JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2007, p. 2754–2755 Vol. 45, No. 8
0095-1137/07/$08.00+0 doi:10.1128/JCM.00579-07

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

Streptococcus agalactiae (group B streptococcus [GBS]) is the most common cause of neonatal sepsis (9). Penicillin is used 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 clinical isolates (including the original 512) from nine countries by using the same mPCR/RLB, 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 *alp1* and insertion sequence IS*1381* (8, 10). Two of these isolates had *alp1* but, instead of IS*1381*, 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

Primer or probe[a]	Target	T_m (°C)[b]	GenBank accession no.	Sequence (5′–3′)[c]
mefA/E primers and new *mefA*- and *mefE*-specific probes for mPCR/RLB				
mefAESb	*mefA/E*	63.41	AF227521/ AY064721	3314/50 GGC AGG GCA AGC AGT ATC 3331/67
mefAAP	*mefA*	64.33	AF227521	3453 GTC CAA AGA CCG CAT AGG G 3435
mefASP	*mefA*	61.08	AF227521	3529 CTG GTT CGG TGC TTA CTA TTG 3549
mefEAP	*mefE*	64.17	AY064721	192 CAG GTC CCA AAA TCG CAT AG 173
mefESP	*mefE*	62.95	AY064721	265 CTG GTG CAG TGC TTG CTA TT 284
mefAEAb	*mefA/E*	59.76	AF227521/ AY064721	3674/410 CTG TTC TTC TGG TAC TAA AAG TGG 3651/387
Primers for whole *mef* gene amplification and sequencing				
mef-102S[d]	*mefA/E*	65.21	AJ971089	180 GACCAAAAGCCACATTGTGG 199
mef1[d]	*mefA/E*	57.78	AY064721	6 ATG GAA AAA TAC AAC AAT TGG 26
mef523S[e]	*mefA/E*	62.93	AY064721	528 GTA TTG GGT GCT GTG ATT GC 547
mef680A[e]	*mefA/E*	51.84	AY064721	685 AA(/G)G AGT AAT AAA(/G) GCA AAC(/T) AAT C 664
mef1218[d]	*mefA/E*	46.63	AY064721	1223 TTA TTT TAA ATC TAA TTT TCT 1203
mef1329A[d]	*mefA/E*	61.02	AJ971089	1606 CCTCCTGTCTATAATCGCATG 1586

[a] A b suffix indicates a 5′ biotin-labeled primer; a p suffix indicates a 5′ amine-labeled probe.
[b] The primer T_m values were provided by the primer synthesizer (Sigma-Aldrich).
[c] 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).
[d] This primer is a modified form of one described by Klaassen and Mouton (7).
[e] This primer was designed by us as a sequencing primer.

TABLE 2. Characteristics and comprehensive genotyping results of three *mef* variants

Identification[a]	GenBank accession no.	Type[b]	MIC (mg/liter)[c]			Genotype (MS-pgp-mge-AR)[d]
			Erythromycin	Clindamycin	Tetracycline	
HK-115	DQ445269	S	8	0.19	0.25	Ia-*mefB*
HK-99	DQ445270	S	12	0.094	48	Ia-*alp1*-GBSil-*tetM*-*mefG*
HK-121	DQ445271	S	12	0.125	48	Ia-*alp1*-GBSil-*int*-Tn-*mefG*

[a] HK, Hong Kong.
[b] The isolate type was superficial (S) or colonizing.
[c] MICs were measured by E-test and interpreted (with incubation in CO_2) according to the kit instructions. For erythromycin and clindamycin, the categories were as follows: ≤0.5 mg/liter, susceptible; 1 mg/liter, intermediate; ≥2 mg/liter, resistant. For tetracycline, the categories were as follows: ≤2 mg/liter, susceptible; 4 mg/liter, intermediate; ≥8 mg/liter, resistant.
[d] Genotypes are reported as the MS; protein gene profile (pgp) for *bca* (Cα/Bca gene), *bac* (Cβ/Bac gene), *rib* (Rib gene), *alp1* (Alp1/Alp5/epsilon gene), *alp2* (Alp2 gene), and *alp3* (Alp3 gene); mobile genetic elements (mge) (including IS*1381* and GBSi1, among others) (8, 10); and antibiotic resistance (AR) genes (12).

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-hemolytic 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.

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▽ Published ahead of print on 27 June 2007.