Susceptibilities of Oral and Nasal Isolates of Streptococcus mitis and Streptococcus oralis to Macrolides and PCR Detection of Resistance Genes

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The susceptibility of viridans group streptococci to macrolides was determined. Thirteen isolates (17%) were resistant to erythromycin. Five strains carried an erm gene that was highly homologous to that in Tn917. Four strains had mefE genes that coded erythromycin efflux ability.

Viridans group streptococci, commensal bacteria in the human oral and nasal cavities, are associated with systemic diseases, including infective endocarditis, bacteremia, and pneumonia (2, 5, 6, 10, 12). Macrolide resistance has spread in many oral and nasal cavities, are associated with systemic diseases, including infective endocarditis, bacteremia, and pneumonia (2, 5, 6, 10, 12). Macrolide resistance has spread in many

TABLE 1. In vitro activity of macrolide antibiotics for S. oralis and S. mitis

<table>
<thead>
<tr>
<th>Organism</th>
<th>Antibiotic</th>
<th>MIC (μg/ml)</th>
<th>Range</th>
<th>50%</th>
<th>90%</th>
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<tbody>
<tr>
<td>S. oralis</td>
<td>Erythromycin</td>
<td>0.016–512</td>
<td>0.125</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>0.016–2048</td>
<td>0.031</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roxithromycin</td>
<td>≤0.016–512</td>
<td>0.25</td>
<td>8</td>
<td></td>
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<tr>
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<td>0.25</td>
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<tr>
<td>Rokitamycin</td>
<td>0.015–4</td>
<td>0.125</td>
<td>0.5</td>
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<tr>
<td>S. mitis</td>
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<td>0.125</td>
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<td>Clarithromycin</td>
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<tr>
<td>Azithromycin</td>
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<td>Rokitamycin</td>
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<td>0.125</td>
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</table>

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The erm (23S rRNA methylase) gene were designed based on the sequence of corresponding genes from other organisms. The highly resistant strains gave a distinct band of the expected 530-bp size from position 103 to position 633 of the erm gene. PCR products from these strains and S. salivarius E37 were sequenced (Fig. 1). Sequences from strains E2, E21, and E30 were identical to those of the gene encoding the rRNA methylase on transposon Tn917 (4). Those from other strains had ~98% homology to Tn917. The nucleotide changes in strains SO12, SO13, and E37 resulted in six, four, and two amino acid alterations, respectively, but did not affect the reading frame. Primer sets for the mefE gene were designed based on the sequence of that gene in S. pneumoniae (GenBank U83667).

The intermediate resistant strains O24, E17, and E3 and highly resistant strain O14 gave the expected band (approximately 1,200 bp) for the mefE gene encoding the macrolide efflux pump (18). The sequences of DNA obtained from PCR amplification for mefE in strains O24, E3, and E17 were analyzed. These sequences were identical to those at positions 30 to 1190 of the corresponding mefE gene. Less erythromycin accumulated in mefE-positive strain E17 than in mefE-negative strain E115 min after the addition of [N-methyl-14C]erythromycin (data not shown). Accumulation of erythromycin in the mefE-positive strain was restored by addition of proton conductors, suggesting that a macrolide efflux system exists in strain E17. For genes encoding macrolide-inactivating enzyme,
ereA, ereB, and mphE and macrolide efflux genes msr and mefA (17), PCR amplification was performed with DNA from macrolide-resistant strains; however, no PCR products have been detected.

Although erm genes encoding rRNA methylase are present in various organisms, such as Escherichia coli, Bacillus subtilis, and Staphylococcus aureus, and macrolide resistance is widespread in bacteria associated with humans (4, 15, 17), the potential reservoir for erm genes is unknown. It has been reported that the genes lie on various transposable elements or conjugal plasmids (4, 11, 14). In the present study, we showed that the nucleotide sequences of PCR products obtained with erm primers from some strains were identical to the rRNA methylase gene in Enterococcus faecalis transposon Tn917, while those from S. oralis SO12 and S. mitis SO13 were highly homologous with the ermB gene from conjugal plasmid pIP501 of Streptococcus agalactiae (3, 4). In the upstream region, the sequence homologous to LR, an internal sequence of Tn917, while those from ermB, ermA, and ermC, are major species in the oral and nasal normal flora. The results of this study suggest the transmission of macrolide-resistant genes between oral streptococci and other more virulent streptococci.

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REFERENCES


