**Change of antimicrobial susceptibility of group B streptococci over 15 years in Japan**

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We compared antimicrobial susceptibility patterns of 206 group B streptococcal (GBS) strains isolated from pregnant women and six from neonates/infants with invasive infection during the two periods 1985–1986 and 1999–2000. All strains in both periods were susceptible to the penicillins, cephalosporins and carbapenem tested. Seven (3\%) isolates were resistant to erythromycin and three (1\%) were resistant to clindamycin. There were no significant differences between the two study periods in the incidence of GBS resistant to the 14 antibiotics tested. These results showed that penicillins are still the first choice to prevent vertical transmission of GBS in Japan.

**Introduction**

Group B streptococci (GBS) are pathogens causing invasive infections in pregnant women, neonates and young infants. Considerable mortality and morbidity associated with such infections has prompted the development of prevention strategies.\textsuperscript{1} Recent guidelines issued by the Centers for Disease Control,\textsuperscript{2} American Academy of Pediatrics\textsuperscript{3} and American College of Obstetricians and Gynecologists\textsuperscript{4} resulted in a significant decrease in early-onset GBS infections in the USA.\textsuperscript{1} These strategies expose a large number of pregnant women to penicillin G (or ampicillin) or, for women with penicillin allergies, to erythromycin or clindamycin.\textsuperscript{2–4} Despite consistent susceptibility of GBS to penicillin/ampicillin,\textsuperscript{5–9} recent studies demonstrated an increase in strains resistant to erythromycin and clindamycin.\textsuperscript{6–10}

In Japan, debate remains about universal introduction of these recommendations mainly because the incidence of early-onset GBS infection in newborns is lower than one-tenth of that in the USA.\textsuperscript{11} However, as the use of antibiotics continues to increase, changes in antimicrobial patterns of GBS in Japan are also of great concern. There are few data on susceptibility patterns of GBS in Japan.

We sought to establish the antimicrobial susceptibility patterns of GBS from pregnant women and from invasively infected neonates/infants over the past 15 years. We also investigated possible trends in GBS capsular serotypes and compared the differences in susceptibility among capsular serotypes, especially those between the newer serotypes found exclusively in Japan (VI and VIII) and more common serotypes (I–III).

**Materials and methods**

**Patients**

The subjects included pregnant women attending Nishi-Kobe Medical Center and Meijo Hospital, and neonates and infants invasively infected by GBS who were hospitalized at the two hospitals during the following two periods. The first period was set from January 1985 to July 1986, when the capsular serotypes VI and VIII, isolated exclusively in Japan, emerged and became prevalent. The second period was from August 1999 to July 2000. Both hospitals are located in central Japan. Informed consent to this study was obtained either from the pregnant women themselves or from the infant’s parents. One hundred and three isolates (102 pregnant women and one neonate) were retrieved during the first period, and 109 (104 pregnant women and one neonate) during the second period.
women and five neonates/infants) during the second period. The six neonates/infants were all born at hospitals other than the two study hospitals and in hospitals where a prevention strategy was not carried out. They were referred to the study hospitals to have their infections treated. We adopted an intrapartum screening-based preventative strategy using ampicillin in 1984 at Meijo Hospital and in 1997 at Nishi-Kobe Medical Center. Universal chemoprophylaxis was administered to all pregnant women found to be carriers of GBS.

Isolation and preservation of GBS, and determination of serotype

Specimens for culture were obtained from pregnant women between 28 and 36 weeks gestation. Cotton swabs in Amies transport medium were used to obtain samples from the distal vagina. The yield of GBS from vaginal swabs was 12.4% and 14.3% of pregnant women in the first and second periods, respectively. Each swab was inoculated into a selective Todd–Hewitt medium broth (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA), incubated at 35°C in 5% CO₂ for 24 h, and subcultured to a 5% sheep blood agar plate. Resultant colonies were confirmed as GBS with a grouping kit reagent (Slidex Strepto-kit; bioMérieux, Lyons, France). GBS serotyping was determined using a commercially available kit (Denka Seiken, Tokyo). After replating, pure colonies were stored at −80°C in cryopreservation.

Test for antimicrobial susceptibility

Frozen isolates of GBS were thawed, inoculated in Mueller–Hinton agar (Becton Dickinson) with 5% defibrinated sheep blood and incubated in ambient air at 35°C overnight. After overnight incubation, the culture was diluted to achieve the turbidity of a 0.5 McFarland standard. Then the suspension was diluted 1:10 to an inoculum of 10⁷ cfu/mL. The antimicrobial susceptibility test was performed in duplicate by the agar dilution method in Mueller–Hinton agar with 5% defibrinated sheep blood. The plates were incubated in ambient air at 35°C for 24 h. The final inocula contained c. 3 × 10⁴ cfu/spot. The control strain (Staphylococcus aureus ATCC 29213) was included in each test. The MIC was defined as the lowest concentration of antibiotic that completely inhibited bacterial growth. The following 14 antimicrobial agents were investigated: penicillin G, ampicillin, cefazolin, cefotiam, cefotaxime, ceftiraxone, cefepime, meropenem, erythromycin, clindamycin, chloramphenicol, tetracycline, gentamicin and vancomycin. Interpretation breakpoints were those of the NCCLS.¹²

Statistical analysis

The two-tailed Fisher’s exact test was used for analysis, and a P value of <0.05 was considered statistically significant.

Results

The antimicrobial susceptibilities of a total of 212 GBS isolates are shown in the Table. All strains were susceptible to vancomycin, and the tested penicillins, cephalosporins and carbapenem. Penicillin G, cefotaxime, ceftiraxone and meropenem were the most active agents (MIC₉₀ 0.06 mg/L). While MICs of erythromycin and clindamycin were comparable to those of penicillins, seven (3%) isolates were resistant to erythromycin (MIC ≥ 1 mg/L) and three (1%) were resistant to clindamycin (MIC ≥ 1 mg/L). Of the seven erythromycin-resistant strains, three also exhibited resistance to clindamycin and tetracycline and one additional strain showed intermediate susceptibility to clindamycin (MIC 0.5 mg/L). Seventeen (8%) isolates were resistant to chloramphenicol (MIC ≥ 16 mg/L) and 55 (26%) were resistant to tetracycline (MIC ≥ 8 mg/L). Gentamicin exhibited the least activity against GBS with all the strains having MIC ≥ 16 mg/L.

We then analysed patterns of susceptibility of GBS by period of isolation and serotype. MIC₉₀s and MIC₅₀s of almost all antibiotics were consistent between the two periods except for the MIC₉₀ of cefepime and MIC₅₀ of ampicillin, erythromycin and chloramphenicol (Table). However, there were no significant differences between the two periods in the incidence of GBS resistant to the 14 antimicrobial agents: 3% (3/103) versus 4% (4/109) for erythromycin (P = 1.0), 1% (1/109) versus 2% (2/109) for clindamycin (P = 1.0), 10% (10/103) versus 7% (7/109) (P = 0.45) for chloramphenicol and 29% (30/103) versus 23% (25/109) (P = 0.38) for tetracycline. The serotypes isolated from pregnant women, in decreasing order, were: Ia (33), VIII (23), III (17), Ib (10), II (10), VI (6), V (2) and IV (1) in the first period, and VIII (33), VI (26), Ib (15), III (12), Ia (10), II (5), non-typeable (5), V (3) and 7271 (3) in the second period. One strain from neonates/infants in the first period was serotype VIII, and the five isolates in the second period included Ia (2), Ib (1), III (1) and VIII (1). No significant differences in the resistance patterns were found between the newer serotypes (VI and VIII) and more common serotypes (I–III).

Discussion

One of our objectives in this investigation was to determine the changes in in vitro antimicrobial susceptibility patterns of GBS over the last 15 years in Japan. The isolates during the second period were obtained from hospitals in which a preventive approach to vertical transmission had been used consistently for at least 2 years. Thus this investigation offered the potential to detect emerging resistant patterns resulting from the implementation of an early-onset neonatal GBS prevention strategy. However, our results showed that there were no significant differences between the first and second period in the incidence of GBS resistant to the 14 antibiotics tested.
Antibiotic susceptibility patterns of GBS

We did not find any strain showing resistance or intermediate susceptibility to the penicillins or cephalosporins. These results confirm those reported by several investigators.6–9 Cefotaxime, ceftriaxone and meropenem were as active as penicillin G, all having MIC₉₀'s of 0.06 mg/L. These three antibiotics are potential alternatives for the treatment of neonates and infants with meningitis. However, once GBS has been identified as the causative organism, penicillin G is preferable because of its demonstrated safety, narrow spectrum and low cost.5

We found that the incidence of resistance to erythromycin and clindamycin were 3% and 1%, respectively. These figures are less than those noted by recent investigators.6–10 Resistance to erythromycin and clindamycin was found in 15–25% and 7–16% of isolates, respectively, in the USA,7–9 and in 30% and 24%, respectively, in Taiwan.10 These authors raised concern about the possibility of inadequate prophylaxis using currently recommended alternatives in penicillin-allergic patients.7–10 However, because of small proportions of resistant strains in our study, these two agents are still the second-line antibiotics in Japan. Although the precise mechanisms are not yet fully known, erythromycin resistance is usually due to target modification involving the shared 23S rRNA target site with lincosamides and streptogramins.10

This investigation is also unique in that GBS isolates included newer serotypes VI and VIII. These serotypes emerged approximately two decades ago and at present have become the leading isolates from pregnant women in Japan.11 However, limited data have been available on their antimicrobial susceptibility patterns. Our results showed that there were no significant differences in the incidence of resistance to the antibiotics tested between the newer serotypes and more common serotypes. However, erythromycin and clindamycin resistance has been found recently among serotype V strains.5,8,9 Thus, continued susceptibility monitoring as well as serotyping remains necessary.

Acknowledgement
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References

Table. In vitro susceptibility of 212 GBS strains to 14 antibiotics

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MIC₉₀ (mg/L)</th>
<th>MIC₉₀ (mg/L)</th>
<th>MIC₉₀ (mg/L)</th>
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<tr>
<td>Penicillin G</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
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<tr>
<td>Amoxicillin</td>
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<td>0.125</td>
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<tr>
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<td>0.125</td>
<td>0.125</td>
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<td>0.06–0.125</td>
<td>0.06–0.125</td>
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<td>0.125–0.25</td>
<td>0.125–0.25</td>
</tr>
<tr>
<td>Meropenem</td>
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<td>0.06–0.125</td>
<td>0.06–0.125</td>
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<td>0.25–0.5</td>
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<td>0.25–0.5</td>
<td>0.25–0.5</td>
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<tr>
<td>Vancomycin</td>
<td>0.25–0.5</td>
<td>0.25–0.5</td>
<td>0.25–0.5</td>
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MICs in mg/L. Isolates were considered resistant according to the MICs of the NCCLS in 2000.12


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