Original Article

Characteristics of Group B Streptococcus Isolated from Infants with Invasive Infections: A Population-Based Study in Japan

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SUMMARY: Group B Streptococcus (GBS) is one of the leading causes of neonatal bacterial infections. Population-based surveillance of GBS-related invasive diseases among newborns and infants from 10 prefectures in Japan was performed between 2007 and 2012. The characteristics of cases and isolated GBS are described in this study. The incidence rate of GBS-related invasive diseases was 0.13 per 1,000 live births. Analysis of GBS samples obtained from 60 invasive cases showed that the most frequent serotypes were III (48.3%), Ia (30.0%), and Ib (10.0%). All isolates were susceptible to penicillin G, ampicillin, cefotaxime, imipenem, and panipenem. However, 14, 2, and 7 isolates were resistant to erythromycin, clindamycin, and both erythromycin and clindamycin, respectively. Multilocus sequence typing revealed that GBS sequence type (ST) 23, ST17, and ST335 caused higher incidences of meningitis. These data show that serotypes III, Ia, and Ib together caused more than 80% of invasive infections in Japanese infants, and that GBS strains are still susceptible to β-lactam antibiotics.

INTRODUCTION

Streptococcus agalactiae (group B Streptococcus [GBS]) infects mainly infants and causes pneumonia, sepsis, and meningitis (1–3). Invasive neonatal GBS infections can be classified as either early-onset disease (EOD) that occurs within the first week of life, or late-onset disease (LOD) that develops after the first week of life (4). EOD is often caused by maternal transmission of GBS to the neonate through the birth canal during labor (1–3). On the other hand, the route of infection for many LOD cases remains unknown. After the adoption of new preventative measures (i.e., universal antenatal screening and intrapartum antibiotic prophylaxis for culture-positive and high-risk pregnant women) recommended by the Centers for Disease Control and Prevention (CDC) in the United States (4), a reduction in the incidence of EOD, but not LOD, was observed (4,5).

Until now, 10 GBS serotypes have been classified based on specific capsular polysaccharides (6,7). In Japan, serotypes VIII and VI have been mainly isolated from the vagina of pregnant/ puerperal women (8). However, a broad survey of GBS serotyping, which causes invasive neonatal infections, has never been performed in Japan; hence, important disease-associated serotypes have not been well established.

Population-based surveillance of invasive GBS diseases in children aged <5 years was conducted in 10 prefectures (i.e., Hokkaido, Niigata, Fukushima, Chiba, Mie, Okayama, Kochi, Fukuoka, Kagoshima, and Okinawa) in Japan between 2007 and 2012 (9). The clinical features of 60 invasive infection cases that occurred in 8 prefectures (i.e., Niigata, Fukushima, Chiba, Mie, Kochi, Fukuoka, Kagoshima, and Okinawa) were collected, and the results of serotyping, antimicrobial susceptibility, and multilocus sequence typing (MLST) analysis of these GBS isolates are reported here.

MATERIALS AND METHODS

Invasive GBS cases and bacterial isolates: Between July 2007 and December 2012, population-based surveillance efforts included collection of data on invasive
infections in children aged <5 years who reside in the 10 prefectures in Japan. Invasive infections caused by GBS were confirmed by isolation of the pathogenic agent obtained from a body site that was originally sterile. Information regarding invasive infections and isolates from hospitals in the 10 prefectures was provided to the Department of Bacteriology I, National Institute of Infectious Diseases. EOD and LOD were classified according to the recommendations by the CDC (i.e., EOD was classified as infections occurring within the first week of life, and LOD was classified as infections occurring in infants aged >1 week) (4). The identity of isolated bacteria was confirmed again to be \textit{S. agalactiae} using the API 20 Strep Kit (bioMérieux, Marcy l’Étoile, France). Serotypes were determined using a latex agglutination test. All GBS strains were initially serotyped using the Group B Streptococci Typing Antisera “SEIKEN” (Denka Seiken, Tokyo, Japan) that contains 9 types of specific antisera for GBS. For strains that were not serotyped, the \textit{Streptococcus} ImmuLex Strep-B Kit (Statens Serum Institut, Copenhagen, Denmark) containing serotype IX antiserum was used.

**Antimicrobial susceptibility test:** The antimicrobial susceptibility of GBS isolates to 7 antibiotics (i.e., penicillin G, ampicillin, cefotaxime, imipenem, panipenem, erythromycin, and clindamycin) was analyzed using dry plates (Eiken Chemistry, Tokyo, Japan) by the broth microdilution method described by the Clinical and Laboratory Standards Institute (10). Antimicrobial susceptibility breakpoints were defined according to the recommendations by the CDC (i.e., penicillin, ampicillin, cefotaxime, imipenem, erythromycin, and clindamycin) (11,12).

**MLST analysis:** Genomic DNA of GBS was purified using the High Pure PCR template purification kit (Roche Diagnostics, Tokyo, Japan). MLST was performed using primers that were designed as previously described (13), and both strands of amplicons were sequenced. The allelic numbers and sequence types (STs) of all isolates were determined by comparing their sequences with those in the \textit{S. agalactiae} MLST database (http://pubmlst.org/sagalactiae/).

**Statistical analyses:** Statistical analyses were performed using the Fisher’s exact test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate the ability of different STs of GBS to invade the neonatal central nervous system.

**RESULTS**

**Clinical features and bacterial characteristics:** During the investigation period, a total of 151 sporadic GBS-related invasive diseases, including 90 meningitis cases in the investigation period, a total of 151 sporadic GBS-related invasive diseases, including 90 meningitis cases were identified. The characteristics of the 60 invasive infection cases and their isolated bacteria from 8 of the 10 prefectures are listed in Table 1. All 60 cases were analyzed independently in an epidemiological survey. Fifteen cases (25.0%) and 45 cases (75.0%) were EOD and LOD, respectively. The exact date of infection in 2 cases was unclear; however, symptoms presented within the first week of life. Among these 60 cases, 38 (6 EOD and 32 LOD) were diagnosed as meningitis, and 22 as bacteremia/sepsis. Two cases also presented with symptoms of pneumonia.

A total of 85 isolates were obtained from the blood and/or cerebrospinal fluid of 60 patients. All isolates were confirmed to be \textit{S. agalactiae}. In 23 cases, more than 1 strain was isolated from different specimens. GBS isolated from the same patient showed identical serotypes and STs and similar antimicrobial susceptibility profiles (data not shown); therefore, it was considered as a single strain.

**Serotype distribution of GBS isolates:** Serotypes of 60 GBS isolates were determined using an agglutination kit. As shown in Table 1, the most frequently identified GBS isolates from 15 EOD patients included serotypes III (5 cases; 33.3%) and Ia (4 cases; 26.7%). One case (6.7%) each of the serotypes Ib, II, IV, V, VI, and VIII was also identified among EOD patients. Among 45 LOD cases, serotype III was most frequently observed (24 cases; 53.3%), other serotypes identified were Ia (14 cases; 31.1%), Ib (5 cases; 11.1%), II (1 case; 2.2%), and IV (1 case; 2.2%). When EOD and LOD isolates were analyzed together, the most frequently observed serotypes were III (29 cases; 48.3%), Ia (18 cases; 30.0%), and Ib (6 cases; 10.0%). Furthermore, 2 cases (3.3%) each of GBS serotypes II and IV and 1 case (1.7%) each of serotypes V, VI, and VIII were detected. No cases of serotypes VII or IX were found among 60 analyzed cases. Overall, we found no obvious difference in serotype distribution between the different prefectures and isolated years (data not shown). The association between serotypes and the ability to invade the neonatal central nervous system was analyzed, but high invasive activity related to meningitis was not shown by any serotype (Fig. 1).

**Antimicrobial susceptibility of GBS isolates:** All GBS isolates were susceptible to the \(\beta\)-lactam antibiotics used in this study (i.e., penicillin G, ampicillin, cefotaxime, imipenem, and panipenem) (data not shown). \(\beta\)-lactams
Table 1. Characteristics of GBS isolates from invasive infections

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serotype</th>
<th>Age of onset</th>
<th>Diagnosis</th>
<th>Specimen</th>
<th>ST</th>
<th>CC</th>
<th>EM (µg/ml)</th>
<th>CLDM (µg/ml)</th>
<th>ermA</th>
<th>ermB</th>
<th>mefA/E</th>
<th>linB</th>
</tr>
</thead>
<tbody>
<tr>
<td>KSP7</td>
<td>II</td>
<td>0 d</td>
<td>Meningitis</td>
<td>Blood</td>
<td>ST1</td>
<td>CC1</td>
<td>0.25</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KSP8</td>
<td>Ib</td>
<td>EOD(1)</td>
<td>Meningitis</td>
<td>Spinal fluid</td>
<td>ST571</td>
<td>CC10</td>
<td>0.25</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KSP21</td>
<td>II</td>
<td>3 m</td>
<td>Bacteremia</td>
<td>Blood</td>
<td>ST28</td>
<td>CC19</td>
<td>0.12</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KSP39</td>
<td>III</td>
<td>27 d</td>
<td>Meningitis</td>
<td>Spinal fluid</td>
<td>ST17</td>
<td>CC17</td>
<td>0.12</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KSP50</td>
<td>III</td>
<td>1 m</td>
<td>Meningitis</td>
<td>Spinal fluid</td>
<td>ST335</td>
<td>CC19</td>
<td>2</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KSP66</td>
<td>III</td>
<td>2 m</td>
<td>Meningitis, bacteremia</td>
<td>Spinal fluid</td>
<td>ST335</td>
<td>CC19</td>
<td>1</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KSP76</td>
<td>la</td>
<td>1 d</td>
<td>Meningitis, bacteremia</td>
<td>Spinal fluid</td>
<td>ST23</td>
<td>CC23</td>
<td>0.12</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KSP77</td>
<td>III</td>
<td>19 d</td>
<td>Bacteremia</td>
<td>Blood</td>
<td>ST335</td>
<td>CC19</td>
<td>1</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1): Date of occurrence was unclear, but was within the first week after birth.

+; positive; -; negative; ST, sequence type; CC, clonal complex; DIC, disseminated intravascular coagulation; PROM, premature rupture of the membrane.

EM, erythromycin; CLDM, clindamycin.
were identified in KSP652 (MIC of erythromycin, 1). However, none of the 4 resistance-related genes erythromycin-resistant GBS isolates, different between CC17 and CC19 (OR respectively (Table 1). The incidence of meningitis was not meningitis caused by all 3 STs was 69.4\% (13/19). The incidence rates of EOD and LOD were 0.08 and 0.10 per 1,000 live births, respectively, and only approximately one-third of all cases had meningitis (16). The reasons for the high incidence rates of GBS infection (0.13/1,000 live births) and meningitis (59.6\%) in the 10 prefectures remain unknown. In addition, only 1 case of EOD caused by serotype V was found among all cases in the present study; however, serotype V was the third most commonly isolated serotype among patients with GBS invasive diseases in England, Wales, and the United States (14,15). In several regions and countries, the CC17 GBS strain has been shown to have an increased ability to invade the neonatal central nervous systems and cause meningitis (17–22). However, we did not find a high incidence of meningitis caused by the CC17 GBS strain in the present study. Although the reason remains unknown, the GBS isolates ST23, ST17, and ST335 seem to be involved in the development of meningitis. The small sample size is a limitation of our study and may have contributed to our statistical findings. Recently, Morozumi et al. reported that GBS ST335 containing the mefA/E gene gradually increased the frequency of invasive GBS infections in Japanese infants between 2006 and 2011 (23). GBS ST335 was also isolated at a high rate in our investigation (20.0\%; 12/60 cases). However, most of the ST335 strains (10/12) possessed the erm gene, whereas only 1 strain possessed the mefA/E gene (Table 1). Although it remains unclear, differences in the isolation region may have contributed to the variation in the ST335 strain.

Of 60 investigated cases, the number of LOD cases was almost 2-fold greater than that of EOD cases. The source of infection and pathways of most LODs still remain unknown, and no effective means for disease prevention are available. Therefore, vaccination may be
the best preventive measure against not only LOD but also EOD GBS infections. Epidemiological studies of capsular polysaccharide-protein conjugate vaccines for prevention of GBS infections in infants have been conducted since the 1990s (24). The development of a trivalent polysaccharide-protein conjugate vaccine, which includes Ia, Ib, and III serotype polysaccharides, has advanced to a phase II clinical trial, and phase III of this study is currently being planned (25). In the present surveillance study, serotype analysis revealed that GBS serotypes Ia, Ib, and III caused 88.3% of 60 invasive cases. Therefore, the trivalent polysaccharide-protein conjugate vaccine could have theoretically prevented most cases of GBS-related invasive disease among Japanese infants. Hence, we highly recommend the early licensing of GBS vaccines. Ongoing monitoring of the incidence and distribution of serotypes for disease surveillance is important to determine the serotype components that would be most beneficial for inclusion in a GBS conjugate vaccine. Furthermore, ongoing monitoring will enable the continued evaluation of these preventive measures after the distribution of the vaccine. Further population-based surveillance studies of bacterial invasive infections in Japanese children are required.

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Conflict of interest None to declare.

REFERENCES