Three New Macrolide Eﬄux (mef) Gene Variants in Streptococcus agalactiae

Streptococcus agalactiae (group B streptococcus [GBS]) is the major cause of neonatal sepsis (9). Penicillin is the most common choice for intrapartum prophylaxis, but erythromycin or clindamycin is recommended for patients allergic to penicillin (3). There are two major mechanisms of erythromycin resistance in S. agalactiae: (i) erythromycin ribosomal methylase, mediated by ermB, ermA, ermTR, or ermC, which confers cross-resistance to macrolides, lincosamides, and streptogramin B (MLS-B phenotype), and (ii) a less common macrolide eﬄux pump, mediated by mef (7), which confers resistance to 14- and 15-membered macrolides only (M phenotype). The major mef variants, mefE and mefA, were originally identiﬁed in S. pneumoniae and S. pyogenes, respectively (5, 11); both are found in S. agalactiae (2), although mefE is much more common (4, 12).

Recently, we tested 512 GBS isolates from Australia, Hong Kong, and South Korea by using a multiplex PCR-based reverse line blot (mPCR/RLB) assay to identify nine resistance markers and identiﬁed mef in 22 (12). However, we did not distinguish mef variants. Subsequently, we tested a total of 1,629 GBS isolates, which included the 512 isolates, from nine countries by using the same mPCR/RLB assay, except that two new probe pairs, speciﬁc for mefA and mefE, were added (Table 1). Isolates were typed by using a three-set genotyping system which identiﬁes the molecular serotype (MS), surface protein genes, and mobile genetic elements, as described previously (8). Antibiotic susceptibilities to erythromycin, clindamycin, and tetracycline were measured by E-test (AB Biodisk; Australia Laboratory Services Pty. Ltd.) and interpreted as recommended by the Clinical and Laboratory Standards Institute (12).

Forty-ﬁve (2.7%) of 1,629 isolates were positive for mef, and of these, 35 contained mefE, 7 contained mefA, and 3 gave weak or variable signals with mefE- and mefA-specific probes. These three isolates were among 16 mef-positive isolates from Hong Kong. Their genotypes and phenotypic susceptibilities to erythromycin, clindamycin, and tetracycline are shown in Table 2. All three had the M phenotype and MS Ia but atypical genotypes. MS Ia usually has the surface protein gene alpl and insertion sequence IS1381 (8, 10). Two of these isolates had alpl, but, instead of IS1381, carried the type II intron GBSi1, usually found in MS III but rarely in other serotypes (10). The other isolate had neither the surface protein gene nor the insertion sequence.

From each of these three isolates, mef was ampliﬁed and sequenced with the primers shown in Table 1. The full sequences indicated that all were novel mef variants not previously described in a GBS. They were deposited in GenBank with accession numbers DQ445269 to DQ445271. DQ445271 and DQ445270 were 99% similar to each other but only 88% and 89% homologous with mefE (GenBank accession no. AF227521) and mefA (GenBank accession no. AY064721), respectively. They shared 99 to 100% homology with a mef variant recently identiﬁed in Streptococcus dysgalactiae (a group G streptococcus) (GenBank accession no. AM168138 and AY355405) (1). DQ445269 has not been described before; it had 89% homology with mefA (GenBank accession no. AY064721) and the novel group G streptococcus mef gene (GenBank accession no. AY355405), 91% homology with mefE (GenBank accession no. AY227521), and 92% homology with another mef variant, mefI, described in Streptococcus pneumoniae (GenBank accession no. AJ971089) (6). The inconsistent mPCR/RLB results for these isolates can be explained by mutations in the mefAESb and mefAEAb regions. New primers and probes will be required to detect them reli-

### Table 1. Oligonucleotide primers and probes used in this study

<table>
<thead>
<tr>
<th>Primer or probe</th>
<th>Target</th>
<th>$T_m$ (°C)</th>
<th>GenBank accession no.</th>
<th>Sequence (5’–3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mefA/E primers and new mefA- and mefE-specific probes for mPCR/RLB</td>
<td>mefA/E</td>
<td>63.41</td>
<td>AF227521/AY064721</td>
<td>3314/50 GGC AGG AGG GCA AGC AGT ATC 3331/67</td>
</tr>
<tr>
<td>mefA/E primers and new mefA- and mefE-specific probes for mPCR/RLB</td>
<td>mefE</td>
<td>66.33</td>
<td>AF227521</td>
<td>3453 TGC CAA AGA CCG CAT AGG G 3435</td>
</tr>
<tr>
<td>mefA/E primers and new mefA- and mefE-specific probes for mPCR/RLB</td>
<td>mefA</td>
<td>61.08</td>
<td>AF227521</td>
<td>3529 CTT GTC CGG TGC TTA CTA TTA TTA 3549</td>
</tr>
<tr>
<td>mefA/E primers and new mefA- and mefE-specific probes for mPCR/RLB</td>
<td>mefE</td>
<td>61.08</td>
<td>AF227521</td>
<td>192 CAG GTG CCA AAA TCG CAT AGT 173</td>
</tr>
<tr>
<td>mefA/E primers and new mefA- and mefE-specific probes for mPCR/RLB</td>
<td>mefE</td>
<td>61.08</td>
<td>AF227521</td>
<td>265 CGT GTG CAG TGC TGA TTA TT 284</td>
</tr>
<tr>
<td>mefA/E primers and new mefA- and mefE-specific probes for mPCR/RLB</td>
<td>mefE</td>
<td>59.76</td>
<td>AF227521/AY064721</td>
<td>3674/410 CTG TCC TCC TCC TCC TCC TCC TAA AAT TGG TGG 3651/387</td>
</tr>
</tbody>
</table>

| Primers for whole mef gene amplification and sequencing | mef-1025 | 65.21 | AJ971089 | 180 GACCCCAAGCCACACTGGG 199 |
| Primers for whole mef gene amplification and sequencing | mef1 | 57.78 | AJ064721 | 6 ATG GAA AAA AAC AAT TGG 26 |
| Primers for whole mef gene amplification and sequencing | mef5235 | 62.93 | AJ064721 | 528 GTA TGT GGT GCT GTG AAT GC 547 |
| Primers for whole mef gene amplification and sequencing | mef608A | 51.84 | AJ064721 | 685 AA/G/G AGT AAT AAAA (G) GCA AAC/T ATG C 664 |
| Primers for whole mef gene amplification and sequencing | mef1218 | 46.63 | AJ064721 | 1223 TTA TTG TTA ATC TAA TTA TTA TCT 1203 |
| Primers for whole mef gene amplification and sequencing | mef1329A | 61.02 | AJ971089 | 1666 CCT CTG CTA TCA ATG CAT 1586 |

* A b suffix indicates a 5’ biotin-labeled primer; a p suffix indicates a 3’ amine-labeled probe.
* The primer $T_m$ values were provided by the primer synthesizer (Sigma-Aldrich).
* Numbers represent the numbered base positions at which primer sequences start and finish (numbering start point 1 refers to start point 1 of the gene with the corresponding GenBank accession number).
* This primer is a modiﬁed form of one described by Klaassen and Mouton (7).
* This primer was designed by us as a sequencing primer.
ably by mPCR/RLB. For these novel mef variants, we propose the names mefG (for DQ445270 and DQ445271) and mefB (for DQ445269) to reflect the beta-haemolytic streptococcus groups in which they were first identified.

These findings and the atypical genotype patterns suggest that these strains have arisen by recombination. Further investigation will be required to determine their clinical significance.

We sincerely thank Margaret Ip, Department of Microbiology, The Chinese University of Hong Kong, and the Prince of Wales Hospital, Hong Kong, for allowing us to study their isolates.

This work was partially supported by National Health and Medical Research Council grant 358351 to G.L.G. and F.K.

We thank Christine Norman, Centre for Infectious Diseases and Microbiology (CIDM) Westmead, New South Wales, Australia for help with figure preparation.

REFERENCES


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*Published ahead of print on 27 June 2007.