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Trends in Antimicrobial Resistance of *Streptococcus pneumoniae* in Japan

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A total of 184 isolates of *Streptococcus pneumoniae* were recovered from the sputa of patients over a 5-year period in the Nagasaki area and were examined. A total of 30 strains were resistant to penicillin (MIC, ≥0.10 μg/ml), 13 of which belonged to serotype 19B. These strains showed decreased susceptibility to other antimicrobial agents. Vancomycin, cefpirome, and imipenem were the most active agents tested.

Streptococcus pneumoniae is a major cause of pneumonia, together with *Haemophilus influenzae* and *Moraxella catarrhalis*. *S. pneumoniae* remains the leading cause of death in patients with pneumonia, despite antibiotic therapy. It has been estimated that 150,000 to 570,000 cases of pneumococcal pneumonia occur in the United States and that 5% of individuals with pneumococcal pneumonia die each year (2). Recently, penicillin-resistant pneumococci (PRP) have been isolated all over the world, and the incidence of these strains has risen alarmingly (1, 7–10, 14, 16). Little information on PRP has been reported in Asia (3–5, 11, 15).

Consecutive clinical isolates of *S. pneumoniae* recovered from the sputa of colonized patients in Nagasaki University Hospital between 1988 and 1992 were studied. Numerous isolates recovered from the same patient were considered one isolate. Identification was based on colony morphology, results of a Gram staining, bile solubility, and optochin susceptibility (12). Serotyping was performed by detection of the quellung reaction with specific antisera from the Staten Seruminstitut (Copenhagen, Denmark).

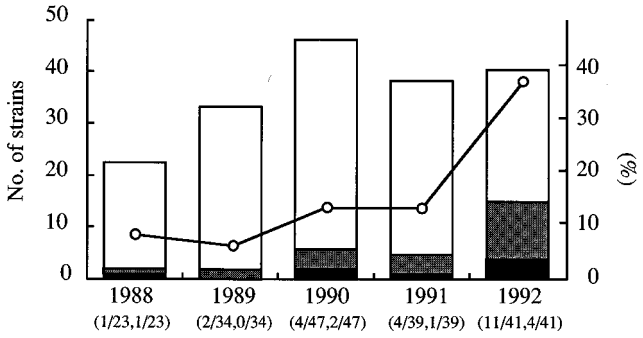

FIG. 1. Frequency of occurrence of PSP, PIR, and PRP isolates in hospitalized patients. Open, shaded, and solid bars represent the total numbers of PSP, PIR, and PRP strains, respectively, isolated from 1988 to 1992. Open circles represent the percentages of PIR and PRP among isolated pneumococci. The ratios below each year are ratios of PIR and PRP strains to the total numbers of pneumococcal strains isolated. One strain isolated in 1989 and two strains isolated in 1990 failed to grow in subculture and were not recovered for further study (see Table 1).

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MICs for the isolates of *S. pneumoniae* were determined by the National Committee for Clinical Laboratory Standards-recommended broth microdilution method (13) with Mueller-Hinton broth (Difco) plus 3% lysed horse blood. Inocula were prepared by suspending organisms grown during 20 to 24 h of incubation in 5% CO_2 on sheep blood agar plates. The MICs for penicillin-susceptible pneumococci (PSP) were ≤0.06 μg/ml, the MICs for intermediate-resistant pneumococci (PIR) ranged from 0.1 to 1.0 μg/ml, and the MICs for PRP were ≥2.0 μg/ml. In all our studies, *S. pneumoniae* ATCC 49619, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922 were used as the quality control strains.

The following antimicrobial agents were tested: penicillin G (0.008 to 16 μg/ml), ampicillin (0.008 to 16 μg/ml), cefaclor (0.06 to 128 μg/ml), cefazolin (0.016 to 32 μg/ml), cefotiam (0.016 to 32 μg/ml), cefpirome (0.008 to 16 μg/ml), imipenem (0.004 to 8 μg/ml), erythromycin (0.03 to 64 μg/ml), clarithromycin (0.03 to 64 μg/ml), minocycline (0.06 to 128 μg/ml), vancomycin (0.004 to 8 μg/ml), and ofloxacin (0.06 to 64 μg/ml).

A total of 184 isolates were isolated over a 5-year period. Twenty-two isolates were PIR and eight were PRP. The number and the prevalence of PIR and PRP have been increasing for 5 years (Fig. 1). 2 (8.7%), 2 (5.9%), 6 (12.8%), 5 (12.8%), and 15 (36.6%) of the total number of strains isolated in 1988, 1989, 1990, 1991, and 1992, respectively, were resistant to pen-

TABLE 1. Trends in serotypes of 27 PRP clinical strains isolated from patients in Nagasaki University Hospital between 1988 and 1992

Serotype	No. (%) of strains	No. of strains belonging to each serotype isolated in:				
		1988	1989	1990	1991	1992
6B	1 (3.7)	0	0	0	0	1
14	1 (3.7)	0	0	0	0	1
19B	13 (48.1)	1	0	2	3	7
19C	3 (11.1)	0	0	2	1	0
19F	3 (11.1)	0	0	0	0	3
23F	6 (22.2)	1	1	0	1	3
Total	27 (100)	2	1	4	5	15

TABLE 2. Antimicrobial susceptibilities of *S. pneumoniae* isolated from patients in Nagasaki University Hospital between 1988 and 1992

Antimicrobial agent	MIC (μg/ml)[a]					
	PSP (20 strains)			PRP (27 strains)		
	Range	50%	90%	Range	50%	90%
Penicillin G	0.016–0.06	0.03	0.06	0.13–2	1	2
Ampicillin	0.016–0.13	0.03	0.06	0.06–4	1	2
Cefaclor	0.25–2	0.5	1	0.5–≥128	16	64
Cefazolin	0.06–0.5	0.13	0.25	0.5–4	2	4
Cefotiam	0.13–1	0.25	0.5	0.25–8	4	8
Cefpirome	0.016–0.25	0.03	0.13	0.06–1	0.5	0.5
Imipenem	≤0.004–0.03	0.008	0.016	0.016–0.25	0.06	0.25
Erythromycin	0.03–64	0.13	64	0.03–64	2	4
Clarithromycin	0.03–64	0.06	64	0.03–64	1	2
Minocycline	0.06–32	16	32	0.06–32	8	32
Vancomycin	0.25–0.5	0.25	0.5	0.13–0.5	0.25	0.5
Ofloxacin	1–64	2	2	1–16	1	2

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively.

icillin. There were no significant differences regarding age, sex, length of hospitalization, β-lactam therapy, and underlying conditions among 30 patients with PRP and 154 patients with PSP (data not shown).

The serotype distribution of the 27 PIR and PRP isolates that were recovered is presented in Table 1. Of the 27 isolates which were tested, 48.1 and 22.2% belonged to serotypes 19B and 23F, respectively. The increase of PIR and PRP corresponded to an increase of strains which belonged to serogroup 19B.

For further testing of susceptibility to other antimicrobial agents, 20 strains were picked at random from PSP. Data on the susceptibilities of these 20 PSP strains are summarized in Table 2. Ampicillin exhibited activity similar to that of penicillin G. The MICs of the four cephalosporins, imipenem, and vancomycin were low for all 20 PSP strains. A total of 4 strains were intermediately resistant to erythromycin (MIC, 1.0 to 2.0 μg/ml), and 6 strains were resistant to erythromycin (MIC, ≥4.0 μg/ml). Clarithromycin exhibited activity similar to that of erythromycin. Minocycline was active against 7 strains (MICs, 0.06 to 0.5 μg/ml), but 13 strains were resistant (MICs, 8 to 32 μg/ml). The MIC range of ofloxacin was 1 to 16 μg/ml for 20 PSP strains.

The antibiotic susceptibilities of 27 PIR and PRP strains are shown in Table 2. These strains also showed decreased susceptibility to other β-lactam agents, although the MICs of cefpirome (MIC at which 50% of the isolates are inhibited [MIC_{50}] and MIC_{90}, 0.5 μg/ml) were two to four times lower than those of penicillin G. A total of 7 strains (25.9%) were intermediately resistant to erythromycin (MIC, 1 to 2 μg/ml), and 13 strains (48.1%) were resistant to erythromycin (MIC, ≥4 μg/ml). One strain was intermediately resistant to minocycline (MIC, 4 μg/ml), and 22 strains (81.5%) were resistant to minocycline (MIC, ≥8.0 μg/ml). Pneumococci with resistance to at least three different classes of antibiotics are defined as multiply resistant (6). Twenty strains (74.1%) of PIR and PRP were resistant to erythromycin and minocycline. All 27 PIR and PRP strains were susceptible to vancomycin (MICs, ≤1.0 μg/ml), and the MICs of imipenem for all were low (MIC_{50}, 0.06 μg/ml, and MIC_{90}, 0.25 μg/ml). Four strains were intermediately resistant to imipenem (MICs, 0.25 to 0.5 μg/ml) (13). The range of MICs for ofloxacin was 1 to 64 μg/ml for 27 PIR and PRP strains.

Ten of the serogroup 19B isolates were multiply resistant (resistant to penicillin, erythromycin, and minocycline). These data revealed the possibility that the serotype 19B strains could have been transmitted among our patients or that they were derived from a common source, similar to the multiply resistant strain of serotype 6B, which spread from Spain to Iceland (17). We need to examine the epidemic spread of serotype 19B isolates in our hospital by molecular techniques, and we also need to investigate the similarities between pneumococci isolated in our hospital and isolates from other hospitals in Japan and nearby countries.

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