Phenotypic and Molecular Characterization of Erythromycin Resistance in Four Isolates of Streptococcus-Like Gram-Positive Cocci Causing Bacteremia

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Among nine patients with bacteremia caused by Granulicatella or Gemella in a 6-year period (July 1995 to June 2001), three had bacteremia caused by erythromycin-resistant Granulicatella adiacens and one had bacteremia caused by erythromycin-resistant Gemella haemolysans. All four isolates possessed mef genes, whereas none possessed ermT, ermTR, or ermB genes.

Macrolides constitute an important group of drugs because of their antimicrobial and immunomodulatory activities (17). Macrolide resistance has been increasingly reported in various species of Streptococcus (5–7, 11, 20, 21) and is mediated through two major mechanisms, target site modification and efflux pumps. Target site modification is mediated through the acquisition of erm (erythromycin resistance methylase) genes (2–5, 12, 13, 15, 20). These genes encode enzymes that N6 dimethylate a specific adenine residue in the peptidyl transferase circle of 23S rRNA domain V of the bacteria, leading to cross-resistance to macrolides, lincosamides, and streptogramin B. As for efflux pumps, they are mediated through mef genes, which encode membrane proteins responsible for active efflux of macrolides, hence reducing the intracellular macrolide concentration to subtoxic levels.

No study describing the phenotypic and molecular characterization of macrolide resistance in Streptococcus-like gram-positive cocci (Granulicatella and Gemella) was found in the literature. In this study, we report phenotypic and genotypic characterization of the erythromycin resistance in these Streptococcus-like gram-positive cocci recovered from blood cultures of patients in a 6-year period.

The patients in this study were hospitalized at the Queen Mary Hospital in Hong Kong during a 6-year period (July 1995 to June 2001). All clinical data were collected prospectively. Clinical specimens were collected and handled according to standard protocols. The BACTEC 9240 blood culture system (Becton Dickinson, Sparks, Md.) was used. All suspect colonies were identified by standard conventional biochemical methods (9), and Streptococcus-like gram-positive cocci were further identified using the API system (20 STREP) (bioMerieux Vitek, Hazelwood, Mo.).

16S rRNA gene sequencing was used to confirm the identities of Granulicatella and Gemella (18, 19). MICs of penicillin, erythromycin, clindamycin, and vancomycin were determined by using the agar dilution method, and results were interpreted according to the NCCLS criteria for viridans streptococci (9, 10).

Bacterial DNA extraction and PCR amplification and DNA sequencing of the ermT, ermTR, ermB, and mef genes were performed according to our previous publications (7, 16, 20). The sequences of the PCR products were compared with known erm and mef gene sequences in GenBank by multiple sequence alignment using the CLUSTAL W program (14), and phylogenetic tree construction was performed using the PileUp method with GrowTree (Genetics Computer Group, Inc.).

The characteristics of the nine patients with bacteremia caused by Streptococcus-like gram-positive cocci (Granulicatella [n = 7] and Gemella [n = 2]) have been reported previously (18, 19). The MICs of the 11 Streptococcus-like gram-positive cocci isolates were summarized in Table 1. The isolates recovered from five patients (56%) (four Granulicatella adiacens isolates and one Gemella morbillorum isolate) were sensitive to penicillin, erythromycin, clindamycin, and vancomycin. One isolate of Gemella haemolysans was resistant to penicillin (MIC, 0.25 μg/ml) and erythromycin (MIC, 1 μg/ml), one G. adiacens isolate was resistant to penicillin (MIC, 0.25 μg/ml) and erythromycin (MIC, 8 μg/ml), and two G. adiacens isolates were resistant to erythromycin (MICs of 8 μg/ml for both isolates) but sensitive to penicillin, clindamycin, and vancomycin. All four Streptococcus-like gram-positive cocci isolates that were resistant to erythromycin possessed mef genes (Fig. 1), whereas none of them possessed ermT, ermTR, or ermB genes.

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TABLE 1. Antibiotic susceptibility patterns of Streptococci-like gram-positive cocci

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/sex</td>
<td>M55</td>
<td>F77</td>
<td>M66</td>
<td>M80</td>
<td>M82</td>
<td>M85</td>
<td>M47</td>
<td>M41</td>
<td>M43</td>
</tr>
<tr>
<td>Underlying disease(s)</td>
<td>Lymphoma, neutropenic fever</td>
<td>Recurrent pyogenic cholangitis</td>
<td>Abdominal aortic aneurysm</td>
<td>Polyoid cystitis, benign prostatic hypertrophy</td>
<td>Ischemic heart disease, polycystic kidney disease, cerebrovascular accident</td>
<td>Aortic regurgitation, chronic renal failure</td>
<td>Acute myeloid leukemia, neutropenic fever</td>
<td>Lymphoma, bone marrow transplant recipient, neutropenic fever</td>
<td>Lymphoma, bone marrow transplant recipient, neutropenic fever</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Primary bacteremia</td>
<td>Infective endocarditis with septic thromboemboli</td>
<td>Infective endocarditis with dissection</td>
<td>Acute prostatitis, hematuria</td>
<td>Infective endocarditis</td>
<td>Primary bacteremia</td>
<td>Primary bacteremia</td>
<td>Primary bacteremia</td>
<td>Primary bacteremia</td>
</tr>
<tr>
<td>Identification by 16S rRNA sequencing</td>
<td>Granulicatella adiacens</td>
<td>Granulicatella adiacens</td>
<td>Gemella morbilorum</td>
<td>Granulicatella adiacens</td>
<td>Granulicatella adiacens</td>
<td>Granulicatella adiacens</td>
<td>Gemella hiemalis</td>
<td>Granulicatella adiacens</td>
<td>Granulicatella adiacens</td>
</tr>
<tr>
<td>MIC (μg/ml)</td>
<td>0.03</td>
<td>0.03</td>
<td>0.04</td>
<td>&lt;0.002</td>
<td>&lt;0.002</td>
<td>&lt;0.002</td>
<td>0.25</td>
<td>0.25</td>
<td>0.15</td>
</tr>
<tr>
<td>Penicillin</td>
<td>8</td>
<td>0.125</td>
<td>0.06</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
<td>8</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.25</td>
<td>0.06</td>
<td>0.06</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.15</td>
<td>0.06</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.25</td>
<td>0.5</td>
<td>0.5</td>
<td>&lt;0.015</td>
<td>0.25</td>
<td>0.25</td>
<td>0.5</td>
<td>0.5</td>
<td>0.125</td>
</tr>
</tbody>
</table>

In our locality, erythromycin resistance in the prevalent gram-positive cocci was predicted through erm and mef genes, respectively. For Streptococcus pneumoniae, 27 and 73% of our isolates possessed the respective genes.

Recently, we noticed that 36.5% of the isolates associated with invasive Streptococcus bovis infection were erythromycin resistant. For Streptococcus pneumoniae, nine and 11 of these 24 erythromycin-resistant isolates possessed both genes. This observation of the presence of the mefA and ermTR genes, respectively, is in line with the evidence from a study which showed that it is possible to move the mefA gene from all 11 erythromycin-susceptible Streptococcus pneumoniae isolates to S. pyogenes, 

Macrolide resistance in Streptococcus-like as well as other gram-positive cocci may have resulted from horizontal gene transfer of mef genes, mainly among different species of grammegative bacteria. For example, in our laboratory, 24% of the isolates possessed the mefA gene, whereas none of the isolates possessed the ermTR gene. In contrast to S. pneumoniae, S. pyogenes, and S. bovis, S. epidermidis is resistant to erythromycin-producing tools.

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lates have been lodged within the GenBank sequence database under accession numbers AY422726, AY422727, AY422728, and AY422729.

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REFERENCES