

Antimicrobial Susceptibility and Macrolide Resistance Inducibility of *Streptococcus pneumoniae* Carrying *erm(A)*, *erm(B)*, or *mef(A)*

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Erythromycin-resistant *Streptococcus pneumoniae* isolates from young carriers were tested for their antimicrobial susceptibility; additionally, inducibility of macrolide and clindamycin resistance was investigated in pneumococci carrying *erm(A)*, *erm(B)*, or *mef(A)*. Of 125 strains tested, 101 (81%) were multidrug resistant. Different levels of induction were observed with erythromycin, miocamycin, and clindamycin in *erm(B)* strains; however, in *erm(A)* strains only erythromycin was an inducer. Induction did not affect macrolide MICs in *mef(A)* strains.

The predominant mechanisms of resistance to erythromycin and the other macrolides in *Streptococcus pneumoniae* are through target site modification by methylation that prevents the binding of the antibiotic to its ribosomal target, encoded by the *erm(B)* gene (16), or through efflux of the antibiotic, mediated by the *mef(A)* gene (13). Methylation of the ribosomal target of the antibiotics leads to cross-resistance to macrolides (M), lincosamides (L), and streptogramin B (S_B), the so-called MLS_B phenotype. Erm methylase synthesis can be inducible or constitutive (10, 11). Alterations in ribosomal proteins L4 and L22 or 23S rRNA have been reported to cause resistance in *S. pneumoniae* (1). Antibiotic efflux confers resistance only to the 14- and 15-member macrolides (1, 4, 5, 7).

Recently, *S. pneumoniae* isolates resistant to erythromycin due to carriage of the *erm(A)* gene have been reported (15). The macrolide resistance determinant *erm(A)* gene was previously described as *erm(TR)* in *S. pyogenes* (12) and now is reclassified as *erm(A)* (9).

The present study was undertaken to compare differences in antimicrobial susceptibility between *S. pneumoniae* isolates harboring *erm(A)* and those carrying either the *erm(B)* or *mef(A)* gene and to investigate the effect of induction on the expression of macrolide resistance in *erm(A)*-, *erm(B)*-, or *mef(A)*-positive pneumococci. The presence of genes coding for macrolide resistance was studied by PCR as described previously (8, 12, 13).

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One hundred twenty-five erythromycin-resistant *S. pneumoniae* isolates carrying the *erm(A)*, *erm(B)*, or *mef(A)* gene, recovered from 2,448 carriers younger than 2 years between February 1997 and February 1999 (14, 15), were evaluated for

their susceptibility to 10 antibiotics representing different classes of antimicrobial agents. These agents were 14-member (erythromycin and clarithromycin), 15-member (azithromycin), and 16-member ring macrolides (miocamycin), lincosamides (clindamycin), streptogramins (quinupristin/dalfopristin), ketolides (telithromycin), penicillin G, tetracycline, and chloramphenicol. Antimicrobial susceptibility tests were performed by the agar dilution method as previously described (3). Plates were incubated in ambient air at 37°C. Antibiotics were obtained from their respective manufacturers. Miocamycin was from Meiji Seika Kaisha (Tokyo, Japan). Except for those of miocamycin and telithromycin, MICs were interpreted according to the criteria of the National Committee for Clinical Laboratory Standards (NCCLS) (6). European break-points were used for miocamycin (≤ 1 $\mu\text{g/ml}$) (2) and telithromycin (≤ 0.5 $\mu\text{g/ml}$) (C. J. Soussy, F. Goldstein, A. Bryskier, H. Drugeon, J. Andrews, F. Baquero, O. Cars, D. Felmingham, B. Olsson-Liljequist, A. Rodloff, G. C. Schito, B. Wiedemann, and R. Wise, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 321, 2000).

The effect of induction by erythromycin, miocamycin, or clindamycin on the expression of resistance to erythromycin, clarithromycin, azithromycin, miocamycin, clindamycin, and telithromycin was evaluated. For induction studies, strains were incubated overnight on separate blood agar plates containing no antibiotic, 0.05 μg of erythromycin/ml, 0.05 μg of miocamycin/ml, or 0.01 μg of clindamycin/ml. MIC agar plates contained the same concentration of inducer to continue the effect of induction in addition to the concentration of antibiotic tested, except for MIC testing of the inducer. The concentration of antibiotic for induction was 1/5 to 1/10 of the lowest MIC level for a given antibiotic. The four *erm(A)*, five *erm(B)*, and two *mef(A)* pneumococci were tested for inducibility of resistance. All strains studied for induction were tested for the presence of mutation in the fifth domain of 23S rRNA and ribosomal proteins L4 and L22 as described previously (1). Only one strain with *mef(A)* had a mutation in L22 (K94R). In the macrolide MICs no significant differences were observed

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TABLE 1. Susceptibility of erythromycin-resistant pneumococci carrying the *erm(A)*, *erm(B)*, or *mef(A)* gene to various antimicrobial agents

Macrolide resistance determinant (no. of strains)	MIC range (MIC ₉₀) (μg/ml) ^a									
	ERY ^b	CLR	AZM	MIO	CLI	Q/D	TEL	PEN	TET	CHL
<i>erm(A)</i> (4)	1-2	0.5	2-4	0.5	0.06	0.5	0.016	0.03	4-16	4
<i>erm(B)</i> (84)	4->64 (>64)	1->64 (>64)	64->64 (>64)	1->16 (>16)	0.25->64 (>64)	0.125-1 (1)	0.008-0.25 (0.06)	0.016-4 (2)	0.25-64 (64)	2-16 (16)
<i>mef(A)</i> (37)	0.5-16 (16)	0.125-16 (8)	0.5-16 (8)	0.03-0.5 (0.25)	≤0.008-0.06 (0.06)	0.125-1 (1)	0.016-0.5 (0.25)	≤0.008-4 (2)	0.125-64 (32)	≤1-16 (4)

^a ERY, erythromycin; CLR, clarithromycin; AZM, azithromycin; MIO, miocamycin; CLI, clindamycin; Q/D, quinupristin/dalfopristin; TEL, telithromycin; PEN, penicillin G; TET, tetracycline; CHL, chloramphenicol. MIC₉₀, MIC at which 90% of isolates tested are inhibited.

between the *mef(A)* strain with a mutation in a highly conserved region of L22 and other strains without any mutation.

Of the 125 erythromycin-resistant pneumococci, 4 (3%), 84 (67%), and 37 (30%) isolates harbored the *erm(A)*, *erm(B)*, or *mef(A)* gene coding for macrolide resistance, respectively. None of the *S. pneumoniae* isolates was found to carry more than one macrolide resistance gene.

Of 125 erythromycin-resistant strains, 101 (81%) were also intermediate or resistant to one or more classes of antimicrobial agents other than macrolides and lincosamides. The four *erm(A)* pneumococci had decreased susceptibility to tetracycline; one isolate was intermediate and three were resistant (Table 1). Of the 84 *erm(B)* isolates, 78 were also resistant to tetracycline, 68 were resistant to chloramphenicol, and 39 were intermediate or resistant to penicillin. Of the 37 *mef(A)* pneumococci, 16 were also intermediate or resistant to penicillin, 14 were resistant to tetracycline, and 1 was resistant to chloramphenicol.

The susceptibilities to 14-, 15-, and 16-member macrolides and clindamycin of the pneumococcal isolates carrying an *erm(A)*, *erm(B)*, or *mef(A)* gene, appear in Table 1. Macrolide and clindamycin resistance levels were high in *S. pneumoniae* with the *erm(B)* gene but were low in the strains with *erm(A)* or *mef(A)*. Two of the *S. pneumoniae* strains were not resistant to clindamycin despite having an *erm(B)* gene. The MICs of clindamycin were 0.25 and 0.5 μg/ml for these strains, and the MIC of miocamycin was 1 μg/ml. The MICs of erythromycin, clarithromycin, and azithromycin (>64 μg/ml) were high for these strains. Among pneumococci that harbored *erm(B)*, 5 (6%) and 1 (1.2%) were susceptible and 16 (19%) and 1 (1.2%) were intermediate to miocamycin and clindamycin, respectively. Among pneumococci that harbored *mef(A)*, 0, 1 (3%), and 1 (3%) were susceptible and 1 (3%), 7 (19%), and 4 (11%) were intermediate to erythromycin, clarithromycin, and azithromycin, respectively. All strains tested were susceptible to telithromycin at ≤0.5 μg/ml, regardless of their macrolide resistance mechanism.

The results of inducibility studies are summarized in Table 2. In *erm(A)* strains, induction with clindamycin or miocamycin did not affect MICs for the antimicrobials tested. Induction with erythromycin did not significantly increase the MICs of erythromycin, clarithromycin, azithromycin, or telithromycin; after induction, MICs did not increase or increased a maximum of fourfold for these antibiotics. However, erythromycin induction caused a very high level of increase, at least 512-fold, in clindamycin MICs. For strains with *erm(A)*, erythromycin was the only inducer, and the erythromycin induction was especially increased in clindamycin MICs.

Among the five *S. pneumoniae* isolates carrying an *erm(B)* gene that were tested, two already had high-level resistance (>64 μg/ml) to erythromycin, clarithromycin, and azithromycin. In these strains induction with erythromycin increased the MICs for miocamycin and clindamycin 8- to 16-fold and 4- to more than 128-fold, respectively. Clindamycin induction increased the MICs for miocamycin and clindamycin to 8 and 32 to 64 μg/ml regardless of the initial MICs. Induction with miocamycin did not significantly affect the level of resistance for miocamycin among 2 *erm(B)* strains with high-level erythromycin resistance. Miocamycin induction increased the MIC of clindamycin for *erm(B)* strain 129. For the three *erm(B)* strains

TABLE 2. Detection of inducible resistance to antimicrobial agents of *S. pneumoniae* isolates: susceptibility testing with and without induction with erythromycin, miocamycin, or clindamycin

Macrolide resistance determinant	Strain with or without inducer ^a	MIC (µg/ml) ^b					
		ERY	CLR	AZM	MIO	CLI	TEL
<i>erm(A)</i>	SP215	4	1	8	0.5	0.125	0.03
	SP215 + ERY	4	1	16	2	>64	0.016
	SP215 + CLI	2	1	8	0.5	0.125	0.016
	SP215 + MIO	2	1	8	0.5	0.125	0.016
<i>erm(A)</i>	SP357	1	0.5	4	0.5	0.125	0.03
	SP357 + ERY	4	2	16	4	>64	0.016
	SP357 + CLI	2	1	8	0.5	0.125	0.016
	SP357 + MIO	1	0.5	4	0.5	0.06	0.016
<i>erm(A)</i>	SP16	1	0.5	4	0.5	0.06	0.03
	SP16 + ERY	4	2	16	4	>64	0.016
	SP16 + CLI	2	1	8	0.5	0.125	0.016
	SP16 + MIO	1	0.5	4	0.5	0.125	0.016
<i>erm(A)</i>	SP96	1	0.5	2	0.5	0.06	0.03
	SP96 + ERY	4	2	16	4	>64	0.016
	SP96 + CLI	2	1	8	0.5	0.125	0.016
	SP96 + MIO	1	0.5	4	0.5	0.06	0.016
<i>erm(B)</i>	SP129	>64	>64	>64	2	0.5	0.03
	SP129 + ERY	>64	>64	>64	16	>64	0.06
	SP129 + CLI	>64	>64	>64	8	64	0.03
	SP129 + MIO	>64	>64	>64	2	16	0.016
<i>erm(B)</i>	SP139	>64	>64	>64	2	16	0.03
	SP139 + ERY	>64	>64	>64	32	>64	0.03
	SP139 + CLI	>64	>64	>64	8	32	0.03
	SP139 + MIO	>64	>64	>64	4	16	0.06
<i>erm(B)</i>	SP62	2	1	32	8	16	0.03
	SP62 + ERY	>64	>64	>64	64	>64	0.06
	SP62 + CLI	>64	>64	>64	64	32	0.03
	SP62 + MIO	4	1	>64	8	16	0.03
<i>erm(B)</i>	SP406	1	1	4	0.5	0.25	0.03
	SP406 + ERY	>64	>64	>64	>64	>64	0.125
	SP406 + CLI	>64	>64	>64	8	32	0.03
	SP406 + MIO	4	1	>64	8	16	0.016
<i>erm(B)</i>	SP665	2	1	32	8	16	0.03
	SP665 + ERY	>64	>64	>64	>64	>64	0.125
	SP665 + CLI	>64	>64	>64	16	32	0.03
	SP665 + MIO	>64	8	>64	64	16	0.03
<i>mef(A)</i> +, mutation in L22 ^c	SP289	8	8	8	0.125	0.03	0.25
	SP289 + ERY	8	8	8	0.06	0.03	0.25
	SP289 + CLI	8	16	8	0.125	0.03	0.25
	SP289 + MIO	8	4	2	0.125	0.016	0.125
<i>mef(A)</i>	SP380	16	8	8	0.125	0.06	0.5
	SP380 + ERY	16	8	16	0.25	0.06	0.5
	SP380 + CLI	16	8	8	0.125	0.06	0.5
	SP380 + MIO	16	8	8	0.06	0.016	0.125

^a MICs were determined with and without induction. Strains were grown overnight on agar plates containing no antibiotic, 0.05 µg of erythromycin per ml, 0.01 µg of clindamycin per ml, or 0.05 µg of miocamycin per ml. MIC plates contained the same amount of inducer as the overnight plates.

^b ERY, erythromycin; CLR, clarithromycin; AZM, azithromycin; MIO, miocamycin; CLI, clindamycin; TEL, telithromycin.

^c This strain had a *mef(A)* gene and a K94R mutation in ribosomal protein L22.

(62, 406, and 665) with low-level resistance to erythromycin, induction with erythromycin and clindamycin significantly increased the MICs for erythromycin, clarithromycin, azithromycin, and miocamycin. Clindamycin MICs increased from 0.25 or 16 µg/ml to 32 µg/ml and increased to >64 µg/ml after

induction with clindamycin and erythromycin, respectively. Miocamycin induction increased MICs for erythromycin, azithromycin, miocamycin, and clindamycin in strains 406 and 665.

No significant changes in MICs for macrolides and lincos-

amides were noted in the two isolates carrying the *mef(A)* gene. In *erm(A)*-, *erm(B)*-, and *mef(A)*-carrying *S. pneumoniae* strains tested with or without induction, telithromycin MICs were in the susceptible range.

The degrees of resistance to erythromycin, clarithromycin, and azithromycin of the *erm(A)* pneumococci were within the more commonly quoted MIC range for *mef(A)* isolates (4, 5, 7). The *erm(A)* as well as the *mef(A)* pneumococci could easily be distinguished from the *erm(B)* isolates, most reliably by the significantly higher MICs observed for the 14-, 15-, and 16-member macrolides and the lincosamides by the majority of the *erm(B)* pneumococcal isolates.

The results of the present study indicate that the ability of erythromycin, miocamycin, and clindamycin to induce resistance was different in *erm(A)*- or *erm(B)*-carrying *S. pneumoniae* isolates. Macrolide and clindamycin resistances were found to be inducible by erythromycin, miocamycin, and clindamycin in *erm(B)* strains. By contrast, in *S. pneumoniae* isolates with the *erm(A)* gene, erythromycin was the only inducer and clindamycin MICs were most influenced by erythromycin induction. The high level of resistance to clindamycin in induced *erm(A)* pneumococci may show that methylation by *erm(A)*-encoded methylases affect clindamycin susceptibility more than macrolide susceptibility. This finding is in contrast with the MICs of drugs obtained for strains with methylated adenine at position 2058 of 23S rRNA. Methylation or base substitution at position 2058 of 23S rRNA affects mainly 14- and 15-member macrolides, and substitution of this base moderately increases the level of MICs for clindamycin (1). The affect of *erm(A)*-encoded methylase seems to be different from that of the other *erm*-encoded methylases that methylate adenine at position 2058. In *erm(B)* strains, erythromycin, clindamycin, and miocamycin appeared to be inducers of resistance. Erythromycin is the most potent inducer, followed by clindamycin and miocamycin. Miocamycin induced its resistance significantly in only two strains with *erm(B)*. Clindamycin induction, regardless of the initial MIC, increased the level of clindamycin resistance to a certain level (32 to 64 $\mu\text{g/ml}$). Occasionally *erm(B)* isolates appeared to be resistant to 14- and 15-member macrolides and susceptible or intermediate to 16-member macrolides and/or to clindamycin according to the applied criteria (2, 6). Especially in *erm(B)* strains, the ability of macrolides and clindamycin to induce their resistance should be taken into consideration when choosing antibiotics for treatment. Telithromycin can be distinguished from the other antibiotics tested: the constitutive or inducible expression of *erm*-type resistance or induction by erythromycin, clin-

damycin, or miocamycin did not change the activity of telithromycin on the *S. pneumoniae* strains tested.

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