

## 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 N<sup>6</sup> 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 conven-

tional 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  $\mu\text{g/ml}$ ) and erythromycin (MIC, 1  $\mu\text{g/ml}$ ), one *G. adiacens* isolate was resistant to penicillin (MIC, 0.25  $\mu\text{g/ml}$ ) and erythromycin (MIC, 8  $\mu\text{g/ml}$ ), and two *G. adiacens* isolates were resistant to erythromycin (MICs of 8  $\mu\text{g/ml}$  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 *Streptococcus*-like gram-positive cocci

Characteristic	Data for patient								
	1	2	3	4	5	6	7	8	9
Year of isolation	1995	1997	1997	1999	1999	2000	2000	2000	2001
Age/sex	M/55	F/77	M/66	M/80	M/62	M/85	M/47	F/41	F/43
Underlying disease(s)	Lymphoma, neutropenic fever	Recurrent pyogenic cholangitis	Abdominal aortic aneurysm	Polypoid cystitis, benign prostatic hypertrophy	Ischemic heart disease, polycystic kidney disease, cerebrovascular accident	Aortic regurgitation, chronic renal failure	Acute myeloid leukemia, neutropenic fever	Lymphoma, bone marrow transplant recipient, neutropenic fever	Lymphoma, bone marrow transplant recipient, neutropenic fever
Diagnosis	Primary bacteremia	Acute cholangitis	Infective endocarditis with septic thromboemboli	Acute prostatitis, hematuria	Infected aortic aneurysm with dissection	Infective endocarditis	Primary bacteremia	Primary bacteremia	Primary bacteremia
Identification by 16S rRNA sequencing	<i>Granulicatella adiacens</i>	<i>Granulicatella adiacens</i>	<i>Gemella morbillitorum</i>	<i>Granulicatella adiacens</i>	<i>Granulicatella adiacens</i>	<i>Granulicatella adiacens</i>	<i>Granulicatella adiacens</i>	<i>Gemella haemolysans</i>	<i>Granulicatella adiacens</i>
MIC ( $\mu\text{g/ml}$ ) of:									
Penicillin	0.03	0.03	0.004	<0.002	<0.002	<0.002	0.25	0.25	0.015
Erythromycin	8	0.125	0.06	0.125	0.125	0.125	8	1	8
Clindamycin	0.25	0.06	0.06	0.5	0.25	0.06	0.125	0.25	0.06
Vancomycin	0.25	0.5	0.5	0.06	<0.015	0.25	0.5	0.5	0.125

In our locality, erythromycin resistance in the prevalent alpha-hemolytic and beta-hemolytic streptococci was mediated through *erm* and/or *mef* genes. For *Streptococcus pneumoniae*, 27 and 73% of our erythromycin-resistant isolates possessed *erm* and *mef* genes, respectively (5). As for *Streptococcus bovis*, recently we noticed that 24 (65%) out of 37 *S. bovis* strains isolated from patients with *S. bovis* bacteremia were erythromycin resistant (7). Fourteen and 11 of these 24 erythromycin-resistant isolates possessed *ermB* and *ermT* genes, respectively, with one isolate possessing both *ermB* and *ermT* genes, and none possessed *mef* genes. For *Streptococcus pyogenes*, it was noticed that 36.5% of the isolates associated with invasive *S. pyogenes* infections in our locality were resistant to erythromycin, and the resistance was mediated through the possession of an *ermTR* gene, a *mef* gene, both an *ermTR* and a *mef* gene, or an *ermB* gene (2). As for beta-hemolytic Lancefield group G streptococci, among 100 patients with beta-hemolytic group G streptococcal bacteremia in a 6-year period, seven (7%) had bacteremia caused by erythromycin-resistant beta-hemolytic group G streptococci (20). Five of the seven isolates possessed *mef* genes only, whereas one possessed an *ermTR* gene and one possessed both *mef* and *ermB* genes. In contrast to *S. pneumoniae*, *S. bovis*, *S. pyogenes*, and beta-hemolytic group G streptococci, the present study showed that erythromycin resistance in *Streptococcus*-like gram-positive cocci in our locality was mediated by the presence of *mef* genes, whereas none of the isolates possessed an *ermT*, *ermTR*, or *ermB* gene. This observation of the presence of *mef* genes but no *erm* genes is in line with the phenotypic resistance profiles of the isolates, in that all four isolates were resistant to erythromycin but sensitive to clindamycin.

Macrolide resistance in *Streptococcus*-like as well as other gram-positive cocci may have resulted from horizontal transfer of *mef* genes, mainly among different species of gram-positive cocci (2, 4, 5, 8, 20). From the available sequence information, it can be observed that the *mef* genes of the three strains of *G. adiacens* and one strain of *G. haemolysans* shared more than 99% amino acid identities with those of *S. pneumoniae* (U83667, AF274302, AB011259, and AF376746), *Streptococcus salivarius* (AJ318993), *Staphylococcus aureus* (AY064721), *Streptococcus intermedius* (AY064722), an *Enterococcus* species (AY071836), and *Streptococcus dysgalactiae* (AY355410 and AY355408) (Fig. 1). These implied that there could be horizontal gene transfer of *mef* genes among the various gram-positive cocci. This is in line with the evidence from a study which showed that it is possible to move the *mef* gene from all 11 erythromycin-resistant *S. pneumoniae* isolates tested to erythromycin-susceptible *S. pneumoniae* and/or *E. faecalis* recipients (8). Since the *mef* genes of *S. pneumoniae* have been documented experimentally to be of the *mef*(E) type and that of *S. pyogenes* was of the *mef*(A) type (1), it is most likely that the *mef* genes of the four *Streptococcus*-like gram-positive cocci in the present study were of the *mef*(E) type, since their amino acid sequences shared more than 99% sequence identity with those of *S. pneumoniae*.

**Nucleotide sequence accession numbers.** The *mef* gene sequences of the four *Streptococcus*-like gram-positive cocci iso-

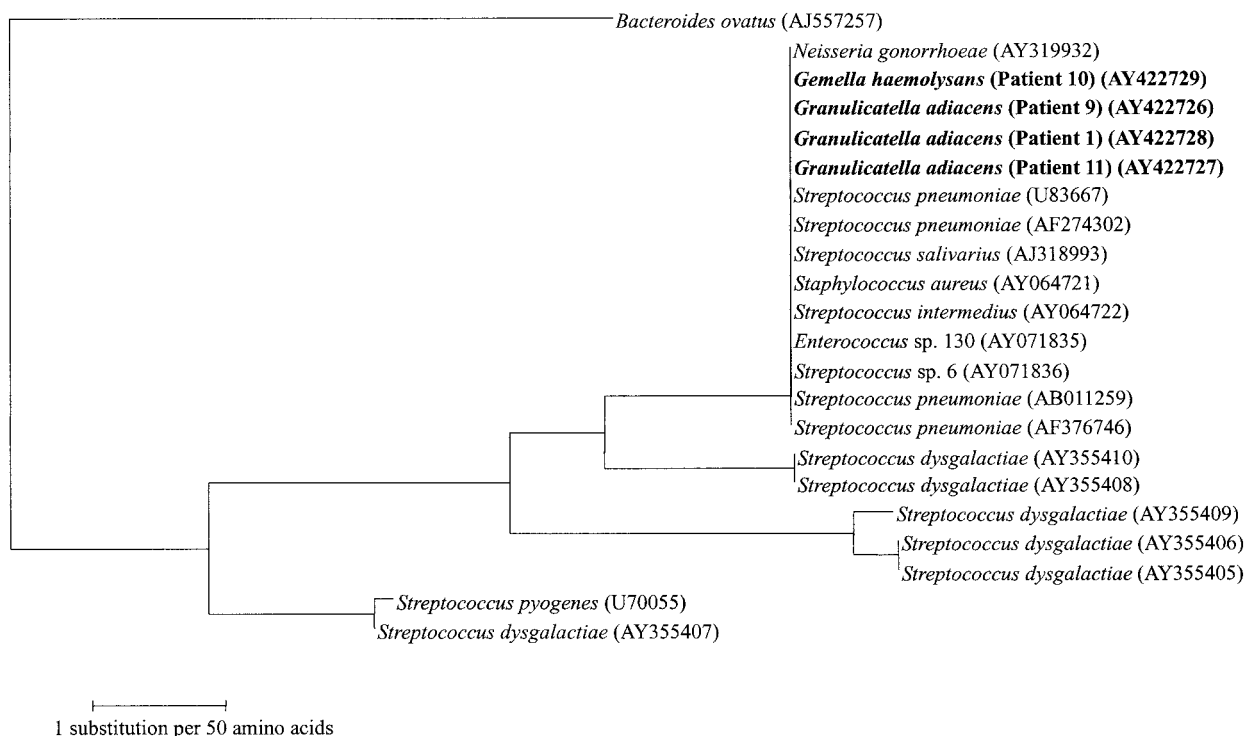


FIG. 1. Phylogenetic tree showing the relationships of the amino acid sequences of the *mef* genes of the four *Streptococcus*-like gram-positive cocci (highlighted in bold) to those of other *mef* genes. The tree was inferred from the 318-amino-acid sequence data by the neighbor-joining method. The scale bar indicates the estimated number of substitutions per 50 amino acids using the Kimura correction. Names and accession numbers are given as cited in the GenBank database.

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

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