ผลมีความไวน้อยต่อยา erythromycin (35.3%), penicillin (31.1%), tetracycline (28.8%) และ trimethoprim/sulfamethoxazole (24.1%) แบบแผนของการดื้อยาต้านจุลชีพพร้อมกันหลายชนิด ที่พบบ่อยที่สุด คือ erythromycin–penicillin–tetracycline–trimethoprim/sulfamethoxazole (39%) และที่พบรองลงมาคือ chloramphenicol–erythromycin–penicillin–tetracycline–trimethoprim/sulfamethoxazole (29.5%) สำหรับผลการศึกษาค่า MIC ของเชื้อต่อยาต้านจุลชีพ 2 ชนิด คือ penicillin และ cefotaxime ด้วยวิธี broth microdilution นั้นพบว่าเชื้อที่แยกได้จาก sterile site จำนวน 51 สายพันธุ์ มีความไวต่อยา penicillin 92% และมีความไวต่อยา cefotaxime 90% ถ้าเป็นกรณีของผู้ป่วยชนิด meningitis ส่วนเชื้อที่แยกได้จาก nonsterile site จำนวน 26 สายพันธุ์มีความไวต่อยา penicillin 26.9% และ cefotaxime 76.9% สำหรับค่า MIC ของเชื้อที่แยกได้จาก nonsterile site ต่อยาต้านจุลชีพทั้ง 2 ชนิดนี้พบว่าสูงกว่าค่า MIC ของเชื้อที่แยกได้จาก sterile site