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Nasal Carriage in Vietnamese Children of *Streptococcus pneumoniae* Resistant to Multiple Antimicrobial Agents

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Resistance to antimicrobial agents in *Streptococcus pneumoniae* is increasing rapidly in many Asian countries. There is little recent information concerning resistance levels in Vietnam. A prospective study of pneumococcal carriage in 911 urban and rural Vietnamese children, of whom 44% were nasal carriers, was performed. Carriage was more common in children <5 years old than in those ≥5 years old (192 of 389 [49.4%] versus 212 of 522 [40.6%]; *P*, 0.01). A total of 136 of 399 isolates (34%) had intermediate susceptibility to penicillin (MIC, 0.1 to 1 mg/liter), and 76 of 399 isolates (19%) showed resistance (MIC, >1.0 mg/liter). A total of 54 of 399 isolates (13%) had intermediate susceptibility to ceftriaxone, and 3 of 399 isolates (1%) were resistant. Penicillin resistance was 21.7 (95% confidence interval, 7.0 to 67.6) times more common in urban than in rural children (35 versus 2%; *P*, <0.001). More than 40% of isolates from urban children were also resistant to erythromycin, trimethoprim-sulfamethoxazole, chloramphenicol, and tetracycline. Penicillin resistance was independently associated with an urban location when the age of the child was controlled for. Multidrug resistance (resistance to three or more antimicrobial agent groups) was present in 32% of isolates overall but in 39% of isolates with intermediate susceptibility to penicillin and 86% of isolates with penicillin resistance. The predominant serotypes of the *S. pneumoniae* isolates were 19, 23, 14, 6, and 18. Almost half of the penicillin-resistant isolates serotyped were serotype 23, and these isolates were often multidrug resistant. This study suggests that resistance to penicillin and other antimicrobial agents is common in carriage isolates of *S. pneumoniae* from children in Vietnam.

Streptococcus pneumoniae is a major cause of morbidity and mortality in children and adults in developing countries. It is a common cause of otitis media, sinusitis, pneumonia, septicemia, and meningitis. An estimated 1 million children less than 5 years old die each year from pneumococcal pneumonia (17). This situation may deteriorate further as human immunodeficiency virus infection, which is spreading in many tropical countries, increases the risk of invasive pneumococcal disease (6). In Vietnam, *S. pneumoniae* is an important cause of pneumonia and meningitis in children and adults (28; unpublished observations).

S. pneumoniae is part of the normal flora of the nasopharynx. Carriage is higher in preschool children (35%) than in adults without preschool children in the family (2 to 9%) (8). The pneumococcal serotype colonizing a child varies over time. Acquisition of a new serotype may lead to invasive disease, with a risk of 15% in the first month after new acquisition (7), and children may act as a reservoir for the dissemination of new serotypes to others (8). For many years, penicillin and chloramphenicol have been the mainstay of treatment for pneumococcal disease in developing countries, as they are both inexpensive and effective. Unfortunately, the rapid increase in resistance to penicillin and other antimicrobial agents worldwide has made the choice of antimicrobial agent for *S. pneumoniae* infections more difficult and costly (5). As nasopharyngeal *S. pneumoniae* may have a predictive potential for resistance in clinically significant isolates (13, 16, 21), we have undertaken a study to determine the prevalence of nasal carriage of *S. pneumoniae* resistant to penicillin and other antimicrobial agents in Vietnamese children.

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MATERIALS AND METHODS

The children studied were from six separate sites in southern Vietnam. Three sites were urban, one primary and two kindergarten schools in Ho Chi Minh City. Three sites were rural, two primary schools in Dong Nai province about 50 km outside of Ho Chi Minh City and an isolated village about 40 km from Nha Trang in Khanh Hoa province, central Vietnam. Children who attended the schools on the day of the survey were included. From the community in Khanh Hoa, children were invited to attend the village clinic on the day of the survey. Consent was obtained from the parents of children studied. The study was approved by the scientific and ethical committee of the Centre for Tropical Diseases, Ho Chi Minh City.

A nasal swab was obtained from each child with a cotton wool swab (Medical Wire, Corsham, Wiltshire, United Kingdom) premoistened with sterile water and inserted and rotated in each of the anterior nares. The swab samples were plated immediately onto selective *S. pneumoniae* agar (sheep blood agar base, 10% sheep blood, 2 mg of crystal violet per liter, 50 mg of nalidixic acid per liter, and 2 mg of gentamicin per liter) (20), with an optochin (Oxoid, Basingstoke, United Kingdom) disk placed between the initial inoculum and the first streak, and onto heated blood (chocolate) agar. Plates were incubated for 24 to 48 h at 37°C in candle jars. Potential *S. pneumoniae* colonies were selected by colonial morphology and alpha-haemolysis and confirmed by gram staining and susceptibility to optochin. Susceptibility to antimicrobial agents was tested by agar plate incorporation immediately after primary isolation or after storage in glycerol broth at −40°C. Susceptibility testing for trimethoprim-sulfamethoxazole was not performed directly on isolates from the first three schools visited (two urban and one rural). It was performed at a later date on the stored isolates. Unfortunately, many of the isolates from these first three schools did not remain viable for subsequent testing. The number of isolates with a trimethoprim-sulfamethox-

azole susceptibility test result is therefore smaller than the number with susceptibility test results for the other antibiotics.

MICs were determined by an agar plate incorporation method. For penicillin G and ceftriaxone, doubling dilutions over the concentration range of 0.008 to 32 μg/ml were used for all isolates. For the other antimicrobial agents, appropriate breakpoint concentrations were used. Penicillin G, erythromycin, chloramphenicol, trimethoprim-sulfamethoxazole, and tetracycline were added to Mueller-Hinton agar (Oxoid) with 5% defibrinated sheep blood. The antimicrobial agents were purchased from Sigma, Poole, Dorset, United Kingdom, except for ceftriaxone, which was a gift from Roche Pharmaceuticals. The antimicrobial agents were supplied as laboratory powders of known potency, and stock solutions were made as recommended by the manufacturer. Suspensions of *S. pneumoniae* with a turbidity equivalent to that of a 0.5 McFarland standard were prepared by suspending 5 to 10 colonies from a sheep blood agar plate (Oxoid) in saline and then diluting the mixture 10-fold. Bacteria were applied to plates using a multipoint inoculator (Denley Scientific, Billinghurst, Sussex, United Kingdom) to give a final inoculum size of 10^4 CFU per spot. The plates were incubated at 37°C for 18 h in air. The MICs for 160 isolates were determined again but with incubation in 5% CO_2. The concentration of antimicrobial agent inhibiting growth was taken as the MIC. A growth control was included in each MIC run. *S. pneumoniae* ATCC 49619 was used as the quality control strain and gave values within the acceptable range.

Antimicrobial susceptibility breakpoints were defined according to National Committee for Clinical Laboratory Standards criteria (19). For penicillin, an MIC of ≤0.06 μg/ml was considered susceptible, an MIC of ≥0.1 to 1.0 μg/ml was considered intermediate, and an MIC of ≥2.0 μg/ml was considered resistant. The breakpoints for ceftriaxone were as follows: MIC of ≤0.5 μg/ml, susceptible; MIC of 1.0 μg/ml, intermediate; and MIC of ≥2.0 μg/ml, resistant. The breakpoints for the other antimicrobial agents were as follows: erythromycin—MIC of ≤0.25 μg/ml, susceptible; MIC of 0.5 μg/ml, intermediate; and MIC of ≥1 μg/ml, resistant; chloramphenicol—MIC of ≤4 μg/ml, susceptible; and MIC of ≥8 μg/ml, resistant; tetracycline—MIC of ≤2 μg/ml, susceptible; MIC of 4 μg/ml, intermediate; and MIC of ≥8 μg/ml, resistant; and trimethoprim-sulfamethoxazole (ratio, 1:20)—MICs of ≤0.5 and 9.5 μg/ml, susceptible; MICs of 1 to 2 and 19 to 38 μg/ml, intermediate; and MICs of ≥4 and 76 mg/liter, resistant.

Serotyping was performed by use of the Quellung reaction in the checkerboard method with 12 pools of antisera (Pneumotest; Staten Seruminstitut, Copenhagen, Denmark). Pools G and I were not used. About 20 isolates from each location were chosen for serotyping. One colony was tested from each primary isolation plate.

Statistical analysis was performed using the Epi-Info package, version 6.0 (Centers for Disease Control and Prevention, Atlanta, Ga.) and SPSS version 7.5 for Windows (SPSS Inc., Chicago, Ill.). Normally distributed continuous variables were compared using the unpaired *t* test, and categorical variables were compared using the χ^2 test. Associations between penicillin resistance and age or location were examined by calculation of relative risk. The independence of these associations was tested by logistic regression. The MICs obtained with incubation in air were compared to those obtained with incubation in CO_2, considered the reference method. MICs within ±1 dilution were considered the same. For classifying the results, a very major error was defined as a resistant result with the reference method and a susceptible result with incubation in air. A major error was defined as a susceptible result with the reference method and a resistant result with incubation in air. A minor error was defined as an intermediate result with either method and either a susceptible or a resistant result with the other method.

RESULTS

Nasal swabs were taken from 911 children 1 to 16 years old. The mean (range) number of children at each site was 152 (118 to 200). A total of 389 of the 911 children (43%) were <5 years old. A total of 472 of the children (52%) were from urban schools, and 439 of the children (48%) were from rural schools or a rural village. Of the urban children, 332 of 472 (70%) were <5 years old; 57 of 439 (13%) of the rural children were <5 years old (*P*, <0.001). *S. pneumoniae* was isolated from 404 (44%) of the children. Carriage was present in 192 of 389 children (49.4%) <5 years old and in 212 of 522 of those (40.6%) ≥5 years old (*P*, 0.01). The rates of nasal carriage in the urban and rural children were 211 of 472 (45%) and 193 of 439 (44%), respectively. There was no significant difference between the urban and rural rates of carriage when age was controlled for.

Antimicrobial susceptibility testing was carried out on 399 of the *S. pneumoniae* isolates, except that trimethoprim-sulfamethoxazole susceptibility test results were available for only 263 isolates. Five isolates died before complete susceptibility testing could be performed. The MICs tests were repeated for 160 isolates incubated in CO_2. When the MICs obtained under different incubation conditions were compared, the proportions of agreement were 91% for penicillin and 89% for ceftriaxone; there were no very major or major errors of classification, but there were 24 of 160 minor errors (15%) and 18 of 160 minor errors (11%), respectively. The proportions of agreement for the other antibiotics were as follows: erythromycin, 93%; trimethoprim-sulfamethoxazole, 95%; chloramphenicol, 92%; and tetracycline, 94%. There was no systematic misclassification for any antibiotic toward more or less resistance.

Overall, 212 of 399 isolates (51%) showed reduced susceptibility to penicillin (34% intermediate, 19% resistant), 57 of 399 (14%) showed reduced susceptibility to ceftriaxone (13% intermediate, 1% resistant), 201 of 399 (50%) showed resistance to erythromycin, 111 of 263 (42%) showed reduced susceptibility to trimethoprim-sulfamethoxazole (16% intermediate, 26% resistant), 157 of 399 (39%) showed resistance to chloramphenicol, and 285 of 399 (71%) showed reduced susceptibility to tetracycline (1% intermediate, 70% resistant).

The proportions of penicillin-resistant isolates (MIC, >1.0 μg/ml) were 60 of 191 (31%) and 16 of 208 (8%) in children <5 years old and in those ≥5 years old, respectively (*P*, <0.001). The levels of resistance in the isolated pneumococci were very similar for each of the three urban sites and each of the three rural sites. Children from the urban schools were significantly more likely to carry isolates resistant to penicillin, ceftriaxone, erythromycin, chloramphenicol, and trimethoprim-sulfamethoxazole but less likely to carry an isolate resistant to tetracycline (Table 1), even after age was controlled for by logistic regression. In Table 2, the susceptibility results are classified according to penicillin susceptibility. Resistance to penicillin was associated significantly with resistance to erythromycin, trimethoprim-sulfamethoxazole, and chloramphenicol.

Serotyping was performed on 125 of the isolates (Table 3). Serotypes 14, 19, and 23 accounted for 34 of 51 isolates (67%) in children <5 years old, and serotypes 6, 14, 18, 19, and 23 accounted for 52 of 74 isolates (70%) in those ≥5 years old. Of the penicillin-resistant isolates serotyped, 14 of 31 (45%) were serotype 23, and these isolates were always resistant to erythromycin and frequently also resistant to trimethoprim-sulfamethoxazole and chloramphenicol.

DISCUSSION

The high levels of resistance to penicillin and other antimicrobial agents in *S. pneumoniae* in this study in Vietnamese children are consistent with increasing drug resistance in *S. pneumoniae* in some countries in the Asian-Pacific region. The rate of 35% resistance (MIC, >1.0 μg/ml) in the urban children is comparable to the results of a study from Taiwan, in which 41% of 584 carriage isolates from urban children attending day-care centers or kindergarten or outpatients were penicillin resistant (3). Lower prevalences were found in carriage studies in Australia (3%) (25) and China (1.2%) (30). Widely different levels of penicillin resistance have been found in clinical and invasive isolates in different countries. They include more than 20% in Korea (4, 12, 26), Hong Kong (10, 14) and Taiwan (3); 5 to 20% in Singapore (11) and Australia (29); <5% in Japan (31), Bangladesh (23), China (30), and Malaysia (22); and none in Pakistan (15), the Philippines (2), and India (9).

A potential limitation of this study was that we were unable to incubate all the MIC plates in CO_2 as recommended (18).

TABLE 1. Results of susceptibility tests of the *S. pneumoniae* isolates overall and divided according to urban or rural location of the children

Drug	Age group (yr)	No. of isolates resistant/no. tested (%)			RR (95% confidence interval) (urban vs rural)
		Total	Urban	Rural	
Penicillin	All	76/399 (19)	73/211 (35)	3/188 (2)	21.7 (7.0–67.6)
	<5	60/191 (31)	60/163 (37)	0/28 (0)	
	≥5	16/208 (8)	13/48 (27)	3/160 (2)	14.4 (4.3–48.6)
Ceftriaxone	All	3/399 (0.8)	3/211 (1.4)	0/188 (0)	
	<5	3/191 (2)	3/163 (2)	0/28 (0)	
	≥5	0/208 (0)	0/48 (0)	0/160 (0)	
Erythromycin	All	201/399 (50)	168/211 (80)	33/188 (18)	4.5 (3.3–6.2)
	<5	149/191 (78)	142/163 (87)	7/28 (25)	3.5 (1.8–6.6)
	≥5	52/208 (25)	26/48 (54)	26/160 (16)	3.3 (2.1–5.2)
Trimethoprim-sulfamethoxazole	All	69/263 (26)	44/107 (41)	25/156 (16)	2.6 (1.7–3.9)
	<5	45/118 (38)	38/90 (42)	7/28 (25)	1.7 (0.8–3.4)
	≥5	24/145 (17)	6/17 (35)	18/128 (14)	2.5 (1.2–5.4)
Chloramphenicol	All	157/399 (39)	126/211 (60)	31/188 (17)	3.6 (2.6–5.1)
	<5	115/191 (60)	111/163 (68)	4/28 (14)	4.8 (1.9–11.9)
	≥5	42/208 (20)	15/48 (31)	27/160 (17)	1.8 (1.1–3.2)
Tetracycline	All	280/399 (70)	133/211 (63)	147/188 (78)	0.8 (0.7–0.9)
	<5	121/191 (63)	97/163 (60)	24/28 (86)	0.7 (0.6–0.9)
	≥5	159/208 (76)	36/48 (75)	123/160 (77)	1.0 (0.8–1.1)
Three or more antibiotic groups[a]	All	83/263 (32)	64/107 (60)	19/156 (12)	4.9 (3.1–7.7)
	<5	60/118 (51)	57/90 (63)	3/28 (11)	5.9 (2.0–17.4)
	≥5	23/145 (16)	7/17 (41)	16/128 (13)	3.3 (1.6–6.8)

[a] Multidrug-resistant isolates.

All the MICs were, however, determined with a growth control and an *S. pneumoniae* control strain. A comparison of the MICs for 160 isolates incubated in air and CO_2 showed no major classification errors and no systematic bias in the results.

The nasopharynx is the usual source of pneumococci in clinical disease, and it is thought that resistance in carriage isolates is potentially predictive of the emergence of resistance in clinically significant isolates (13, 16, 21). In this study, it was not possible to obtain nasopharyngeal samples. The overall carriage rate of 44%, however, was within the range reported for other studies in which nasopharyngeal isolates were obtained (3, 8, 16, 25), and the distribution of serotypes of *S. pneumoniae* was as expected for nasopharyngeal carriage isolates at this age. It is possible that pneumococci isolated from the anterior nares may have susceptibility patterns different from those in the posterior nasopharynx. In one study, pneumococci from nasal swabs tended to lead to an overestimation of the levels of resistance in invasive pneumococcal isolates (13).

Resistance to penicillin, ceftriaxone, erythromycin, chloramphenicol, and trimethoprim-sulfamethoxazole was significantly more common in isolates from urban children than in those from rural children. Tetracycline resistance was most common in rural children <5 years old. This urban-rural disparity was seen in a similar study in Pakistan (16) and probably reflects differences in availability and usage of antimicrobial agents (1, 21). Antimicrobial agents are available over the counter without prescription in Vietnam and were easily available for urban children in this study. For the children in the three rural sites, the nearest pharmacies were more than 5 km away. Urban overcrowding and use of day-care facilities in Ho Chi Minh City may also be important, as carriage and the spread of resistant strains are associated with overcrowding and day-care facilities (21).

Many of the penicillin-resistant and multidrug-resistant isolates were serotype 23. Penicillin resistance has been predominantly associated with serotypes 6B, 14, 19F, and 23F in Korea (4, 12, 26), 23F and 19F in Taiwan (24), 19F and 23F in Hong Kong (10, 14), and 19 in Singapore (11). Molecular typing studies have identified a number of clones of highly penicillin-resistant pneumococci that are also multidrug resistant; some of them have spread globally (27). The spread of the serotype 6B Spanish clone from Spain to Iceland and the serotype 23F Spanish clone to the United States, Mexico, Portugal, France, Croatia, South Africa, South Korea, and Taiwan is well described (24, 27). It is possible that the multidrug-resistant serotype 23 isolates in this study are related to isolates in other countries in the region and further afield.

If penicillin resistance starts to emerge in invasive isolates, it would have implications for the blind empirical therapy of pneumonia and meningitis. Penicillin cannot be relied on to

TABLE 2. Susceptibilities of the *S. pneumoniae* isolates categorized by penicillin susceptibility

Drug	No. (%) of isolates that were penicillin		
	Susceptible (n = 187)	Intermediate (n = 136)	Resistant (n = 76)
Ceftriaxone	0 (0)	0 (0)	3 (4)
Erythromycin	19 (10)	111 (82)	71 (93)
Trimethoprim-sulfamethoxazole[a]	19 (13)	24 (34)	26 (51)
Chloramphenicol	35 (19)	67 (49)	55 (72)
Tetracycline	147 (79)	103 (76)	30 (40)
Three or more antibiotic groups[a,b]	11 (8)	28 (39)	44 (86)

[a] Results for susceptibility testing of 263 isolates.
[b] Multidrug-resistant isolates.

TABLE 3. Serotypes of a sample of 125 *S. pneumoniae* isolates

Serotype	No. of isolates			
	Total	That were penicillin		
		Susceptible	Intermediate	Resistant
1	4	3	1	0
2	1	0	1	0
3	1	1	0	0
6	12	9	1	2
7	3	3	0	0
8	2	1	0	1
9	2	1	0	1
10	3	2	0	1
12	3	3	0	0
14	19	8	9	2
15	3	2	1	0
18	8	8	0	0
19	26	16	3	7
20	2	2	0	0
22	1	1	0	0
23	24	7	3	14
33	3	2	0	1
Nontypeable	8	0	5	3
Total	125	70	24	31

cure meningitis, although is still probably effective for pneumonia if given in an adequate dosage, and chloramphenicol cannot be recommended as an alternative treatment (5). The high cost of ceftriaxone or vancomycin will make treatment with these drugs unaffordable for many in Vietnam. In our isolates, penicillin resistance was strongly associated with resistance to erythromycin and trimethoprim-sulfamethoxazole, two recommended alternatives for treating acute respiratory infections. These data suggest that neither erythromycin nor co-trimoxazole could be reliably substituted for penicillin in penicillin-resistant pneumococcal respiratory infections unless there is clear evidence of susceptibility to these alternatives.

This study shows that there is a significant reservoir of resistance to antimicrobial agents in *S. pneumoniae* carried by Vietnamese schoolchildren. It is possible that penicillin resistance in clinical isolates of *S. pneumoniae* will become an important problem in Vietnam in the future as it has in several other countries in Asia. Strategies to prevent the emergence of clinically significant disease caused by drug-resistant *S. pneumoniae* in this region are urgently needed.

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