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

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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 N^6 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 antimicrobial 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′-AGTTCCGACCTG CACGAATGGC-3′) and 23S-2 (5′-GTTCGTCCGTCCCGG TCCTCTC-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 ML^s *A. pyogenes* isolate BBR1 being designated the wild type. Of the other ML^s isolates, OX-5 has a wild-type sequence and OX-9 has a G2137T substitution (*E. coli* numbering). As OX-9 is ML^s, 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 ML^s 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

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TABLE 1. *A. pyogenes* strains used in this study

Strain	Characteristics[a]	Source and/or reference
BBR1	Bovine; Tc^r ML^s	1
OX-2	Porcine; Tc^r Erm^r Tyl^s Clm^r	Oxford Laboratories, Worthington, Minn.
OX-5	Porcine; Tc^s ML^s	Oxford Laboratories
OX-7	Porcine; Tc^r ML^r	Oxford Laboratories; 7
OX-9	Porcine; Tc^r ML^s	Oxford Laboratories
JGS583	Bovine; Tc^r ML^r	Pharmacia and Upjohn Animal Health, Kalamazoo, Mich.
JGS881	Avian; Tc^r Erm^r Tyl^s Clm^r	California Animal Health and Food Safety Laboratory System, University of California, Davis
JGS882	Avian; Tc^r Erm^r Tyl^s Clm^s	California Animal Health and Food Safety Laboratory System, University of California
JGS942	Spontaneous Erm^r mutant of OX-9	This study

[a] The R superscript denotes resistance, with MIC breakpoints of ≥4 μg/ml for tetracycline (Tc) and ≥8 μg/ml for ML agents. The S superscript denotes susceptibility, with MIC breakpoints of ≤1 μg/ml for tetracycline and ML agents. Phenotypes are indicated for ERY (Erm), TYL (Tyl), and CLI (Clm).

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 *Brachyspira hyodysenteriae* result in high MICs of ERY and TYL but only intermediate MICs of CLI (4). In contrast, A2058G mutations in *Mycoplasma pneumoniae* result in high MICs of ML agents, with the exception of low MICs of TYL (8, 11). This latter phenotype is similar to that seen in *A. pyogenes* strains containing an A2058T mutation (Table 2). Interestingly, in *A. pyogenes*, an A-to-G transition at base 2058 appears to result in a significantly higher MIC of CLI than an A-to-T transversion at this position, assuming that no additional mutations are present.

As well as mutations at base 2058, a C2611G mutation was identified in strain JGS882, and this mutation confers yet a different MIC pattern. The MICs of ERY, OLM, and SPM are high and the MICs of TYL and CLI are low (Table 2). A C2611G mutation in *Streptococcus pneumoniae* conferred a similar pattern of MICs of ML antimicrobial agents (11). However, C2611G and C2611U mutations in other bacterial species confer a variable spectrum of ML resistance (9, 17).

Conclusions. Mutations at bases 2058 and 2611 of the 23S rRNA gene can result in high MICs of a range of ML antimicrobial agents, although there does appear to be some vari-

ability in the MIC pattern, depending on the bacterial species examined (4, 7–9, 11, 12, 17). This is the first description of these mutations associated with macrolide resistance in *A. pyogenes*, although A2058T or A2058G and C2611G mutations resulted in only low MICs of TYL, compared to the other macrolides tested (ERY, OLM, and SPM). Isolation of *A. pyogenes* strains with this mechanism of resistance is uncommon. Of 36 macrolide-resistant *A. pyogenes* isolates, only 4 (11.1%) do not carry known *erm* genes, and each of the 4 contains mutations in its 23S rRNA genes. Given that this animal commensal and pathogen will most likely encounter TYL, rather than other ML antimicrobial agents, it is perhaps logical that *erm* genes, which confer high-level resistance to TYL, encode the most predominant type of macrolide resistance found in *A. pyogenes*.

Nucleotide sequence accession numbers. 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 (JGS583), AY375324 (JGS881), and AY375325 (JGS882).

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TABLE 2. MICs of ML antimicrobial agents for *A. pyogenes* strains

Strain	Mutation in 23S rRNA gene[b]	MIC[a] 14-Membered ring macrolide ERY	OLM	16-Membered ring macrolide SPM	TYL	Lincosamide, CLI
BBR1	None	≤0.06	0.5	0.125	≤0.06	≤0.06
OX-5	None	≤0.06	0.25	0.25	≤0.06	≤0.06
OX-9	G2137T	≤0.06	0.25	0.5	≤0.06	≤0.06
OX-7[c]	ND	>64	>64	>64	>64	>64
OX-2	A2058T	>64	>64	64	0.5	8
JGS583	A2058T; G2137C	>64	>64	32	8	8
JGS881	A2058G	>64	>64	64	0.25	>64
JGS882	C2611G	>64	>64	8	0.125	1

[a] MICs were determined using modified NCCLS methodology (10, 14).
[b] *E. coli* numbering. ND, not determined.
[c] OX-7 carries the *erm*(B) gene.

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