

## Prevalence and Characterization of the Mechanisms of Macrolide, Lincosamide, and Streptogramin Resistance in Isolates of *Streptococcus pneumoniae*

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Received 19 September 1997/Returned for modification 16 January 1998/Accepted 16 June 1998

**Of a total of 147 erythromycin-resistant *Streptococcus pneumoniae* isolates, 64 (43.5%) were resistant to erythromycin, clindamycin, and streptogramin B (MLS<sub>B</sub> phenotype), 57 of which possessed the *ermB* gene. Eighty-two (55.8%) were resistant to erythromycin alone (M phenotype), 81 of which possessed the *mefE* gene. One was erythromycin and streptogramin B resistant but susceptible to clindamycin (MS phenotype) and possessed neither the *erm* gene nor the *mefE* gene.**

The macrolide, lincosamide, and streptogramin B (MLS<sub>B</sub>) antimicrobials are three chemically distinct, functionally related drug classes which inhibit protein synthesis through alteration of the 50S subunit (2). Resistance may occur by target site modification, active efflux, or enzymatic inactivation.

Target modification arises through the presence of an *erm* methylase, which induces a conformational change in the ribosome. This results in inducible or constitutive cross-resistance to MLS<sub>B</sub> antimicrobials (MLS<sub>B</sub> phenotype) (2). Previously, target modification, due to *ermAM(B)*, was the only reported mechanism of MLS<sub>B</sub> resistance in *S. pneumoniae* (10). More recently, however, a macrolide-specific efflux mechanism, encoded by *mef*, has been described (M phenotype) (9). In this study, we looked at the prevalence of these phenotypes in a large collection of strains and correlated it with the presence of the *erm* and *mef* genes.

A total of 5,029 clinical isolates of *S. pneumoniae* were obtained from 1993 to 1996 from a cross-Canada surveillance study involving 113 hospital and private laboratories in all 10 provinces. These strains were characterized by *in vitro* susceptibility testing and molecular techniques in order to determine the prevalence and mechanisms of macrolide resistance.

Susceptibility testing was carried out by broth microdilution with all isolates and disk diffusion with those isolates subsequently found to be erythromycin resistant, according to the National Committee for Clinical Laboratory Standards guidelines (4). An MLS<sub>B</sub> phenotype isolate was defined as an isolate that was found by disk diffusion to be resistant to erythromycin, clindamycin, and quinupristin (streptogramin B). An M phenotype isolate was an isolate found to be resistant to erythromycin alone. An MS phenotype isolate was an isolate found to be resistant to erythromycin and quinupristin. The antimicrobial concentration of the disks was 15 µg of erythromycin, 2 µg of clindamycin (Oxoid, Nepean, Ontario, Canada), and 7.5 µg of quinupristin (Rhône-Poulenc-Rorer, Collegenille, Pa.).

Genomic DNA was isolated as described by Smith et al. (7). Plasmid isolation was performed with the Quantum Prep plasmid miniprep kit (Bio-Rad, Mississauga, Ontario, Canada). Multiplex PCR was performed with primers specific for *ermA*,

*ermB* (*ermAM*), *ermC*, and *mefE* as described by Sutcliffe et al. (8) for all erythromycin-resistant strains and eight random erythromycin-susceptible strains. Pulsed-field gel electrophoresis (PFGE) (3) with the CHEF DRII apparatus (Bio-Rad) and *SmaI* digestion were performed with 8 to 12 representative isolates of each of the following groups: MLS<sub>B</sub> phenotypes that were *erm* positive, M phenotypes that were *mefE* positive, susceptible strains that were *erm* negative and *mefE* negative, and resistant strains that were *erm* negative and *mefE* negative. Modifications included a lysis time of 2 h and the following electrophoretic parameters: pulse times of 0.2 to 35 s, a temperature of 14°C, and 200 V for 21 h. *S. pneumoniae* strains 3585 (*ermAM*), 02J1175 (*mefE*), and ATCC 49619 were used as controls. Southern blotting of the PFGE gels was performed as described previously, and blots were probed with *ermAM* and *mefE* amplicons (1). Dot blot hybridization was performed by standard methodology on the representative isolates with the addition of plasmid DNA from *E. coli* RN7951 (*ermA*) and *S. aureus* RN4220 (*ermC*), and blots were probed with *ermA* and *ermC* amplicons. To ensure genomic DNA integrity, 16S rRNA was amplified by PCR from *S. pneumoniae* 3585, under the conditions used for *mefE* and *erm*, and was used to confirm the presence of DNA. Probes were purified with the QIAquick PCR purification kit (Qiagen, Mississauga, Ontario, Canada). Hybridization and detection were performed by enhanced chemiluminescence with the ECL direct nucleic acid labelling system (Amersham Life Science, Oakville, Ontario, Canada).

Of 5,029 isolates tested, 147 (2.9%) were found to be erythromycin resistant by broth microdilution. Disk diffusion results (Table 1) demonstrated that 64 isolates were of the MLS<sub>B</sub> phenotype, 82 were of the M phenotype, and 1 was of the MS

TABLE 1. Characterization of 147 erythromycin-resistant *S. pneumoniae* isolates used in this study

Resistance phenotype	No. of strains	No. of isolates with resistance gene(s) detected		
		<i>ermA</i> , -B, or -C	<i>mefE</i>	None
MLS <sub>B</sub>	64	57	1 <sup>a</sup>	7
M	82	0	81	1
MS	1	0	0	1

<sup>a</sup> One constitutive MLS<sub>B</sub> isolate possessed both *erm* and *mefE* genes.

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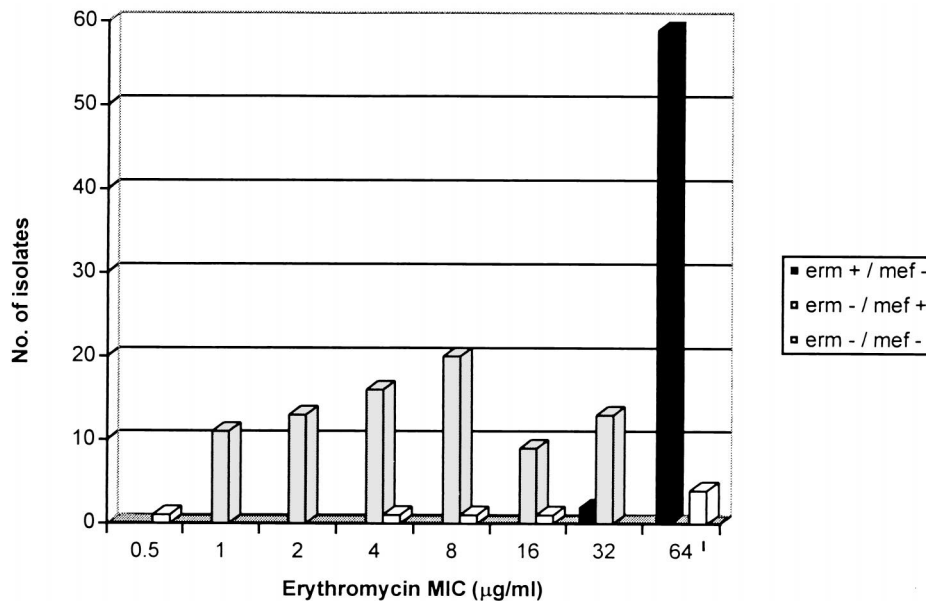


FIG. 1. Distribution of *mefE* and *erm* genes according to the MIC of erythromycin.

phenotype. PFGE demonstrated that of 42 strains tested, 41 were clonally distinct (data not shown).

Multiplex PCR with primers specific for *ermA*, *ermB* (*ermAM*), *ermC*, and *mefE* yielded results predictive of the observed MLS<sub>B</sub> and M phenotypes (Table 1). Of 64 strains possessing an MLS<sub>B</sub> phenotype, 57 yielded an *erm* amplicon. One isolate possessed both *mefE* and *erm* genes. In contrast, of 82 strains bearing the M phenotype, 81 possessed a *mefE* gene. Of nine strains lacking *mefE* and *erm*, seven were of the MLS<sub>B</sub> phenotype, one was of the M phenotype, and one was of the MS phenotype.

When tested by disk diffusion, broth microdilution results were 100% concordant for erythromycin and 97.3% concordant for clindamycin. For the latter, four (2.7%) of the isolates appeared clindamycin susceptible by broth microdilution but were fully resistant by disk diffusion and possessed an *erm* gene. Of the 64 isolates with an MLS<sub>B</sub> phenotype, 57 were found to have an *erm* gene, whereas, of the 82 isolates with an M phenotype, 81 were found to possess the *mefE* gene. Erythromycin resistance was generally found to be greater in the isolates with an *erm* gene (MIC at which 50% of the isolates are inhibited [MIC<sub>50</sub>], ≥64 µg/ml; MIC<sub>90</sub>, ≥64 µg/ml) than in those with a *mefE* gene (MIC<sub>50</sub>, 4.0 µg/ml; MIC<sub>90</sub>, 32 µg/ml) (Fig. 1).

Hybridization studies of selected isolates supported the PCR findings in that only those positive for an *erm* gene by PCR hybridized with the *ermB* probe, but not with the *ermA*, *ermC*, and *mefE* probes, whereas, only those positive for *mefE* by PCR hybridized with a *mefE* probe. Erythromycin-susceptible strains and erythromycin-resistant strains, which were *ermB* *mefE* negative, did not hybridize with any of the probes. Erythromycin-resistant *ermB*-*mefE*-negative isolates were negative by probing for the *ermA*, -B, and -C and *mefE* genes. Thus, PCR was reliable in characterizing the two major mechanisms of resistance. The presence of nine isolates which did not yield amplicons by PCR and did not hybridize with *erm* and *mefE* suggests the presence of novel genes or mechanisms of resistance.

In this population-based study of clinical *S. pneumoniae* isolates from across Canada, we found that 55.8% of all macrolide-resistant isolates possessed the M phenotype, compared to 85 and 42% found by Sutcliffe et al. (9) and Shortridge et al. (6), respectively. Knowledge of the prevalence and type of

macrolide resistance may have therapeutic implications in view of the different levels of resistance, depending on the resistance mechanism and the ability of the macrolides to concentrate at the site of some infections (Fig. 1) (5).

We gratefully acknowledge the following for providing control strains: Barry Kreiswirth (Public Health Research Institute, New York, N.Y.) for *E. coli* RN7951 and *S. aureus* RN4220, Joyce Sutcliffe (Groton, Connecticut) for *S. pneumoniae* 02J1175, and the Centers for Disease Control and Prevention (Atlanta, Ga.) for *S. pneumoniae* R6.

This work was supported in part by a grant from Pfizer Pharmaceutical and the Canadian Bacterial Diseases Network.

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