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Two main mechanisms of macrolide resistance have been described in erythromycin-resistant *Streptococcus pneumoniae* (ERSP): a ribosomal methylase, ErmAM, and a macrolide efflux pump, MefE. In this study, we examined the prevalence of these mechanisms in 114 clinical isolates of ESRP from a 30-center study conducted in the United States between November 1994 and April 1995. The isolates were screened by polymerase chain reaction for the presence of known macrolide resistance genes. Seventy (61%) ESRP contained the macrolide efflux gene (*mefE*), whereas 36 isolates (32%) contained the biosomal methylase gene (*ermAM*). Isolates that were *ermAM*-positive had constitutive macrolide resistance. The minimum inhibitory concentrations (for which 90% of isolates were susceptible) of clarithromycin for the efﬂux-positive strains were much lower than those for the *ermAM*-positive strains (4 µg/mL vs. >128 µg/mL, respectively). The efﬂux mechanism is the predominant form of macrolide resistance in the United States.

Penicillin-resistant *Streptococcus pneumoniae* has become a serious problem in many parts of the world including the United States [1, 2]. A number of these isolates are resistant to one or more additional drug classes such as tetracycline, trimethoprim-sulfamethoxazole, and erythromycin. Much work has been done on the microbiological and clinical implications of intermediate (minimum inhibitory concentration [MIC], 0.1–1 µg/mL) and high-level (MIC, ≥2 µg/mL) penicillin resistance, including an understanding of the molecular basis of resistance [3, 4]. For the macrolides, the first mechanism of erythromycin resistance described in streptococci was production of a ribosomal methylase (encoded by *ermAM*), referred to as MLSB-type resistance [5]. The enzyme methylates a speciﬁc adenine on rRNA, thereby blocking binding of the macrolide to the ribosome. MICs of erythromycin for strains with inducibly regulated *ermAM* are 1–16 µg/mL, and these strains have apparent susceptibility to clindamycin until induction by a macrolide such as erythromycin [6]. Once expression of the gene is fully induced, or it is already constitutively expressed, the organism has complete cross-resistance to all macrolides, lincosamides, and streptogramin B (MLSB-R); most *S. pneumoniae* isolates with *ermAM* express it constitutively—that is, they have MLSB resistance with MICs of erythromycin and clindamycin of ≥16 µg/mL.

Efﬂux as a mechanism of macrolide resistance in streptococci has been recently described in *S. pneumoniae* and *Streptococcus pyogenes* and is encoded by *mefE* and by *mefA*, respectively [7–10]; *mefA* and *mefE* are 90% identical on the basis of their DNA sequence. *S. pneumoniae* strains with *mefE* are resistant to macrolides (with an MIC of erythromycin generally between 1 and 16 µg/mL) but are susceptible to clindamycin [8]. Mef is believed to be a membrane protein that is sufﬁcient for the energy-dependent efﬂux of 14- and 15-membered macrolides from the cell [10]. This efﬂux pump is much less effective (perhaps ineffective) on 16-membered macrolides, such as josamycin, and against ketolides and lincosamides [9, 11].

In this study, we determined the prevalence of *mefE* and *ermAM* in 114 erythromycin-resistant *S. pneumoniae* isolates from a 30-center antibiotic resistance survey performed in the United States during 1994–1995 [12].

**Methods**

The bacterial strains used in this study were obtained from a 30-center study that collected consecutive isolates from nonhospitalized adult and pediatric patients [12]. The isolates were obtained from either the lower respiratory tract or normally sterile body sites. The most common specimen type was sputum (42%) followed by blood (36%). MICs of erythromycin and clindamycin (Sigma, St. Louis) for all survey isolates were determined by the reference standard method for microbroth dilution testing of the National Committee for Clinical Laboratory Standards [13]; the test used Mueller-Hinton broth (Becton Dickinson Microbiology Systems, Cockeysville, MD) with 3% lysed horse blood.

One hundred fourteen clinical isolates of *S. pneumoniae* that were not susceptible to erythromycin (MIC, ≥0.5 µg/mL) were available for further study. Erythromycin-resistant isolates were screened for the presence of *ermAM* by PCR amplification with use of gene-
specific internal primers, as described elsewhere [8]. These isolates were also screened for the presence of \textit{mef}A and \textit{mef}E with use of the following primers: upper primer (5’ position 206), ATGCA-GACCAAAAGCCACCAC; and lower primer (3’ position 439), GCCTAGACAAGCCATCGC [9]. The primers were chosen from identical regions of \textit{mef}A and \textit{mef}E with Oligo 5.0 (NBI Software, Plymouth, MN) from sequences deposited in GenBank (Bethesda, MD). Macrolide-resistant isolates that did not produce an amplicon with primers for \textit{erm}AM or \textit{mef} were also screened with primers for \textit{erm}A, \textit{erm}C, \textit{ere}A, \textit{ere}B, and \textit{msr}A, as described elsewhere [8].

**Results**

A multicenter surveillance study was conducted in 1994–1995 [12]. A total of 1527 \textit{S. pneumoniae} isolates were prospectively collected from 30 different medical centers throughout the United States. One hundred fifty-two isolates were identified as not susceptible to erythromycin by microbroth dilution susceptibility testing. Erythromycin-resistant isolates were found at all 30 centers; macrolide resistance at each center ranged from 2.1%–23.0% (unpublished data, G. V. Doern et al.). Of these isolates, 114 were available for study of their erythromycin resistance mechanism. The isolates were retested to confirm macrolide resistance and screened for the presence of \textit{erm}AM and \textit{mef}, the two resistance mechanisms described in streptococci. Those isolates that were negative for \textit{erm}AM and \textit{mef} were screened for other resistance mechanisms. The results are shown in table 1.

Most strains (61%) had the macrolide efflux gene \textit{mef}. MICs of erythromycin for the \textit{mef}-positive isolates ranged from 1 to 16 \textmu g/mL, and these isolates were uniformly susceptible to clindamycin (figure 1). Thirty-two percent of the strains were \textit{erm}AM-positive. MICs of erythromycin for strains with \textit{erm}AM ranged from 1 to >128 \textmu g/mL; MICs for 32 of 36 strains were >16 \textmu g/mL. MICs of clindamycin ranged from 0.12 to >128 \textmu g/mL, and MICs for 32 of 36 strains were >16 \textmu g/mL. All but four strains had a constitutive phenotype (i.e., resistance to erythromycin and clindamycin). MICs of erythromycin for the four clindamycin-resistant \textit{erm}AM-positive strains were 1–2 \textmu g/mL, and the MIC of clindamycin was ≤0.12 \textmu g/mL.

Tests of these 4 isolates for induction with erythromycin and clindamycin disks showed multiple inner colonies, but these isolates were not inducible as defined by blunting of the inhibition zone of clindamycin [14]. The presence of inner colonies around the erythromycin disk suggests a mixed population of cells. It is possible that the inner colonies produce large amounts of methylase constitutively, while the apparently susceptible colonies may produce very low or no methylase constitutively. The variability of methylase production in \textit{S. pneumoniae} is under investigation.

Five strains had both \textit{mef} and \textit{erm}AM and phenotypically appeared to be constitutive producers of methylase. Three strains lacked all the macrolide resistance markers that were screened for, which included \textit{mef}A, \textit{mef}E, \textit{ere}A, \textit{ere}B, \textit{erm}A, \textit{erm}C, and \textit{erm}AM. One of these strains had a phenotype that resembled macrolide efflux, and two had high-level resistance to macrolides and lincosamides that was consistent with the constitutive production of a methylase. These strains suggest that although 97% of macrolide resistance in streptococci is due to \textit{erm}AM and \textit{mef}, other less common genes remain to be described.

The correlation of macrolide resistance to penicillin resistance was examined. Macrolide efflux was equally distributed among isolates that were susceptible, intermediate susceptible, and resistant to penicillin and was the most common resistance mechanism in all 3 classes. ErmAM methylase was found only in strains intermediate susceptible or resistant to penicillin but accounted for <50% of penicillin-resistant and macrolide-resistant isolates.

**Discussion**

Before the first description of the efflux phenotype in \textit{S. pyogenes} by Seppala et al. [7], macrolide-resistant streptococci were assumed to have methylase and were either inducible or
constitutive. The genes *mefA* and *mefE* were subsequently cloned and shown to be required for expression of the efflux phenotype in *S. pyogenes* and *S. pneumoniae*, respectively [9, 10]. It has been previously reported that *mef* is a common macrolide resistance mechanism among *S. pneumoniae* clinical isolates (45%–56%) [8, 15, 16]. In this study, we confirmed that *mef* is the most common mechanism of macrolide resistance among *S. pneumoniae* isolates in the United States. We also showed that isolates with Mef are uniformly susceptible to clindamycin and that MICs of erythromycin for these isolates are lower. The nature of the efflux mechanism and its lack of inducibility to higher MICs suggests that these strains may be inhibited if sufficiently high drug levels can be obtained [8].

Many respiratory infections are treated empirically with new macrolides, such as clarithromycin, that have higher concentrations at the site of infection. Clarithromycin levels in epithelial lining fluid of the lung (c_{max}, 34 μg/mL) and alveolar macrophages (c_{max}, 2000 μg/mL) exceed the MIC_{90} for susceptible *S. pneumoniae* strains (MIC_{90}, 0.03 μg/mL) as well as strains with *mef* (MIC_{90}, 4 μg/mL) [11, 17]. Knowledge of resistance mechanisms and their prevalence may impact the choice of empirical treatment if the most common resistance type, *mef*, responds to clarithromycin treatment. The efficacy of clarithromycin treatment for respiratory infections caused by *mef*-containing *S. pneumoniae* is currently being investigated.

References