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Canadian National Survey of Prevalence of Antimicrobial Resistance among Clinical Isolates of *Streptococcus pneumoniae*

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The antimicrobial susceptibilities of 1,089 clinical isolates of *Streptococcus pneumoniae* obtained from 39 laboratories across Canada between October 1994 and August 1995 were determined. A total of 91 isolates (8.4%) demonstrated intermediate resistance (MIC, 0.1 to 1.0 µg/ml) and 36 (3.3%) had high-level resistance (MIC, ≥2.0 µg/ml) to penicillin. Penicillin-resistant strains were more likely to have been recovered from normally sterile sites ($P = 0.005$) and to be cross-resistant to several β-lactam and non-β-lactam antimicrobial agents ($P < 0.05$). These results indicate that there has been a recent significant increase in the prevalence of antibiotic-resistant *S. pneumoniae* in Canada.

In the past few years, the incidence of invasive infections such as bacteremia and meningitis due to *Streptococcus pneumoniae* has been rising (2, 6). These infections continue to be associated with considerable morbidity and mortality. There has also been a recent worldwide increase in the incidence of pneumococcal resistance to several antimicrobial agents, including penicillin and other β-lactams (1, 3, 4, 7, 10, 12, 14). Until very recently, resistant pneumococci were infrequently identified in Canada. Three large Canadian surveys of pneumococcal susceptibility in the 1970s and 1980s found rates of resistance to penicillin of 2.4, 1.3, and 1.5% in Alberta (5), Quebec (13), and Ontario (15), respectively. Furthermore, only intermediate resistance to penicillin (MIC, 0.1 to 1.0 µg/ml) had been detected; high-level resistance to penicillin (MIC, ≥2.0 µg/ml) was not detected in any of these studies. An increase in penicillin resistance was first noted in southern Ontario in late 1993 (20). Therefore, we set out to determine the prevalence of antimicrobial resistance among clinical isolates of *S. pneumoniae* in Canada.

A total of 30 hospital-based and 9 privately owned community microbiology laboratories from across Canada participated in the study by submitting consecutive isolates of *S. pneumoniae* recovered from patients between October 1994 and August 1995. Only single isolates from different patients were included in the study. Identification of isolates as *S. pneumoniae* was confirmed by using standard techniques, including Gram stain characteristics, colonial morphology, ethylhydrocuprein susceptibility, bile solubility, and the Pneumoslide Test

(Becton Dickinson Microbiology Systems, Cockeysville, Md.). Pneumococcal isolates were serotyped by the National Reference Centre for Streptococcus (Edmonton, Alberta, Canada) on the basis of capsular swelling with type-specific antisera.

All isolates were stored frozen at −70°C. Prior to susceptibility testing, isolates were thawed and subcultured onto blood agar twice. In vitro susceptibility testing was done by a broth microdilution procedure in accordance with National Committee for Clinical Laboratory Standards guidelines (16, 17), by using cation-adjusted Mueller-Hinton broth (Becton Dickinson Microbiology Systems) with 5% lysed horse blood and an inoculum of 10^5 CFU/ml. MICs (defined as the lowest concentration of antibiotic that completely inhibited visible growth) were determined after incubation for 20 to 24 h at 35°C in ambient air. *Streptococcus pneumoniae* ATCC 6303 (penicillin susceptible), *S. pneumoniae* ATCC 49619 (penicillin resistant), *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212 were used as control strains. The 17 antimicrobial agents tested (penicillin G, cefuroxime, cefotaxime, ceftriaxone, ceftazidime, cefpirome, cefpodoxime, imipenem, erythromycin, clindamycin, tetracycline, chloramphenicol, ciprofloxacin, ofloxacin, levofloxacin, trimethoprim-sulfamethoxazole,

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TABLE 1. Sources of 1,089 *S. pneumoniae* isolates collected across Canada from October 1994 to August 1995

Specimen origin (no.)	No. (%) of isolates	
	Penicillin susceptible	Intermediately or highly resistant to penicillin
Sterile sites (120)	96 (80.0)	24 (20.0)[a]
Blood (97)	79	18
Other (23)	17	6
Nonsterile sites (913)	817 (89.5)	96 (10.5)[a]
Upper respiratory tract (539)	492	47
Lower respiratory tract (365)	316	49
Other (9)	9	0
Not known (56)	49	7
Total	962 (88.3)	127 (11.7)

[a] $P = 0.005$ for sterile-site isolates versus nonsterile-site isolates.

TABLE 2. Antimicrobial susceptibilities of 962 penicillin-susceptible, 91 intermediately penicillin-resistant, and 36 highly penicillin-resistant *S. pneumoniae* isolates collected across Canada from October 1994 to August 1995

Antimicrobial agent	Penicillin MIC (µg/ml)	No. of isolates for which the MIC (µg/ml) of penicillin was:										
		0.03	0.06	0.12	0.25	0.5	1.0	2.0	4.0	8.0	16	32
Penicillin	<0.12	866	96									
	0.12–1.0			40	21	8	22					
	≥2.0							26	10			
Cefuroxime[a]	<0.12		651	297	8	5	1					
	0.12–1.0			5	30	23	13	10	7	2	1	
	≥2.0							3	12	20	1	
Cefpodoxime[b]	<0.12		935	21	2	1	3					
	0.12–1.0		15	14	25	19	12	4	2			
	≥2.0					2	5	10	18	1		
Cefotaxime[a]	<0.12	869	70	19	4							
	0.12–1.0	10	12	25	17	20	6	1				
	≥2.0			1	3	4	18	10				
Ceftriaxone[a]	<0.12	869	87	5	1							
	0.12–1.0	4	21	14	27	19	5	1				
	≥2.0					8	20	8				
Ceftazidime[b]	<0.12	61	40	347	470	36	2	5	1			
	0.12–1.0			11	3	26	21	10	13	7		
	≥2.0							1	10	25		
Cefpirome[b]	<0.12	915	41	3	2	1						
	0.12–1.0	34	6	31	10	9	1					
	≥2.0				7	20	9					
Imipenem[a]	<0.12	958	3	1								
	0.12–1.0	51	12	8	19	1						
	≥2.0			12	14	9	1					
Erythromycin[a]	<0.12			906	36	4	3	2	5	4	2	
	0.12–1.0				80	2		2	4	1	1	1
	≥2.0				27	3		3	2			1
Clindamycin[a]	<0.12				954				3	3	2	
	0.12–1.0				86				2	1	1	1
	≥2.0				32						1	3
Tetracycline[a]	<0.12							944		2	14	2
	0.12–1.0							72		1	5	13
	≥2.0							27		2	3	4
Chloramphenicol[a]	<0.12							902	55	2	3	
	0.12–1.0							74	12	2	3	
	≥2.0							29		3	4	
Ciprofloxacin[b]	<0.12			94	21	571	253	16	4	3		
	0.12–1.0				4	22	55	12	1	2		
	≥2.0				1	9	20	4	1	1		
Ofloxacin	<0.12				7	60	673	219	1	2		
	0.12–1.0				1	2	19	66	2	1		
	≥2.0					1	8	24	1	2		
Levofloxacin[b]	<0.12			38	19	626	272	5		2		
	0.12–1.0				1	31	56	1	1	1		
	≥2.0					12	20	3		1		
Trimethoprim-sulfamethoxazole[a]	<0.12				658	187	39	48	12	12	6	
	0.12–1.0				20	22	5	7	6	10	18	3
	≥2.0					1	1	1	3	7	21	2
Vancomycin	<0.12					962						
	0.12–1.0					91						
	>2.0					36						

[a] $p \leq 0.05$ for antimicrobial resistance of penicillin-susceptible versus penicillin-resistant strains.
[b] No current National Committee for Clinical Laboratory Standards-recommended criteria for intermediate and high-level resistance categories.

and vancomycin) were supplied by the respective manufacturers.

The chi square test or Fisher's exact test was used, as appropriate, to determine the significance of differences between groups.

A total of 1,089 clinical isolates of *S. pneumoniae* were examined. The number of isolates contributed by each of the 39 participating laboratories varied from a low of 11 to a maximum of 124 isolates, with a mean of 30 isolates per laboratory. A total of 443 isolates (41%) were obtained from children under the age of 16 years, whereas 502 isolates (46%) were from those 16 years of age or older. The age of the patient was unknown for 144 (13%) of the isolates.

Reduced penicillin susceptibility was detected in 127 (11.7%) of the isolates: 91 (8.4%) with intermediate resistance and 36 (3.3%) with high-level resistance. Resistance (MIC, ≥ 2.0 μg/ml) to cefotaxime or ceftriaxone was detected in 1% of isolates; 2% of isolates had intermediate susceptibility (MIC = 1.0 μg/ml). Resistance to these extended-spectrum cephalosporins occurred only in strains with reduced susceptibility to penicillin. Antimicrobial resistance rates varied across the country, increasing from east to west. In three Atlantic provinces (Prince Edward Island, New Brunswick, and Nova Scotia), 13 (7.4%) of 176 isolates were resistant to penicillin. Resistance rates increased to 10.3% (58 of 565) for isolates recovered in central Canada (Quebec, Ontario, and Manitoba), and to 16.1% (56 of 348) for isolates from Western Canada (Saskatchewan, Alberta, and British Columbia) ($P = 0.001$). As shown in Table 1, 20% of 120 sterile-site isolates of *S. pneumoniae* were resistant to penicillin, compared with 10.5% of 913 nonsterile-site isolates ($P = 0.005$).

The in vitro activities of the antimicrobial agents tested against the 1,089 study isolates categorized by penicillin susceptibility are summarized in Table 2. Penicillin-susceptible strains of *S. pneumoniae* were generally also susceptible to the other antimicrobial agents tested, whereas penicillin-resistant strains were much more likely to be resistant to other antibiotics. For example, all penicillin-susceptible pneumococcal isolates were also susceptible to the other β-lactam agents tested. However, 100, 28, and 22% of the strains with high-level penicillin resistance were resistant to cefuroxime, cefotaxime, and ceftriaxone, respectively. Resistance to many of the non-β-lactam antibiotics was also seen much more frequently in penicillin-resistant isolates (Table 2). Isolates were uniformly susceptible only to vancomycin.

Forty-six randomly selected penicillin-resistant pneumococcal strains were serotyped and found to belong to eight different serotypes: 9V (11 strains), 6B (9 strains), 23F (8 strains), 19A (7 strains), 19F (6 strains), 14 (3 strains), 6A (1 strain), and 21 (1 strain). There was no apparent geographic clustering of any particular serotype.

The results of this investigation confirm that the prevalence of antimicrobial resistance in clinical isolates of *S. pneumoniae* has increased dramatically in Canada in just a few years. Although resistance was detected in all parts of the country, the prevalence of resistance was not uniformly distributed, with a trend for somewhat higher resistance rates in the western prairie provinces. A diversity of serotypes was present, including several which are included in the currently available polyvalent pneumococcal vaccine.

Pneumococcal strains from normally sterile sites (blood, cerebrospinal fluid, pleural fluid, etc.) were almost twice as likely to be resistant to penicillin as were isolates from nonsterile sites ($P = 0.005$), confirming the importance of drug resistance in invasive *S. pneumoniae* infections (12). Slightly more than one-quarter of the resistant isolates demonstrated high-level

resistance (MIC, ≥ 2.0 μg/ml) to penicillin. Not surprisingly, since the mechanism of resistance is related to altered penicillin-binding proteins with reduced affinity for β-lactam drugs (9, 11), these strains are also more likely to have reduced susceptibility to other β-lactams, including extended-spectrum cephalosporins. As previously described (1, 12, 14, 18), increased resistance to other classes of antimicrobial agents, such as macrolides, tetracyclines, chloramphenicol, and trimethoprim-sulfamethoxazole, was also detected in the current study.

Limited clinical experience and the lack of prospective controlled trials have hindered the development of recommendations for the management of pneumococcal infection due to penicillin-resistant strains. The treatment of otitis media is hampered by the uncertainty of adequate antibiotic penetration into middle ear effusions (18). For bacterial meningitis that may be due to penicillin-resistant *S. pneumoniae*, recommended empiric treatment is either with ceftriaxone plus vancomycin or with cefotaxime plus vancomycin (8, 19). However, vancomycin therapy may be suboptimal because its penetration into cerebrospinal fluid is variable and treatment failure with vancomycin therapy has been reported (22). Treatment with high doses of cefotaxime has been used successfully in a small number of adult patients with meningitis due to *S. pneumoniae* with decreased susceptibilities to penicillin and extended-spectrum cephalosporins (21). Further studies are required to determine whether treatment with newer investigational compounds or combinations of antimicrobial agents would be beneficial.

The recent increase in the prevalence of penicillin- and cephalosporin-resistant *S. pneumoniae* as a cause of serious and life-threatening infection in Canada and the United States emphasizes the need for continued surveillance to determine local antimicrobial susceptibility data and to identify changing patterns of resistance. These data are essential for developing appropriate guidelines for empiric treatment of pneumococcal infections. Greater use of pneumococcal vaccine, especially for those at high risk of developing severe pneumococcal infections, should also be promoted while ongoing efforts are directed at the development of a new conjugate vaccine with improved efficacy.

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