Short Communication

Active Screening of Group B Streptococci with Reduced Penicillin Susceptibility and Altered Serotype Distribution Isolated from Pregnant Women in Kobe, Japan

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SUMMARY: Group B streptococcus (GBS; *Streptococcus agalactiae*) is a leading cause of neonatal invasive infections and was believed to be fully susceptible to penicillin. However, we recently identified several clinical GBS isolates with reduced penicillin susceptibility (PRGBS), which were mainly isolated from respiratory specimens of elderly people. An investigation of both the isolation rate of PRGBS and the serotype distribution among pregnant women is crucial to decisions regarding optimal prevention and strategies for GBS treatment in neonates. We collected 141 GBS isolates from vaginal specimens of 122 pregnant women in a hospital in Kobe, Japan, from 2007 to 2008. Of the 141 GBS isolates, 139 were subjected to antimicrobial susceptibility testing based on the results of screening for PRGBS by the disk diffusion method. All 139 isolates were susceptible to penicillin G, ampicillin, cefotaxime, cefepime, and meropenem; no PRGBS isolates were detected. However, the rates of erythromycin and clindamycin resistance in the isolates were 10.1% and 5.0%, respectively, which are much higher than the values previously reported in Japan. Serotypes VI and VIII accounted for 26% of GBS; a markedly decreased percentage from the rates observed around the year 2000. These findings suggested that penicillin remains an effective means of intrapartum antibiotic prophylaxis in Japan.

Group B streptococcus (GBS; *Streptococcus agalactiae*) is a leading cause of neonatal sepsis and meningitis (1–3). Some neonatal GBS infections occur due to vertical GBS transmission from the vaginal tracts of pregnant women to neonates during delivery; moreover, no GBS vaccines are available (4). Penicillins are used as first-line agents for intrapartum antibiotic prophylaxis and treatment because clinical GBS isolates are uniformly susceptible to β -lactams (3,5). However, in 2008, we identified and molecularly characterized GBS isolates that demonstrated reduced penicillin susceptibility (PRGBS); the isolates contained multiple amino acid substitutions in the penicillin-binding protein 2X (6,7). Similar PRGBS isolates were later reported in the United States (8), Canada (9,10), and Japan (11–13). PRGBS isolates primarily originate from respiratory specimens, blood, decubitus ulcers, and adult hip joint fluid (6–14). The isolation rate of PRGBS in pregnant women in Japan remains unknown.

Multivalent polysaccharide conjugate vaccines against 5 major serotypes (Ia, Ib, II, III, and V) have been proposed (4). A total of 10 GBS serotypes (Ia, Ib, II, III, IV, V, VI, VII, VIII, and IX) have been identi-

fied; serotypes Ia and III represent typical serotypes of neonatal invasive infections isolates (12). In the 1990s and early in the 2000s, GBS serotypes VI and VIII accounted for 60% of all GBS serotypes isolated from pregnant women's vaginal specimens in Japan (15–17). GBS serotypes VI and VIII have been reported to have a pathogenicity comparable to GBS serotypes Ia–III and V (18,19).

All of the clinical isolates in this study were recovered from vaginal specimens obtained from 3 groups of pregnant women who attended the Nishi-Kobe Medical Center between 2007 and 2008. The first group included 34 pregnant women; 34 GBS isolates were obtained between the 23rd and 25th gestational weeks. The second group included 61 pregnant women; 61 GBS isolates were obtained between the 34th and 36th gestational weeks. The third group included 27 pregnant women; a total of 48 GBS isolates were obtained during both of the periods stated above. We selected no more than 1 isolate per woman per time frame. All clinical isolates were confirmed as *S. agalactiae* by β -hemolysis on sheep blood agar plates and agglutination with Lancefield grouping anti-serum (Denka-Seiken, Tokyo, Japan).

The disk diffusion test using oxacillin, ceftizoxime, and ceftibuten disks for detecting PRGBS (20), was performed in accordance with Clinical and Laboratory Standards Institute (CLSI) recommendations (5).

The minimum inhibitory concentrations (MICs) of penicillin G, ampicillin, oxacillin, cefazolin, cefotaxime, cefepime, ceftizoxime, meropenem, erythromy-

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cin, and clindamycin were determined by CLSI agar dilution methods (5). *Streptococcus pneumoniae* ATCC49619 was used as the quality control strain.

The serotypes of 141 clinical isolates were determined by the agglutination method with anti-sera (Denka-Seiken) against the Ia, Ib, II, III, IV, V, VI, VII, and VIII capsular types. The serotypes of the 31 clinical isolates that were not typable by the agglutination method were determined genetically by polymerase chain reaction (PCR), as reported elsewhere (21), with minor modifications.

To detect PRGBS, we performed a disk diffusion test developed previously by our group (20); we used 3 Kirby-Bauer disks of oxacillin, ceftizoxime, and ceftibuten. The diameters of the growth-inhibitory zones around each disk for PRGBS were smaller than those for penicillin-susceptible GBS. Although an apparent growth-inhibitory zone appeared around the ceftibuten disk for penicillin-susceptible GBS, similar zones did not appear around the ceftibuten disk for most PRGBS isolates (20). We therefore used this disk diffusion test for the initial PRGBS screening of the 139 clinical isolates. In all 139 isolates, the growth-inhibitory zones around the oxacillin, ceftizoxime, and ceftibuten disks were 17–23, 29–35, and 19–24 mm, respectively; we did not observe any particularly small growth-inhibitory zones around any of the 3 disks. These results imply the absence of PRGBS in these 139 isolates.

We determined the MICs of the 3 antibiotics for each of the 139 isolates by the agar dilution method (Table 1). Previously, we demonstrated considerably high MICs of oxacillin and ceftizoxime for PRGBS (6). We therefore determined the MICs of oxacillin and ceftizoxime as a method of detecting PRGBS. The distributions of the MICs of 8 antibiotics are listed in Table 1. No established criteria exist for the evaluation of oxacillin, cefazolin, and ceftizoxime susceptibility in the CLSI protocol, but all 139 isolates were classified as “susceptible” to penicillin G, ampicillin, cefotaxime, cefepime, and meropenem. This result confirmed the absence of PRGBS in the 139 GBS isolates.

According to the CLSI criteria, 14 clinical isolates (14/139, 10.1%) were resistant to erythromycin and 7 (7/139, 5.0%) to clindamycin. Two isolates were intermediate to erythromycin and 2 to clindamycin. Among the remaining isolates, 123 were susceptible to erythromycin and 130 to clindamycin.

We determined the serotypes of 141 clinical isolates by agglutination methods using anti-serum. The number of GBS isolates for each of the serotypes Ia, Ib, II, III, IV, V, VI, VII, VIII, and non-typable serotype were 10 (7%), 17 (12%), 15 (11%), 14 (10%), 0 (0%), 21 (15%), 19 (13%), 1 (1%), 13 (9%), and 31 (22%), respectively (Fig. 1A). The 31 non-typable isolates were subjected to PCR in order to confirm their genetic serotype. The number of the 31 non-typable GBS isolates for each of the serotypes listed above were 0 (0%), 9 (29%), 3 (10%), 3 (10%), 2 (6%), 8 (26%), 3 (10%), 0 (0%), 2 (6%), and 1 (3%), respectively (Fig. 1B). We combined the results from the agglutination and PCR methods and determined that the number of the 139 isolates for each of the serotypes listed above were 10 (7%), 26 (18%), 18 (13%), 17 (12%), 2 (1%), 29 (21%), 22

Table 1. Distribution of minimum inhibitory concentrations (MICs), MIC₅₀, and MIC₉₀ for 8 β -lactams on 139 clinical isolates

Antibiotic	Distribution of		
	MIC (μ g/ml)	MIC ₅₀ (μ g/ml)	MIC ₉₀ (μ g/ml)
Penicillin G	0.015–0.06	0.03	0.06
Ampicillin	0.03–0.12	0.06	0.12
Oxacillin	0.12–0.5	0.25	0.5
Cefazolin	0.03–0.25	0.12	0.12
Cefotaxime	$\leq$ 0.03–0.25	0.06	0.06
Cefepime	0.03–0.5	0.12	0.12
Ceftizoxime	0.06–1	0.25	0.25
Meropenem	0.015–0.06	0.03	0.06

Fig. 1. Serotype distribution. (A) Serotype distribution determined by agglutination methods is represented in a circle graph. (B) Serotype distribution of clinical isolates determined non-typable by agglutination methods was determined by PCR methods. (C) The combined results of serotype distributions determined by agglutination and PCR methods are shown. (D) Serotype distribution of clinical isolates obtained from pregnant women between 1999 and 2000 as reported in ref. 16 is shown.

(15%), 1 (1%), 15 (11%), and 1 (1%), respectively (Fig. 1C). The 2 GBS isolates that were recovered during the middle and late stages of pregnancy from each of the 18 women who had tested positive for GBS exhibited identical serotypes.

Our study did not detect PRGBS (penicillin MIC, $>0.12 \mu$ g/ml) in any of the 139 GBS isolates, which suggests that the isolation rate of vaginal PRGBS was relatively low ($\leq 0.7\%$) in Japan between 2007 and 2008. However, continuous screening for PRGBS in GBS isolates obtained from pregnant women’s vaginal specimens may become increasingly important if neonatal invasive PRGBS emerges.

Erythromycin was the alternative antibiotic to prevent from GBS infections in pregnant women who were

allergic to penicillin. The rates of GBS resistance to erythromycin and clindamycin in vaginal specimens in Japan around 2000 reportedly were 3% and 1%, respectively (16). However, in the present study, the rates of resistance against these agents were 10.1% and 5.0%, respectively, which implies recent increases in GBS resistance to these antibiotics.

Figure 1 illustrates the serotype distributions as determined by the agglutination and PCR methods. Serotypes VI and VIII accounted for 60% of GBS recovered from pregnant women in Japan in the 1990s and early 2000s (15–17; Fig. 1D). In the present study, however, the incidence of serotypes VI and VIII clearly have decreased, and account for only 26% of GBS between 2007 and 2008 (Fig. 1C). The development of multivalent polysaccharide conjugate GBS vaccines, which are effective against 5 major serotypes (Ia, Ib, II, III, and V), was proposed in the United States (4). According to our results, this serotype coverage of these vaccines would account for 71%.

Despite the inclusion of a few GBS isolates from only 1 hospital in this study, our results provide important information concerning GBS isolates from pregnant women in Japan. The serotype shift among GBS isolates from pregnant women may continue; therefore, serotype distribution among GBS isolates from neonates and pregnant women should be monitored continuously. Careful monitoring of antimicrobial susceptibility also will become increasingly necessary among these types of patients.

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Conflict of interest None to declare.

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