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Erythromycin and Clindamycin Resistance in Group B Streptococcal Clinical Isolates†

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Erythromycin (EM) and clindamycin (CM) susceptibility testing was performed on 222 clinical isolates of group B *Streptococcus*. A multiplex PCR assay was used to detect the *ermB*, *ermTR*, and *mefA*/*E* antibiotic resistance genes. These results were compared to the phenotypes as determined by the standard EM/CM double disk diffusion assay.

Group B *Streptococcus* (GBS) is one of the leading causes of neonatal bacterial infection. This type of infection commonly leads to pneumonia, septicemia, or meningitis. Because of the serious nature of neonatal GBS infections, the suggested standard protocol for the obstetrician/gynecologist is that pregnant women should be tested for the presence of GBS at 35 to 37 weeks of gestation (7, 15). Once GBS colonization is diagnosed, the typical treatment for these patients is penicillin, to which there is no known resistance. However, there is a significant population of penicillin-allergic patients, a reported 12% of pregnant women (12), for whom the macrolide (erythromycin [EM]) or lincosamide (clindamycin [CM]) class of drugs needs to be administered, in particular, for those patients who are at high risk for anaphylactic shock. Previous reports have cited resistance of GBS to EM and CM of up to 37% and 17%, respectively (7). The resistance is commonly caused by three genes: *ermB*, *ermTR*, and *mefA*/*E* (1, 9, 10). The *ermB* and *ermTR* genes encode 23S rRNA methylases, which alter the binding of the antibiotic target site. The expression of these genes leads to the *c*onstitutively expressed and the erythromycin-*i*nduced *m*acrolide, *l*incosamide, and *s*treptogramin B (cMLS and iMLS, respectively) resistance phenotypes (9). The *mefA* and *mefE* genes, which are 90% identical, encode 14- and 15-member macrolide efflux pumps and lead to the macrolide only (M) resistance phenotype (1). Because of the presence of *ermB*, *ermTR*, *mefA*/*E*, and other antibiotic resistance genes on plasmids and/or transposons, these genes can pass from organism to organism, and the monitoring of the antibiotic resistance of GBS should occur regularly (13). We used a multiplex PCR assay to screen for the prevalence of the *ermB*, *ermTR*, and *mefA*/*E* genes in GBS clinical isolates from 222 patients for whom physicians ordered GBS testing. The samples, representing 20 states in the United States and 60% of which were from Florida, New Jersey, and Texas, were chosen at random. Patient ages ranged from 15 to 82 years, with an average of 31.3 ± 11.8 years. These results were compared to the antibiotic resistance phenotypes as determined by the standard EM/CM double disk diffusion assay (3, 11, 15) to determine clinical correlations.

Cervicovaginal-rectal swabs in transport media (Cellmatics [Becton Dickinson, Sparks, MD] and *OneSwab* [Medical Diagnostic Laboratories, L.L.C., Hamilton, NJ]) were collected between December 2004 and June 2005. GBS strains were isolated by streak plating 1 to 10 μl of transport medium- or Todd-Hewitt broth-inoculated cultures for single colonies onto a NEL-GBS agar plate (Northeast Laboratory Services, Waterville, Maine) (NEL-P8000). The plates were incubated in an anaerobic chamber (BBL GasPak 100 Anaerobic System) at 37°C for 18 to 24 h. GBS was selected by the production of an orange pigment when grown anaerobically on NEL-GBS agar. A tryptic soy agar plate with 5% sheep blood (NEL-P1100) was used for streak purification, verification of beta-hemolysis, and CAMP testing of all clinical GBS strains. The *Streptococcus agalactiae* ATCC 12386 and the *Streptococcus pyogenes* ATCC 19615 strains were used as GBS-positive and -negative controls, respectively, and the *Staphylococcus aureus* ATCC 25923 strain was used in the CAMP test.

GBS strains were tested for EM and CM susceptibility using the double disk diffusion assay as described previously to identify the cMLS, iMLS, M, and L (lincosamide) resistance phenotypes (3, 11, 15) (see the supplemental material). A multiplex PCR was used to identify the *ermB*, *ermTR*, and *mefA*/*E* genes from the GBS strains, using primers (Table 1) and conditions previously reported, and a separate PCR was used to amplify the *linB* gene (2, 4, 5, 16) (see the supplemental material).

Of 222 clinical GBS strains, 38% were resistant to EM and 21% were resistant to CM as determined by the standard double disk diffusion assay. Specifically, there were 40 cMLS, 19 iMLS, 25 M, and 6 L resistance phenotypes. The multiplex PCR assay proved to be an effective method to detect the resistance genes, as well as to predict the susceptibility phenotype of the double disk diffusion assay (Table 2). We also identified a GBS strain containing the *linB* gene, encoding a lincosamide nucleotidyltransferase, which confers the L phenotype. The *linB* gene was originally identified in *Enterococcus faecium* (2), and two recent studies of GBS antibiotic resis-

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† Supplemental material for this article may be found at http://aac.asm.org.

TABLE 1. Primers and products

Primer name	Sequence (reference)	Gene target(s)	Product size (bp)
ermB1 ermB2	5′-GAA AAG GTA CTC AAC CAA ATA-3′ (forward) 5′-AGT AAC GGT ACT TAA ATT GTT TAC-3′ (reverse) (16)	*ermB*	639
ermTR1 ermTR2	5′-GAA GTT TAG CTT TCC TAA-3′ (forward) 5′-GCT TCA GCA CCT GTC TTA ATT GAT-3′ (reverse) (5)	*ermTR*	395
mefA1 mefA2	5′-AGT ATC ATT AAT CAC TAG TGC-3′ (forward) 5′-TTC TTC TGG TAC TAA AAG TGG-3′ (reverse) (16)	*mefA* and *mefE*	346
linB1 linB2	5′-CCT ACC TAT TGT TTG TGG AA-3′ (forward) 5′-ATA ACG TTA CTC TCC TAT TC-3′ (reverse) (2)	*linB*	944

tance mechanisms of macrolides and lincosamides each identified a strain that contained the *linB* gene (4, 5).

GBS strains containing the *ermTR* gene resulted in a variety of phenotypes: 17 iMLS, 1 cMLS, 2 EM-intermediate, and 3 novel L (EM-intermediate and CM-resistant) phenotypes. The *mefA*/*E*-containing strains also differed in their expression, resulting in 25 EM-resistant, 5 EM-intermediate, and 1 EM-susceptible strains (Table 2). Whether it is possible for these intermediate or sensitive *ermTR* and *mefA*/*E* strains to become resistant upon environmental stimulus or over time is unknown. The mechanism(s) of the phenotypic variation or expression of the *ermTR*- and *mefA*/*E*-containing strains is under investigation.

TABLE 2. Comparison of phenotypes and genotypes of 222 GBS clinical isolates

Phenotype[a]	Total no. of strains (% resistant strains)	Resistance genotype (no. of strains)
cMLS (EM-R, CM-R)	40 (44)	*ermB* (37) *ermB* and *ermTR* (1) *ermTR* (1) Unknown (1)
iMLS (EM-R, CM-R induced, or D phenotype)	19 (21)	*ermTR* (17) *ermB* (1) Unknown (1)
M (EM-R, CM-S)	25 (28)	*mefA*/*E* (25)
L		
EM-S, CM-R	2 (2)	*linB* (1) Unknown (1)
EM-I, CM-R	4 (4)	*ermTR* (3) (novel phenotype) Unknown (1)
Intermediate		
EM-I, CM-S	7	*mefA*/*E* (5) *ermTR* (2)
EM-S, CM-I	1	Unknown (1)
Sensitive (EM-S, CM-S)	123 1	None *mefA*/*E* (1)

[a] CLSI (NCCLS) 2005 disk diffusion breakpoints (3, 11, 15). For EM: ≥21 mm, susceptible (S); 16 to 20 mm, intermediate (I); ≤15 mm, resistant (R). For CM: ≥19 mm, susceptible (S); 16 to 18 mm, intermediate (I); ≤15 mm, resistant (R).

Four strains were isolated that demonstrated EM and/or CM resistance and one that demonstrated a CM-intermediate phenotype, all with unidentified antibiotic resistance genotypes. The four unknown resistant strains were found to have different phenotypes: cMLS, iMLS, L, and a novel L phenotype. One possible mechanism of the cMLS strain could be a point mutation(s) of the 23S rRNA gene (i.e., A2058G/C, A2059G/C, or C2611G) or the riboproteins L4 and L22, which have previously been found in gram-positive and gram-negative organisms (6, 17). We were unable to identify the 2058 or 2059 point mutation, the most common ribosomal mutations that confer resistance, in these five strains by pyrosequencing using the methods and primers previously described by Haanpera et al. (8) (data not shown).

Since many antibiotic resistance genes are found on mobile genetic elements, such as plasmids or transposons, GBS has the potential to acquire these genes from the cervicovaginal-rectal environment (14). The frequent monitoring of the antibiotic susceptibility of GBS by multiplex PCR and double disk diffusion assays is necessary, not only to characterize and enumerate known resistance genotypes and phenotypes for effective patient management, but also to identify potentially newly acquired and/or unidentified resistance mechanisms.

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