Antimicrobial-resistant *Streptococcus pneumoniae* in Canadian hospitals: Results from the 2007 CANWARD study

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BACKGROUND: The Canadian Ward Surveillance Study (CANWARD 2007) tested isolates collected from January to December 2007 from 12 Canadian hospitals to a range of antimicrobial agents. The present paper focuses on antimicrobial resistance in *Streptococcus pneumoniae* in Canadian hospitals, with an emphasis on macrolide resistance.

METHODS: Minimum inhibitory concentrations of antimicrobial agents were determined using the broth microdilution method and interpreted according to Clinical and Laboratory Standards Institute breakpoints. Macrolide-nonsusceptible strains (clarithromycin minimum inhibitory concentrations 0.5 µg/mL or greater) were analyzed by multiplex polymerase chain reaction for the presence of *mef*A and *erm*B genes.

RESULTS: *S pneumoniae* represented 9.0% (706 of 7881) of all isolates collected in CANWARD 2007. Of the 706 *S pneumoniae* isolates collected, 33.1% (234) were from blood and 66.9% (472) were from respiratory specimens. The overall resistance (resistant and intermediate) rates for *S pneumoniae* isolated from respiratory and blood specimens, respectively, were: penicillin (23.9%, 14.4%), clarithromycin (22.1%, 12.6%), trimethoprim-sulfamethoxazole (14.7%, 11.5%), doxycycline (7.8%, 5.1%) and clindamycin (7.1%, 3.3%). Multidrug resistance (resistance to penicillin, clarithromycin and trimethoprim-sulfamethoxazole) accounted for 2% (n=9) and 0.5% (n=1) of respiratory and blood isolates, respectively. Susceptibility of 95% or greater was found with amoxicillin-clavulanic acid (99.5%, 99.3%), ceftriaxone (99.5%, 100%), cefuroxime (95.0%, 96.8%), ertapenem (99.8%, 100%), meropenem (96.1%, 99.5%) and levofloxacin (99.1%, 100%) for respiratory and blood specimens, respectively. No resistance to vancomycin, tigecycline, cethromycin or telithromycin was found. *mef*A was present in 53.6% (52 of 97) of respiratory and 59.3% (16 of 27) of blood macrolide-nonsusceptible *S pneumoniae*, while *erm*B was present in 38.1% (37 of 97) of respiratory and 37% (10 of 27) of blood isolates. Eight of 97 (8.2%) respiratory and one of 27 (3.7%) blood isolates contained both *mef*A and *erm*B genes.

CONCLUSIONS: *S pneumoniae* is a common organism isolated from clinical specimens in Canadian hospitals. Resistance was highest to penicillin and clarithromycin, while ceftriaxone and levofloxacin susceptibility were both greater than 99%. No isolates resistant to vancomycin, tigecycline, linezolid or the ketolides were found. Resistance rates were higher among respiratory tract isolates of *S pneumoniae* than among blood isolates. Macrolide efflux, *mef*A, was the predominant mechanism of macrolide resistance among both respiratory and blood clarithromycin-nonsusceptible isolates.

Key Words: *Antibiotics; Clarithromycin;* ermB*; Hospital; Macrolides;* mefA*; Penicillin; Resistance;* S pneumoniae

Streptococcus pneumoniæ résistant aux antibiotiques dans les hôpitaux canadiens : Résultats de l'étude CANWARD 2007

HISTORIQUE : L'étude de CANWARD (*Canadian Ward Surveillance Study*) 2007 a testé la résistance d'isolats recueillis entre janvier et décembre 2007 dans 12 hôpitaux canadiens à différents antibiotiques. Le présent article s'attarde à la résistance de *Streptococcus pneumoniæ* aux antibiotiques dans les hôpitaux canadiens, et plus particulièrement, sa résistance aux macrolides.

MÉTHODES : Les concentrations minimales inhibitrices (CMI) des antibiotiques ont été déterminées à l'aide de la méthode de microdilution et interprétées conformément aux paramètres établis par le *Clinical and Laboratory Standards Institute*. Les souches non sensibles aux macrolides (CMI de la clarithromycine 0,5 µg/mL ou plus) ont été analysées par PCR multiplex pour y déceler la présence de gènes *mef*A et *erm*B.

RÉSULTATS : *S. pneumoniæ* représentait 9,0 % des isolats recueillis (706 sur 7 881) dans le cadre de l'étude CANWARD 2007). Parmi les 706 isolats de *S. pneumoniæ*, 33,1 % (234) provenaient de spécimens de sang et 66,9 % (472), de spécimens d'expectorations. Les taux globaux de résistance (résistance et résistance intermédiaire) des isolats de *S. pneumoniæ* provenant des spécimens respiratoires et sanguins, respectivement, étaient les suivants : pénicilline (23,9 %, 14,4 %), clarithromycine (22,1 %, 12,6 %), triméthoprime-sulfaméthoxazole (14,7 %, 11,5 %), doxycycline (7,8 %, 5,1 %) et clindamycine (7,1 %, 3,3 %). La multirésistance (à la pénicilline, à la clarithromycine et au triméthoprime-sulfaméthoxazole) a été observée avec 2 % (n = 9) et 0,5 % (n = 1) des isolats respiratoires et sanguins, respectivement. Une sensibilité de 95 % ou plus a été observée avec l'amoxicilline-acide clavulanique (99,5 %, 99,3 %), la ceftriaxone

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(99,5 %, 100 %), le céfuroxime (95,0 %, 96,8 %), l'ertapénem (99,8 %, 100 %), le méropénème (96,1 %, 99,5 %) et la lévofloxacine (99,1 %, 100 %) pour les spécimens respiratoires et sanguins, respectivement. Aucune résistance à la vancomycine, à la tigécycline, à la céthromycine ni à la télithromycine n'a été observée. Le gène *mef*A était présent dans 53,6 % des isolats de *S. pneumoniæ* respiratoires (52 sur 97) et 59,3 % des isolats sanguins (16 sur 27) résistants aux macrolides, tandis que le gène *erm*B était présent dans 38,1 % des isolats respiratoires (37 sur 97) et 37 % des isolats sanguins (10 sur 27). Huit isolats respiratoires sur 97 (8,2 %) et 1 isolat sanguin sur 27 (3,7 %) contenaient les deux gènes, *mef*A et *erm*B.

CONCLUSION : L'agent pathogène *S. pneumoniæ* est couramment isolé dans les spécimens cliniques des hôpitaux canadiens. Sa résistance a été la plus forte à l'endroit de la pénicilline et de la clarithromycine, tandis que sa sensibilité à la ceftriaxone et à la lévofloxacine dépassait 99 %. On n'a recensé aucun isolat résistant à la vancomycine, à la tigécycline, au linézolide ou aux kétolides. Les taux de résistance de *S. pneumoniæ* étaient plus élevés dans les isolats respiratoires que dans les isolats sanguins. La pompe à efflux *mef*A a été le mécanisme prédominant de la résistance aux macrolides, tant pour les isolats respiratoires que pour les isolats sanguins résistants à la clarithromycine.

Community-acquired pneumonia (CAP) is an opportunistic infection associated with significant morbidity and mortality (1-3). It disproportionately affects young, elderly and immunocompromised persons (3,4). *Streptococcus pneumoniae* is the most common bacterial pathogen, responsible for at least 15% of CAP cases; 20% of patients with pneumococcal CAP become bacteremic and require hospitalization (3,5-8). Fatality rates for pneumonia remain high, in the range of 15% to 36%, and are highest among bacteremic patients with pneumococcal pneumonia. *S pneumoniae* is also an important cause of meningitis, otitis media and bacteremia (3,6-8).

Treatment of CAP is usually empirical, and knowledge of local resistance patterns and predominant mechanisms of resistance are important for successful antimicrobial therapy (5). Macrolides are among the principal therapeutic agents used for the empirical treatment of outpatient CAP, while combinations of beta-lactams and macrolides are used for inpatient CAP; however, this choice is complicated by the increasing prevalence of antimicrobial resistance (4,5). Until the 1960s, resistance to penicillin was uncommon among *S pneumoniae* isolates, and beta-lactams served as agents of choice for treatment of documented or presumed *S pneumoniae* infections (3,4). During the 1980s, infections due to penicillin-nonsusceptible pneumococci became widespread (3,4). Today, *S pneumoniae* has acquired resistance to several classes of antimicrobial agents, including penicillins, macrolides, trimethoprim-sulfamethoxazole (SXT) and fluoroquinolones, and by variety of mechanisms (1,2,6,9). More alarming is the fact that pneumococcal strains that are not susceptible to penicillin are likely to be resistant to other agents, including macrolides (1,2,6,7).

Clinical outcomes may depend on the level of macrolide resistance (high versus low minimum inhibitory concentration [MIC]) in the pneumococcal strain causing the infection (5,7). There are two common mechanisms of macrolide resistance, which differ in the level of resistance conferred (10,11). The first, most common globally and predominant in Europe, involves modification of the ribosomal macrolide target site by methylases encoded by the *erm*B gene, and is associated with high-level macrolide resistance (MICs of 64 µg/mL or greater) (11). The second mechanism of resistance, prevalent in North America, involves drug efflux encoded by *mef*A gene and is associated with low level of macrolide resistance (MICs of 16 µg/mL or less) (11).

The purpose of the present study was to assess the antibiotic resistance in *S pneumoniae* isolates in hospitals across Canada. Although we reported previously (4) on antimicrobial resistance among *S pneumoniae* isolates, including in patients in Canadian intensive care units, this is the first national surveillance study assessing the antimicrobial resistance in *S pneumoniae* blood and respiratory specimens of patients in Canadian hospitals. In addition, because macrolides are among the first-line therapeutics used for treatment of CAP, resistance genotypes of macrolide-nonsusceptible *S pneumoniae* were determined.

METHODS

Study design and isolate collection

Isolates were collected, shipped and tested as a part of the the Canadian Ward Surveillance Study (CANWARD 2007); details of this process are provided by Zhanel et al (pages 9A-19A) in the present supplement. In total, 706 (9.0%) of a total of 7881 (472 [67%] respiratory and 234 [33%] blood) isolates received by CANWARD 2007 were *S pneumoniae*.

Antibiotic susceptibility

MICs of antibiotics commonly used in empirical treatment of pneumococcal respiratory and blood infections were determined using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method (12). MICs were interpreted and isolates were categorized as susceptible, intermediate and resistant based on the 2006 CLSI-defined breakpoints (12). Antimicrobial susceptibility testing was performed on 445 respiratory and 219 blood isolates.

Genotyping

Macrolide-nonsusceptible (clarithromycin MIC of 0.5 µg/mL or greater) respiratory (n=97) and bacteremic (n=27) *S pneumoniae* isolates were analyzed for the presence of *erm*B and/or *mef*A genes using a multiplex polymerase chain reaction assay as described by Monaco et al (13) and Pantosti et al (14). *mef*A, *erm*B and dual *mef*A and *erm*B positive controls as well as ATCC 49619 *S pneumoniae* (susceptible to macrolides; negative control) were used.

Statistical analysis

All statistical tests were performed using EpiInfo StatCals2 version 6 statistical program (Centers for Disease Control and Prevention, USA). Results were reported as statistically significant when $P<0.05$.

RESULTS

Patient demographics and specimen types

Respiratory tract specimens were isolated from patients in all ward types, ranging from surgical wards (5.6%) to medical wards (27.9%), while blood isolates were mostly isolated from patients in emergency rooms (59.8%) (Table 1). Among patients aged 17 years and younger, 18 to 64 years, and 65 years or older, pneumococci accounted for similar percentages of

TABLE 1
Demographics of patients with respiratory or blood isolates of *Streptococcus pneumoniae*

Characteristic	Respiratory (n=445), n (%)	Blood (n=219), n (%)
Sex		
Male	273 (61)	134 (61)
Female	172 (39)	85 (39)
Age group, years		
≤17	67 (15)	48 (22)
18–64	236 (53)	123 (56)
≥65	142 (32)	48 (22)
Region		
British Columbia/Alberta	75 (16.8)	54 (24.7)
Saskatchewan/Manitoba	121 (27.2)	64 (29.3)
Ontario	111 (25.0)	46 (21.0)
Quebec/Nova Scotia	138 (31.0)	55 (25.0)
Hospital ward type		
Emergency room	106 (23.8)	131 (59.8)
Hospital clinic	102 (22.9)	12 (5.4)
Intensive care unit	88 (19.8)	19 (8.7)
Medical ward	124 (27.9)	51 (23.3)
Surgical ward	25 (5.6)	6 (2.8)

isolates from respiratory tract and blood specimens (P>0.05). A greater trend toward male patients (61%) than female (39%) was observed for both respiratory and blood isolates (P<0.05).

Antibiotic susceptibility

Resistance rates (intermediate and resistant) for the 445 respiratory isolates of pneumococci were as follows: penicillin (23.9%), clarithromycin (22.1%), SXT (14.7%), doxycycline (7.8%), clindamycin (7.1%) and cefuroxime (5.0%) (Table 2). Greater than 95% of isolates were susceptible to amoxicillin-clavulanate (99.3%), ceftriaxone (99.5%), ertapenem (99.8%), meropenem (96.1%) and levofloxacin (99.1%). Among the 219 bacteremic *S pneumoniae* isolates, no resistance to levofloxacin, ertapenem or ceftriaxone was observed; resistance rates (resistant and intermediate) were as follows: penicillin (14.4%), clarithromycin (12.6%), SXT (11.5%), doxycycline (5.1%), clindamycin (3.3%) and cefuroxime (3.2%). Among other beta-lactam agents, resistance (intermediate) to amoxicillin-clavulanate (0.5%) and meropenem (0.5%) was observed (data not shown). A comparison of antimicrobial susceptibility patterns of respiratory and blood *S pneumoniae* isolates showed that blood isolates demonstrated lower rates of resistance than respiratory isolates to: penicillin (23.9% versus 14.4%; P=0.004), clarithromycin (22.1% versus 12.6%; P=0.003) and clindamycin (7.1% versus 3.3; P=0.049). SXT (14.7% versus 11.5%; P=0.3), doxycycline (7.8% versus 5.1%; P=0.2) and cefuroxime (5.0% versus 3.2%; P=0.3) resistance rates were similar among both respiratory and blood isolates (P>0.05). Both respiratory and blood isolates had similar, low resistance rates to other beta-lactam agents, carbapenems and fluoroquinolones. No resistance to vancomycin, tigecycline, telithromycin or cethromycin (no CLSI breakpoints; telithromycin breakpoints applied) was observed for either respiratory or blood isolates.

TABLE 2
Activity of various antimicrobial agents against 445 respiratory tract *Streptococcus pneumoniae* isolates collected by the CANWARD 2007 study

Antimicrobial agent	MIC (µg/mL)			Isolate susceptibility		
	50%	90%	Range	% S	% I	% R
Penicillin	0.06	0.5	≤0.03–>8	76.1	17.8	6.1
Amoxicillin-clavulanate	≤0.06	0.25	≤0.06–8	99.3	0.5	0.2
Ceftriaxone	≤0.06	0.12	≤0.06–4	99.5	0.3	0.2
Cefuroxime	≤0.25	0.5	≤0.25–>16	95.0	2.3	2.7
Clarithromycin	≤0.03	2	≤0.03–>32	77.9	6.4	15.7
Clindamycin	≤0.12	≤0.12	≤0.12–>8	92.9	0.2	6.9
Ertapenem	≤0.06	≤0.06	≤0.06–4	99.8		0.2
Meropenem	≤0.06	≤0.06	≤0.06–2	96.1	3.4	0.5
Levofloxacin	0.5	1	≤0.06–32	99.1		0.9
Moxifloxacin	0.12	0.25	≤0.06–8	98.6	0.5	0.9
Linezolid	0.5	1	≤0.12–2	100		
Telithromycin	0.015	0.03	≤0.008–0.5	100		
Cethromycin	0.015	0.015	≤0.008–0.12	NB		
Doxycycline*	≤0.25	1	≤0.25–>16	92.2	2.3	5.5
Tigecycline	≤0.03	≤0.03	≤0.03–0.12	NB		
SXT	≤0.12	2	≤0.12–>8	85.3	6.8	7.9
Vancomycin	≤0.25	≤0.25	≤0.25–0.5	100		

**Tetracycline breakpoints were applied to doxycycline. I Intermediate; MIC Minimum inhibitory concentration; NB No breakpoint defined; R Resistant; S Susceptible; SXT Trimethoprim-sulfamethoxazole*

Macrolide resistance mechanisms and antibiotic susceptibility

The MIC_{50}, MIC_{90} (MICs needed to inhibit 50% and 90% of organisms, respectively), range and percentages of isolates susceptible, intermediate and resistant to clarithromycin, clindamycin and ketolides telithromycin and cethromycin for all macrolide-nonsusceptible, *erm*B-positive, *mef*A-positive and dual *erm*B- and *mef*A-positive isolates are shown in Table 3. Of the 445 respiratory isolates, 97 (22%) were nonsusceptible (MICs 0.5 µg/mL or greater) to clarithromycin. Of these, 46.4% (45 of 97) isolates were resistant to clindamycin (MIC 1 µg/mL or greater). Clarithromycin MIC_{50} and MIC_{90} for all nonsusceptible respiratory *S pneumoniae* were 2 µg/mL and 64 µg/mL, respectively, and the MICs ranged from 0.5 µg/mL to 64 µg/mL. Of the 97 isolates, 37 (38%) were *erm*B-positive and 52 (54%) were *mef*A-positive. Clarithromycin MIC_{50} and MIC_{90} for *erm*B-positive were both 64 µg/mL, and the MICs ranged from 0.5 µg/mL to 64 µg/mL. Two of 37 (5.4%) isolates were intermediate to clarithromycin (MIC 0.5 µg/mL). All isolates were cross-resistant to clindamycin. Clarithromycin MIC_{50}s and MIC_{90}s for *mef*A-positive were 1 µg/mL and 4 µg/mL, respectively, and the MICs ranged from 0.5 µg/mL to 4 µg/mL. Twenty-one of 52 (40.4%) of *mef*A-positive isolates were intermediate (MIC 0.5 µg/mL) to clarithromycin. All isolates were susceptible to clindamycin. Eight of the 97 (8.2%) contained both *erm*B and *mef*A genes. Clarithromycin MIC_{50}s and MIC_{90}s for dual *erm*B and *mef*A-positive isolates were 64 µg/mL, and the MIC ranged from 2 µg/mL to 64 µg/mL. All isolates resistant clindamycin (MICs of 1 µg/mL or greater).

Of the 219 bacteremic *S pneumoniae* isolates, 27 (12%) were nonsusceptible to clarithromycin (MICs of 0.5 µg/mL or greater).

TABLE 3
Genotypic and phenotypic data for 97 macrolide-nonsusceptible respiratory tract *Streptococcus pneumoniae* isolates collected by the CANWARD 2007 study

Organism and drug	MIC (μg/mL)			Isolate susceptibility		
	MIC_{50}	MIC_{90}	Range	% S	% I	% R
***S pneumoniae*, resistant (n=97)**						
Clarithromycin	2	64	0.5–≥64	0	23.7	76.3
Clindamycin	0.12	16	0.12–16	53.6	0	46.4
Cethromycin	0.015	0.06	0.008–0.12	100	0	0
Telithromycin	0.03	0.25	0.008–0.5	100	0	0
***S pneumoniae ermB* (n=37)**						
Clarithromycin	64	64	0.5–≥64	0	5.4	94.6
Clindamycin	16	16	8–16	0	0	100
Cethromycin	0.015	0.015	0.008–0.03	100	0	0
Telithromycin	0.015	0.015	0.008–0.06	100	0	0
***S pneumoniae mefA* (n=52)**						
Clarithromycin	1	4	0.5–4	0	40.4	59.6
Clindamycin	0.12	0.12	0.12–1	98.1	0	1.9
Cethromycin	0.015	0.06	0.008–0.06	100	0	0
Telithromycin	0.03	0.25	0.015–0.25	100	0	0
***S pneumoniae ermB + mefA* (n=8)**						
Clarithromycin	64	64	2–64	0	0	100
Clindamycin	16	16	16–16	0	0	100
Cethromycin	0.015	0.12	0.015–0.12	100	0	0
Telithromycin	0.06	0.5	0.03–0.5	100	0	0

I Intermediate; MIC Minimum inhibitory concentration; $MIC_{50/90}$ MICs required to inhibit 50%/90% of organisms; R Resistant; S Susceptible

Clarithromycin MIC_{50}s and MIC_{90}s for all nonsusceptible bacteremic *S pneumoniae* were 4 μg/mL and 64 μg/mL, and the MICs ranged from 0.5 μg/mL to 64 μg/mL. Of the 27 isolates, 10 (37%) were *erm*B-positive and 16 (59%) were *mef*A-positive. Clarithromycin MIC_{50}s and MIC_{90}s for *erm*B-positive were both 64 μg/mL, and the MICs ranged from 8 μg/mL to 64 μg/mL. All *erm*B-positive isolates were cross-resistant to clindamycin (MICs of 1 μg/mL or greater). Clarithromycin MIC_{50}s and MIC_{90}s for *mef*A-positive were 0.5 μg/mL and 8 μg/mL, and the MICs ranged from 0.5 μg/mL to 8 μg/mL. Eleven of the 16 (68.8%) *mef*A-positive isolates were intermediate to clarithromycin (MIC 0.5 μg/mL). All isolates were susceptible to clindamycin (MIC 0.25 μg/mL or less). Of the 27 nonsusceptible isolates, one (3.7%) was positive for both *erm*B and *mef*A genes. This isolate was resistant to clarithromycin (32 μg/mL) and clindamycin (16 μg/mL). A comparison of respiratory and blood isolates of *S pneumoniae* showed that *mef*A was the predominant genotype in clarithromycin-resistant and -intermediate (MIC of 0.5 μg/mL or greater) isolates (data not shown).

Resistant phenotypes

The resistance rates to a variety of antimicrobials were also assessed against penicillin-susceptible, -intermediate and -resistant as well as clarithromycin-resistant *S pneumoniae*. For penicillin-susceptible isolates, resistance rates (resistant and intermediate) for respiratory (n=334) versus blood (n=185) isolates were as follows: clarithromycin (12.7% versus 9.8%), SXT (7.5% versus 8.1%), doxycycline (3.9% versus 2.7%), clindamycin (2.4% versus 1.6%) and levofloxacin (0.9% versus 0.0%). Among penicillin-intermediate isolates (respiratory n=78 and blood n=25), resistance rates were as follows: clarithromycin (57.2% versus 28.0%), SXT (28.2% versus 16.0%), doxycycline (20.8% versus 20.0%), clindamycin (20.8% versus 12.0%), cefuroxime (2.6% versus 4.0%) and levofloxacin (1.3% versus 0%). Among isolates resistant to penicillin (respiratory n=27 and blood n=6), resistance rates were as follows: clarithromycin (40.7% versus 33.3%), SXT (66.7% versus 83.3%), doxycycline (18.5% versus 16.7%) and clindamycin (25.9% versus 16.7%). Resistance to other beta-lactam agents was as follows: cefuroxime (74.1% versus 100%), meropenem (63.0% versus 16.7%), amoxicillin-clavulanate (11.1% versus 16.7%), ceftriaxone (7.4% versus 0%) and ertapenem (3.7% versus 0%). Among macrolide-resistant respiratory (n=69) and blood (n=15) isolates, resistance (resistant and intermediate) rates were as follows: penicillin (62.62% versus 46.67%) clindamycin (43.8% versus 46.7%), SXT (40.6% versus 20.0%), doxycycline (39.1% versus 46.7%) meropenem (13.0% versus 6.7), cefuroxime (11.6% versus 13.3%) and amoxicillin-clavulanate (4.4% versus 6.7%).

Nine (2%) of respiratory and one (0.5%) of blood *S pneumoniae* isolates exhibited a MDR phenotype. Among MDR isolates, resistance rates (resistant and intermediate) for respiratory and blood isolates, respectively, were as follows: meropenem (88.9% versus 100%), cefuroxime (77.8% versus 100%), amoxicillin-clavulanate (33.3% versus 100%), ceftriaxone (22.2% versus 0%), ertapenem (11.1%) and doxycycline (11.1% versus 0%).

DISCUSSION

CANWARD 2007 is the first study focusing on pathogens isolated from patients in Canadian hospitals. The goal of the current study was to analyze the antibiotic susceptibility profile of *S pneumoniae* present among patients attending Canadian hospitals. The least active (based upon MIC only) agents against *S pneumoniae* collected during CANWARD 2007 study were penicillin (23.9%), clarithromycin (22.1%) and SXT (14.7). Against blood isolates, penicillin (14.4%), clarithromycin (12.6%) and SXT (11.5%) were also least active, although the overall extent of resistance was lower for blood isolates than for respiratory isolates. In comparison, the Canadian Respiratory Organism Susceptibility Study (CROSS) (4,15), which assessed antibiotic resistance in *S pneumoniae* in both inpatients and outpatients from 1998 to 2006, showed that penicillin resistance (resistant and intermediate) ranged from the lowest of 16.1% to highest of 25.0% during 1998 and 2006. The average penicillin resistance (resistant and intermediate) was 21.6% during the nine years of the study (15). During the first five years of the CROSS study, the increase in penicillin non-susceptibility was attributed to an increase in penicillin-resistant (MIC 2 μg/mL or greater) isolates and a decrease in penicillin-intermediate (MIC 0.12 μg/mL to 1 μg/mL) isolates (4). Penicillin-resistant isolates ranged from 6.4% in 1998 to 13.8% in 2002, while penicillin-intermediate isolates decreased from 14.8% to 10.2% during the same period (4). In 2003, the rate of penicillin-intermediate isolates increased to 14.3% while the rate of penicillin-resistant isolates decreased to 6.9%, and they have remained stable at an average 8.1% penicillin-resistant rate and 14.7% penicillin-intermediate rate for the remainder of the study (15). The results from the CANWARD

study showed a penicillin-intermediate rate of 17.8% and penicillin-resistant rate of 6.2%. The susceptibility of *S pneumoniae* to penicillin among patients in CANWARD is similar to patients in CROSS, which suggests that penicillin resistance among *S pneumoniae* in Canada may have stabilized. The observation that increasing penicillin-intermediate isolates affecting the rate of penicillin nonsusceptibility is not unique and has been published by others (8). Compared with our previous studies (4), the resistance rates to all beta-lactams also increased in parallel with increasing resistance to penicillin (4). Among the beta-lactams, ertapenem (99.8%), ceftriaxone (99.5%) and amoxicillin-clavulanate (99.3%) were the most active. Meropenem was less active, with a resistance rate of 3.9%.

Macrolide resistance among pneumococci in Canada increased from 8% in 1998 to 18.7% in 2006 as measured by the CROSS study (10,15). During the CANWARD 2007 study, macrolide resistance was 22.1%. These data speak to the continued increase in macrolide resistance in Canada. Efflux-mediated resistance has been the most predominant mechanism of macrolide resistance in Canada throughout the CROSS study (10,15), and *mef*A-positive isolates were the most prevalent among macrolide-resistant respiratory and blood *S pneumoniae* isolated in CANWARD 2007 as well. Isolates with dual *mef*A and *erm*B genes emerged during the CROSS study (10,15) and have been identified among both respiratory and blood *S pneumoniae* isolates in CANWARD 2007 as well, approaching 10% of macrolide-resistant isolates. The emergence of these dual *mef*A and *erm*B isolates is worrisome because these isolates spread by clonal dissemination and are associated with high-level macrolide resistance as well as resistance to multiple antibiotics (10,15). All isolates were susceptible to telithromycin. Cethromycin, an investigational ketolide, demonstrated potent in vitro activity against macrolide-resistant *S pneumoniae* (10). In addition, cethromycin appears to have slightly greater in vitro activity than telithromycin, particularly against *mef*A strains, which in turn appears to be affecting the activity of ketolides more than *erm*B-positive strains (7,10).

Among fluoroquinolones, the resistance to levofloxacin and moxifloxacin was low (less than 1% and less than 2%, respectively), similar to our previous studies (4). No resistance to vancomycin or linezolid was noted.

In light of the recent changes in the penicillin breakpoints for nonmeningitis *S pneumoniae*, perhaps it is important to note that we applied old breakpoints (meningitis breakpoints: susceptible 0.06 µg/mL or less; intermediate 0.12 µg/mL to 1 µg/mL; resistant 2 µg/mL or greater) (3). In doing so, we are reporting higher than actual penicillin resistance rates among respiratory or blood isolates. The old breakpoints were conservative to ensure accurate interpretation of results for cerebrospinal fluid isolates (3). However, these conservative breakpoints may not have accurately represented lack of response in patients treated with parenteral beta-lactams for pneumococcal pneumonia (3). Application of the new parenteral breakpoints to *S pneumoniae* will result in a significantly lower rate of penicillin nonsusceptibility in nonmeningeal pneumococcal isolates. With the new breakpoints, a penicillin resistance rate of 2.3% was noted, significantly lower than reported with the old breakpoints.

CONCLUSIONS

S pneumoniae is a common organism isolated from clinical specimens in Canadian hospitals. Resistance was highest to penicillin and clarithromycin, while ceftriaxone and levofloxacin susceptibilities were both greater than 99%. No isolates resistant to vancomycin, tigecycline, linezolid or the ketolides were found. Resistance rates were higher among respiratory tract isolates of *S pneumoniae* compared with blood isolates. The macrolide efflux gene *mef*A was the predominant mechanism of macrolide resistance among both respiratory and blood clarithromycin nonsusceptible isolates.

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