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Epidemiology of *Streptococcus pneumoniae* infection in Malaysia

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SUMMARY

During a 1-year period from October 1995 to September 1996, 273 isolations of *Streptococcus pneumoniae* were made from various types of clinical specimens. The majority of the isolates (39·2%) were from sputum whilst 27·5% were from blood, CSF and other body fluids. The organism was isolated from patients of all age groups, 31·1% from children aged 10 years and below, 64·7% of which come from children aged 2 years or below. The majority of the isolates belong to serotypes 1, 6B, 19B, 19F and 23F. Serotypes 1 and 19B were the most common serotypes associated with invasive infection. About 71·9% of the invasive infections were due to serotypes included in the available 23 valent polysaccharide vaccine. The rates of resistance to penicillin and erythromycin were 7·0 and 1·1% respectively. Our findings show that the serotypes of *S. pneumoniae* causing most invasive infections in Malaysia are similar to those in other parts of the world and the available vaccine may have a useful role in this population.

INTRODUCTION

Infections caused by *Streptococcus pneumoniae* continue to be an important cause of morbidity and mortality, particularly among elderly persons with a variety of chronic diseases and in children < 5 years old [1, 2]. In adults, pneumococci are the most frequent cause of community-acquired pneumonia, a disease that carries a mortality of 5–10% despite modern antimicrobial therapy and intensive care [3]. In children, pneumococci are the most common cause of otitis media [4], sinusitis, bacterial pneumonia and are also a frequent cause of meningitis [2]. The incidence of pneumococcal meningitis has been estimated to be close to 1·5 cases per 100000 individuals per year with an average mortality rate of 30–40%. In the United States about 40000 deaths annually are caused by pneumococcal pneumonia or meningitis [5].

The emergence of penicillin-resistant pneumococci complicates treatment strategies and emphasizes the need for an effective vaccine [6]. The pneumococcal capsular polysaccharide vaccine currently in use is composed of 23 different capsular polysaccharides

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representing approx. 90% of the types of pneumococci isolated from patients with an invasive infection. Epidemiological surveillance of *S. pneumoniae* is important in order to detect any changes in the pattern of drug susceptibility among the isolated strains. Surveillance will also determine the distribution of pneumococcal types in different populations. This is important in order to ensure an optimal formulation of the existing polysaccharide vaccine and to help in determination of the composition of future protein conjugated vaccines.

From October 1994 to September 1995 the Bacteriology Division of the Institute for Medical Research (IMR) conducted epidemiological surveillance of *S. pneumoniae* infections among the Malaysian population in order to determine the distribution of the serotypes that caused various types of infections in hospitalized patients and to study the antibiotic susceptibility pattern of these isolates.

MATERIALS AND METHODS

Pneumococcal strains

Clinical isolates were obtained from 6 hospitals representing the 5 geographical regions of the country. Isolates were from patients with clinical infections, samples studied included blood, sputum, body fluid and pus. The identity of all strains was reconfirmed by IMR; all isolates were optochin sensitive.

Antisera

Serotyping was done using a special set of pneumococcal diagnostic antisera, the Pneumotest Kit from Statens Seruminstitut, Copenhagen, Denmark. The kit contains 12 pooled antisera (pool A–I, and P–T). The pooled antisera are intended for typing and/or grouping of 90–95% of the pneumococci commonly isolated from blood or spinal fluid.

Typing and/or grouping of pneumococci

Typing and/or grouping was done by the capsular reaction test carried out with the nine pooled antisera A–I until a positive reaction was observed. Following instructions given with the kit the typing with pool A–I was followed by testing for a positive reaction with the other serum pool P–T. The type or group was then established from the reaction pattern using the table provided. Further typing of certain serogroups into serotypes was done using factor sera.

Antibiotic susceptibility pattern

The antibiotic susceptibility pattern of isolates was determined by a modified Kirby–Bauer disk diffusion method recommended by the National Committee of Clinical Laboratory Standard (NCCLS) [7] using penicillin, cefuroxime, erythromycin, clindamycin, chloramphenicol, tetracycline and cotrimoxazole disks. Susceptibility to penicillin was tested using 1 μg oxacillin disks. A bacterial suspension was prepared using an overnight culture with a turbidity equivalent to 0·5 McFarland standard. A sterile cotton swab was dipped into the suspension and streaked onto Mueller–Hinton blood agar after removing the excess inoculum by pressing the swab onto the inside wall of the tube. Antibiotic disks were dispensed onto the surface of the agar within 15 min of inoculation. The plate were incubated at 35 °C in 5% CO_2 for 24 h. After incubation the diameter of the inhibition zone was measured and compared to a standard table for interpretation.

RESULTS

During the 1-year period, 153285 clinical specimens were processed in the six study centres. From these samples 273 (0·18%) isolates of *S. pneumoniae* were obtained. The majority of the strains were isolated from sputum 107 (39·2%) whilst 75 (27·5%) were invasive strains recovered from blood, cerebrospinal fluid or other body fluids (Table 1). Twenty-seven were recovered from tracheal or nasopharyngeal aspirates of in-patients who were either critically ill or on a ventilator.

Isolates were obtained from patients of all ages; 85 (31·1%) were isolated from children aged $\leqslant$ 10 years (Table 2), and the 55 (64·7%) of these isolates were obtained from children aged $\leqslant$ 2 years. 102 (37·4%) were recovered from patients aged 10–50 years and 58 (21·2%) from patients aged $>$ 50 years. The ages of another 10·2% of the patients were not known. Invasive strains were obtained from patients of all ages, 46·7% from children $<$ 10 years old; 53·6% of the latter were obtained from children $<$ 2 years old.

Community acquired pneumonia was the main clinical manifestation of pneumococcal infections, followed by sepsis, otitis media, conjunctivitis and other clinical syndromes. Most of the patients aged 50 years or more had preexisting chronic conditions such as current or treated pulmonary tuberculosis, chronic obstructive airway diseases, asthma or lung cancer and some were in coma.

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Table 1. *Samples from which* Streptococcus pneumoniae *was isolated.*

Specimen	No. of isolates	Percentage
Sputum	107	39·3
Blood	58	21·3
Tracheal/nasopharyngeal	27	9·9
Nasal/throat swab	23	8·4
Eye swab	17	6·2
Cerebrospinal fluid	12	4·4
Ear swab	9	3·3
Body fluids*	5	1·8
Others†	15	5·5
Total	273	100·0

* Body fluids: peritoneal fluid, pleural fluid, synovial fluid.
† Others: include pus, HVS, corneal scraping, etc.

Table 2. Streptococcus pneumoniae *isolates by age of subject*

Age group (yr)	No. of isolates (%)	Invasive disease (%)*
0–10	85 (31·1)	28 (46·7)
11–20	20 (7·3)	3 (5·8)
21–30	26 (9·5)	5 (8·3)
31–40	31 (11·4)	5 (8·3)
41–50	25 (9·2)	5 (8·3)
51–60	13 (4·8)	4 (6·7)
61–70	17 (6·2)	2 (3·3)
71–80	21 (7·7)	4 (6·7)
81–90	6 (2·2)	2 (3·3)
91 and above	1 (0·4)	–
Unknown	28 (10·2)	2 (3·3)
Total	273 (100)	60 (100)

* Invasive disease: presented with septicaemia or meningitis and *S. pneumoniae* was isolated from either blood, CSF or other body fluid.

Serotyping was performed on 201 viable strains, among which 42 serotypes were detected, 26 serotypes were seen in children (10 years and less) and 33 serotypes were seen in adults. Table 3 shows the serotypes of *S. pneumoniae* obtained; 62·7% of the 201 strains and 71·9% of the invasive strains belonged to the serotypes that are included in the presently available 23 valent polysaccharide vaccine and an additional 22·9% of the isolates and 6·5% of the invasive strains were vaccine related strains. Serotype 1 was the most common serotype associated with invasive infection (26·3%), and was also the most common serotype isolated. This was followed by serotypes 6B, 19B, 19F and 23F. Other common

Table 3. *Serotypes of* Streptococcus pneumoniae *isolates*

Serotype	Invasive strains*	Non-invasive strains†	Total
1‡	15	16	31
2‡	1	0	1
3‡	0	9	9
4‡	2	1	3
5‡	2	0	2
6A	1	2	3
6B‡	3	16	19
6C	0	1	1
7A	1	0	1
7B	1	1	2
7C	0	2	2
7F‡	0	1	1
8‡	1	0	1
9A	1	0	1
9L	0	1	1
9N‡	1	3	4
10A‡	1	3	4
11A‡	0	3	3
11C	0	3	3
11F	0	1	1
12A	1	1	2
12F‡	3	0	3
14‡	3	6	9
15B*‡	0	2	2
17A	0	2	2
18A	0	2	2
18B	0	1	1
18C‡	2	2	4
18F	1	1	2
19A‡	0	1	1
19B	5	6	11
19C	0	2	2
19F‡	2	9	11
20‡	2	3	5
22A	0	1	1
22F‡	0	3	3
23	0	2	2
23A	0	3	3
23B	2	3	5
23F‡	3	7	10
G	1	7	8
I	1	3	4
Auto agglutinated	1	14	15
Total	57	144	201

* Invasive strains: strains isolated from blood, CSF and body fluids.
† Non-invasive strains: strains isolated from other specimens.
‡ Serotypes included in the 23 valent polysaccharide vaccine.

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Table 4. *Distribution of invasive* Streptococcus pneumoniae *capsular types in relation to age group and types in the 23-valent pneumococcal polysaccharides vaccine*

Type	No. of isolates among age groups							Total no. of isolates
	< 2 yr	2–5 yr	6–10 yr	11–20 yr	21–65 yr	> 65 yr	Unknown	
Vaccine types								
1	2	2	2	2	4	1	2	15
2	1							1
4					1	1		2
5	2							2
6B		1		1	1			3
8	1							1
9N					1			1
10A					1			1
12F		1			1		1	3
14	1		2					3
18C				1	1			2
19F			1		1			2
20	1		1					2
23F		1	1				1	3
Vaccine related types								
6A	1							1
7A					1			1
7B							1	1
9A					1			1
12A	1							1
18F					1			1
19B	1	2					2	5
23B	2							2
Non-vaccine related				1				1
Autoagglutinate			1					1

Table 5. *Susceptibility pattern of* Streptococcus pneumoniae *isolates* (%)

Antibiotic	Susceptible*	Intermediate†	Resistant‡
Azithromycin	98·1	—	1·9
Cefuroxime	99·6	—	0·4
Chloramphenicol	95·1	1·5	3·4
Clindamycin	99·2	0·4	0·4
Cotrimoxazole	86·4	3·9	9·7
Erythromycin	98·4	0·4	1·1
Penicillin	93·0	—	7·0
Tetracycline	78·2	0·8	21·0

* Susceptible: the infection may be appropriately treated with the recommended dosage.
† Intermediate: dosage higher than the recommended one may be effective, also include buffer zone.
‡ Resistant: the drug may not be able to inhibit the organism.

serotypes associated with invasive infections included 3, 12F and 14. Fourteen serotypes were associated with invasive infection in children and 12 serotypes in adults (Table 4). Serotype 1 and serotype 19B were equally distributed among the age groups whilst serotype 19F was obtained more from adult patients (81·8%) and serotypes 6B and 23F were isolated more frequently from paediatric patients < 10 years old, 42·1% and 80·0% respectively.

Table 5 shows the susceptibility pattern of the *S.*

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pneumoniae isolates. Rates of resistance were penicillin (7·0%), erythromycin (1·5%) and cefuroxime (0·4%). Penicillin resistance strains were verified by determination of the minimum inhibitory concentration (MIC) of the drug using E-test (8). Among the resistant strains, two strains showed high level resistance with MIC 4 μg/ml and > 32 μg/ml while the rest showed low level resistance with an MIC 0·125–1·5 mg/ml.

DISCUSSION

In 1995, 50584 significant pathogens were isolated from 15 hospitals in Malaysia (unpublished National Surveillance data) comprising 99 different bacteria/group species. *S. pneumoniae* was listed among the 25 most common isolated organisms, comprising 1·09% of the total isolates. As observed in our study, the majority of pneumococci were isolated from sputum, 7·49% were isolated from patients with an invasive infection.

S. pneumoniae infections occurred in all age group but the peak incidence was seen in young children and the elderly. Based on the number of isolations and the total number of admission (361946 patients) to the six hospitals during the study period, the incidence rate of *S. pneumoniae* infections (invasive and non-invasive), irrespective of age, in these hospitals was 75·4 per 100000 patients.

Sankilampi and colleagues [9] reported the overall incidence of invasive pneumococcal infections in Finland was 9·1 per 100000 adults aged $\geqslant 16$ years. As observed by Macracken and colleagues [10] the commonest serotypes detected were included in the current polysaccharide vaccine. In our study 63·3% of pneumococcal infections in children < 2 years were due to serotypes covered by the currently available vaccine. The serotypes associated with invasive infections were not very different from the serotypes reported by Butler and colleagues [11] and Gratten and colleagues [8]. Serotype 19F was one of the most common serotype in those studies but both 19F and 19B were common in our patients and serotype 19B was more frequently associated with invasive infections. Serotype 19B is not included in the available vaccine.

Penicillin resistance among strains of *S. pneumoniae* (PRP) has emerged as an important worldwide problem [12–14]. We observed an increase of PRP from 0·8% in 1988 [15] to 7·0% in 1994/5, 6% of low level and 1% of high level resistance. These were very much lower when compared to those reported by Fenoll and colleagues [16] where 29% of 521 pneumococci in Spain were of low level resistant and 15·3% were highly resistant. The highest MIC of the resistance strains had increased from 8 μg/ml previously (15) to > 32 μg/ml in this study. The PRP strains belonged to serotypes 1, 3, 6B, 7B, 11C, 14, 19B, 19F, 22A, 22F, 23B and three were of undetermined serotype (autoagglutinated). The serotypes were equally distributed among the invasive and non-invasive strains. The pneumococci were still very susceptible to clindamycin (99·2%) an old drug for Gram positive aerobic organisms.

The fatality rate of pneumococcal infections is still high [3, 17] despite modern intensive care and the availability of penicillin, to which most pneumococci are still fully sensitive. The emergence of strains of *S. pneumoniae* resistant to penicillin and other antibiotics has become a major concern for the future management of this infection. Clinical studies [18, 19] showed that vaccination with 23 valent pneumococcal polysaccharide vaccine was able to provide type specific protection in both healthy adults and children. Hospital based case-control study by Sapiro and colleagues [20, 21] have indicated that the vaccine is efficacious in preventing invasive pneumococcal infections. Vaccination had protected many splenectomized children from pneumococcal infection compared to a 4% attack rate in the period when the vaccine was not available [22].

Our results showed that the majority of the serotypes of *S. pneumoniae* causing most of the cases of invasive pneumococcal disease in Malaysia are similar to other regions of the world, and are included in the 23-valent pneumococcal polysaccharide vaccine.

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