Heterogeneity of Genotype-Phenotype Correlation among Macrolide-Resistant Streptococcus agalactiae Isolates

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Seventy-four erythromycin-resistant group B Streptococcus isolates were analyzed regarding their phenotype-genotype and phenotype-serotype correlation. Four different phenotypes were assessed, one of them for the first time. ermB and ermTR were the most frequent genotypes (80%). The most prevalent serotype III showed great phenotypic variability while serotype V was strongly associated only with two different phenotypes.

Streptococcus agalactiae (group B Streptococcus [GBS]) is a well-known cause of invasive infections in neonates (3, 15). For treatment or prevention of GBS disease, erythromycin and clindamycin are recommended second-line drugs for patients with β-lactam allergy (1, 5). In recent years, increasing macrolide resistance rates have been reported for several streptococcal populations worldwide, which is of significant clinical relevance since macrolides are among the most frequently used antibiotics, especially in the pediatric population (9, 11, 12, 13, 14). This resistance is genetically based on either the modification of the ribosome encoded by erm genes (macrolide-lincosamide-streptogramin B [MLSβ] phenotype with inducible [i] or constitutive [c] expression) or the efflux of macrolide antibiotics by a mefA-encoded efflux pump (M phenotype). The correlation of specific antimicrobial susceptibility patterns and the respective macrolede resistance genes in streptococci has been an important subject for investigation worldwide (18, 19). To analyze the phenotype-genotype as well as the phenotype-serotype correlation among macrolide resistant GBS isolates, the present study focused on a collection of 74 erythromycin-resistant GBS strains from a southwestern region of Germany collected between the years 1999 and 2004 with 27 of them described previously (20). Briefly, 74 erythromycin-resistant GBS isolates of neonatal origin (cultured from blood, cerebrospinal fluid, urine, and swab cultures) as well as from vaginal swabs of pregnant women were identified using β-hemolysis testing as well as the Pastorex Strep B agglutination test (Bio-Rad, Marnes-la-Coquette, France). Isolates were subsequently phenotypically analyzed by means of the disk diffusion method and screened by PCR for erythromycin resistance genes (mefA or mefE, ermB, and ermTR) as well as linB using primers previously described (4, 6, 16, 17). PCR assays were reproducibly repeated at least three times for every strain. To differentiate between specific MLS phenotypes, a triple-disk test using erythromycin, clindamycin, and josamycin was performed as previously described (10). Serotyping was performed using an enzymatic extraction method (2).

Results of triple-disk testing revealed four distinct phenotypes are shown in Fig. 1. Thirty-nine isolates (52%) showed the cMLS phenotype (P1) mostly correlated with the ermB resistance gene. Twenty isolates (27%) showed an inducible resistance phenotype (iMLS) with 13 strains exhibiting inducible resistance to clindamycin and josamycin associated with the ermTR gene (P3) and another 7 strains displaying constitutive resistance to clindamycin as well as inducible resistance to josamycin based on either the ermB or the ermTR gene (P2). The M phenotype (P4) could be detected in 15 isolates (20%) strongly associated with the mefA gene (Table 1). All three phenotypes (P1, P3, and P4) correspond well to the ones previously described by different authors (10, 19) while P2, in contrast, has not been described so far. The most prevalent serotypes (ST) among all erythromycin-resistant GBS isolates were ST V (37%) and III (27%) (Table 2). While ST III showed a great variety of different phenotypes, including P2 and P4 (M phenotype), ST V revealed only two different MLS phenotypes (P1 and P3) strongly associated with the ermB or ermTR gene, respectively. Since different studies described the clonal spread of ST V isolates within the GBS population in recent years (8, 20), this might be an explanation for the observed homogeneity of this respective ST V population compared to the ST III isolates in this study. Regarding the newly described P2 phenotype, serotypes Ib and II as well as the predominant serotype III (four of seven isolates) were detected. PCR analysis of these seven isolates revealed either the ermB or the ermTR gene while PCR on linB, a clindamycin resistance gene previously described in GBS by de Azavedo et al. (7) yielded negative results. To our knowledge, there are no data in present literature describing this P2 phenotype as well

### TABLE 1. Distribution of resistance genes among four different phenotypes, P1 to P4

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>ermB</th>
<th>ermTR</th>
<th>mefA</th>
<th>ermB + ermTR</th>
<th>ermTR + mefA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>34</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td></td>
<td>39</td>
</tr>
<tr>
<td>P2</td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>P3</td>
<td>1</td>
<td>9</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>P4</td>
<td>15</td>
<td>15</td>
<td>4</td>
<td>1</td>
<td></td>
<td>74</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>17</td>
<td>15</td>
<td>4</td>
<td>1</td>
<td>74</td>
</tr>
</tbody>
</table>

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as its genetic determinant. It is likely to presume that other to date unknown resistance factors or a certain combination of those with known resistance genes is responsible for the heterogeneity of phenotype-genotype correlation among GBS isolates. Such a variety of resistance factors possibly acquired through horizontal gene transfer might as well be responsible for the reported rapid increase of macrolide resistance among GBS isolates.

TABLE 2. Distribution of serotypes among four different phenotypes, P1 to P4a

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>No. of isolates with serotype:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ia</td>
<td>Ib</td>
</tr>
<tr>
<td>P1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>P2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

a Boldface indicates the predominant serotype in the respective phenotype. NT, nontypeable.

FIG. 1. Phenotypes of erythromycin-resistant S. agalactiae isolates determined by the triple-disk test. The erythromycin disk (30 μg) is at the center, with the clindamycin disk (10 μg) on the right and the josamycin disk (30 μg) on the left. P1, cMLS phenotype; P2 and P3, iMLS phenotypes; P4, M phenotype.

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