JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1994, p. 2572–2575
0095-1137/94/$04.00+0

Vol. 32, No. 10

Pneumococcal Resistance to Antimicrobial Agents in the Province of Québec, Canada

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Received 30 March 1994/Returned for modification 28 June 1994/Accepted 26 July 1994

The serogroup/serotypes (SGTs) and antimicrobial susceptibilities to 10 antimicrobial agents of 110 clinical strains of *Streptococcus pneumoniae* were determined. Strains intermediately resistant or highly resistant to penicillin G (80 of 110) belonged predominantly to SGTs 23 (45.0%), 19 (13.7%), 6 (10.0%), 9 (6.2%), and 14 (3.7%). The MICs of all cephalosporins, tetracycline, trimethoprim-sulfamethoxazole, and chloramphenicol increased along with the MICs of penicillin G. However, erythromycin resistance and clindamycin resistance were observed more frequently among the intermediately penicillin-resistant strains. Multiple resistance was observed for 32 strains, of which 25 were highly resistant to penicillin G and belong to SGT 23F. All strains were susceptible to vancomycin.

Infections due to *Streptococcus pneumoniae* continue to be a significant cause of morbidity and mortality (21, 23). Penicillin is considered the drug of choice for the treatment of these infections (21). However, recent studies have brought attention to the increasing penicillin resistance of *S. pneumoniae* strains worldwide, including in the United States (2, 5, 6, 8, 9, 19). In Canada, only a few cases of serious pneumococcal disease caused by strains with reduced susceptibilities to penicillin G have been reported (1, 7), and previous data for the province of Québec have indicated a prevalence of less than 4% for these strains (12).

Resistance to penicillin may occur in combination with resistance to other antimicrobial agents (9). For pneumococci, multiple resistance was initially reported in 1977 in South Africa (3) and subsequently in Europe (5, 11, 18) as well as the United States (27). In Canada, the first multiresistant strain was reported in 1983 (15). Penicillin-resistant and multiple-resistant strains belonged predominantly to serogroup/serotypes (SGTs) 6, 19, 14, and 23 (11, 14, 28), with SGT 23F being the most associated with multiresistance (17, 25).

The high number of SGT 23 *S. pneumoniae* strains with reduced susceptibilities to penicillin G received during the last 4 years at the Laboratoire de santé publique du Québec (LSPQ) prompted us to determine the susceptibilities of *S. pneumoniae* strains to antimicrobial agents. The objectives of this study were to verify the presence of multiresistant *S. pneumoniae* strains isolated in our province and to identify the SGTs to which they belong.

A total of 110 strains of *S. pneumoniae* were selected from 220 isolates (one per patient from 31 hospitals) received at the LSPQ for serotyping and/or antimicrobial susceptibility testing between January 1989 and December 1992. The penicillin G susceptibilities of 175 of 220 isolates were determined. All the strains confirmed to have reduced susceptibilities to penicillin G (n = 80) were included in the study. In addition, 30 randomly chosen penicillin-susceptible strains were added as a comparative group. Strains were identified by standard bacteriological methods (4). They were kept frozen at −60°C in tryptic soy broth containing 10% (vol/vol) glycerol for further analysis. Serotyping was performed by the capsular swelling method (Quellung reaction) (4) with 46 antisera available from the Statens Serum Institute of Copenhagen, Denmark. The SGTs of 54 strains were confirmed by Marguerite Lovgren, National Centre for *Streptococcus*, University of Alberta, Edmonton, Canada.

Penicillin G susceptibility was determined by the broth microdilution method in cation-adjusted Mueller-Hinton broth supplemented with 3% lysed horse blood, and susceptibility testing for other antimicrobial agents was performed by the agar dilution method, both in accordance with the methods of the National Committee for Clinical Laboratory Standards (24). For the agar dilution method, final inocula of 10^4 CFU were delivered onto the surfaces of Mueller-Hinton agar plates enriched with 5% sheep blood by using a Cathra replicator. For trimethoprim-sulfamethoxazole (TMP-SMX) susceptibility testing, media were supplemented with 5% lysed horse blood. Plates were incubated at 35°C for 20 to 24 h in an ambient air incubator, except for two strains which required an atmosphere of 5 to 7% CO_2 for adequate growth. Standard control strains (24) were included in each run.

The following antimicrobial agents were tested: cefaclor, cefuroxime sodium, cefotaxime, chloramphenicol, erythromycin, clindamycin, ofloxacin, penicillin G, tetracycline, TMP-SMX, and vancomycin. Antibiotic powders were purchased from Sigma Chemical Co., St. Louis, Mo., except for the penicillin G and ofloxacin obtained from GIBCO Laboratories, Grand Island, N.Y., and Ortho McNeil Inc., Don Mills, Canada, respectively.

The MIC was defined as the lowest concentration of antimicrobial agent that completely inhibited growth (24), except in the case of TMP-SMX, for which an obvious 80 to 90% diminution of growth was interpreted as the endpoint (4). The interpretative criteria for the MICs of penicillin G were as follows: ≤0.06 μg/ml, susceptible; 0.12 to 1 μg/ml, intermediately resistant; ≥2 μg/ml, highly resistant. Pneumococcal strains were defined as multiply resistant to antimicrobial

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TABLE 1. SGT distribution of 110 strains of *S. pneumoniae* according to their susceptibilities to penicillin G

SGT	No. of strains that were:		
	Susceptible	Intermediately resistant	Highly resistant
6	8	7	1
9	2	1	4
14	4	1	2
19	3	11	0
23A	0	2	0
23F	3	8	26
Other[a]	8	7	0
Nontypeable	2	9	1

[a] SGTs 3, 4, 8, 10, 11, 15, 20, 22, 29/35, and 34.

agents if they showed resistance to three or more different groups of antibiotics (13).

Strains were isolated from blood (44.5%), cerebrospinal fluid (2.7%), respiratory tract specimens (38.2%), eyes (6.4%), or other sites (8.2%). Among strains isolated from normally sterile body fluids, 20 were intermediately resistant and 11 were highly resistant to penicillin G. Of the 46 intermediately penicillin-resistant and 34 highly penicillin-resistant strains, the most frequently observed SGTs were 23 (45.0%), 19 (13.7%), 6 (10.0%), 9 (6.2%), and 14 (3.7%) (Table 1). The highly resistant strains belonged predominantly (26 of 34) to SGT 23, all of which were confirmed to be SGT 23F.

The resistance of *S. pneumoniae* strains to other antimicrobial agents according to their susceptibilities to penicillin G is presented in Table 2. The MICs of all cephalosporins, tetracycline, TMP-SMX, and chloramphenicol increased along with the MICs of penicillin G. All highly penicillin-resistant strains were also resistant to cefaclor and cefuroxime (second-generation cephalosporins) as well as intermediate (31 of 34) or resistant (3 of 34) to cefotaxime. Most of these strains were also resistant to chloramphenicol (24 of 34), tetracycline (26 of 34), and TMP-SMX (32 of 34). However, erythromycin resistance and clindamycin resistance were observed more frequently among the intermediately penicillin-resistant strains, with 8 of 46 and 5 of 46 strains, respectively. All strains were susceptible to vancomycin and ofloxacin, except for one penicillin-susceptible strain which was intermediate to ofloxacin. Overall, 13.3% of penicillin-susceptible strains, 58.7% of intermediately penicillin-resistant strains, and 100% of highly penicillin-resistant strains were intermediate or resistant to one or more of the other antimicrobial agents tested. Multiple resistance was observed for 32 strains, all with reduced susceptibilities to penicillin G. Six of these strains were intermediately resistant to penicillin and belonged to SGTs 6 (two strains) and 22 (one strain) or were nontypeable (three strains). The other 26 strains were highly resistant to penicillin and belonged to SGTs 23F (25 strains) and 14 (1 strain). Among SGT 23F multiresistant strains, 24 strains showed the same major multiresistance pattern of resistance to cefaclor, cefuroxime, chloramphenicol, tetracycline, and TMP-SMX and intermediate susceptibility to cefotaxime.

TABLE 2. In vitro susceptibilities of 110 strains of *S. pneumoniae* to 10 antimicrobial agents according to their susceptibilities to penicillin G

Susceptibility to penicillin G (no. of isolates)	Antimicrobial agent (resistance breakpoint [μg/ml])	MIC (μg/ml)[a]			% Resistant
		Range	50%	90%	
Susceptible (30)	Cefaclor (≥32)	1–2	1	1	0
	Cefuroxime (≥2)	≤0.03–0.12	≤0.03	≤0.03	0
	Cefotaxime (≥2)	≤0.015–0.06	0.03	0.03	0
	Chloramphenicol (≥16)	1–16	2	4	3.3
	Erythromycin (≥4)	≤0.015–0.06	0.03	0.06	0
	Clindamycin (≥4)	≤0.03–0.06	0.06	0.06	0
	Ofloxacin (≥8)	2–4	2	2	0
	Tetracycline (≥8)	0.12–>32	0.25	0.5	3.3
	TMP-SMX[b] (≥4/76)	≤0.12/2.3–>4/76	0.5/9.5	2/38	10
	Vancomycin (ND)[c]	0.25–0.5	0.5	0.5	0
Intermediately resistant (46)	Cefaclor	0.5–64	2	8	2.2
	Cefuroxime	≤0.03–4	0.25	1	4.3
	Cefotaxime	0.03–0.5	0.12	0.25	0
	Chloramphenicol	1–4	2	4	0
	Erythromycin	0.03–>8	0.06	>8	17.4
	Clindamycin	≤0.03–>16	0.06	2	10.9
	Ofloxacin	1–2	2	2	0
	Tetracycline	0.25–>32	0.5	>32	19.6
	TMP-SMX	≤0.12/2.3–>4/76	1/19	>4/76	41.3
	Vancomycin	0.25–0.5	0.5	0.5	0
Highly resistant (34)	Cefaclor	32–>64	>64	>64	100
	Cefuroxime	2–8	8	8	100
	Cefotaxime	0.5–2	1	1	8.8
	Chloramphenicol	2–16	16	16	70.6
	Erythromycin	0.03–>8	0.03	0.06	2.9
	Clindamycin	≤0.03–>16	0.06	0.06	2.9
	Ofloxacin	2	2	2	0
	Tetracycline	0.25–>32	32	>32	76.5
	TMP-SMX	0.25/4.75–>4/76	>4/76	>4/76	94.1
	Vancomycin	0.25–0.5	0.5	0.5	0

[a] 50% and 90%, MICs for 50 and 90% of the strains tested, respectively.
[b] Ratio of TMP to SMX was 1:19.
[c] ND, not determined. All strains were susceptible, with a MIC of ≤0.5 μg/ml.

Although this study did not determine the prevalence of *S. pneumoniae* strains with reduced susceptibilities to penicillin G, the data clearly suggest an increase in the absolute number of these strains in the province of Québec. Indeed, during the surveillance of invasive *S. pneumoniae* infections in Québec from 1984 to 1986 (12), only 6 of 468 strains isolated from sterile body fluids were found to have reduced susceptibilities to penicillin G. For this study, 31 such strains isolated from blood or cerebrospinal fluid were received at the LSPQ. As reported elsewhere (9, 14, 28), we found that most intermediately resistant and highly resistant strains belong to SGTs 23, 19, 6, 9, and 14. These SGTs were found in 78.7% of the strains with reduced susceptibilities to penicillin G, a proportion similar to those observed in the United States (79.6%) (27), Spain (84.4%) (10), and France (82.0%) (11).

On the basis of serogroups, 85% of the strains with reduced susceptibilities to penicillin G were included in the 23-valent pneumococcal polysaccharide vaccine.

Highly penicillin-resistant strains showed higher rates of resistance to chloramphenicol, tetracycline, and TMP-SMX, and these results are similar to those previously reported in Spain (17) and the United States (25). This report reaffirms that the susceptibilities to all cephalosporins decrease with increasing penicillin G MICs (16, 26). Resistance to erythromycin and clindamycin was observed more frequenly among intermediately penicillin-resistant strains, often in association with tetracycline resistance. This multiresistance pattern (tetracycline, erythromycin, and clindamycin) was also observed by Klugman and Koornhof in South Africa for penicillin-susceptible *S. pneumoniae* strains (14). As expected, all strains were susceptible to vancomycin.

In our study, most of the highly resistant strains belonged to SGT 23F (76.5%), which has already been associated with penicillin resistance as well as multiresistance (17, 22). This serotype was isolated in about 36% of the penicllin-resistant pneumococci in Spain during 1988 and in 49.3% of those isolated in France between 1984 and 1990 (11). The majority of our multiresistant SGT 23F strains showed a similar profile of resistance to penicillin G, decreased susceptibilities to cephalosporins, and resistance to chloramphenicol, tetracycline, and TMP-SMX. To our knowledge, this is the first report in Canada to describe this specific resistance profile for *S. pneumoniae* strains. In addition, these strains differ from the first multiresistant pneumococci isolated in our country, which were resistant to penicillin G, chloramphenicol, and tetracycline and belonged to SGT 6B (15). However, the resistance profile observed in this study has already been reported for SGT 23F strains isolated in Spain (10, 17) and the United States (20, 25). Furthermore, Munoz et al. (22) demonstrated that multiple-antibiotic-resistant SGT 23F pneumococci present in Spain and Ohio were clonally related, suggesting that this antibiotic-resistant clone had spread intercontinentally from Spain to the United States. Further analyses will be necessary to determine if our SGT 23F multiresistant strains are clonally related to those of Spain and Ohio.

In this study, three pneumococcal isolates (two from blood and one from sputum) were interpreted as resistant to cefotaxime, with MICs of 2 μg/ml. Unfortunately, the clinical outcomes and therapeutic regimens of these patients were unknown.

In conclusion, our data clearly indicate the presence and possible increase of penicillin resistance and multiple resistance among clinical isolates of *S. pneumoniae* in the province of Québec. This situation makes it mandatory for all laboratories to put more effort into the antimicrobial susceptibility testing of *S. pneumoniae* strains, particularly those resistant by the oxacillin disk screening method, and to determine promptly the susceptibilities to various agents in cases of serious infections. Finally, most of the SGTs of the strains with reduced susceptibilities to penicillin G were found in the 23-valent pneumococcal polysaccharide vaccine, indicating the potential usefulness of the vaccine in the Québec population.

We acknowledge Johanne Boilard and Martial Demers for their technical assistance, Luc Massicotte for the supervision of medium preparation, and Manon Lorange for identification expertise. We also thank all the technicians in hospital microbiology laboratories who sent us pneumococcal strains. Finally, we acknowledge Marguerite Lovgren of the National Centre for *Streptococcus*, University of Alberta, Edmonton, Canada, for confirmations of the SGTs of 54 strains included in this study.

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