Plasmid-Borne \( \text{erm}(T) \) from Invasive, Macrolide-Resistant \textit{Streptococcus pyogenes} Strains

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Twenty-three isolates of group A streptococci (GAS) recovered from population-based invasive GAS surveillance in the United States were erythromycin resistant, inducibly clindamycin resistant, and lacked known macrolide resistance determinants. These 23 isolates, representing four different clones, contained a broad-host-range plasmid carrying the \( \text{erm}(T) \) methylase gene, which has not been detected in GAS previously.

Penicillins are the antibiotics of choice for treatment of pharyngitis caused by group A streptococci (GAS), with macrolides recommended for patients with penicillin allergy. Macrolide-resistant GAS are an increasing concern worldwide, with a correlation between increasing macrolide resistance and an increase in macrolide consumption (2, 6, 22).

In GAS, macrolide resistance is conferred by the 23S rRNA methylase genes \( \text{erm}(B) \) and \( \text{erm}(T) \), as well as by the efflux determinant \( \text{mef}(A) \) (12, 15). The \( \text{erm}(B) \) and \( \text{erm}(T) \) genes often confer inducible resistance to macrolides, lincosamides, and streptogramin B, but also may encode constitutive resistance to macrolides, lincosamides, and streptogramin B due to upstream attenuator sequence alterations. \( \text{mef}(A) \) encodes efflux pump-mediated resistance to erythromycin (and other 14- or 15-member ring macrolides), while the organism remains susceptible to clindamycin (and other lincosamides) and streptogramin B. Acquisition of macrolide resistance primarily occurs by horizontal gene transfer of \( \text{erm} \) or \( \text{mef} \) genes (4, 13), while more rarely resistance results from mutations within genes encoding ribosomal components (12). The \( \text{erm}(B) \) and \( \text{erm}(T) \) genes often confer cross-resistance to both lincosamides and streptogramins and therefore limit the therapeutic possibilities available to clinicians for the treatment of GAS disease (12).

In this study, we identified the genetic basis of macrolide and inducible clindamycin resistance in 23 invasive GAS isolates that were negative for macrolide resistance determinants known to be disseminated among resistant GAS (5, 16, 18). These 23 isolates were collected among all invasive GAS infections reported during 1999, 2001, and 2003 (3,189 reported cases) from CDC’s population-based Active Bacterial Core surveillance conducted in 10 sites throughout the United States (http://www.cdc.gov/ncidd/dbdn/abc/s/).

Susceptibility to erythromycin was determined by the broth dilution technique with a standard panel of antibiotics, includ-
plasmid, designated pRW35 (GenBank accession no. EU192194). The pRW35 sequence had a G+C content of 37% and three distinct open reading frames (ORFs) (Fig. 1A). One ORF exhibited near identity (98%) to the erythromycin resistance methylase gene \( \text{erm}(T) \) found on a transposon in \textit{Streptococcus gallolyticus} subsp. \textit{pasteurianus} (20) as well as plasmid-borne \( \text{erm}(T) \) determinants from \textit{Lactobacillus reuteri} (19), \textit{Lactobacillus} sp. (23), and \textit{Streptococcus bovis} (NCBI accession no. BAA75016). In addition to \( \text{erm}(T) \), pRW35 contains two ORFs highly similar to plasmid replication and transfer genes found in broad-host-range plasmids. ORF2 predicts a protein highly similar (>60%) to mobilization proteins from \textit{Streptococcus agalactiae}, \textit{Lactococcus lactis}, and \textit{Streptococcus bovis} (14, 21).

An attenuator region upstream of \( \text{erm}(T) \) in pRW35 nearly identical to its counterpart in \textit{S. gallolyticus} subsp. \textit{pasteurianus} was identified (Fig. 1B). This region shares features with the corresponding region upstream of the \textit{S. aureus} plasmid-borne \( \text{erm}(C) \) (7) and is predicted to share the same mechanism of regulating translation of the \( \text{erm}(T) \) message (3, 7). During induction by erythromycin, sensitive ribosomes are stalled upon the upstream leader peptide favoring formation of hairpin structure 5-6 (active conformation). As the proportion of resistant ribosomes increases (originally present in relatively small numbers) to a critical level, translation of the \( \text{erm}(T) \) message greatly decreases due to a shift of the message to the inactive conformation.

**TABLE 1.** PCR primers used in this study for detection of macrolide resistance genes

<table>
<thead>
<tr>
<th>Gene(s)</th>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{erm}(C) )</td>
<td>TR-322U</td>
<td>5'-GGGTCAAGGAAAAAGGACAT-3'</td>
</tr>
<tr>
<td>( \text{erm}(C) )</td>
<td>TR619L</td>
<td>5'-CCTAAACGTCTGATT-3'</td>
</tr>
<tr>
<td>( \text{mef}(A) ) and ( \text{mef}(E) )</td>
<td>mef-3301U</td>
<td>5'-AGGGCAAGCAGTATCATATTAA TCA-3'</td>
</tr>
<tr>
<td>( \text{mef}(A) ) and ( \text{mef}(E) )</td>
<td>mef-3673L</td>
<td>5'-CTGCAAAGACTGACTATAGC CT-3'</td>
</tr>
<tr>
<td>( \text{erm}(T) )</td>
<td>( \text{erm}(T) ) forward</td>
<td>5'-CCGCCATTGAAATAGATCATTCA-3'</td>
</tr>
<tr>
<td>( \text{erm}(T) )</td>
<td>( \text{erm}(T) ) reverse</td>
<td>5'-GCTTGATAAAATTGGTTTTTGGA-3'</td>
</tr>
</tbody>
</table>

**FIG. 1.** (A) Diagram of pRW35. Three ORFs are indicated. (B) Nearly identical attenuator and translational start regions of \( \text{erm}(T) \) from \textit{S. pyogenes} and \textit{Streptococcus pasteurianus}. The sequences encompassing the leader peptide-encoding sequence and \( \text{erm}(T) \) translational start are shown. Perfect and imperfect inverted repeats are indicated with arrows. Pairing of inverted repeats 5 and 6 represents the putative active conformation according to the \( \text{erm}(C) \) model (10). Pairing of inverted repeats 1 and 2 and 3 and 4 represents the putative inactive conformation in which the \( \text{erm}(T) \) ribosome binding site (RBS) and translation initiation codon are sequestered by pairing of inverted repeats 3 and 4. The RBS, translational start codons, and leader peptide translational stop codons are in bold.
(1-2 and 3-4 hairpins) that results in sequestration of the emm(T) translational start and ribosome binding site.

emm typing (2; www.cdc.gov/ncidod/biotech/strep/protocol_emm-type.htm) identified four unique types among the 23 emm(T)-positive isolates, including emm92 (19 isolates), emm3 (2 isolates), emm9, and emm28 (Table 2). The 19 emm92 isolates formed a distinct cluster of highly related pulsed-field gel electrophoresis (PFGE) patterns, and the two emm3 isolates displayed an additional distinct PFGE profile (data not shown). The emm9 and emm28 isolates represented two additional distinct patterns. The T types from all of the isolates and PFGE profiles from types emm92, emm3, and emm28 conferred to patterns typically associated with erythromycin-sensitive strains of the same emm types (9; unpublished data), indicating that the emergence of emm(T)-positive GAS is not associated with newly emerging clonal types.

We examined the ability of pRW35 to transfer resistance to another streptococcal species and to another S. pyogenes strain. Plasmid pRW35 was extracted from S. pyogenes isolates and electroporated into S. agalactiae (group B streptococci) and S. pyogenes strain NZ131 by previously described methods (11, 17). Transformants of both species selected on sheep blood agar plates containing 1 μg/ml erythromycin were subsequently screened for the presence of the emm(T) gene by PCR and tested for inducible clindamycin resistance with the D test. All transformants had identical resistance phenotypes with the 23 GAS isolates in that they were resistant to erythromycin (≥32 μg/ml) and inducibly clindamycin resistant (12 μg/ml). All transformants were positive for emm(T) and the entire circular plasmid pRW35 by PCR analysis. These results indicated that pRW35 conferred erythromycin and inducible clindamycin resistance as a replicative plasmid in the distantly related group B species and in a heterologous S. pyogenes strain.

In summary, our data indicate that plasmid pRW25 is disseminated among multiple unrelated GAS strains, including classical opacity factor-positive (types emm28, emm9, and emm92) and -negative (emm3) strains. Transformation of pRW25 into S. agalactiae and an additional GAS strain as a replicative plasmid is consistent with the hypothesis that pRW35 is capable of dissemination to a broad range of hosts. emm(T)-specific primers may be useful in subsequent PCR screens for macrolide resistance determinants among GAS.

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REFERENCES


### TABLE 2. Features of invasive GAS isolates containing emm(T)

<table>
<thead>
<tr>
<th>emm type</th>
<th>Yr isolated (no. of isolates)</th>
<th>State(s) (no. of isolates)</th>
<th>T pattern (no. of isolates)</th>
<th>PFGE profilea</th>
</tr>
</thead>
<tbody>
<tr>
<td>emm92</td>
<td>1999 (3), 2001 (6), 2003 (10)</td>
<td>California (11), Oregon (7), Connecticut (1)</td>
<td>T/8/25/Imp19b (17), T nontypeable (2)</td>
<td>1</td>
</tr>
<tr>
<td>emm3</td>
<td>2003 (2)</td>
<td>Oregon (2)</td>
<td>T3</td>
<td>2</td>
</tr>
<tr>
<td>emm9</td>
<td>2001 (1)</td>
<td>Georgia (1)</td>
<td>T14</td>
<td>3</td>
</tr>
<tr>
<td>emm28</td>
<td>2001 (1)</td>
<td>Oregon (1)</td>
<td>T28</td>
<td>4</td>
</tr>
</tbody>
</table>

a PFGE patterns within a given profile share identity or diverge from others within the profile by no more than three bands and differ from the other profiles by more than seven bands.

b This pattern includes T/8/25/Imp19, T25/Imp19, T/Imp19, and T/8/25.


