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Serotype Distribution and Antimicrobial Susceptibilities of Nasopharyngeal Isolates of *Streptococcus pneumoniae* from Children Hospitalized for Acute Respiratory Illnesses in Hong Kong▽

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Five hundred nineteen *Streptococcus pneumoniae* isolates from nasopharyngeal aspirates of 3,157 children (age, <16 years) participating in a respiratory surveillance study in Hong Kong in 2005 and 2006 indicated that 64.9% and 37.2% of the isolates were not susceptible to penicillin and cefotaxime, respectively. The rate of potential coverage by the seven-valent conjugate vaccine was 72.3%, and the rate increased to 74.6% for serogroup-specific types.

Penicillin-nonsusceptible *Streptococcus pneumoniae* (PNSP) has evolved to be a global problem in recent decades. The introduction of a seven-valent (types 4, 6B, 9V, 14, 18C, 19F, and 23F) pneumococcal conjugate vaccine (PCV7) in the United States and Europe has demonstrated its impact in reducing invasive disease (1) and otitis media (4). Invasive disease caused by serotypes of PNSP clones have diminished significantly in the United States (15, 17). In addition, the rate of nasopharyngeal carriage of vaccine serotypes in children has declined, thus reducing the rates of transmission and the numbers of infections caused by these serotypes (16).

Pneumococci are "inhabitants" of the oral flora and colonize the nasopharynx, particularly in young children from birth, and the level of colonization gradually declines with age. Although their isolation from the nasopharynx of children with respiratory illnesses does not necessarily represent pneumococcal disease, nasopharyngeal colonization is often the first step in the development of pneumococcal infections. Coinfection or secondary bacterial infection may result from organisms that had colonized the nasopharynx. Invasive diseases with meningitis and bacteremia remain "tip-of-the-iceberg" presentations for pneumococcal disease, but *S. pneumoniae* is infrequently cultured from cerebrospinal fluid and blood, especially when antibiotics have been given. Besides, tympanic aspirate or sputum specimens are rarely obtained from children. We thus sought to examine the serotypes and antibiotic susceptibilities of *S. pneumoniae* strains isolated from nasopharyngeal aspirates (NPAs) obtained in a prospective surveillance study of childhood acute respiratory illness. As PCV7 has been made available in Hong Kong since August 2006, the serotypes and antibiotic susceptibilities of the *S. pneumoniae* isolates obtained in this study represent those that existed prior to the introduction of PCV7.

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Pneumococcal isolates. All children ages >1 month to 15 years who were hospitalized at the Prince of Wales Hospital (PWH) from March 2005 to March 2006 for a suspected respiratory or febrile illness and from whom an NPA was obtained were included in the study. PWH is a 1,350-bed teaching hospital that serves a population of approximately 1,000,000 in the New Territories East Region of Hong Kong. An NPA was obtained from each child and placed in 2 ml of transport medium containing Hanks' balanced salt solution, 5% bovine serum albumin, and 7.5% $NaHCO_3$ without antibiotics; and 1 μl was cultured onto blood agar with or without gentamicin (5 mg/liter). The *S. pneumoniae* isolates were saved in 10% glycerol brain heart infusion broth at −70°C. Duplicate isolates from the same child during the same hospital admission were excluded.

Antibiotic susceptibility testing. The MICs of penicillin G, cefotaxime, chloramphenicol, tetracycline, erythromycin, lincomycin, ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin, and linezolid were determined by the broth microdilution method, as described by the Clinical Laboratory Standards Institute (3).

Capsular typing. Capsular typing was performed with Pneumotest antisera (Statens Seruminstitut, Copenhagen, Denmark), according to the manufacturer's instruction. Isolates that were not typeable by the Pneumotest were classified as nontypeable. Typing of certain serogroups into serotypes was done by using a combination of factor sera and a multiplex PCR that targeted the serotypes of the seven-valent vaccine (13).

The rate of isolation of *S. pneumoniae* from the NPAs of 3,157 children ages 1 month to 15 years was 16.9%. A total of 519 nonduplicate *S. pneumoniae* isolates were available for capsular typing and antibiotic susceptibility testing. The median age of the children from whom *S. pneumoniae* was isolated was 2.9 years (25% interquartile ratio [IR], 1.3 years; 75% IR, 4.5 years), and the ratio of males to females was 1.3:1. The distribution of the serotypes in the different age groups and the percentage of isolates nonsusceptible to penicillin are listed in Table 1. Eighty-two percent of the isolates were type-

TABLE 1. Distribution of capsular types of *S. pneumoniae* by age and percentage of isolates penicillin nonsusceptible[a]

Serotype	No. (%) of isolates from children in the following age (yr) groups:						No. of PNSP isolates (% *S. pneumoniae*)
	<2	2–3	3–4	4–5	≥5–15	Total	
6B	36	31	24	16	20	127 (24)	103 (20)
19F	29	24	20	8	20	101 (19)	81 (16)
23F	17	14	18	4	18	71 (14)	50 (10)
14	10	14	17	6	18	65 (13)	56 (11)
15	4	1	2	3	4	14 (3)	1
18C	3	3	1	1	2	10 (2)	0
11	4	1	1	0	1	7	0
Non-19F	2	1	1	0	2	6	2
3	3	0	1	1	0	5	0
33	5	0	0	0	0	5	0
10	2	0	0	0	1	3	0
Non-6B (6A)	1	0	0	0	1	2	0
Non-23F	1	0	1	0	0	2	0
22	2	0	0	0	0	2	0
18B	0	0	0	0	1	1	0
1	0	0	0	0	1	1	0
4	1	0	0	0	0	1	0
9L	1	0	0	0	0	1	0
Nontypeable	46	7	8	9	25	95 (18)	44 (8)
Subtotal	167	96	94	48	114	519 (100)	337 (64.9)

[a] A total of 519 isolates were tested. PNSP isolates were those for which the penicillin MIC was ≥0.12 μg/ml.

able, and the most common serotypes/serogroups were 6, 19, 23F, 14, 15, and 18. The potential proportions of serotype coverage by the 7-, 9-, and 11-valent vaccines were 72.3%, 72.4%, and 73.4%, respectively. If serogroups were included, the rate of PCV7 coverage increased to 74.4%, while the rates of 9- and 11-valent vaccine coverage increased to 74.6 and 75.7%, respectively.

The antibiotic susceptibilities of the 519 *S. pneumoniae* isolates are listed in Table 2. The prevalence of PNSP was 64.9%, while the prevalence of cefotaxime nonsusceptibility (MIC ≥ 1.0 μg/ml) among the *S. pneumoniae* isolates was 37.2%. High rates of nonsusceptibility to erythromycin (75.7%) and tetracycline (61.8%) were obtained. No resistance to respiratory fluoroquinolones was detected, except that 2.3% of the *S. pneumoniae* isolates had ciprofloxacin MICs of 4.0 μg/ml, suggesting that first-step mutations at the quinolone resistance-determining region may already have been present in some isolates. All isolates were susceptible to linezolid. The highest penicillin MIC remained at 4.0 μg/ml, but high-level cefotaxime resistance (MIC > 4 μg/ml) was identified. A previous report described two fatal cases of pneumococcal bacteremia in children, one of whom also had meningitis, that were caused by strains with cefotaxime MICs of 4 μg/ml (18).

The antibiotic resistance profiles and the serotypes of these *S. pneumoniae* isolates reflect strains which may be associated both with carriage and with acute respiratory illness. Ho et al. (7) compared both invasive and nasopharyngeal carriage isolates from children and did not find significant differences in terms of resistance rates or serotypes. Our potential seven-valent vaccine coverage rate fell between that based on invasive isolates (89.7%) and that based on nasopharyngeal carriage (66.1%) (7). Previous data concerning the serotypes from

TABLE 2. MICs of *Streptococcus pneumoniae* to 11 antibiotics[a]

Antibiotic	MIC range (μg/ml)	No. of isolates			% of isolates nonsusceptible[b]
		Sensitive	Intermediate	Resistant	
Penicillin	0.008–4.0	182	212	125	64.9
Cefotaxime (nonmeningitis)	0.015–≥4.0	451	41	27	13.0
Cefotaxime (meningitis)	0.015–≥4.0	326	125	68	37.2
Erythromycin	0.03–>64.0	126	24	369	75.7
Lincomycin	0.25–>32.0			225	43.4
Tetracycline	0.06–>16.0	198	8	313	61.8
Chloramphenicol	1.0–32	427		92	17.7
Linezolid	0.12–2.0	519		—[c]	
Ciprofloxacin	0.25–4.0			12[d]	2.3
Levofloxacin	0.25–2.0	519	0	0	0
Gatifloxacin	0.06–0.5	519	0	0	0
Moxifloxacin	0.03–0.25	519	0	0	0

[a] A total of 519 isolates were tested.
[b] Nonsusceptible isolates were those that were intermediate and resistant.
[c] No CLSI breakpoint was available.
[d] MIC ≥ 4.0 μg/ml.

the group <2 years of age were very limited and were based on 33 invasive isolates saved over a 6-year period (2, 7). However, a lower rate of carriage based on nasopharyngeal isolates would be expected and was shown in a study from Taiwan (12). The application of DNA amplification by sequential multiplex PCR of the specific capsular genes (14) may further identify the serogroups or serotypes presently nontypeable with the Pneumotest antisera. Our previous findings indicated that PNSP was significantly associated with children, particularly those 2 to 10 years of age (8). A rapid rise in the incidence of PNSP was seen in Hong Kong beginning in the early 1990s as a result of the spread of multidrug-resistant clones of Spain^{23F}-1 and Spain^{6B}-2 (9–11). Based on recent outcomes on the use of PCV7 in populations with a high prevalence of PNSP infection or carriage, PNSP is likely to remain prevalent until the vaccine can be introduced to effectively reduce the incidence of invasive pneumococcal infections caused by these resistant strains.

The pneumococcal population is undergoing strong evolutionary pressure to change as the conjugate vaccine is used more extensively in different parts of the world. This study emphasizes the need for surveillance of the antibiotic resistance rates and serotypes of *S. pneumoniae* isolates with the changing scene as vaccination programs and antibiotic stewardships are being introduced.

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