Characterization of Group B Streptococcus Isolated from Women in Saitama City, Japan

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SUMMARY: *Streptococcus agalactiae* (group B streptococcus; GBS) is a common cause of neonatal sepsis and meningitis. Intrapartum antibiotic prophylaxis is effective in reducing neonatal GBS disease. Penicillin is recommended for intrapartum antibiotic prophylaxis; however, other antibiotics are administered to pregnant women with penicillin allergy. Serotyping and antibiotic susceptibility testing were performed on 376 GBS isolates collected from vaginal swabs in Saitama City. Of the 376 isolates, 328 (87.2%) were obtained from obstetrics and gynecology clinics. Although approximately 80% of the isolates (299/376) were from women of reproductive age (age, 15–49 years), no definite information on their pregnancy status was obtained. The most frequent serotype was V (19.1%) followed by Ib (18.6%), III (16.2%), VI (14.9%), and Ia (14.6%). None of the isolates were resistant to penicillins and cephalosporins. Isolates that were resistant to erythromycin (12.8%), clindamycin (9.0%), ofloxacin (19.4%), levofloxacin (18.4%), and tetracycline (46.5%) were detected. There was a high prevalence of resistance to erythromycin (39.3%) and clindamycin (27.9%) in serotype III. In addition, almost all serotype Ib isolates were resistant to ofloxacin and levofloxacin (both, 91.4%). Pulsed-field gel electrophoresis analysis on certain GBS isolates (serotype Ib, III, and V) indicated that there was genetic diversity among the resistant isolates obtained from a limited area of Japan. In conclusion, present intrapartum antibiotic prophylaxis with penicillins would be effective in Japan; however, performing susceptibility testing before administering other antibiotics is important in order to ensure activity against the relevant isolate.

INTRODUCTION

*Streptococcus agalactiae* (group B streptococcus; GBS) is one of the leading causes of neonatal sepsis and meningitis. Invasive GBS infection in newborns during the first week of life, known as early-onset GBS disease, is mainly caused by vertical transmission of GBS from colonized mothers to their infants during labor or delivery. Approximately 10%–30% of pregnant women are colonized with GBS, and the incidence of early-onset GBS disease is 0.3–2 per 1,000 live births (1–4). Intrapartum antibiotic prophylaxis is effective in reducing early-onset GBS disease (5).

In 1996, the Centers for Disease Control and Prevention (CDC) published consensus guidelines (6) for the prevention of neonatal GBS disease; these guidelines were revised in 2002 (7). The revised guidelines recommended the screening of all pregnant women between 35 and 37 weeks of gestation for vaginal and rectal colonization with GBS. Further, the guidelines recommended intrapartum antibiotic prophylaxis for colonized pregnant women. In 2008, the Japan Society of Obstetrics and Gynecology also presented guidelines (8) for the prevention of neonatal GBS disease.

Penicillins are the antibiotics of choice for intrapartum antibiotic prophylaxis. GBS has been considered to be uniformly susceptible to penicillins; however, clinical GBS isolates with reduced penicillin susceptibility have been reported (9). On the other hand, alternative antibiotics are administrated for pregnant women with penicillin allergy, such as clindamycin, erythromycin, cefazolin, or vancomycin. In several countries, both clindamycin and erythromycin resistance has been reported in GBS isolates (10–17).

There are 10 GBS serotypes (Ia, Ib, and II to IX) based on variations in the capsular polysaccharide, a major virulence factor that helps the microorganism to evade the host’s defense mechanisms. The GBS serotype IX was recently identified (18); hence, little epidemiological data are available for serotype IX at present. GBS vaccines based on capsular polysaccharide expression have been investigated as a tool for reducing maternal colonization and preventing transmission to neonates (19,20). Although the predominant serotypes obtained from colonized pregnant women are different in each country (21), the serotypes commonly causing neonatal GBS disease are Ia, III, and V (10,12,14). Other studies (10–12,22) have also identified serotype V that is associated with erythromycin resistance.

In this study, we reported the phenotypic characteristics of GBS isolates collected from vaginal swabs by serotyping and antibiotic susceptibility testing. In addition, we performed pulsed-field gel electrophoresis...
(PFGE) in order to determine the clonal relationship of the GBS isolates.

**MATERIALS AND METHODS**

**GBS isolates:** From April 2007 to March 2010, 376 GBS isolates were collected from vaginal swabs of women who visited 11 clinics in Saitama City. Of the 376 isolates, 328 (87.2%) were obtained from obstetrics and gynecology clinics. Although almost 80% of the isolates (299/376) were from women of reproductive age (age, 15–49 years), no definite information on their pregnancy status was obtained. Each isolate was confirmed as GBS based on the colony characteristics and determination of the Lancefield group by a latex agglutination grouping kit (Prolex Streptococcal Grouping Latex Kit; Iwaki Co., Tokyo, Japan).

**Serotyping and molecular capsule typing:** Serotyping was performed by slide agglutination tests with rabbit antisera (Group B Streptococci Typing Sera; Denka Seiken, Tokyo, Japan) following the Clinical and Laboratory Standards Institute (CLSI) guidelines (25). We confirmed the accuracy by using the quality control strain, Streptococcus pneumoniae ATCC 49619, which was recommended by the CLSI. GBS isolates were tested for susceptibility to benzylpenicillin, ampicillin, erythromycin, clindamycin, cefazolin, cefotaxime, ceftriaxone, and tetracycline.

**PFGE:** PFGE analysis was performed on GBS isolates serotyped as Ib, III, and V. GBS cells in agarose plugs were lysed with mutanolysin (50 U) for 24 h at 37°C, and chromosomal DNA was digested with the restriction enzyme Smal (80 U) for 16 h at 30°C. Electrophoresis was performed as described previously (26) by using the CHEF-DRII system (Bio-Rad, Hercules, Calif., USA). The PFGE banding patterns were analyzed with Fingerprinting II Software (Bio-Rad). Dice’s coefficient was used to determine the similarity between each banding pattern, and a dendrogram was constructed by using the unweighted-pair group method with arithmetic averages with a tolerance coefficient of 0.72%.

**RESULTS**

Of the 376 isolates, 323 were serotyped by slide agglutination tests. Multiplex PCR assay could determine capsular type for 50 of the 53 isolates, which were not typeable by slide agglutination tests. Of the remaining 3 isolates, 1 isolate was typed by the serotype IX-specific PCR. Finally, based on the serotyping and molecular capsule typing, 374 isolates were determined to be from 10 serotypes in this study (Fig. 1).

The most frequent serotype was V (19.1%) followed by Ib (18.6%), III (16.2%), VI (14.9%), and Ia (14.6%). Serotypes IV (0.5%), VII (0.8%), and IX (0.3%) were less common.

MICs of the antibiotics were determined in all 376 GBS isolates (Table 1). None of the isolates were resistant to penicillins such as benzylpenicillin and ampicillin. MIC of benzylpenicillin was ≤ 0.06 µg/ml in all isolates. Furthermore, all isolates were susceptible to cefoxime, ceftriaxone, ceftizoxime, meropenem, vancomycin, and linezolid. Although the CLSI guidelines do not specify susceptibility breakpoints for cefpodoxime, MIC of cefpodoxime were ≤ 0.25 µg/ml in all isolates.

Isolates resistant to erythromycin, clindamycin, ofloxacin, levofloxacin, and tetracycline were found. In total, 48 isolates (12.8%) were resistant to erythromycin, including 24 isolates (6.4%) with high MIC (> 32 µg/ml); 34 isolates (9.0%) were resistant to clindamycin, including 28 isolates (7.4%) with high MIC (≥ 32 µg/ml). With respect to fluoroquinolones, 73 (19.4%) and 69 (18.4%) isolates were resistant to levofloxacin and ofloxacin, respectively. All of the 69 isolates with high MIC (> 32 µg/ml) to ofloxacin were also highly resistant to levofloxacin (> 32 µg/ml). Regarding ofloxacin, 101 isolates (26.9%) showed intermediate susceptibility, remarkably more than that to levofloxacin (0.8%). Further, 175 GBS isolates (46.5%), i.e., nearly half of the tested isolates, were resistant to tetracycline.

Table 2 shows the number of isolates resistant to antibiotics by the serotype. There was a high prevalence of isolates that were resistant to erythromycin (39.3%) and clindamycin (27.9%) in serotype III. Almost all of the serotype Ib isolates were resistant to ofloxacin and levofloxacin (both, 91.4%). In both serotype VI and VIII, few isolates were resistant to these antibiotics.

Serotype Ib, III, and V showed higher prevalence of isolates that were resistant to erythromycin, clindamycin, ofloxacin, or levofloxacin than the other serotypes. Therefore, PFGE analysis was performed on 55 isolates including 18 isolates from serotype Ib, 23 isolates from serotype III, and 14 isolates from serotype V. These 55
Table 1. MICs of the 376 GBS isolates to 14 antibiotics

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (µg/ml)</th>
<th>≤0.06</th>
<th>0.12</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>&gt;32</th>
<th>No. of resistance isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzylpenicillin</td>
<td>376</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48 (12.8)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>13</td>
<td>342</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>34 (9.0)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>95</td>
<td>222</td>
<td>7</td>
<td>4†</td>
<td>8‡</td>
<td>3©</td>
<td>9©</td>
<td>4©</td>
<td>24©</td>
<td>48 (12.8)</td>
<td>48 (12.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clindamycin</td>
<td>7</td>
<td>288</td>
<td>45</td>
<td>2†</td>
<td>1©</td>
<td>1©</td>
<td>2©</td>
<td>2©</td>
<td>28©</td>
<td>34 (9.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefazolin¹</td>
<td>329</td>
<td>107</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>73 (19.4)</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>344</td>
<td>31</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>69 (18.4)</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>306</td>
<td>69</td>
<td>1</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>69 (18.4)</td>
<td></td>
</tr>
<tr>
<td>Cefepine</td>
<td>323</td>
<td>52</td>
<td>1</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>69 (18.4)</td>
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<tr>
<td>Meropenem</td>
<td>374</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>69 (18.4)</td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td>252</td>
<td>124</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>69 (18.4)</td>
<td></td>
</tr>
<tr>
<td>Linezolid</td>
<td>261</td>
<td>115</td>
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<td></td>
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<td>69 (18.4)</td>
<td></td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>3</td>
<td>199</td>
<td>101¹</td>
<td>4©</td>
<td>69²</td>
<td>73 (19.4)</td>
<td>73 (19.4)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>1</td>
<td>130</td>
<td>173</td>
<td>3¹</td>
<td>24²</td>
<td>45²</td>
<td>69 (18.4)</td>
<td>69 (18.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>3</td>
<td>149</td>
<td>41</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>26</td>
<td>63</td>
<td>81²</td>
<td>175 (46.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹: The Clinical and Laboratory Standards Institute guidelines do not specify susceptibility breakpoints for cefazolin.
†, intermediate susceptibility; ‡, resistant.

Table 2. Distribution of isolates resistant to each antibiotic by the serotype

<table>
<thead>
<tr>
<th>Serotype (No. of isolates)</th>
<th>Erythromycin</th>
<th>Clindamycin</th>
<th>Tetracycline</th>
<th>Ofloxacin</th>
<th>Levofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia (55)</td>
<td>5 (9.1)</td>
<td>43 (78.2)</td>
<td>1 (1.8)</td>
<td>64 (91.4)</td>
<td>64 (91.4)</td>
</tr>
<tr>
<td>Ib (70)</td>
<td>5 (7.1)</td>
<td>5 (7.1)</td>
<td>64 (91.4)</td>
<td>64 (91.4)</td>
<td></td>
</tr>
<tr>
<td>II (15)</td>
<td>2 (13.3)</td>
<td>1 (6.7)</td>
<td>13 (86.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III (61)</td>
<td>24 (39.3)</td>
<td>17 (27.9)</td>
<td>53 (86.9)</td>
<td>4 (6.6)</td>
<td>4 (6.6)</td>
</tr>
<tr>
<td>IV (2)</td>
<td></td>
<td>1 (50)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V (72)</td>
<td>11 (15.3)</td>
<td>10 (13.9)</td>
<td>56 (77.8)</td>
<td>2 (2.8)</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>VI (56)</td>
<td></td>
<td>1 (1.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VII (3)</td>
<td></td>
<td>1 (1.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIII (39)</td>
<td>1 (2.6)</td>
<td>1 (2.6)</td>
<td>1 (2.6)</td>
<td>1 (1.8)</td>
<td></td>
</tr>
<tr>
<td>IX (1)</td>
<td></td>
<td>1 (100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT (2)</td>
<td></td>
<td>1 (50)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (376)</td>
<td>48 (12.8)</td>
<td>34 (9.0)</td>
<td>175 (46.5)</td>
<td>73 (19.4)</td>
<td>69 (18.4)</td>
</tr>
</tbody>
</table>

Isolates consisted mainly of resistant isolates. We successfully obtained the PFGE banding patterns for all 55 isolates. Dendrogram of the PFGE banding patterns is shown in Fig. 2.

PFGE banding patterns were roughly divided into 4 groups by serotype, and identical banding patterns were found in some isolates. In isolates with a different serotype, the banding patterns were not similar (≥70% dendrogram similarity). With respect to the relationship between banding pattern and susceptibility, almost all isolates with similar banding patterns showed the same susceptibility to erythromycin, clindamycin, and tetracycline. In contrast, some isolates with identical banding patterns showed different susceptibility to fluoroquinolones.

DISCUSSION

Epidemiological study of GBS serotypes is important because the GBS serotype is associated with pathogenicity (10,12,14) and antibiotic susceptibility (10–12,22). In addition, the development and formulation of current multivalent GBS vaccines depends on accurate population data of the serotype distribution (20).

In this study, to determine the serotype of GBS isolates, we used a two-step identification scheme. First, we applied the serotype-specific slide agglutination test, which determined the serotypes of 85.9% isolates. The percentage of non-typeable isolates (14.1%) was relatively higher than that reported elsewhere (21). As for these untyped isolates, 51 of 53 isolates were identified successfully by PCR-based molecular capsule typing method. Although we did not evaluate the PCR-based method, PCR-based capsular typing is an essential tool for analyzing the seroprevalence and serotypic shift of GBS from pregnant women. Additionally, the new serotype, IX, was easily identified using the molecular method. The GBS serotype IX was isolated in this study, but it was still rare in Japan (1/376 GBS). Even with the combination of serotyping and molecular capsule typing, 2 out of the 376 isolates remained untyped. For precise analysis, the region involved in capsular synthesis should be genetically determined.

Previous studies (27–30) in Japan showed that sero-
types VI (24%–27%) and VIII (19%–36%) are most frequently isolated from vaginal cultures of pregnant women (1992–2009). Global GBS serotype distribution as revealed by a meta-analysis indicated that the seroprevalence in Japan (VI and VIII predominant) is unique. In this study, the prevalence of serotypes VI and VIII was 14.9% and 10.4%, respectively, indicating lower prevalence than that shown in previous reports. Another difference was the prevalence of serotype V, which was reported to be 3%–9% by previous reports on Japanese GBS. However, this study showed that serotype V was the most frequently observed serotype (16.0% by slide agglutination test, 19.1% by a combination of serotyping and molecular capsule typing). Although all of our isolates were from vaginal specimens, and approximately 80% of the isolates were from women of reproductive age, our data set lacked information on the pregnancy status. Thus, it is possible that
the pregnancy status might affect the seroprevalence. Additionally, temporal and/or geographical difference might influence the prevalence.

In Japan, clinical GBS isolates with reduced penicillin susceptibility have been reported (9). However, all tested isolates in this study were susceptible to penicillins, indicating that they would be effective as the first-line agents on intrapartum antibiotic prophylaxis.

Conversely, some women with penicillin allergy require other antibiotics such as clindamycin, erythromycin, cefazolin, or vancomycin, which are recommended in the guidelines (7,8). All isolates in this study were susceptible to cefazolin and vancomycin. However, 12.8% and 9.0% of the tested isolates were resistant to erythromycin and clindamycin, respectively. These results are higher than that of previous studies (3%) and 1% in Japan (28). Susceptibility testing should be recommended before administering erythromycin or clindamycin in order to ensure activity against the isolate. In the USA, the rates of resistance are relatively high (10,11). Consequently, the revised CDC guidelines in 2010 (31) excluded erythromycin from the recommended alternative antibiotics.

The majority of erythromycin- and clindamycin-resistant GBS were from serotype III, one of the most frequent serotype causing neonatal GBS disease (10,12,14,32). This result differs from previous studies (10-12,22) that identified serotype V to be associated with erythromycin resistance.

Most of the fluoroquinolone-resistant isolates from vaginal swabs were serotype Ib, which was in agreement with previous studies on invasive GBS isolates (33). Although small variations in the PFGE profile were observed in the serotype Ib isolates, some isolates with identical banding patterns showed different susceptibility to fluoroquinolones. Previous reports (34-37) indicated that the majority of quinolone resistance in GBS was caused by point mutations in the quinolone resistance-determining regions (QRDRs). It is believed that the PFGE banding patterns are not affected by point mutations, because these mutations in QRDRs would affect neither the whole genome size nor digestion with restriction enzyme.

Conversely, regarding erythromycin, clindamycin, and tetracycline, isolates with the same resistance patterns in a serotype did not always show similar banding patterns. This observation indicates that the resistant isolates did not originate from a single clone and that there was genetic diversity among resistant isolates obtained from a limited area of Japan.

There are some cases of neonates being born with early-onset GBS disease to women who received appropriate intrapartum antibiotic prophylaxis (4). This suggests that appropriate intrapartum antibiotic prophylaxis will not eliminate all cases of early-onset GBS disease. Although no licensed vaccine is available currently, immunization with a GBS vaccine has the potential to overcome these problems, including antibiotic resistance.

There is a possibility that some of the GBS isolates in this study were from non-pregnant women. Thus, we should be aware of this when using the data. However, these results can be useful as a basis of information on GBS.

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Conflict of interest None to declare.

REFERENCES
women at a tertiary care military medical center relative to global serotype distribution. BMC Infect. Dis., 10, 336.


