J Microbiol Immunol Infect
2003;36:175-181

Rate of nasopharyngeal carriage, antimicrobial resistance and serotype of *Streptococcus pneumoniae* among children in northern Taiwan

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Received: December 25, 2002 Revised: March 20, 2003 Accepted: April 22, 2003

Multiple-antibiotic-resistant strains of *Streptococcus pneumoniae* are isolated from clinical specimens in Taiwan with increasing frequency. This study aimed to define the carriage rate of *S. pneumoniae* among children in northern Taiwan, and to determine the antibiotic susceptibility and the serotype incidence of these isolates. Nasopharyngeal swabs were taken from a total of 478 children (age, 1 month-14 years) who sought medical care only for non-infectious disease or routine vaccination at our hospital between July 1998 and November 1999. *S. pneumoniae* was isolated from 95 patients, and the collected isolates were available for analysis. All pneumococcal isolates were serotyped and their antimicrobial susceptibility tested by standard methods. The total rate of pneumococcal carriage in the study population was 19.9% and the isolation rate was higher in children aged between 2 and 5 years. Only 10 (10.5%) of the isolates were susceptible to penicillin (minimum inhibitory concentration [MIC], ≤0.06 μg/mL); 47 (49.5%) isolates were intermediately resistant (MIC, 0.12-1 μg/mL) and 38 (40%) were highly resistant (MIC, ≥2 μg/mL). Among the 95 *S. pneumoniae* isolates, the common serotypes were 23F (22%), 6B (18.9%), 19F (18.9%), and 14 (8.4%). Evaluation of the results showed that serotypes 23F (24. 7%), 19F (21.2%), 6B (15.3%), and 14 (9.4%) composed 70.6% of all penicillin-non-susceptible *S. pneumoniae* isolates. The significant rate of isolation of penicillin-non-susceptible *S. pneumoniae* from children indicates that both the judicious use of antibiotics and the availability of conjugate pneumococcal vaccines are the most appropriate strategy to reduce the carriage of resistant pneumococci.

Key words: Antimicrobial resistance, nasopharyngeal carriage, serotype, *Streptococcus pneumoniae*

Streptococcus pneumoniae infections cause substantial morbidity and mortality worldwide [1]. This organism is not only the most common cause of otitis media and sepsis in children under the age of 2 years, but also a leading cause of meningitis and pneumonia [2-4]. The first case of a clinically important isolate not susceptible to penicillin (PCN) was reported in Australia in 1967 [5]. Recent evidence suggests that the prevalence of PCN-non-susceptible *S. pneumoniae* (PNSSP) is growing rapidly in Taiwan [6-12], as has been reported in many other geographic areas, including North America, Europe, Africa, and Asia [13-18]. Development of a rational antimicrobial policy requires data on antimicrobial susceptibility patterns and their changes over time. The serotype distribution of invasive isolates in Taiwan and many countries has been established [12,18-22]. These epidemiological data can provide valuable information for assessing the formulations of conjugate vaccines.

The nasopharynx as a source of pneumococci has obvious predictive potential for the emergence of resistance in clinically significant isolates, and has therefore been used to assess antibiotic resistance among pneumococci from different population groups [7,23]. This study was designed to (1) further investigate the prevalence PNSSP strains carriage among children in northern Taiwan; (2) determine the prevalence of *S. pneumoniae* strains with reduced susceptibility to PCN; (3) establish the incidence of cross-resistance to other antimicrobials; and (4) obtain data on the serotype of *S. pneumoniae*, which was not provided in a previous study [7].

Materials and Methods

Survey of nasopharyngeal carriage of *S. pneumoniae*

Nasopharyngeal swab specimens were obtained from 478 children. The survey was conducted from July 1998

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to November 1999. The study population comprised children who sought medical care only for non-infectious disease or routine vaccination in a tertiary-care referral center in northern Taiwan. Children between 1 month and 14 years of age were enrolled. Consent for participation in the study was obtained from their parents or guardians.

Specimen collection

Nasopharyngeal swab specimens for culture were collected by a single investigator who used a cotton swab (Copan, Italy) placed 1 to 1.5 inch into the nasopharynx. The specimens were immediately plated onto 5% sheep blood (Becton Dickinson Microbiology System, Cockeysville, MD, US).

Isolation and identification of *S. pneumoniae*

All plates were incubated for 24 to 48 h at 35°C in 5% CO_2. *S. pneumoniae* isolates were identified based on typical colonial appearance, α-hemolysis, and gram staining. Confirmatory tests included Optochin sensitivity and bile solubility tests. All isolates were kept frozen in tryptic soy broth at –70°C until further analysis. On thawing, the isolates were checked for purity and Optochin sensitivity.

Antimicrobial susceptibility tests

The minimum inhibitory concentration (MIC) of PCN, amoxicillin/clavulanate, cefuroxime, cefotaxime, erythromycin, azithromycin, chloramphenicol, tetracycline, ofloxacin, trimethoprim/sulfamethoxazole (TMP-SMZ), vancomycin, and imipenem were determined using a broth microdilution method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [24]. Suspensions with a turbidity equal to a 0.5 McFarland standard were prepared in Mueller-Hinton broth, with a final inoculum of 5×10^5 cfu/mL. All plates were incubated in ambient air at 35°C for 20 to 24 h. The MIC was defined as the lowest concentration of a particular agent tested that yielded no visible evidence growth of the test isolate. *S. pneumoniae* (American Type Culture Collection [ATCC] 49619) was used as control organism throughout this study. Interpretation of result was performed according to recommendations of the Centers for Disease Control and Prevention (CDC) for non-meningitis isolates of *S. pneumoniae*. Isolates for which the MIC was ≤1 μg/mL were considered susceptible, whereas those for which the MIC was ≥4 μg/mL were defined as highly resistant. Multidrug non-susceptibility is defined by intermediate susceptibility or resistance to 2 or more drug classes that are used to treat *S. pneumoniae* infections [15].

Serotyping of the isolates

Serotyping of the isolates was done with Quellung reaction by using antisera from Statens Seruminstitut (Copenhagen, Denmark) [25,26]. Specific types were confirmed using pooled sera, the single individual group serum, and the single type serum. Isolates reacting positively with the omni-serum but negatively with pooled sera were reported as non-typable [12].

Results

Prevalence of carriage

A total of 478 children were enrolled in this study; 95 (19.9%) of their nasopharyngeal swab cultures were positive for *S. pneumoniae*. The age-specific carrier rate of *S. pneumoniae* isolates is shown in Table 1. The rate of pneumococcal carriage in the study population ranged from 14.4% to 27.2% and the isolation rate was associated with age, being higher in children aged between 2 to 5 years.

Antimicrobial susceptibility

The antibacterial activities of 12 antimicrobial agents against 95 isolates of *S. pneumoniae* are shown in Table 2. Based on the criteria of NCCLS and CDC, 85 (89.5%) and 38 (40%) of 95 isolates were PNSSP, respectively. The PNSSP carrier rate ranged from 80% to 95.9% and was also higher in children aged between 2 to 5 years (Table 3). The percentages of PNSSP isolates that were also resistant to erythromycin, azithromycin, and TMP-SMZ were all more than 90% (Table 4).

Serotyping

Serotyping was performed on 95 viable isolates. A total

Table 1. Age-specific carrier rate of *S. pneumoniae* isolates

Age group (year)	No. of childern	No. of isolates	Carrier rate (%)
<2	180	26	14.4
2-5	180	49	27.2
>5	118	20	16.9
Total	478	95	19.9

Table 2. Susceptibility of 95 isolates of *S. pneumoniae* to 12 antimicrobial agents

Antimicrobial agent	MIC (μg/mL)			No. (%) of isolates		
	50%	90%	Range	Susceptible	Intermediate	Resistant
Penicillin	1.0	2.0	0.015-4.0	10 (10.5)	47 (49.5)	38 (40)
Amoxicillin/clavulanate (non-meningitis)	1.0	2.0	0.06-8.0	66 (69.5)	22 (23.1)	7 (7.4)
Cefuroxime	2.0	8.0	0.06-8.0	21 (22.1)	7 (7.4)	67 (70.5)
Cefotaxime (meningitis)	0.5	1.0	0.06-4.0	59 (62.1)	28 (29.5)	8 (8.4)
Cefotaxime (non-meningitis)	0.5	1.0	0.06-4.0	87 (91.6)	7 (7.4)	1 (1.0)
Erythromycin	8.0	8.0	0.03-8.0	8 (8.4)	1 (1.1)	86 (90.5)
Azithromycin	8.0	16.0	0.06-16.0	8 (8.4)	7 (7.4)	80 (84.2)
Chloramphenicol	2.0	16.0	0.5-32.0	68 (71.6)	0	27 (28.4)
Tetracycline	16.0	32.0	0.5-128.0	11 (11.6)	6 (6.3)	78 (82.1)
Ofloxacin	1.0	2.0	0.5-32.0	94 (98.9)	0	1 (1.1)
Trimethoprim/sulfamethoxazole	16.0	32.0	0.12-32.0	11 (11.6)	21 (22.1)	63 (66.3)
Vancomycin	0.25	0.5	0.06-0.5	95 (100)	0	0
Imipenem	0.12	0.25	0.03-1.0	68 (71.6)	26 (27.3)	1 (1.1)

Abbreviation: MIC = minimum inhibitory concentration

of 10 serotypes were found, with 17 isolates being non-typable (Table 5). Ten serotypes (6A, 6B, 9V, 10A, 12F, 14, 15B, 19A, 19F, 23F) accounted for 82.1% of all isolates. The predominant serotypes were 23F (22%), 6B (18.9%), 19F (18.9%), and 14 (8.4%). In addition, there were differences in the relative distribution of some serotypes among the different age groups. In children aged below 2 years or above 5 years, serotype 23F was the most common, followed by serotypes 19F and 6B. In children aged between 2 to 5 years, serotype 6B was the most common, followed by serotypes 19F, 14, and 23F. Among the 10 major serotypes, 23F, 19F, 14, 9V, 15B, 6A, and 19A showed the highest rate (100%) of PCN resistance; and serotypes 10A and 12F showed the lowest (0%) (Table 6). Among 85 PNSSP isolates, serotypes 23F (24.7%), 19F (21.2%), 6B (15. 3%), and 14 (9.4%) composed 70.6% of all isolates.

Discussion

This study has provided data on the epidemiological features of *S. pneumoniae* seen in children visiting a tertiary-care referral center in northern Taiwan. The results of this surveillance study indicated a continuing increase in the prevalence of PCN resistance among respiratory tract isolates of *S. pneumoniae* in Taiwan. Resistance to PCN among *S. pneumoniae* strains is an emerging problem worldwide [6-18]. Carriage of *S. pneumoniae* has been correlated with the emergence of clinical disease [2,7,27-29]. Thus, the characteristics of carriage isolates could serve as an indicator of the

Table 3. Age-specific carrier rate of penicillin-non-susceptible *S. pneumoniae* isolates

Age group (year)	No. of isolates	No. (%) of isolates		
		Susceptible	Intermediate	Resistant
<2	26	4 (15.4)	15 (57.7)	7 (26.9)
2-5	49	2 (4.1)	25 (51)	22 (44.9)
>5	20	4 (20)	7 (35)	9 (45)
Total	95	10 (10.5)	47 (49.5)	38 (40)

Table 4. Activity of erythromycin, azithromycin, and trimethoprim/sulfamethoxazole against penicillin-non-susceptible *S. pneumoniae* strains

Antimicrobial agent	No. (%) of isolates		
	PCN-susceptible strains	PCN-intermediate strains	PCN-resistant strains
Erythromycin	6 (60)	45 (95.7)	36 (94.7)
Azithromycin	7 (70)	44 (93.6)	36 (94.7)
Trimethoprim/sulfamethoxazole	6 (60)	45 (95.7)	36 (94.7)

Abbreviation: PCN = penicillin

Table 5. Serotype distribution of *S. pneumoniae* isolates by age-group

Serotype	No. (%) isolates in different age group			
	<2 year	2-5 year	>5 year	Total
6A	0	3 (6)	0	3 (3.2)
6B	5 (20.8)	9 (18)	4 (19)	18 (18.9)
9V	1 (4.2)	3 (6)	0	4 (4.2)
10A	0	0	1 (4.8)	1 (1.1)
12F	1 (4.2)	0	0	1 (1.1)
14	0	7 (14)	1 (4.8)	8 (8.4)
15B	0	3 (6)	0	3 (3.2)
19A	1 (4.2)	0	0	1 (1.1)
19F	5 (20.8)	8 (16)	5 (23.8)	18 (18.9)
23F	9 (37.5)	6 (12)	6 (28.6)	21 (22)
Non-typable	2 (8.3)	11 (22)	4 (19)	17 (17.9)
Total	24 (100)	50 (100)	21 (100)	95 (100)

prevalence of resistant strains in the community [7,30-33]. According to the reference breakpoints of CDC for PCN susceptibility to non-meningitis isolates of *S. pneumoniae*, a decrease in the rate of PNSSP was observed (40.0%). However, with reference to the guidelines of NCCLS, the high prevalence (89.5%) of resistance reported here is particularly troubling in light of previous study that found a lower prevalence (71%) in southern Taiwan [7]. We have detected an extraordinarily high prevalence of resistance to PCN among *S. pneumoniae* strains isolated from the nasopharynges of children visiting our hospital. The prevalence of highly resistant strains was also high.

Rates of resistance to other β-lactams are also clearly increasing. These include amoxicillin/clavulanate (30.5%; MIC, >2/1 μg/mL) and the 2 cephalosporins (cefuroxime [77.9%] and cefotaxime [meningitis, 37.9%; non-meningitis, 8.4%]) examined in this study. Stratification of cephalosporin activity on the basis of PCN resistance level of *S. pneumoniae* isolates has been well documented, and is explained by common PCN-binding-protein targets of action [34-39]. It is not surprising, therefore, that as rates of PCN resistance increase, so do rates of cephalosporin resistance. Among the cephalosporins tested in this surveillance study, only cefotaxime remains relatively sufficiently active for empirical management of non-meningeal infections with *S. pneumoniae* as the known or suspected cause.

Macrolide resistance in *S. pneumoniae* has remained at a low level in most countries. In South Africa [13] and Hungary [14], the rate of resistance to erythromycin was reported to be about 50%. However, recent surveillance data in Taiwan [8,9] demonstrated the continuing upsurge of *S. pneumoniae* isolates non-susceptible to clarithromycin (from 89% in 1996-1997 to 95% in 1998-1999). This resistance was particularly high among nasopharyngeal isolates from children in southern Taiwan (95% in 1995-1997) [7]. In this survey, the nasopharyngeal isolates also showed a strikingly high incidence of resistance to erythromycin (91.6%) and azithromycin (91.6%). Although azithromycin is a newer macrolide, results of this study indicate that the susceptibility of *S. pneumoniae* to this drug was no

Table 6. Susceptibility of penicillin against different serotypes of *S. pneumoniae*

Serotype (no.)	No. (%) of isolates		
	Susceptible	Intermediate	Resistant
6A (3)	0	3	0
6B (18)	5 (27.8)	3 (16.7)	10 (55.5)
9V (4)	0	0	4
10A (1)	1	0	0
12F (1)	1	0	0
14 (8)	0	7	1
15B (3)	0	1	2
19A (1)	0	0	1
19F (18)	0	15 (83.3)	3 (16.7)
23F (16)	0	9 (42.9)	12 (57.1)
Non-typable (17)	3 (17.7)	9 (52.9)	5 (29.4)

better than to erythromycin. Macrolide resistance was thought to have evolved in response to different antibiotic pressures in the community. Moreover, a decrease in the rate of non-susceptibility to TMP-SMZ for *S. pneumoniae* isolates in Taiwan has been reported (from 87% in 1996-1997 to 65% in 1998-1999) [8,9]. However, another remarkable finding of this study is the high TMP-SMZ resistance (88.4%). In recent years, resistance to TMP-SMZ has been emerging among isolates of *S. pneumoniae*, and it is now recommended that the use of TMP-SMZ should immediately be discontinued in areas with a high prevalence of resistant strains [13]. In this study, vancomycin resistance was not observed.

Among the non-β-lactam antimicrobial agents examined in this study, rates of resistance to the macrolides and TMP-SMZ were highest among strains of *S. pneumoniae* that were PCN-resistant. For example, the overall rates of erythromycin resistance among PCN-susceptible, intermediate, and highly-resistant strains were 60%, 95.7%, and 94.7%, respectively. This relationship has been observed previously [40-42], and since resistance mechanisms are different, it may be explained by antimicrobial selective pressure [43]. This also means that physicians in Taiwan have a limited choice of drugs that can be used against *S. pneumoniae* due to multiple drug resistance.

Prevalence studies worldwide have shown that serotypes 6, 19, and 23 are the most common infecting children. This prevalence was also observed in this study. Recent surveillance of the distribution of serotypes in Taiwan during the period from 1998 to 1999 revealed that the most prevalent serotypes encountered in the invasive isolates were 23, 6, 14, 19, and 3 [12]. This study demonstrated that the most frequent serotypes of *S. pneumoniae* among children in northern Taiwan were, in order of decreasing frequency, 23F, 6B, 19F, and 14. The most common serotypes in the present study were similar among different age groups, except for rank order. Conjugate polyvalent *S. pneumoniae* vaccines are being developed with the aims of improving efficacy to prevent disease in all age groups as compared with the current polysaccharide vaccine, and also of reducing nasopharyngeal carriage to prevent transmission in healthy carriers [44,45]. Although coverage of the serotypes in this study by the current 23-valent vaccine was high, careful consideration would be required in the selection of serotypes to be included in the new conjugate vaccines. Heptavalent conjugate vaccines contain capsular polysaccharides of serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. These 7 serotypes and the potentially cross-reactive serotypes (6A, 9A, 9L, 18B, and 18F) accounted for 65% to 85% of invasive *S. pneumoniae* disease [46-49] and comprised 76% of the serotypes in this study. Thus, these new vaccines may have the potential to reduce carriage of the majority of *S. pneumoniae* in our population, including the most common important serotypes causing invasive disease.

In conclusion, the results of this current study indicate that the problem of antimicrobial resistance of *S. pneumoniae* continues to grow in Taiwan, with nearly all antimicrobial classes being affected. The increasing rates of antimicrobial resistance in *S. pneumoniae* are a major problem for therapy of invasive disease, especially of meningitis. Promoting the judicious use of antibiotics remains an important component of strategies to control antibiotic resistance. Moreover, hospital-based surveillance of community-acquired infections can provide important data to inform health-policy decisions. The findings of this study further emphasize the necessity to continuously monitor *S. pneumoniae* strains isolated from across Taiwan for their antimicrobial resistance patterns and serotypes.

Acknowledgments

This work was supported by a grant (TSGH-C88-26) from the Tri-Service General Hospital, National Defense Medical Center, Taiwan, ROC.

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