Short Communication

Screening for Group B Streptococci with Reduced Penicillin Susceptibility in Clinical Isolates Obtained between 1977 and 2005

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SUMMARY: Group B streptococcus (GBS; Streptococcus agalactiae) is a leading cause of neonatal invasive infections, and until recently, it was thought to be completely susceptible to penicillin. However, we recently identified several clinical GBS isolates with reduced penicillin susceptibility (PRGBS) whose minimum inhibitory concentrations of penicillin were >0.12 μg/ml, which is above the susceptibility breakpoint set by the Clinical and Laboratory Standards Institute. These PRGBS were isolated between 1995 and 2005 in Japan; whether these PRGBS existed in Japan before 1995 is unknown. In the study described here, we screened for PRGBS among 349 clinical GBS isolates obtained in Japan between 1977 and 2005 using the previously developed disk diffusion method for the detection of PRGBS. With this method, we selected 6 PRGBS candidates and confirmed that 1 isolate was PRGBS, using agar dilution method, including oxacillin, ceftizoxime, and penicillin-binding protein 2X (PBP2X) gene sequencing analysis. This isolate was obtained from sputum in 2005, and we could not detect PRGBS isolates before 1995 in this investigation.

Group B streptococcus (GBS; Streptococcus agalactiae) is a leading cause of neonatal sepsis and meningitis and is also an important pathogen causing illness among elderly people and those suffering from underlying medical disorders (1–4). The highest GBS mortality and morbidity result from invasive infections in neonates, particularly in those with very low-birth weight (5–7). Approximately 5% of GBS-infected infants die, and survivors often suffer from severe neurological sequelae such as mental retardation and vision and/or auditory disabilities (8). Vaccination against GBS is still under investigation (9,10). Therefore, intrapartum antibiotic prophylaxis has been recommended by the Center for Disease Control and Prevention (8), and the rate of early onset GBS infection, during the first postnatal week, has declined.

Penicillins are the first-line agents in the prophylaxis and treatment of GBS infections because all clinical GBS isolates have been considered to be uniformly susceptible to β-lactams, including penicillins (8,11). However, we recently identified and molecularly characterized several clinical GBS isolates obtained between 1995 and 2005 that demonstrated reduced penicillin susceptibility (PRGBS) through acquisition of multiple mutations in the penicillin-binding protein 2X (PBP2X) gene (12), and similar PRGBS isolates were recently reported from the United States (13), Canada (14,15), and Japan (from our group [16–20] and other group [21]). However, it is unclear whether PRGBS has existed undetected for several years or emerged only recently.

In this study, we used the previously reported disk diffusion method (22) to detect PRGBS among 349 clinical isolates that were collected from various sources including blood and cerebrospinal fluid in Japan between 1977 and 2005. A total of 349 clinical isolates (324 clinical isolates between 1977 and 1994, and 25 clinical isolates between 1995 and 2005) were recovered from patients who visited Meijyo Hospital in Japan between 1977 and 2005. These were isolated from various sources such as vaginal specimens, respiratory specimens, blood, and cerebrospinal fluid. Lancefield grouping was performed using Lancefield grouping anti-serum (Denka-Seiken, Tokyo, Japan). The disk diffusion method for detecting PRGBS using oxacillin, ceftizoxime, and cefitubone disks was used as reported previously (22). The minimum inhibitory concentrations (MICs) of penicillin G, oxacillin, and ceftizoxime were determined by the agar dilution using method Streptococcus pneumoniae ATCC49619 as a quality control for MIC measurements, in accordance with Clinical and Laboratory Standards Institute (CLSI) recommendations (11). Sequencing analysis of PBP2X genes was performed as reported previously (12).

The disk diffusion method uses 3 Kirby-Bauer disks of oxacillin, ceftizoxime, and cefitubone. The diameters of the growth-inhibitory zones around each Kirby-Bauer disk for PRGBS isolates are smaller than those for penicillin-susceptible GBS. Although penicillin-susceptible GBS produce an apparent growth-inhibitory
Fig. 1. Diameters of the growth-inhibitory zones and the number of clinical isolates. The diameters of the growth-inhibitory zones measured by the disk diffusion method using oxacillin (A), ceftizoxime (B), and cefitibuten disks (C) (X-axis) and the number of isolates (Y-axis) are plotted. The numbers on the bars indicate the number of clinical isolates. Diameters of growth-inhibitory zones are given in millimeters. The vertical lines indicate the tentative cutoff values for the growth-inhibitory zones around oxacillin (<17 mm), ceftizoxime (<29 mm), and cefitibuten (<20 mm) disks.
Table 1. Results of disk diffusion method for detecting PRGBS and MICs of penicillin G, oxacillin, and ceftizoxime for PRGBS candidates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Diameters of growth-inhibitory zones around Kirby-Bauer disks (mm)</th>
<th>MICs determined by the agar dilution method (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxacillin disk</td>
<td>Ceftriaxone disk</td>
</tr>
<tr>
<td>MRY08-469</td>
<td>17</td>
<td>26</td>
</tr>
<tr>
<td>MRY08-470</td>
<td>17</td>
<td>28</td>
</tr>
<tr>
<td>MRY08-471</td>
<td>19</td>
<td>28</td>
</tr>
<tr>
<td>MRY08-472</td>
<td>18</td>
<td>28</td>
</tr>
<tr>
<td>MRY08-474</td>
<td>17</td>
<td>27</td>
</tr>
<tr>
<td>MRY08-517</td>
<td>7</td>
<td>17</td>
</tr>
</tbody>
</table>

Mics, minimum inhibitory concentrations.

zone around the cefitobuten disk, most PRGBS strains do not produce these zones.

We applied this disk diffusion method to the 349 clinical isolates described above. Fig. 1 illustrates the distribution of the isolates in terms of the diameters of the growth-inhibitory zones around the oxacillin (Fig. 1A), ceftriaxone (Fig. 1B), and cefitobuten disks (Fig. 1C), which were 7–24 mm, 17–39 mm, and 6–29 mm, respectively. We previously established tentative cutoff values for the growth-inhibitory zones around oxacillin (< 17 mm), ceftriaxone (< 29 mm), and cefitobuten (< 20 mm) disks (22). We selected 6 PRGBS candidates with diameters lower than these cutoff values for more than 2 disks (Table 1).

The MICs of penicillin G for PRGBS (0.25–1 µg/ml) are close to the susceptibility breakpoint (≤ 0.12 µg/ml) set by the CLSI. However, the MICs of oxacillin (2–8 µg/ml) and ceftriaxone (4–128 µg/ml) for PRGBS are higher than those of penicillin-susceptible GBS (12). Therefore, the 6 PRGBS candidates, selected by the disk diffusion method, were subjected to the MIC determination of penicillin G, oxacillin, and ceftriaxone by the agar dilution method (Table 1). Five isolates, MRY08-469, 470, 471, 472, and 474, did not exhibit high MICs of penicillin G, oxacillin, and ceftriaxone. However, MRY08-517 showed high MICs of oxacillin (8 µg/ml), ceftriaxone (16 µg/ml), and penicillin G (0.5 µg/ml), which were above the CLSI breakpoint. Therefore, we thought that MRY08-517 might be a PRGBS. We performed sequencing analysis on the PBP2X gene of MRY08-517. This gene harbored 3 amino acid substitutions and one silent mutation—C1201T (H401Y), T1214C (V405A), C1408A (R470S), and C1617T (A539A) (GenBank accession no. AB775806), compared with that (SAG0287) of S. agalactiae strain ATCC BAA-611/2603 V/R (Fig. 2). Among these, V405A amino acid substitution is a typical amino acid substitution of PRGBS located near the conserved motif of PBP2X. Therefore, we concluded that MRY08-517 was a PRGBS.

In this study, we detected 1 PRGBS among 349 clinical isolates obtained between 1977 and 2005. This indicates that the disk diffusion method is useful for detecting PRGBS, and the advantage of this method is that it does not require special reagents or equipment. Therefore, this method may be useful for detecting PRGBS in clinical laboratories worldwide.

We have previously compared the disk diffusion method for detection of PRGBS with the agar dilution method for determination of the MICs of penicillin G (22,23). These studies confirmed that isolates that did not exhibit small diameters of growth-inhibitory zones around the 3 disks were penicillin-susceptible GBS. This suggests that it is unlikely that any PRGBS remained undetected in this study.

The only PRGBS detected in this study was isolated in 2005. The oldest PRGBS previously analyzed was isolated in 1995; no PRGBS was detected prior to 1995. To the best of our knowledge, the oldest PRGBS was isolated in 1995, suggesting that PRGBS emerged after the mid-1990s in Japan. One limitation of this study was that isolates were obtained from patients admitted to only one hospital. However, obtaining old clinical isolates can be challenging, and the samples that were procured for this study provided valuable evidence concerning the emergence of PRGBS.

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**Conflict of interest** None to declare.

**REFERENCES**


