Ribosomal Mutations in *Arcanobacterium pyogenes* Confer a Unique Spectrum of Macrolide Resistance

B. Helen Jost,* Hien T. Trinh, J. Glenn Songer, and Stephen J. Billington

Department of Veterinary Science and Microbiology, The University of Arizona, Tucson, Arizona 85721

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Four macrolide-resistant *Arcanobacterium pyogenes* isolates contained A2058T, A2058G, or C2611G (Escherichia coli numbering) mutations in their 23S rRNA genes. While these mutations conferred resistance to erythromycin, oleandomycin, and spiramycin, they did not confer resistance to tylosin.

*Arcanobacterium pyogenes* is a commensal and an opportunistic pathogen of economically important animals, causing liver abscesses in feedlot cattle (5) and arthritis (15) and pneumonia in pigs (1). In livestock, the use of subtherapeutic levels of antimicrobial agents, such as the macrolide tylosin (TYL), to promote growth is common in the United States. Of U.S. feedlot cattle, 42.3% receive TYL, primarily for the prevention of liver abscesses (16). A total of 29.1% of *A. pyogenes* isolates are resistant to TYL, and we have characterized two prevalent determinants of TYL resistance in *A. pyogenes*, Erm X and Erm B (2, 3).

*erm* genes encode methylases that methylate the N⁶ position of Escherichia coli A2058 in the 23S rRNA. This modification confers protection against the action of a variety of macrolide, lincosamide, and streptogramin B antimicrobial agents (6). However, target alteration can also occur by mutation of residues within domain V, the peptidyltransferase loop of the 23S rRNA ribosomal subunit (17). Depending on the exact location and nature of the mutation, resistance to a single antibiotic agent or a broad class of agents is conferred (17).

**Bacterial strains and antimicrobial susceptibility determination.** The bacterial strains used in this study are shown in Table 1. *A. pyogenes* isolates are very susceptible to macrolide-lincosamide (ML) agents, with MICs of erythromycin (ERY), TYL, and clindamycin (CLI) for three non-erm-carrying isolates, BBR1, OX-5, and OX-9, being ≤0.06 μg/ml and those of oleandomycin (OLM) and spiramycin (SPM) being 0.125 to 0.5 μg/ml (Table 2). For *erm*(B)-containing isolates, MICs of all ML agents tested are >64 μg/ml (3) (Table 2). However, we identified four *A. pyogenes* isolates for which ERY, OLM, and SPM MICs were high and for which MIC patterns of TYL and CLI were unique but which did not carry *erm*(B) or *erm*(X) genes (data not shown). OX-2 is resistant to CLI, but the MIC of TYL for this strain is 0.5 μg/ml, which, while considered to indicate susceptibility, is an approximately 10-fold increase over the MICs for known susceptible isolates (Table 2). JGS583 exhibits low-level resistance to both TYL and CLI. JGS881 shows high-level resistance to CLI, but the MIC of TYL for it is elevated. For JGS882, MICs of TYL and CLI are low, although both are elevated compared to MICs for susceptible strains (Table 2).

**Identification of ribosomal mutations.** As base substitutions within domain V of the 23S rRNA can result in ML resistance (17), sequencing of this gene region in these *A. pyogenes* isolates was undertaken. Primers 23S-1 (5′-AGTTCGACCTG CACGAATGGC-3′) and 23S-2 (5′-GTTCGTCCGTCCGG TCCTCCTC-3′) were used to amplify a product of 728 bp, equivalent to bases 1953 to 2680 of the *E. coli* 23S rRNA gene (GenBank accession no. U70214), from the four macrolide-resistant and three macrolide-susceptible *A. pyogenes* isolates. The sequences of the PCR products were determined by using automated DNA sequencing.

Mutations were identified by aligning the sequences using CLUSTAL W (13), with the sequence of the ML₆ *A. pyogenes* isolate BBR1 being designated the wild type. Of the other ML₆ isolates, OX-5 has a wild-type sequence and OX-9 has a G2137T substitution (*E. coli* numbering). As OX-9 is ML₆, it is unlikely that the G2137T change contributes to ML resistance, and it probably represents a naturally occurring polymorphism in the 23S rRNA gene.

OX-2 contains an A2058T mutation which results in high MICs of ERY, OLM, SPM, and CLI but only a slightly elevated MIC of TYL (0.5 μg/ml) (Table 2). JGS583 also contained this mutation and an additional mutation, G2137C. The MIC pattern for this strain is similar to that for OX-2, with the exception of the TYL MIC (8 μg/ml) (Table 2). It is unlikely that mutations at base 2137 results in the increased TYL MIC, as the ML₆ isolate OX-9 also has a mutation at this location. However, it is possible that JGS583 contains one or more additional mutations, either in the 23S rRNA gene or in genes encoding ribosomal proteins L4 or L22, which can also confer ML resistance (17). Gene sequences for *A. pyogenes* ribosomal proteins are not available, so this hypothesis was not tested. JGS881 contains an A2058G mutation which results in only a slightly increased TYL MIC (0.25 μg/ml) (Table 2). However, the CLI MIC for this isolate also is higher than that for strains with the A2058T mutations. This may be due to the specific base substitution at this position or to additional mutations at other sites. While the mutations at base 2058 in the *A. pyogenes* 23S rRNA gene resulted in high MICs of ERY, OLM, SPM, and CLI, their presence resulted in a lower range of TYL MICs.

TYL is used exclusively in veterinary medicine and is not
often used in MIC determinations for human pathogens, which predominate in the antimicrobial drug resistance literature (17). As a result, it has been somewhat accepted as dogma that mutations at base 2058 confer resistance to all macrolides (17), although there is scant experimental evidence to confirm that mutations at this position actually confer resistance to TYL. A2058T or A2058G mutations in Mycoplasma pneumoniae result in high MICs of ERY and TTYL but only intermediate MICs of CLI (4). In contrast, A2058G mutations in Mycoplasma pneumoniae result in high MICs of ML agents, with the MICs of CLI (4). A2058T or A2058G mutations in other bacterial species result in high and the MICs of TYL and CLI are low (Table 2). A

<table>
<thead>
<tr>
<th>Strain</th>
<th>Characteristics*</th>
<th>Source and/or reference</th>
</tr>
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<tbody>
<tr>
<td>BBR1</td>
<td>Bovine; Tc r ML*</td>
<td>1</td>
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<tr>
<td>OX-2</td>
<td>Porcine; Tc r Erm*; Ty*; Clm*</td>
<td>Oxford Laboratories, Worthington, Minn.</td>
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<td>OX-5</td>
<td>Porcine; Tc r ML*</td>
<td>Oxford Laboratories</td>
</tr>
<tr>
<td>OX-7</td>
<td>Porcine; Tc r ML*</td>
<td>Oxford Laboratories; 7</td>
</tr>
<tr>
<td>OX-9</td>
<td>Porcine; Tc r ML*</td>
<td>Oxford Laboratories</td>
</tr>
<tr>
<td>JGS583</td>
<td>Bovine; Tc r ML'</td>
<td>Pharmacia and Upjohn Animal Health, Kalamazoo, Mich.</td>
</tr>
<tr>
<td>JGS881</td>
<td>Avian; Tc r Erm*; Ty*; Clm*</td>
<td>California Animal Health and Food Safety Laboratory System, University of California, Davis</td>
</tr>
<tr>
<td>JGS882</td>
<td>Avian; Tc r Erm*; Ty*; Clm*</td>
<td>California Animal Health and Food Safety Laboratory System, University of California</td>
</tr>
</tbody>
</table>

* The R superscript denotes resistance, with MIC breakpoints of \( \geq 4 \mu g/mL \) for tetracycline (Tc) and \( \geq 8 \mu g/mL \) for ML agents. The S superscript denotes susceptibility, with MIC breakpoints of \( \leq 2 \mu g/mL \) for tetracycline and ML agents. Phenotypes are indicated for ERY (Erm), TYL (Tyl), and CLI (Clm).

The sequence data obtained in this study were submitted to the GenBank database under accession numbers AY375319 (BBR1), AY375320 (OX-2), AY375321 (OX-5), AY375322 (OX-9), AY375323 (JGS883), AY375324 (JGS881), and AY375325 (JGS882).

References


