

Macrolide-Resistant *Streptococcus pneumoniae* in Canada during 1998–1999: Prevalence of *mef(A)* and *erm(B)* and Susceptibilities to Ketolides

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In this study (1998–1999), we collected 215 macrolide-resistant *Streptococcus pneumoniae* isolates from an ongoing Canadian Respiratory Organism Surveillance Study involving 23 centers representing all regions of Canada. The prevalence of erythromycin-resistant *S. pneumoniae* was 8% (215 of 2,688). Of the 215 isolates, 48.8% (105 of 215) were PCR positive for *mef(A)* and 46.5% (100 of 215) were PCR positive for *erm(B)*. The ketolides telithromycin and ABT-773 demonstrated excellent activity against both *mef(A)* (MIC for 90% of strains [MIC₉₀], 0.06 and 0.03 µg/ml, respectively) and *erm(B)* (MIC₉₀, 0.06 and 0.03 µg/ml, respectively) strains of *S. pneumoniae*.

Macrolides, especially the newer agents (azithromycin and clarithromycin) are used extensively for the treatment of respiratory infections due to their broad-spectrum activity against both typical and atypical respiratory pathogens (5, 25). However, emergence of erythromycin resistance (Ery-R) in *Streptococcus pneumoniae* is a growing concern because of the importance of this pathogen in infections of the respiratory tract (6, 13, 15, 25). Although the prevalence of resistant strains varies geographically and temporally, antimicrobial resistance is widespread (6, 9, 10, 26). Macrolide resistance in *S. pneumoniae* has increased during the 1990s to the extent that over 30% of clinical isolates are now resistant in some communities (2, 3, 21, 23, 27).

Macrolide resistance in *S. pneumoniae* may be encoded by the *erm(B)* gene, which reduces the binding affinity of all macrolides for the 23s rRNA (domain V) and leads to cross-resistance to macrolides, lincosamides, and streptogramin B (MLS_B) (16, 19, 27). Cross-resistance occurs as a result of methylation of the adenine residue (A2058) within the overlapping binding sites for the three chemically distinct antimicrobial classes (16, 21, 23, 27). *S. pneumoniae* strains possessing the *erm(B)* gene have an MLS_B phenotype and usually express high-level resistance (MIC for 90% of strains [MIC₉₀], ≥64 µg/ml) to MLS_B antibiotics (23, 27). The second macrolide resistance mechanism in *S. pneumoniae* is the efflux of the antibiotic (22). *S. pneumoniae* strains with this type of resistance mechanism are classified as possessing an M-phenotype. An efflux pump encoded by the *mef(A)* gene in *S. pneumoniae* pumps out 14- and 15-membered macrolides only (20; K. Gay and D. S. Stephens, Program Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., 2000, abstr. 1929). *S. pneumoniae* with an M-phenotype express low-level resistance (MIC₉₀, ≤4

µg/ml) to macrolides but demonstrate no cross-resistance to lincosamides and streptogramin B (20, 22).

The prevalence of the MLS_B and M-phenotypes varies both geographically and temporally (9, 10, 13, 21). In the United States and Canada, where macrolide resistance in *S. pneumoniae* is 20 and 9%, respectively, the M-phenotype [product of the *mef(A)* gene] predominates (9; J. A. Karlowsky, D. J. Hoban, and G. G. Zhanel, Program Abstr. 5th Int. Conf. Macrolides, Azalides, Streptogramins, 2000, abstr. 7.11, p. 65). In Europe the prevalence of macrolide resistance varies from low (<5%) to high (>30%) but the MLS_B phenotype [product of the *erm(B)* gene] is the more prevalent in comparison to the M-phenotype (10, 13; Karlowsky et al., abstr. 7.11).

Ketolides are third-generation, semisynthetic macrolides derived from clarithromycin (12). These 14-membered antibiotics are made by the replacement of the cladinose at C-3 with a keto group (12). This semisynthetic alteration of the natural erythromycin A molecule renders the drug more stable in acidic environments and reduces the induction of MLS_B resistance phenotype (7, 8, 12). Ketolides have activity against a broad range of respiratory tract pathogens, including *S. pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Mycoplasma* species, *Legionella* species, and some anaerobic bacteria (4, 7, 12). Previous work has demonstrated that ketolides are active against macrolide-resistant *S. pneumoniae*, whether possessing the *erm(B)* or *mef(A)* genotype (12). Ketolides bind to domain II as well as domain V of 23s rRNA (13). In addition to the additional ribosomal contact, the potency of ketolides may also be due to slow dissociation from the ribosome (11). The purpose of this study was to determine the incidence of *erm(B)* and *mef(A)* among erythromycin-resistant *S. pneumoniae* isolated in Canada (1998–1999) during an ongoing national respiratory surveillance program. Second, the activity of telithromycin and ABT-773, two new ketolides, was assessed against *erm(B)* and *mef(A)* *S. pneumoniae*.

S. pneumoniae isolates were collected between 1998 and 1999 from an ongoing national surveillance study (Canadian

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TABLE 1. Genotypic and phenotypic results for macrolide-resistant *S. pneumoniae*

Strains	No. (%) of isolates	MIC ($\mu\text{g/ml}$)							
		Erythromycin		Clindamycin		Telithromycin		ABT-773	
		MIC ₉₀	Range	MIC ₉₀	Range	MIC ₉₀	Range	MIC ₉₀	Range
<i>erm(B)</i> ⁺	100 (46.5)	≥ 64	1– ≥ 64	>16	0.12–>16	0.06	≤ 0.02 –1	0.03	≤ 0.002 –0.5
<i>mef(A)</i> ⁺	105 (48.8)	4	1– ≥ 64	0.12	0.12–0.5	0.06	≤ 0.02 –1	0.03	≤ 0.002 –0.5
<i>erm(B)</i> [–] <i>mef(A)</i> [–]	4 (1.9)		1–32		0.12		≤ 0.002 –0.004		≤ 0.002
<i>erm(B)</i> ⁺ <i>mef(A)</i> ⁺	6 (2.8)		16– ≥ 64		0.12–>16		≤ 0.002 –0.06		≤ 0.002 –0.03
Susceptible controls ^a	24 (10)	0.25	0.06–0.25	0.12	<0.12	0.008	≤ 0.002 –0.008	0.004	≤ 0.002 –0.008

^a Included *erm(B)*-positive, *erm(B)*-negative, and *mef(A)*-negative strains.

Respiratory Organism Susceptibility Study [CROSS] [28]). Specifically, isolates were obtained from 23 medical centers in 9 of 10 Canadian provinces (26). Consecutive isolates, one per patient, were collected from respiratory tract specimens only. Isolates were identified by conventional methodology and deemed to be respiratory pathogens by individual laboratory protocols. All isolates were shipped to a central laboratory (Health Sciences Centre, Winnipeg, Manitoba) on Amies charcoal swabs. The identity of each *S. pneumoniae* was confirmed by the central reference laboratory, and it was then stored in skim milk and stored at -80°C (26).

Antibiotics for this study were supplied by the manufacturers: erythromycin (Hoechst Marion Rousell, Romainville, France), ABT-773 (Abbott, Abbott Park, Ill.), telithromycin (Hoechst Marion Rousell, Romainville, France) and clindamycin (Pharmacia and Upjohn, Mich.). Antibiotics were reconstituted according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines (18). Susceptibility to erythromycin, clindamycin, telithromycin, and ABT-773 was determined using the NCCLS M7-A4 broth microdilution method (20). Each final-panel well volume was 100 μl , with a bacterial inoculum of 5×10^5 CFU/ml. Panels were read following 20 to 24 h of incubation at 35°C in ambient air. The MIC was defined as the lowest concentration of antibiotic inhibiting visible growth. Colony counts were performed periodically to confirm inocula.

S. pneumoniae cultures were grown overnight on Trypticase soy agar with 5% sheep blood, and two to five colonies were resuspended in 1 ml of sterile saline. Following centrifugation at 13,000 rpm for 10 min, supernatants were removed, and the resulting bacterial pellet was resuspended in 300 μl of lysis buffer containing 0.1 M NaOH, 2.0 M NaCl, and 0.5% sodium dodecyl sulfate (SDS). Cell suspensions were then boiled for 15 min, and 200 μl of 0.1 M Tris-HCl (pH 8.0) was added. For extraction of genomic DNA, 500 μl of phenol-chloroform-isoamyl alcohol (25:24:1) was added, and the mixture was centrifuged at 13,000 rpm for 10 min; 1 ml of cold (-20°C) anhydrous alcohol was then added, and DNA was precipitated at -80°C for a minimum of 30 min. The precipitated DNA was collected by centrifugation at 13,000 rpm for 15 min at 4°C , and the pellets were allowed to air dry for no less than half an hour. Pellets containing the purified DNA were subsequently resuspended in sterile distilled water and used as the DNA template (26).

DNA was amplified in a total volume of 50 μl containing 5 μl of template DNA, 5 μl of 40 mM MgCl_2 –10 \times PCR buffer,

1.25 mM each of dCTP, dGTP, dATP, and dTTP (Amersham Pharmacia Biotech), 100 mM each primer (Gibco-BRL), 2.5 U of *Taq* DNA polymerase (Amersham Pharmacia Biotech), and 30.5 μl of sterile distilled water. Primers used for amplification of *erm(B)* and *mef(A)* were 5'-GAAAAGGTACTAAACCAA ATA-3' and 5'-AGTAACGGTACTTAAATTGTTTAC-3' (PCR product, 616 bp) and 5'-ACTATCATTAACTACTAG TGC-3' and 5'-TTCTTCTGGTACTAAAAGTGG-3' (PCR product, 346 bp), respectively (16). Amplification of *erm(B)* and *mef(A)* was performed using a Perkin-Elmer GeneAmp PCR system 9700 and consisted of initial denaturation at 95°C for 2 min, 30 cycles at 95, 52, and 72°C for 1 min each, and a final extension at 72°C for 10 min. Amplified DNA fragments were analyzed by electrophoresis through 2% agarose gels containing ethidium bromide and visualized under UV transillumination. *S. pneumoniae* ATCC 49619 and a *mef(A)* or *erm(B)* strain were consistently included as negative and positive controls, respectively.

An ongoing national surveillance study representing all regions in Canada (CROSS) was initiated in 1997 (26). In 1998–1999, 2,688 *S. pneumoniae* isolates from respiratory tract specimens were prospectively collected from 23 different medical centers throughout Canada, and 215 of these isolates were identified as being resistant to erythromycin. Thus, the national macrolide resistance rate in *S. pneumoniae* in Canada was approximately 8%. The incidence of macrolide-resistant *S. pneumoniae* from 1997 to 2000 has consistently remained at approximately 8% over the 3 years of the study (26; Karlowsky et al., abstr. 7.11). The 215 macrolide-resistant *S. pneumoniae* isolates were screened for the presence of *mef(A)* and *erm(B)*, the two major macrolide resistance genes described in *S. pneumoniae*. The results of the PCR amplification studies for *mef(A)* and *erm(B)* are displayed in Table 1.

The quality control strains tested [ATCC 49619, *mef(A)* positive, and *erm(B)* positive] were in control (data not shown). As shown in Table 1, 48.8% of macrolide-resistant *S. pneumoniae* were PCR positive for *mef(A)*, demonstrating the presence of a macrolide efflux pump. The erythromycin MICs for these strains ranged from 1 to ≥ 64 $\mu\text{g/ml}$; however, the majority of these isolates had erythromycin MICs of 1 to 4 $\mu\text{g/ml}$ (see Table 3). None of the *mef(A)*-positive strains were resistant to clindamycin, and 46.5% of all macrolide-resistant *S. pneumoniae* were PCR positive for *erm(B)*. Erythromycin MICs for *erm(B)*-positive strains ranged from 1 to ≥ 64 $\mu\text{g/ml}$; however, the majority of the isolates demonstrated very high erythromycin MICs of ≥ 32 $\mu\text{g/ml}$ (See Table 3). The majority

TABLE 2. MIC distribution data for erythromycin-resistant *S. pneumoniae*

Strain	No.	No. (cumulative %) of strains for which the MIC ($\mu\text{g/ml}$) was:								
		0.06	0.25	1	2	4	8	16	32	≥ 64
<i>erm(B)</i> ⁺ <i>mef(A)</i> ⁻	100			2 (2)	2 (4)	2 (6)	1 (7)	2 (9)	41 (50)	50 (100)
<i>erm(B)</i> ⁻ <i>mef(A)</i> ⁺	105			45 (42.9)	33 (74.3)	18 (91.4)	2 (93.3)	6 (99)		1 (100)
<i>erm(B)</i> ⁻ <i>mef(A)</i> ⁻	4			2 (50)	1 (75)				1 (100)	
<i>erm(B)</i> ⁺ <i>mef(A)</i> ⁺	6							1 (17)	1 (34)	4 (100)
Susceptible controls ^a	24	1 (4.2)	23 (100)							

^a See Table 1, footnote a.

of *erm(B)*-positive *S. pneumoniae* were resistant to clindamycin. A small number (2.8%) of macrolide-resistant *S. pneumoniae* were *erm(B)* and *mef(A)*. All of these strains had high MICs to erythromycin, and the majority were concomitantly resistant to clindamycin (Tables 2 and 3); 1.9% of strains were both *mef(A)* and *erm(B)* negative, generally displayed very low MICs to erythromycin, and were susceptible to clindamycin.

Table 1 describes the susceptibility of macrolide-resistant *S. pneumoniae* to both telithromycin and ABT-773. As can be seen from Table 1, telithromycin was very active against both *mef(A)*-positive and *erm(B)*-positive *S. pneumoniae*, with MIC₉₀s of 0.06 $\mu\text{g/ml}$. Telithromycin was not as active against *mef(A)*-positive or *erm(B)*-positive strains as it was against macrolide-susceptible controls, which demonstrated MIC₉₀s of 0.008 $\mu\text{g/ml}$. Telithromycin was very active against *erm(B)*-positive and *mef(A)*-positive strains, as well as macrolide-resistant *S. pneumoniae* that were both *mef(A)* and *erm(B)* negative. ABT-773 was also very active against both *mef(A)*-positive and *erm(B)*-positive macrolide-resistant *S. pneumoniae*, with MIC₉₀s of 0.03 $\mu\text{g/ml}$. Like telithromycin, ABT-773 was not as active against *mef(A)* or *erm(B)* strains as it was against macrolide-sensitive *S. pneumoniae*, which demonstrated MIC₉₀s of 0.004 $\mu\text{g/ml}$. ABT-773 was very active against *erm(B)*-positive and *mef(A)*-positive strains, as well as macrolide-resistant *S. pneumoniae* that were both *mef(A)* and *erm(B)* negative.

Table 3 describes the telithromycin and ABT-773 distributions with macrolide-susceptible and -resistant *S. pneumoniae*. As can be seen, the majority of telithromycin MICs against *erm(B)*-positive *S. pneumoniae* clustered between 0.004 and 0.008 $\mu\text{g/ml}$. Telithromycin MICs for *mef(A)*-positive strains were more disparate, 0.004 to 0.06 $\mu\text{g/ml}$ (Table 3). Occasional *erm(B)*-positive or *mef(A)*-positive strains demonstrated higher MICs to telithromycin, with two strains as high as 1 $\mu\text{g/ml}$. With ABT-773, the majority of MICs against *erm(B)*-positive *S. pneumoniae* ranged from 0.002 to 0.008; however,

occasional strains demonstrated higher MICs, including one strain as high as 0.5 $\mu\text{g/ml}$. Against *mef(A)*-positive *S. pneumoniae*, the majority of ABT-773 MICs ranged from 0.002 to 0.015 $\mu\text{g/ml}$.

Presently the prevalence of macrolide resistance in Canada is low at approximately 8%. Since 1997, in our ongoing national surveillance program, which assesses macrolide resistance in over 1,000 respiratory isolates of *S. pneumoniae* per year from all regions of Canada, macrolide resistance has been approximately 8% and appears to have remained stable over the last 3 years (26; Karlowky et al., abstr. 7.11). The low and stable incidence of macrolide-resistant *S. pneumoniae* in Canada has occurred despite large increases (29.5% over 1995 to 1998) in macrolide use in all regions of Canada, especially with the new macrolides, such as azithromycin and clarithromycin (5). Perhaps the reason why macrolide resistance is stable in Canada despite large increases in use of newer macrolides may be that total antibiotic use (total number of prescriptions for all antibiotics per year) is decreasing in Canada (14%) over the last 5 years (1995 to 1999) (G. G. Zhanel, A. Carrie, L. Hoban, K. Weiss, D. E. Low, and A. S. Gin, 40th ICAAC, p. 508).

Presently, 48.8% of macrolide-resistant *S. pneumoniae* in Canada harbor *mef(A)*, while 46.5% of macrolide-resistant *S. pneumoniae* harbor *erm(B)*. These data are consistent with previous Canadian and U.S. data and suggest that approximately 50% of macrolide-resistant *S. pneumoniae* possess a macrolide efflux pump and this has not changed over the last 7-year period (13, 21; Karlowky et al., abstr. 7.11). The importance of knowing whether macrolide-resistant *S. pneumoniae* have *mef(A)* or *erm(B)* is that low-level macrolide resistance might be cured by drug concentrations that are clinically achievable in tissues (1). On the other hand, strains demonstrating high MICs to erythromycin may lead to microbiological and clinical failure with macrolides in patients with community-acquired pneumonia (14).

TABLE 3. Telithromycin and ABT-773 MIC distributions with *S. pneumoniae*^a

Ketolide	Genotype	No. of isolates	No. of isolates for which the MIC ($\mu\text{g/ml}$) was:								
			0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.5	1
Teli	<i>erm(B)</i> ⁺	100	6	38	30	8	8	3	5	1	1
	<i>mef(A)</i> ⁺	105	7	21	5	20	22	21	8		1
	MacS	24	5	13	6						
ABT	<i>erm(B)</i> ⁺	100	22	28	34	6	4	4	1	1	
	<i>mef(A)</i> ⁺	105	16	25	11	35	8	9		1	
	MacS	24	8	14	2						

^a Teli, telithromycin; ABT, ABT-773; MacS, macrolide susceptible (erythromycin MIC, ≤ 0.25 $\mu\text{g/ml}$).

In this study we demonstrated that both telithromycin and ABT-773 had excellent activity against macrolide-resistant *S. pneumoniae* possessing the *mef(A)* or *erm(B)* phenotype. In light of their excellent activity against macrolide-resistant *S. pneumoniae*, ketolides may be an attractive alternative for the treatment of respiratory tract infections caused by this important pathogen.

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