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Macrolide, lincosamide, and ketolide mechanisms of resistance and clonal relationships were characterized in a collection of 79 resistant group B streptococcus isolates obtained from neonates or pregnant women. The *erm*(B), *erm*(TR), and *mef*(A) genes were present in 62%, 30.4%, and 3.8% of the isolates, respectively. There was considerable clonal diversity among them.

*Streptococcus agalactiae* (group B streptococcus [GBS]) is the main cause of neonatal sepsis. In Spain, 10% to 18.5% of pregnant women are colonized by GBS in the vagina or lower rectum (3, 8; A. Andreu et al., Abstr. 12th Eur. Congr. Clin. Microbiol. Infect. Dis., Milan, Italy). For prophylactic purposes, colonized women receive penicillin G intrapartum, except for those allergic to penicillin, who receive erythromycin or clindamycin, as recommended by Spanish and U.S. guidelines (16, 17). A recent multicenter study conducted in Spain investigating GBS susceptibility has shown that penicillin, ampicillin, vancomycin, and levofloxacin are always active; however, resistance to erythromycin and azithromycin has risen to 12.45%, resistance to clindamycin has risen to 11.80%, and resistance to telithromycin has risen to 1.80% (9).

Macrolide resistance in streptococci is mainly due to a macrolide-specific efflux mechanism encoded by the *mef*(A) gene, ribosomal modification by a methylase associated with *erm* (erythromycin ribosome methylase) genes, and mutations in 23S rRNA and ribosomal proteins L4 and L22 (5, 15, 19, 20). (erythromycin ribosome methylase) genes, and mutations in

<table>
<thead>
<tr>
<th>Gene</th>
<th>No. of isolates of phenotype:</th>
<th>Total (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Telithromycin susceptible</td>
<td>Telithromycin resistant</td>
</tr>
<tr>
<td><em>erm</em>(B)</td>
<td>39</td>
<td>10</td>
</tr>
<tr>
<td><em>erm</em>(TR)</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td><em>mef</em>(A)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>11</td>
</tr>
</tbody>
</table>

* cMLS<sub>b</sub>, constitutive resistance to macrolides, lincosamides, and streptogramin B; iMLS<sub>b</sub>, inducible resistance to macrolides, lincosamides, and streptogramin B; M, resistance to 14- and 15-member ring macrolides and susceptibility to lincosamides; L', lincosamide resistance and macrolide susceptibility.

† Contributing members of the Spanish Group for the Study of Perinatal Infection from the Spanish Society for Clinical Microbiology and Infectious Diseases are listed in Acknowledgments.

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TABLE 2. Association between phenotype, genotype, and MICs among 76 GBS isolates resistant to erythromycin and 11 resistant to telithromycin.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>Erythromycin</th>
<th>Telithromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MIC &lt; 32</td>
<td>MIC &gt; 32</td>
</tr>
<tr>
<td>cMLS_B</td>
<td>erm(B)</td>
<td>49</td>
<td>&gt;32</td>
</tr>
<tr>
<td></td>
<td>erm(TR)</td>
<td>20</td>
<td>&gt;32</td>
</tr>
<tr>
<td>iMLS_B</td>
<td>erm(B)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>erm(TR)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>M</td>
<td>mef(A)</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

All MICs are given in μg/ml.

clusters and macrolide resistance genes were analyzed using Fisher's exact test. P values of less than 0.05 were considered statistically significant.

The 79 GBS isolates studied include 76 macrolide- and lincosamide-resistant isolates: 69 displayed a cMLS_B phenotype of resistance, 4 an iMLS_B phenotype, and 3 the efflux pump phenotype. The remaining three isolates were clindamycin resistant but macrolide susceptible. Among the 69 isolates with a cMLS_B phenotype, 11 were telithromycin resistant, with MICs ranging from 4 to >32 mg/liter (see Table 2). The interpreta-
tive categories used for each antibiotic followed NCCLS recommen-
dations (13). The MIC breakpoint for telithromycin was taken from Comité de l’Antibiogramme de la Société Française de Microbiologie recommendations (6).

The distribution of genes encoding macrolide-lincosamide resistance according to phenotype is reported in Table 1. The erm(B) gene was present in 62% of macrolide-resistant isolates, erm(TR) in 30.4%, mef(A) in 3.8%, and lin(B) in 0%. Among GBS isolates with constitutive resistance to MLS_B antibi-
tics, 71% showed the erm(B) gene and 29% the erm(TR) gene. All four GBS isolates with an inducible resistance phenotype presented the erm(TR) gene. The three isolates with a phenotype typical of efflux pump had the mef(A) gene. Among the 11 telithromycin-resistant isolates, 10 harbored the erm(B) gene and 1 harbored the erm(TR) gene.

Among GBS isolates presenting the cMLS_B Resistance phenotype, those associated with erm(B) had MICs of erythromycin identical to those associated with erm(TR) (Table 2). How-
ever, isolates with erm(TR) and an inducible phenotype presented lower MICs.

Erythromycin- and lincosamide-resistant GBS isolates showed high clonal diversity. Among the 79 isolates studied, 48 different PFGE patterns were found. Fourteen isolates were repeatedly nontypeable by PFGE, because of incomplete DNA digestion. Four main clusters were defined at 50% homology (CI to CIV): cluster I contained 64.7% of the isolates, CII contained 32.3%, and CIII and CIV contained one isolate each. The CI clusters mainly included those with the erm(B) gene (P = 0.012, χ² test), and the CIII isolates mainly included those with the erm(TR) gene (P = 0.002, χ² test). Resistant isolates causing colonization and sepsis were distributed equally in the different clusters. The three isolates resistant to clindamycin and susceptible to erythromycin and the 11 telithromycin-resistant isolates showed no cluster association.

Our results agree with previous studies conducted in Spain reporting that Erm(B) methylase is the main cause of macrolide resistance in GBS, followed by Erm(TR) (1, 14). This mechanism also predominates in other countries, except in Canada and the United States, where Erm(TR) methylase is the main mechanism (2, 7, 11). In our study, none of the isolates harbored more than one gene for macrolide resistance. However, Betriu et al. (1) found that 26.92% of isolates showed various combinations, mainly erm(B) with erm(A); nonetheless, the origin of the isolates studied was related not only to neonatal sepsis but also to skin and soft tissues, urine, respiratory tract, and others.

The uncommon phenotype of resistance to clindamycin but susceptibility to macrolides has been found by other groups in Spanish isolates (A. B. Campo-Esquibel, E. Ugalde, A. Portillo, M. A. Martinez-Bernal, and L. Martinez-Martinez, Abstr. 11th Spanish Congr. Clin. Microb. Infect. Dis, abstr. 4, 2004). In 2001, da Azavedo et al. reported a GBS isolate with this type of resistance encoded by the lin(B) gene of Enterococcus fae-
cium, which codes for a lincosamide-inactivating nucleotidyltransferase (7). However, our three resistant isolates did not present the lin(B) gene or the other genes conferring resistance to macrolides and lincosamides.

The presence of the previously unreported 11 (1.4%) telithromycin-resistant GBS isolates implies the need to investigate the mechanism of resistance involved and its dissemination.

We are grateful to R. Leclercq for providing Streptococcus pyogenes harboring the mef(A) gene, Streptococcus pneumoniae with erm(TR), and Streptococcus pneumoniae with erm(B) and J. C. S. de Azavedo for Streptococcus agalactiae with lin(B), which were used as control iso-
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REFERENCES


