

Prevalence of Macrolide Resistance Genes in Clinical Isolates of the *Streptococcus anginosus* (“*S. milleri*”) Group

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Twenty-two unrelated erythromycin-resistant anginosus group strains (3.2% resistance rate) were assessed for mechanisms of resistance. *Streptococcus anginosus* accounted for 16 of the 22 isolates. Fifteen isolates harbored the *erm*(B) gene. The *erm*(TR) and the *mef*(E) genes were carried by two isolates each. In three isolates, none of these resistance genes was detected by PCR.

The antimicrobial susceptibility patterns of the *Streptococcus anginosus* group (AG) of streptococci, previously designated the “*Streptococcus milleri*” group, vary according to geography (7, 11, 16), and periodic review of local susceptibility data has been recommended (7, 11). Some authors have reported increasing resistance rates of AG isolates against macrolides and clindamycin (7, 16, 25).

Macrolides, lincosamides, and streptogramins are grouped together as MLS antibiotics. Two MLS antibiotic resistance phenotypes are recognized in streptococci (20, 23, 24). The first confers resistance to macrolides, lincosamides, and streptogramin B and is caused by methylation of the target site in the 23S rRNA subunit by an adenine-*N*⁶-methyltransferase encoded by an *erm* (erythromycin ribosome methylase) gene (15). The phenotypic expression of MLS_B resistance may be inducible (MLS_{Bi} phenotype) or constitutive (MLS_{Bc} phenotype). The other phenotype, designated the M phenotype, causes resistance to 14- and 15-membered macrolides only and is caused by an active drug efflux system (23) encoded by the genes *mef*(A) (macrolide efflux) and *mef*(E) in *Streptococcus pyogenes* and *Streptococcus pneumoniae* respectively (3, 24).

In the present study, we wanted to determine the prevalence of MLS resistance among our collection of AG isolates and to assess the mechanisms of this resistance.

The streptococci studied were nonrepetitive AG isolates consecutively collected from clinical specimens submitted for culture at the microbiology department of the University Hospital of Maastricht from September 1995 to June 1999. They were recovered and identified as previously described (8), and species were identified by hybridization with 16S rRNA oligonucleotide probes in a reverse line blot assay (9, 10).

Screening for erythromycin and clindamycin resistance was carried out by the agar dilution method as recommended by the NCCLS (11, 17, 18). Isolates that were intermediate or resistant to one or both antibiotics were selected for determi-

nation of resistance type, together with the erythromycin-resistant AG isolates described previously (11).

For determination of the resistance phenotype, MICs of erythromycin, roxithromycin, azithromycin, and clindamycin were determined with E-test strips (Oxoid, Basingstoke, United Kingdom) and susceptibility to lincomycin and tylosin was assessed by a disk method (Rosco, Taastrup, Denmark). Inducible resistance was assessed with the double-disc test (20).

For resistance genotype determination, DNAs of the isolates were amplified with primers specific to the *erm*(A), *erm*(B), and *erm*(C) genes (12), the *mef*(A) and *mef*(E) genes (23), and the *erm*(TR) gene (14). For all PCR amplifications, PCR buffer containing 1.5 mM MgCl₂ was used. Nucleotide triphosphates were used at a concentration of 0.04 mM, and 0.01 U of *Taq* polymerase was added to each reaction mixture. Amplifications were performed in a Perkin-Elmer Cetus GeneAmp PCR System 9600 (Perkin-Elmer, Norwalk, Conn.), and the PCR protocols used were those described previously (12, 14, 23). The PCR products generated by the *mef*(A)-*mef*(E) and *erm*(TR) primers were directly sequenced by a fluorescent-sequence method (Amersham Pharmacia Biotechnology, Piscataway, N.J.). To assess the clonal identities of the isolates, typing was performed by amplified fragment length polymorphism (AFLP) analysis as previously described (22).

By the agar dilution method, 11 (3.2%) of 342 isolates were resistant to erythromycin. This resistance rate was slightly higher than the 2.6% resistance rate previously found in our laboratory (11). It was comparable to data reported from Argentina (2) but lower than those reported from Spain (14.3 and 17.7%) and the United States (13.6%) (7, 16, 25).

The 11 previously identified erythromycin-resistant isolates (11) were added to the present ones, resulting in a total of 22 isolates included for further characterization. Table 1 lists the resistance phenotypes and genotypes matched by species. *S. anginosus* accounted for the largest proportion of erythromycin-resistant isolates ($P = 0.015$). Other studies of smaller numbers of isolates demonstrated only slight differences among the three AG species in terms of MLS resistance rates (2, 7, 16, 25).

For 15 isolates assigned to the MLS_{Bc} phenotype, the MICs

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TABLE 1. Identification to the species level and characterization of 22 erythromycin-resistant AG strains

Species	No. of strains ^a with:				Total
	<i>erm</i> (B)	<i>erm</i> (TR)	<i>mef</i> (A)- <i>mef</i> (E)	No resistance gene	
<i>S. anginosus</i>	10 ^b	2 ^c	2 ^d	2 ^c	16
<i>S. constellatus</i>	4 ^b			1 ^b	5
<i>S. intermedius</i>	1 ^b				1
Total	15	2	2	3	22

^a In none of the strains was *erm*(A) or *erm*(C) detected.

^b MLS_{Bc} phenotype.

^c MLS_{Bi} phenotype.

^d M phenotype.

of erythromycin were >256 mg/liter and for 1 isolate the MIC was 8 mg/liter. For four MLS_{Bi} phenotype isolates, the MICs of erythromycin were 1, 3, 12, and >256 mg/liter, respectively, and these isolates showed inducible resistance to both clindamycin and tylosin. For the two M phenotype isolates, the MIC of erythromycin was 4 mg/liter. For all MLS_{Bc} phenotype strains, the MICs of clindamycin were >256 mg/liter.

Among the erythromycin-resistant isolates analyzed in this study, the *erm*(B) gene was predominant, which is in agreement with a previous study (4). The results of the sequence analysis showed that the PCR products generated by the *mef*(A)-*mef*(E) and *erm*(TR) primers were identical to the *mef*(E) and *erm*(TR) genes, respectively. *mef* genes have been demonstrated in a limited number of isolates of the viridans group, including one AG isolate (1, 19). *mef* genes have accounted for the rapid emergence of erythromycin resistance in *S. pyogenes* and *S. pneumoniae* in several countries (6, 21), and therefore their presence in AG isolates is of concern.

The *erm*(TR) gene has been found in *S. pyogenes* (5) and in large-colony group G streptococci (13), but, to our knowledge, it has not been demonstrated in AG isolates before. Three isolates assigned to the MLS_B phenotype did not react with any of the primers chosen in the PCR amplification. We are conducting further studies to reveal the genetic mechanism of this resistance.

The numbers of isolates recovered from various body sites were as follows: abdomen, eight; genital tract, five; head and neck region, five; thoracic cavity, two; blood, one. For one isolate, complete clinical data were not available. AFLP analysis showed that all of the isolates had unique genotypes. There was no relationship between the Lancefield group, pattern of hemolysis, or site of isolation of the isolates on the one hand and the resistance genotype on the other hand.

In conclusion, this study demonstrated the presence of different erythromycin resistance genes, including the *erm*(B), *erm*(TR), and *mef*(E) genes, in a series of clinical AG isolates. The present data highlight the need for periodic surveillance of the rates of the antimicrobial resistance and its mechanism in these streptococci.

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many, for *S. aureus* control strains [*erm*(A) and *erm*(C) genes]; L. Jensen, Danish Veterinary Laboratory, Copenhagen, Denmark, for *E. faecalis* JH2-2::Tn1545 [control strain for *erm*(B) detection], J. Sutcliffe, Department of Infectious Diseases, Pfizer Central Research, Groton, Conn., for *S. pyogenes* 02C1064 and *S. pneumoniae* 02J1175 [for *mef*(A)-*mef*(E) gene detection]. We thank C. Schot, E. Cornelissen, and K. Spee for technical assistance.

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