Macrolide Resistance Determinants of Invasive and Noninvasive Group B Streptococci in a Turkish Hospital

Ziya Cibali Acikgoz,1,* Ebru Almayanlar,2 Sohret Gamberzade,1 and Safiye Gocer1

Department of Microbiology and Clinical Microbiology, Fatih University Faculty of Medicine, Fatih Üniversitesi Hastanesi, 06510 Emek,1 and METIS Biotechnology Ltd., Ostim,2 Ankara, Turkey

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Macrolide resistance in 156 consecutive group B streptococcal isolates was investigated. Thirty-five isolates (22.4%) had inducible (80%) or constitutive (20%) erythromycin resistance. The genes responsible were erm(B), erm(A) subclass erm(TR), and erm(B) plus erm(TR) in 62.9, 2.9, and 8.6% of isolates, respectively. Nine isolates (25.7%) harbored neither mef nor detectable erm genes.

Group B streptococci (GBS), namely, Streptococcus agalactiae, are one of the most important causes of neonatal meningitis and sepsis. GBS also cause other infections in pregnant women and the elderly. The treatment of choice for S. agalactiae infections is penicillin or its congeners. However, if there is a penicillin allergy or a lack of clinical response, macrolides are the major substitutes. Unfortunately, macrolide resistance among gram-positive cocci has been increasing worldwide. Macrolide resistance in streptococci arises mainly from three mechanisms: active drug efflux controlled by the mef(A) gene, modification of the drug target on the rRNA through methylases encoded by erm genes, and mutational changes in the target rRNA or protein. The resistance is expressed as either a macrolide-restricted (M) phenotype or inducible and constitutive macrolide-lincosamide-streptogramin B (MLSb) cross-resistance phenotypes (iMLS and cMLS, respectively). Although there are numerous reports of macrolide resistance mechanisms in Streptococcus pyogenes and Streptococcus pneumoniae, studies of these mechanisms in S. agalactiae are very rare (3, 5, 6, 8, 11–13). In this study, the macrolide resistance determinants of S. agalactiae isolates in Turkey were defined for the first time.

We collected a total of 156 S. agalactiae isolates from 110 vaginal or vaginoanorectal swabs (from women of reproductive age for another GBS screening study) and 46 urine samples (from patients with urinary infections) via standard methods. To avoid any duplication, only one isolate per patient was included in the study. Identification of the isolates was performed with a commercial latex agglutination kit (Avipath-Strep; Omega Diagnostics, Alloa, Scotland, United Kingdom) in addition to the conventional catalase and CAMP tests. All isolates were initially screened for penicillin and erythromycin resistance by the NCCLS disk diffusion method. Macrolide resistance phenotypes were investigated by a double-disk test (9) with erythromycin and clindamycin disks (Oxoid, Basingstoke, United Kingdom). The MICs of erythromycin and clindamycin were measured by the NCCLS agar dilution method for all the resistant isolates. The susceptibilities of these isolates to tetracycline and other antibiotics routinely used for streptococcal infections (azithromycin, clarithromycin, vancomycin, chloramphenicol, and levofloxacin) were also determined by the NCCLS disk diffusion method. All the erythromycin-resistant isolates were analyzed by PCR for the presence of mef(A), erm(A), erm(B), and erm(A) subclass erm(TR) gene determinants, as described elsewhere (2, 4). Two reference Escherichia coli strains carrying erm(A) and erm(BP) genes, respectively, and one S. pyogenes strain positive for mef(A/E) (kindly provided by Helena Seppälä, National Public Health Institute, Turku, Finland) were used as positive PCR controls. An S. pyogenes isolate harboring erm(TR) as confirmed by PCR and sequence analysis was used as the subclass erm(TR) control. For statistical analysis of the results, Fisher’s exact test was used and P values of <0.05 were considered significant.

All isolates were susceptible to penicillin, while 23 (26%) vaginal and 12 (20.9%) urinary isolates (total, 35 [22.4%] (P = 0.6) were resistant to erythromycin. None of the resistant isolates had the M phenotype. A total of 28 (80%) isolates (8 urinary and 20 vaginal isolates) expressed the iMLS phenotype, and the remaining 7 (20%; 5 urinary and 2 vaginal isolates) expressed the cMLS phenotype. In nine (25.7%) isolates none of the investigated genes were detected. None of the isolates carried the mef(A) or erm(A) gene. erm(TR) and erm(B) coexisted in three (8.6%) vaginal iMLS isolates. This coexistence was confirmed by repeating the PCR tests for subcultures of these three isolates. Separately, erm(A) subclass erm(TR) and erm(B) gene determinants were detected in 1 (2.9%) and 22 (62.9%) isolates, respectively. Corresponding phenotypes and genotypes are shown in Table 1. All isolates resistant to erythromycin were also resistant to azithromycin, clarithromycin, and tetracycline but were susceptible to vancomycin and levofloxacin. The chloramphenicol resistance rate was 44.2% overall, 43% in cMLS isolates, and 46.4% in iMLS isolates. The difference noted between cMLS and iMLS isolates for chloramphenicol resistance was statistically insignificant (P > 0.05). MIC test results are summarized in Table 2.

The English-language literature dealing with the macrolide resistance phenotypes and genotypes of S. agalactiae is very limited (3, 5, 6, 8, 11–13). In Turkey as well there is no previously published report on this issue. The macrolide resistance...
All cMLS-expressing isolates had the erm gene with MICs of clindamycin (128 μg/ml). Note that we did not detect the mef gene in any isolates. The isolates from which we could not amplify any of the resistance genes investigated might have harbored other erm genes not investigated or mutants of the investigated genes. Mutations of some ribosomal proteins such as L4 and L22 are other possible resistance mechanisms for the above isolates (10).

The high rate of tetracycline resistance (100%) that we observed among the erythromycin-resistant S. agalactiae isolates was also noted in some previous reports (>80% in Canada, 89.1% in France, 87% in Spain, and 99.2% in Taiwan). However, the mechanisms of this coexistence are yet not clear (5). On the other hand, the chloramphenicol resistance rate that we detected (44.2%) was considerably higher than those previously reported from Taiwan (20.3%) (13) and the United States (1%) (11). This rate was also considerably higher than that detected in Turkish S. pyogenes isolates (5.6%), in which tetracycline resistance was 39% (1). However, to assess the statistical significance of erythromycin-chloramphenicol coreistance detected in our isolates, the erythromycin-susceptible isolates should be compared with the resistant ones.

In conclusion, the MLS resistance rate is high and accompanied by very high rates of tetracycline and chloramphenicol resistance in Turkish S. agalactiae isolates. Vancomycin and levofloxacin are the two reliable substitutes for erythromycin for GBS infections in patients with a penicillin allergy.

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REFERENCES

TABLE 1. Distribution of MLS resistance phenotypes according to genotype

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>No. of isolates with genotype</th>
<th>erm(B)</th>
<th>erm(TR)</th>
<th>erm(B) + erm(TR)</th>
<th>Unknown</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>cMLS</td>
<td>7</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iMLS</td>
<td>15</td>
<td>1</td>
<td>3</td>
<td>9</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>1</td>
<td>3</td>
<td>9</td>
<td></td>
<td>35</td>
</tr>
</tbody>
</table>

TABLE 2. MICs of erythromycin and clindamycin for the erythromycin-resistant isolates

<table>
<thead>
<tr>
<th>Phenotype, (n)</th>
<th>Drug</th>
<th>MIC (μg/ml)</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>cMLS (7)</td>
<td>Erythromycin</td>
<td>&gt;128</td>
</tr>
<tr>
<td></td>
<td>Clindamycin</td>
<td>&gt;128</td>
</tr>
<tr>
<td>iMLS (28)</td>
<td>Erythromycin</td>
<td>&gt;128</td>
</tr>
<tr>
<td></td>
<td>Clindamycin</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>a</sup> MIC<sub>50</sub>: MIC at which 50% of the isolates tested are inhibited.  
<sup>b</sup> MIC<sub>90</sub>: MIC at which 90% of the isolates tested are inhibited.
