

Increasing Antimicrobial Resistance in Clinical Isolates of *Streptococcus pneumoniae*[†]

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Abstract

The presence of antibiotic-resistant Streptococcus pneumoniae has become a major clinical problem in several parts of the world. However, there is a lack of data from Southeast Asia. We therefore initiated a study to determine the serogroups/serotypes and antimicrobial susceptibilities of clinical strains of S. pneumoniae isolated in our laboratory.

In 1995, we isolated 144 strains of S. pneumoniae. Thirty-six (25.0%) strains were resistant to penicillin of which 19 (13.2%) were highly resistant (minimal inhibitory concentration >1.0 µg/ml). Thirty-eight (26.4%) strains were resistant to erythromycin, 75 (52.1%) were resistant to tetracycline, 48 (33.3%) were resistant to sulfamethoxazole-trimethoprim and 29 (20.1%) were resistant to chloramphenicol. Twenty of the penicillin-resistant strains also had diminished susceptibility to ceftriaxone. Strains resistant to penicillin belonged to serogroups/serotypes 6 (n = 1), 9 (n = 1), 23 (n = 4), 14 (n = 6) and 19 (n = 20). Children under 5 years of age were more likely than older children and adults to have isolates resistant to penicillin (52.9% versus 16.2%, P = 0.00002). Penicillin-resistant strains were more likely than penicillin-susceptible strains to be multidrug-resistant (86.1% versus 7.4%, P < 0.000005). We concluded that there is a high prevalence of penicillin-resistant pneumococci in our isolates especially among very young children. Most penicillin-resistant strains belong to serogroup 19 and are also resistant to multiple drugs.

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Introduction

The resistance of *Streptococcus pneumoniae* to penicillin and other antimicrobial agents is becoming an increasing problem in many parts of the world. Spain,¹ Hungary² and South Africa³ were the first few countries to report very high prevalence rates of resistant strains. Since then, the problem has become more widespread. In Atlanta,⁴ 25% of all invasive isolates of *S. pneumoniae* in 1994 were resistant to penicillin. This compares with the national figure of 0.02% in the United States less than a decade ago.⁵

The resistance of *S. pneumoniae* to penicillin may be defined as intermediate or high depending on the minimal inhibitory concentration of penicillin (the minimal concentration of antibiotic required to inhibit growth of the organism). This distinction is clinically significant because patients with pneumonia and sepsis caused by intermediately resistant strains will usually still respond to high doses of penicillin or other β -lactam antibiotics.⁶

In contrast, infections with highly resistant strains have been associated with therapeutic failure. Treatment may also fail when intermediately resistant strains infect the middle ear and meninges, sites where the penetration by antibiotics is poor.⁶

The determination of prevalent serogroups/serotypes and antimicrobial susceptibilities of local strains of *S. pneumoniae* are essential for developing treatment and prevention strategies. While several such studies have been conducted in Europe and North America, there is little data from Asian countries.⁷

We have observed an increase in the number of penicillin-resistant *S. pneumoniae* being isolated in our laboratory over the past 4 years. The objectives of this study were to determine the prevalence, distribution and degree of penicillin resistance in our clinical isolates of *S. pneumoniae*. We also sought to determine if penicillin resistance was associated with resistance to other antibiotics. Finally, we serotyped our isolates to deter-

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mine if there were any predominant clones of resistant strains. The serogroups/serotypes obtained would also be relevant to the formulation of a pneumococcal vaccine for local use.

Materials and Methods

Bacterial Strains

A total of 154 isolates of *S. pneumoniae* were isolated at the Department of Pathology, Singapore General Hospital, between 1 January 1995 and 31 December 1995. Specimens were from the Singapore General Hospital and Alexandra Hospital. The clinical sources included respiratory tract ($n = 82$), blood ($n = 45$), eye ($n = 8$), nose ($n = 8$), ear ($n = 3$), cerebrospinal fluid ($n = 3$), wound ($n = 2$), pleural fluid ($n = 1$), vitreous ($n = 1$), and genital tract ($n = 1$). After excluding repeated isolates from the same patient, 144 strains were included in our study.

Identification

Pneumococci were identified by colony morphology, Gram stain, optochin sensitivity and bile solubility.⁸ The strains were kept frozen at -70°C in fetal calf serum for further analysis. Serotyping was performed by the capsule swelling method (Quellung reaction) with the Pneumotest kit available from the Statens Serum Institute of Copenhagen, Denmark.⁹ This is a new simplified chessboard system for the typing of *S. pneumoniae* isolates based on 12 pooled antisera. The pooled antisera are composed in such a way that enable all 21 vaccine-related types and groups to be typed. This represents 90% to 95% of the pneumococci commonly isolated from blood or cerebrospinal fluid.

Antimicrobial Susceptibility Testing

All strains were screened for susceptibility to penicillin with a 1 μg oxacillin disk. The E-test (AB Biodisk, Solna, Sweden) was used to determine the minimal inhibitory concentrations of benzylpenicillin, and ceftriaxone for resistant strains. Susceptibility to erythromycin, tetracycline, sulfamethoxazole-trimethoprim, chloramphenicol and vancomycin were tested by the Kirby-Bauer disk diffusion method using BBL SensiDiscs (Becton Dickinson, Cockeysville, USA).¹⁰ For both methods, the microorganisms were inoculated into a sterile saline solution and the turbidity adjusted to match that of a 0.5 McFarland standard. The suspension was then streaked onto Mueller Hinton Agar supplemented with 5% sheep blood (Becton Dickinson, Cockeysville, USA) and incubated at 36°C overnight in 5% carbon dioxide. The zone sizes and minimal inhibitory concentrations were interpreted according to the guidelines of the National Committee for Clinical Laboratory Standards.¹¹

Multidrug resistance was defined as resistance to three or more different classes of antibiotics.

Statistical Analysis

The Chi-square test was performed where indicated using SPSS version 6.1 for Windows. A P value of less than 0.05 was considered to be significant.

Results

The type distribution of 144 strains of pneumococci is given in Table I. Penicillin resistance was seen in 36 (25%) strains with 19 (13.2%) strains highly resistant. Four resistant strains were isolated from blood and one from both blood and pleural fluid, representing together 10.6% of all isolates from sterile sites. Other resistant strains were isolated from the respiratory tract ($n = 26$), nose ($n = 3$), and ear ($n = 2$). Resistant strains belonged to serogroups/serotypes 19 ($n = 20$), 14 ($n = 6$), 23 ($n = 4$), 6 ($n = 1$) and 9 ($n = 1$). Three resistant strains were untypable because of autoagglutination and one strain of intermediate resistance did not react with the pool of antisera.

Table II shows the distribution of penicillin-resistant isolates in children and adults. Resistance was significantly more common in isolates from children under 5 years of age compared with older children and adults (52.9% versus 16.2%, $\chi^2 = 18.4$, $P = 0.00002$).

The antimicrobial resistance profiles of *S. pneumoniae* strains showing resistance to at least one drug is given in Table III. Thirty-eight (26.4%) strains were resistant to erythromycin, 75 (52.1%) were resistant to tetracycline, 48 (33.3%) were resistant to sulfamethoxazole-trimethoprim and 29 (20.1%) were resistant to chloramphenicol. All strains were susceptible to vancomycin.

TABLE I: SEROGROUP/SEROTYPE DISTRIBUTION OF 144 STRAINS OF *S. pneumoniae* ACCORDING TO THEIR SUSCEPTIBILITIES TO PENICILLIN

Serogroup/Serotype	No. of strains		
	Susceptible	Intermediately resistant	Highly resistant
	3		
	12		
4	7		
6	19	1	
7	4		
8			
9	2		1
10	2		
11	5		
14	4	5	1
17	3		
19	12	7	13
20			
22	2		
23	15	1	3
Non typable	2	2	1
Non vaccine-related	14	1	

TABLEII: DISTRIBUTION OF *S. pneumoniae* STRAINS ACCORDING TO AGE OF PATIENTS

Age group (years)	No. of strains		
	Susceptible	Intermediately resistant	Highly resistant
0 to 4	16	7	11
5 to 9	3	2	1
10 to 19	5	1	1
20 to 29	7	1	0
30 to 39	5	3	1
40 to 49	6	0	0
50 to 59	15	0	1
60 to 100	47	3	3
Unknown	4	0	1

TABLEIII: ANTIMICROBIAL RESISTANCE PROFILES OF *S. pneumoniae* STRAINS

Antibiotic(s)	No. of strains
P	2
E	2
Te	16
SXT	6
P/Te	1
P/SXT	2
E/Te	6
Te/SXT	4
Te/C	10
SXT/C	1
P/E/Te	3
P/E/SXT	1
P/Te/SXT	5
E/Te/SXT	5
E/Te/C	1
Te/SXT/C	2
P/E/Te/SXT	7
P/Te/SXT/C	2
P/E/Te/SXT/C	13
Total	89

C: chloramphenicol; E: erythromycin; P: penicillin;
SXT: sulfamethoxazole-trimethoprim; Te: tetracycline

Penicillin-resistant strains were significantly more likely than penicillin-susceptible strains to be multidrug-resistant (86.1% versus 7.4%, $\chi^2 = 84.7$, $P < 0.00005$).

Strains that were resistant to penicillin were also tested for resistance to ceftriaxone. Twenty strains were resistant to ceftriaxone with 7 strains showing high level resistance.

Discussion

During the period from 1977 to 1986, only 0.5% of invasive *S. pneumoniae* isolates in Singapore were resistant to penicillin.¹² Since then, we have noticed an increasing number of penicillin-resistant pneumococci isolated from both sterile (blood, pleural fluid and cerebro-

spinal fluid) and non-sterile (sputum, ear, eye and nasal) sites (Figs. 1 and 2).¹³ The rapid changes in resistance since 1993 are reflected by an increase in the overall prevalence of resistant strains as well as the appearance of highly resistant strains.

In this study, we have demonstrated a high prevalence (25.0%) of penicillin-resistant pneumococci. This is in contrast to the low prevalence rates reported in Malaysian¹⁴ (2.0% of clinical isolates from September 1984 to August 1985) and Filipino¹⁵ (4.6% of invasive isolates from January 1988 to December 1992) children. A recent survey of community acquired infections in Malaysia showed that 8% of pneumococcal isolates were resistant to penicillin.¹⁶ It is difficult to comment on the significance of this result as the number of isolates was small ($n = 24$). The differences in reported penicillin resistance may be due to local variation in antibiotic usage, the timing of the studies or differences between the study populations. Our study suggests that in parts of Southeast Asia, the prevalence of penicillin-resistant pneumococci may be approaching the figures seen in Spain, South Africa and Eastern Europe.¹⁷

The serogroups/serotypes of penicillin-resistant strains in our population were similar to those reported elsewhere. In contrast to studies from France,¹⁸ Spain* and

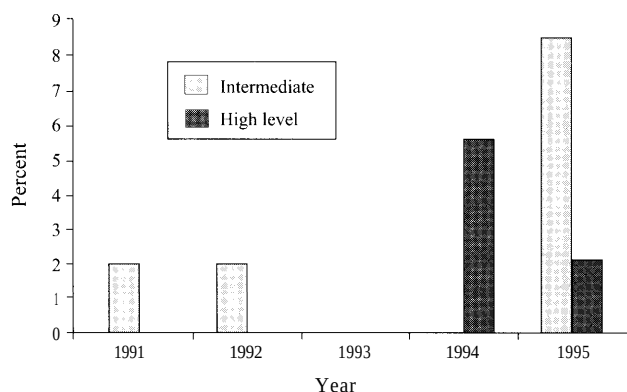


Fig. 1. Percentage of strains from sterile sites that were resistant to penicillin

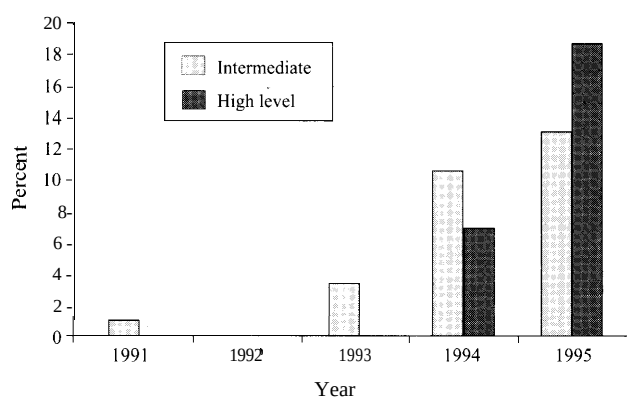


Fig. 2. Percentage of strains from non-sterile sites that were resistant to penicillin.

Canada," only a few of our resistant strains belonged to serogroup 23 ($n = 4$). In our study, the majority of resistant strains belonged to serogroup 19 ($n = 20$).

Overall, 91.3% of pneumococcal strains isolated from sterile sites belonged to serogroups/serotypes included in the 23-valent pneumococcal polysaccharide vaccine. The increasing resistance of *S. pneumoniae* to antibiotics may see a greater role for vaccines in the future. The present polysaccharide vaccine is generally not immunogenic in children under 2 years of age.²⁰ However, a new polysaccharide-protein conjugate vaccine currently being developed may provide protection for this age group.²¹

In Atlanta, Hofmann et al⁴ found no significant difference in the proportion of penicillin-resistant isolates from children under 6 years of age compared to all other age groups. In our study, children under 5 years of age were 3 times as likely as older children and adults to have isolates resistant to penicillin. This finding is in accordance with other studies.^{2,22} The isolation of resistant pneumococci in this age group is consistent with the overwhelming representation of resistant strains in serogroups/serotypes common in young children.⁵ This may be due to the greater use of β -lactam antibiotics in young infants and children. The spread of multidrug-resistant pneumococci between children in day care centres has been documented in the United States.^{23,24}

We have also found high rates of resistance to antibiotics other than penicillin. Penicillin-resistant strains were more likely to be resistant to multiple antibiotics than penicillin-susceptible strains. This finding has also been noted by studies from Spain²⁵ and the United States.²⁶ Particularly alarming is the number of strains showing resistance to 5 different classes of antibiotics; penicillin, erythromycin, tetracycline, sulfamethoxazole-trimethoprim and chloramphenicol ($n = 13$). This resistance profile is similar to that found in multiresistant serotype 19A isolates in Hungary²⁷ and serotype 6B isolates in Spain and Iceland.²⁸

Clones belonging to serotypes 23F²⁹ and 6B²⁸ have been implicated in the spread of multidrug-resistant pneumococci between countries. The majority of our multidrug-resistant strains belonged to serogroup 19.

Although cephalosporins are often used in the treatment of pneumococcal infections, therapeutic failure despite the use of these drugs has been reported.³⁰ Twenty of our penicillin-resistant strains also showed resistance to ceftriaxone. This represents a prevalence of ceftriaxone-resistant pneumococci of at least 13.9% which is more than the rates of cephalosporin resistance reported in the United States⁴ and Spain (9%).³¹

In this study, we included isolates from all clinical sources. Unfortunately, we were unable to determine the clinical significance of strains isolated from non-

sterile sites. Nevertheless, the high prevalence of penicillin-resistant isolates from non-sterile sites is a cause for concern as it gives some indication of the prevalence of resistant strains in the community.

Conclusion

We concluded that there is a high prevalence of penicillin resistance among our pneumococcal isolates, especially those from young children. Most penicillin-resistant strains belong to serogroup 19 and are resistant to multiple antibiotics.

The increase in the number of resistant isolates since the last survey was conducted underlines the importance of continued monitoring of our pneumococcal isolates for changes in antimicrobial susceptibility and serogroup/serotype prevalence. This will allow the development of rational guidelines for the empirical treatment of pneumococcal infections and optimal formulation of vaccines. The judicious use of antimicrobial agents and encouraging vaccination in appropriate settings remain the best strategies for combating the spread of antibiotic-resistant *S. pneumoniae*.

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