SEROTYPES AND ANTIMICROBIAL SUSCEPTIBILITIES OF *STREPTOCOCCUS PNEUMONIAE* ISOLATED FROM HOSPITALIZED PATIENTS IN THAILAND

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Abstract. Two hundred seven *Streptococcus pneumoniae* isolates were obtained from patients admitted to Siriraj Hospital. One hundred two, and 105 isolates were from sterile sites and non-sterile sites, respectively. They were serotyped by Quellung reaction with specific antisera from Statens Serum Institut; 81.6% of these pneumococci were typeable. These serotypes were included in the 23-valent pneumococcal polysaccharide vaccine. The five most common serotypes were serotype 6 (22.5%), followed by serotype 23 (18.9%), serotype 19 (16.6%), serotype 3 (7.7%) and serotype 11 (5.3%). Among typeable pneumococci (169 isolates), 52.7% were from sterile sites and 47.3% were from non-sterile sites. Serotypes 6, 23 and 19 were the predominant serotypes isolated from sterile sites. Of the 9 drugs tested, pneumococcal isolates were sensitive to ofloxacin (99%), ciprofloxacin (81.5%), meropenem (80%), imipenem (66.5%), ceftriaxone (65%), cefotaxime (63%), erythromycin (58%), penicillin (48%), and trimethoprim-sulfamethoxazole (34.5%).

INTRODUCTION

Streptococcus pneumoniae is the most important cause of community-acquired pneumonia and acute otitis media, and one of the most important pathogens leading to bacteremia and meningitis in children and adults (Peter and Klein, 2003; Bruinsma *et al*, 2004). Pneumococcus produces significant morbidity, mortality and long term sequelae in small children, the elderly and in high-risk patients with underlying illness (Breiman *et al*, 1994; Ulloa-Gutierrez *et al*, 2003). The high incidence of pneumococcal infections and the widespread increase in drug-resistant isolates, such as penicillin, trimethoprim-sulfamethoxazole, and macrolides, cause a concern in using empirical treatment and the need to perform antibiotic susceptibility testing (Whitney *et al*, 2000; Di Fabio *et al*, 2001; Jette *et al*, 2001; Reinert *et al*, 2001; Verhaegen *et al*, 2002; Farrell *et al*, 2005; Goldstein *et al*, 2005; Johnson *et al*, 2005; McEllistrem *et al*, 2005; Miller *et al*, 2005; Metlay *et al*, 2006).

Currently there are at least 90 different capsular types, also known as serotypes, classified according to polysaccharide structure and immunogenicity. Protection depends on an antibody which is serotype-specific. Despite this diversity, a relatively small number of serotypes (less than 30 types) are associated with the majority of diseases, although there is substantial variation in the rank order of serotypes that cause disease in different geographic regions. The selection of antigens to include in the pneumococcal vaccine was based on the predominant serotypes that cause disease. Some serotypes (1, 5, 6, 9, 14, 18, 19 and 23) appear regularly in nearly every region of the world (Scott *et al,* 1996; Hausdorff *et al*, 2000). The currently available 23-valent vaccine,

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licensed in the United States in 1983, consists of 23 purified capsular polysaccharide of serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F. At present, the Advisory Committee on Immunization Practices in the United States recommends this vaccine for all persons >65 years and for high risk persons age >2 years, such as immunocompetent persons with chronic disease, splenectomy and other immunocompromized patients (ACIP, 1997).

The objectives of this study were to determine the prevalence of serotypes causing disease, to assess the percent coverage of the 23-valent vaccine and the *in vitro* susceptibility to commonly used antibacterial agents against Thai *S. pneumoniae* isolates.

MATERIALS AND METHODS

Pneumococci were isolated from hospitalized patients from June 1997 to June 2000 at the Bacteriology Laboratory, Department of Microbiology, Faculty of Medicine, Siriraj Hospital. Duplicate isolates from a single patient were identified and only one was kept. Pneumococci were identified by standard microbiological techniques (Ruoff *et al*, 2003) and serotyped by Quellung reaction with a Pneumotest kit from Statens Serum Institut, Copenhagen, Denmark. Serotyping was performed for the 23 vaccine serotypes [1, 2, 3, 4, 5, 6, 7, 8, 9 (9N, 9V), 10, 11, 12, 14, 15, 17, 18, 19 (19A, 19F), 20, 22, 23 and 33]. The isolates that were not one of the 23 serotypes were labeled as nontypeable.

Antimicrobial susceptibility tests for 9 drugs (penicillin, cefotaxime, ceftriaxone, ciprofloxacin, erythromycin, imipenem, meropenem, ofloxacin and trimethoprim-sulfamethoxazole) were performed by standard microbroth dilution using cation-adjusted Mueller Hinton broth supplemented with 3% lysed horse blood as described by the Clinical and Laboratory Standards Institute (CLSI, 2005). *S. pneumoniae* ATCC 49619 was used for quality control in each batch tested.

RESULTS

Siriraj Hospital is a tertiary care university hospital, the largest hospital in Thailand. The number of hospital beds and annual number of patients admitted are about 2,600 and 100,000, respectively.

Over the study period, a total of 207 pneumococcal isolates were collected from various clinical specimens (Table 1). One hundred two isolates were cultured from normally sterile sites, including 70 isolates (33.8%) from the blood and 6 from the cerebrospinal fluid (2.9%). One hundred five isolates were cultured from non-sterile sites, mostly from sputum (88 isolates, 42.5%). Based on the Danish serotyping system, the five most common serotypes were 6 (22.5%), 23 (18.9%), 19 (16.6%), 3 (7.7%) and 11 (5.3%) (Table 2). We did not find serotypes 2, 12 and 22 even though these serotypes are included in the 23-valent vaccine.

The serotype distribution by specimen source, *ie* normally sterile sites and non-sterile sites, is shown in Table 3. One hundred sixty-nine isolates (81.6%) were typeable; 38 isolates (18.4%) were nontypeable. Among typeable pneumococci, 89 strains (52.7%) were from normally sterile sites and 80 (47.3%) were from non-sterile sites. Serotypes 6, 23 and 19 were most frequently isolated from normally sterile sites and serotypes 19, 6 and 23 were most frequently isolated from non-sterile sites.

Antimicrobial susceptibilities are shown in Table 4. Forty-eight percent were sensitive to penicillin, 20.5% were intermediately sensitive and 31.5% were highly resistant. Sixty-three percent were sensitive to cefotaxime, 65% to ceftriaxone, 66.5% to imipenem, 80% to meropenem, 81.5% to ciprofloxacin, 99% to ofloxacin, 58% to erythromycin, and 34.5%

Table 1
Distribution of pneumococcal isolates by site.

Site	No. of isolates	Percentage
Normally sterile sites		
Blood	70	33.8
Pus[a]	15	7.2
Bronchial washing	11	5.3
CSF	6	2.9
Non-sterile sites		
Sputum	88	42.5
Throat	8	3.9
Others[b]	9	4.4
Total	207	100

[a]Pus from ankle, brain, elbow joint, hip joint, lung and peritoneum
[b]Others: eye, ear, gastric washing and nasopharyn.

to trimethoprim-sulfamethoxazole.

Antimicrobial susceptibilities and serotypes of pneumococci are shown in Table 5. Serotypes 6, 23 and 19 were more drug-resistant than other serotypes. Only serotype 19 was resistant to ofloxacin.

The patients' ages ranged from 4 months to 96 years. Table 6 shows the distribution of pneumococcal serotypes in relation to age groups. Fifty-nine isolates (34.9%) were in patients ≥60 years, and 49 isolates (29.0%) in patients age 0-19 years. There were fewer isolates from patients age 20-39 years and 40-59 years. The ages of 3 patients (1.8%) were unknown. For patients age 0-19 years, the three most common serotypes in decreasing order were 6, 23 and 19, whereas for patients ≥60 years, the most common serotypes isolated in decreasing order were 6, 19 and 23.

DISCUSSION

Our results confirm that from normally sterile sites, pneumococci were mostly frequently isolated from blood but rarely from cerebrospinal fluid, which is consistent with the results observed in other studies (Siu *et al*, 2002; Bruinsma *et al*, 2004; Oteo *et al*, 2004; Orrett, 2005).

Table 2
Serotypes of pneumococcal isolates.

Order of frequency	Serotype	No. of isolates	Percentage
1	6	38	22.5
2	23	32	18.9
3	19[a]	28	16.6
4	3	13	7.7
5	11	9[b]	5.3
6	18	8	4.7
7	9	6	3.6
8	14	6	3.6
9	10	5	3.0
10	1	4	2.3
11	20	4	2.3
12	8	4	2.3
13	4	3	1.8
14	7	3	1.8
15	5	2	1.2
16	17	2	1.2
17	15	1	0.6
18	33	1	0.6
19	2	-	-
20	12	-	-
21	22	-	-
Total		169	100

[a]19A, 19F
[b]9N, 9V

Pneumococcal serotypes may vary according to geographic location. In general, geographical differences in serotype distribution interfere with the development of one unique vaccine for worldwide use. Previous reports of 10,298 pneumococcal isolates showed that serotypes 3, 1, 14, 7F, 4, 6A, 6B, 8, 23F, 9V and 19F were most common (Nielsen and Henrichsen, 1992). In Latin America, serotypes 1 and 5 were common but these serotypes were rare in the USA and Canada (Scott *et al*, 1996). In some European countries, such as Denmark and Germany, serotype 1 is common (Reinert *et al*, 1995; Nielsen and Henrichsen, 1996). Serotype 4 is

Table 3
Distribution of pneumococcal serotypes by type of site.

Serotypes	No. of pneumococcal isolates		
	Normally sterile sites (%)	Non-sterile sites (%)	Total (%)
6	19 (50)	19 (50)	38
23	17 (53.1)	15 (46.9)	32
19	8 (28.6)	20 (71.4)	28
3	7 (53.8)	6 (46.2)	13
11	5 (55.5)	4 (44.5)	9
18	6 (75)	2 (25)	8
9	2 (33.3)	4 (66.7)	6
14	6 (100)	0	6
10	1 (20)	4 (80)	5
1	4 (100)	0	4
8	1 (25)	3 (75)	4
20	3 (75)	1 (25)	4
4	3 (100)	0	3
7	1 (33.3)	2 (66.7)	3
5	2 (100)	0	2
17	2 (100)	0	2
15	1 (100)	0	1
33	1 (100)	0	1
Typeable	89 (52.7)	80 (47.3)	169 (81.6)
Nontypeable	13 (34.2)	25 (65.8)	38 (18.4)
Total	102 (49.3)	105 (50.7)	207 (100)

Table 4
Antimicrobial susceptibilities of pneumococci isolated from patients.

Antimicrobial	MICs (μg/ml)			Percentage of isolates		
	Range	MIC_{50}	MIC_{90}	S	I	R
Penicillin	≤0.06-4	0.06	2	48.0	20.5	31.5
Cefotaxime	≤0.06-4	0.12	1	63.0	30.0	7.0
Ceftriaxone	≤0.06-4	0.12	1	65.0	29.0	6.0
Imipenem	≤0.06-4	0.06	0.25	66.5	32.0	1.5
Meropenem	≤0.06-4	0.06	0.50	80.0	19.0	1.0
Ciprofloxacin	≤0.12-8	1	2	81.5	-	18.5
Ofloxacin	≤0.12-8	2	2	99.0	1.0	-
Erythromycin	≤0.06-4	0.06	≥4	58.0	0.5	41.5
Trimethoprim-sulfamethoxazole	≤0.06-4	1	≥4	34.5	23.0	42.5

S = sensitive; I = intermediate; R = resistant

more common in North America than in Europe or South America. Serotypes 19 and 23 are less common in Brazil and Uruguay than elsewhere. Serotype 3 is uncommon in many countries (Butler *et al*, 1995b; Hedlund *et al*, 1995).

In our study, the five most common serotypes (6, 23, 19, 3, 11) were different from

Table 5
Antimicrobial susceptibilities and serotypes of pneumococci.

Serotypes	No. of isolates	Percentage of sensitive isolates								
		PGS	CTX	CRO	IPM	MEM	CIP	OFX	E	SXT
6	35	28.6	42.8	48.6	74.3	85.7	91.4	100	22.8	5.7
23	31	13	25.8	25.8	29	61.3	87.5	100	18.8	12.5
19	28	7.1	36.8	28.6	14.3	32	67.8	96.4	14.3	7.1
3	13	84.6	92.3	92.3	84.6	92.3	92.3	100	100	100
11	9	100	100	100	100	100	55.5	100	100	55.5
18	8	100	100	100	100	100	75	100	100	62.5
14	6	16.7	100	100	100	100	100	100	83.3	16.6
9	5	40	60	60	60	100	80	100	100	0
10	5	100	100	100	100	100	100	100	100	100
1	4	100	100	100	100	100	25	100	75	50
20	4	100	100	100	100	100	75	100	100	100
8	3	100	100	100	100	100	50	100	75	25
4	3	100	100	100	100	100	100	100	100	100
7	3	100	100	100	100	100	100	100	100	66.6
Nontypeable	37	59.5	81.1	83.8	78.4	91.9	82.9	100	88.6	51.4

PGS = penicillin; CTX = cefotaxime; CRO = ceftriaxone; IPM = imipenem; MEM = meropenem
CIP = ciprofloxacin; OFX = ofloxacin; E = erythromycin; SXT = trimethoprim-sulfamethoxazole

Table 6
Distribution of pneumococcal serotypes in relation to age groups.

	Total		Age groups (year)				
Serotypes	n	%	0-19 (%)	20-39 (%)	40-59 (%)	≥ 60 (%)	Unknown (%)
6	38	22.5	15 (39.5)	8 (21)	3 (7.9)	12 (31.6)	-
23	32	18.9	14 (43.8)	2 (6.2)	7 (21.9)	9 (28.1)	-
19	28	16.6	8 (28.6)	5 (17.8)	3 (10.7)	11 (39.3)	1 (3.6)
3	13	7.7	1 (7.7)	1 (7.7)	4 (30.8)	7 (53.8)	-
11	9	5.3	3 (33.4)	2 (22.2)	2 (22.2)	2 (22.2)	-
18	8	4.7	1 (12.5)	2 (25)	-	5 (22.2)	-
9	6	3.6	2 (33.3)	-	1 (16.7)	3 (50)	-
14	6	3.6	3 (50)	2 (33.3)	-	1 (16.7)	-
10	5	3.0	-	2 (40)	-	3 (60)	-
1	4	2.3	-	-	1 (25)	3 (75)	-
8	4	2.3	-	-	3 (75)	1 (25)	-
20	4	2.3	-	1 (25)	2 (50)	1 (25)	-
4	3	1.8	2 (66.7)	1 (33.3)	-	-	-
7	3	1.8	-	1 (33.3)	1 (33.3)	-	1 (33.3)
5	2	1.2	-	2 (100)	-	-	-
17	2	1.2	-	-	1 (50)	1 (50)	-
15	1	0.6	-	-	-	-	1 (100)
33	1	0.6	-	1 (100)	-	-	-
Total	169	100	49 (29.0)	30 (17.7)	28 (16.6)	59 (34.9)	3 (1.8)

those of western countries. Taken together, our data and those reported by others support the notion that serotypes in developed countries are different from those found in developing countries (Riley *et al*, 1991). However, a study by the Asian Network for Surveillance of Resistant Pathogens (ANSORP), in 1996-1997, found that serotypes 23F and 19F were more common in Asian countries (Song *et al*, 1999). In Singapore in 1997-1999 the five most common serotypes were 19F, 23F, 6, 14 and 15 (Soh *et al*, 2001). A study in India in 1996-2000, found the most common serotypes were 1, 6 and 23 (Reba, 2001). It has been hypothesized that environment, crowding in particular, favors the transmission of some serotypes (Austrian, 1989).

The absence of serotypes 2, 12 and 22 in our study is surprising because they were included in the 23-valent vaccine. This vaccine covers approximately 85-90% of invasive pneumococcal disease among children and adults in the USA but cannot be used in children <2 years old (Butler *et al*, 1995a).

The Pneumotest kit used in this study detected 81.6% of isolates. Untypeable serotype means a non-vaccine serotype. Eighty-nine of 102 isolates (87.3%) from normally sterile sites were typeable (Table 3), and in decreasing order were serotypes 6, 23, 19, 3, 11, 18, 9, 14, 10, 1, 8, 20, 4, 7, 5, 17, 15 and 33. Thus, the coverage of this vaccine for invasive pneumococcal diseases may be as high as 87.3% for Thai isolates. For non-sterile site, the percentage coverage for this vaccine would be approximately 76.2% (80 out of 105).

Penicillin non-susceptible (intermediate and resistant) *S. pneumoniae* or PNSP has been reported in many countries, including Spain, Hungary, France, and Romania. In contrast, penicillin resistance remains low in some countries, such as the United Kingdom, Denmark, Sweden, Germany and Italy (Baquero *et al,* 1992; Marton *et al,* 1992; Appelbaum, 1995, Hedlund *et al,* 1995; Nielsen and Henrichsen, 1996; Sahm *et al,* 2000).

In Spain, PNSP increased from 5% in the 1980s to 35% in 1996 (Fenoll *et al,* 1998). From 11 Asian countries in 1996-1997, Korea had the highest percentage of PNSP (79.7%), followed by Japan (65.3%) (Song *et al*, 1999).

This study suggests a high prevalence of drug resistance among pneumococcal isolates in Thailand. More than 50% of all isolates were PNSP. The association of penicillin resistance with specific serotypes has been reported (Song *et al*, 1999). We found that most PNSP were serotypes 19, 23 and 6. In Hong Kong, 20% of PNSP were serotype 23F in 1993 and 1994, rising to 62.2% in 1995 (Lyon *et al*, 1996). This may be due to excessive use of ampicillin to treat upper respiratory tract infections (Lee *et al*, 1995). In Europe, serotypes 6, 14, 19 and 23 were consistently resistant to penicillin and were often resistant to other drugs (Reinert *et al*, 1995).

In the United States, 20% are erythromycin-resistant pneumococci (Metlay, 2006) and 21% are cefotaxime-resistant pneumococci (Miller *et al*, 2005). In Belgium, PNSP was found in 21%, and pneumococci resistant to erythromycin in 30%, cefotaxime in 7.3%, imipenem in 3.8%, and ciprofloxacin in 11% (Vanhoof *et al*, 2003). Our data suggest that Thai isolates were more drug resistant than those of the US and Belgium.

Most erythromycin- or fluoroquinolone-resistant pneumococci belonged to only some serotypes (Perez-Trallero *et al*, 2003). We found erythromycin-resistance was commonly found in serotypes 6,23 and 19. Even though fluoroquinolone resistance in pneumococci is still infrequent, our study found resistance to ciprofloxacin is increasing. Interestingly, we found only one ofloxacin resistant isolate, serotype 19. This is different from a report of serotype 6A being resistant to ciprofloxacin and

levofloxacin (Pletz *et al*, 2006).

The increasing incidence of PNSP as well as pneumococci resistant to erythromycin, third generation cephalosporins, carbapenem drugs (imipenem, carbapenem) and trimethoprim-sulfamethoxazole emphasizes the need to implement preventive measures, such as a limited empirical use of drugs. In light of the increasing prevalence of pneumococci resistant to multiple antibiotics, the need for an effective pneumococcal vaccine is urgent.

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