Effects of Intrapartum Antibiotic Prophylaxis on Neonatal Acquisition of Group B Streptococci

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Objectives To assess the incidence of colonization with group B streptococci (GBS) among neonates as influenced by maternal GBS carriage and intrapartum antibiotic prophylaxis (IAP).

Study design Between October 2014 and May 2015, nasopharyngeal and rectal swab samples were collected from 730 neonates at 1 week and 1 month after birth. GBS and capsular serotype were identified by real-time polymerase chain reaction and by culture. IAP at delivery was determined retrospectively from hospital records.

Results Sixty-four neonates (8.8%) were GBS-positive by real-time polymerase chain reaction and culture. Among neonates born to mothers who were GBS carriers (n = 107), 94.4% (101/107) had maternal IAP; 19.6% nonetheless were GBS-positive, compared with 6.5% of neonates born to noncarrier mothers ($P < .01$). Among neonates born to mothers receiving IAP, more were positive only at 1 month of age than at both 1 week and 1 month. The frequency of GBS in neonates born to mothers receiving IAP was significantly lower than that in neonates born to mothers not receiving IAP ($P < .05$). Capsular serotypes V (25%) and III (23.4%) were common, followed by Ib (15.6%), Ia (14.1%), II (7.8%), IV (6.3%), nontypeable (4.7%), and VI and VIII (each 1.6%).

Conclusions Delayed colonization with GBS occurs in infants born to GBS carrier mothers receiving IAP. GBS should be considered in all infants at 1 month after birth with signs of infection. *(J Pediatr 2017;190:169-73).*

Group B streptococcus (GBS) is a leading cause of severe infections in early infancy. These infections include early-onset disease (EOD), occurring within 6 days and late-onset disease (LOD), occurring from 7 to 89 days. Among pregnant women, the GBS carrier rate was estimated to be near 13% (range, 12%-22%).[1] In approximately one-half of carriers, GBS is transmitted to the infant, with invasive GBS disease developing in 1%-2%.

To prevent EOD due to GBS, a guideline was introduced by the US Centers for Disease Control and Prevention and the American College of Obstetricians and Gynecologists in 1996. Universal screening for maternal GBS colonization and intrapartum antibiotic prophylaxis (IAP) strategies implemented according to the guideline have reduced the incidence of EOD but had no impact on LOD.[2,3]

In Japan, a revised guideline for obstetric practice published in 2014 recommended universal GBS screening by conventional culture for all pregnant women at 33-37 weeks of gestation.[4] IAP during labor or after premature rupture of membranes was recommended for women with any of the following characteristics: GBS culture positivity, GBS infection during infancy in previous offspring, or incidental detection of GBS in a urine culture during the current pregnancy. If GBS status in the current pregnancy is unknown, IAP should be given to mothers with symptoms at delivery that might be attributable to GBS.

In this study, we investigated prevalence of GBS colonization in neonates examined at 1 week and 1 month after birth, influences of IAP and GBS carriage in the mother, and capsular serotype and sequence type (ST) of GBS isolates from neonates.

Methods

We conducted a prospective longitudinal cohort study at 4 hospitals, which were located in Hokkaido, Gunma, and Kanagawa prefectures and in Tokyo. The study was performed from October 2014 to May 2015. Nasopharyngeal and rectal swabs

EOD	Early-onset disease
GBS	Group B streptococcus
IAP	Intrapartum antibiotic prophylaxis
LOD	Late-onset disease
MLST	Multilocus sequence typing
PCR	Polymerase chain reaction
ST	Sequence type

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were collected from all eligible infants at 1 week and 1 month after birth and transported immediately to our laboratory. All samples were tested to identify GBS by amplification of the *dltS* gene, which encodes a histidine kinase, and also to identify capsular types Ia, Ib, and III using real-time polymerase chain reaction (PCR) as developed by Morozumi et al.[5] Correlation between results of real-time PCR and bacterial culture also was examined.

As shown in **Figure 1** (available at www.jpeds.com), samples were collected via cotton-tipped swabs (BBL CultureSwab Plus, Copan, Italy). Immediately after receipt, swab samples were suspended in 0.5 mL of Todd-Hewitt broth (Becton, Dickinson, Franklin Lakes, New Jersey) and centrifuged at 2000*g* for 5 minutes at 4°C to collect bacterial cells. After the supernatant was discarded, 55 μL of pellet was stirred and used for direct DNA extraction (50 μL) and for culture (5 μL).

Each sample of 50 μL was placed in 45 μL of a lysis solution that included SimplePrep reagent for DNA (Takara Bio, Shiga, Japan) and 2 U of mutanolysin (Sigma Aldrich, St. Louis, Missouri). The lytic reaction was carried out for 10 minutes at 37°C, followed by 3 minutes at 95°C. The lysate was added to each of the tubes containing PCR mixtures for the following gene identifications: the *dltS* gene, which encodes a histidine kinase specific to GBS; and capsular types Ia, Ib, and III. Total volume (30 μL) of the PCR mixture included 20 pmol of each primer, 25 pmol of each probe, 2× Multiplex Powermix (Bio-Rad, Hercules, California), and DNase- and RNase-free distilled water. DNA amplification was carried out for 45 cycles as follows: 95°C for 10 seconds, 50°C for 30 seconds, and 72°C for 20 seconds.

The remaining 5 μL of each sample was inoculated on sheep blood agar (Nippon Becton, Dickinson, Tokyo, Japan), which then was incubated at 37°C for 20 hours in an atmosphere containing 5% CO_2. Colonies of β-hemolytic streptococci grown on the blood agar plates underwent identification of capsular type (Ia, Ib, II, III, IV, V, VI, VII, and VIII) and the *dltS* gene by real-time PCR.

For multilocus sequence typing (MLST) analysis, primer sets corresponding to 7 housekeeping genes (*adhP*, *atr*, *glnA*, *glcK*, *pheS*, *sdhA*, and *tkt*) used for MLST were constructed with reference to the MLST Web site (http://pubmlst.org/sagalactiae/). MLST was applied to the sequences for these 7 genes according to previously described methods,[6] with alleles and ST assignments determined using the *Streptococcus agalactiae* MLST database.

Statistical Analyses

Statistical analyses were performed with Ekuseru-Toukei 2012 software for statistics (Social Survey Research information, Tokyo, Japan). The Mann-Whitney *U* test, the χ^2 test, Fisher exact test, the Wilcoxon rank sum test, or univariate logistic regression analysis was used as appropriate. A P value < .05 was considered to indicate statistical significance.

Field workers explained the purpose of the study to mothers of eligible infants, and each mother signed an informed consent form just before discharge or at 1 week after delivery. The study was approved by the Yokohama Rosai Hospital Ethics Committee (approval number 26-51).

Results

Figure 2 is a flowchart showing transmission of GBS among 730 neonates according to GBS status of their mothers as determined by prenatal screening. GBS screening via bacterial cultures was performed at 33-37 weeks of gestation for 710 mothers (97.3%) receiving care at institutions participating in the study, and 107 mothers (14.7%) proved to be GBS carriers. Twenty mothers (2.7%) did not undergo screening.

GBS-positive neonates numbered 64, including 46 positive by both real-time PCR and culture and 18 positive only by real-time PCR. Twenty-one neonates born to the 107 GBS carriers were positive for GBS (19.6%); among neonates born to the 603 noncarriers, 39 (6.5%) were positive for GBS. Frequency of GBS positivity in neonates differed significantly between carrier and noncarrier mothers (P < .001). Four neonates born to mothers without GBS screening were positive for GBS (20%). No infants developed invasive GBS infection during the study period.

Relationships between time points of GBS positivity in neonates and maternal IAP are shown in **Table I**.

GBS-positive neonates were divided into 3 groups: GBS persistence (n = 16, 25%), indicating GBS positivity at both 1 week and 1 month after birth; GBS clearance (n = 7, 10.9%),

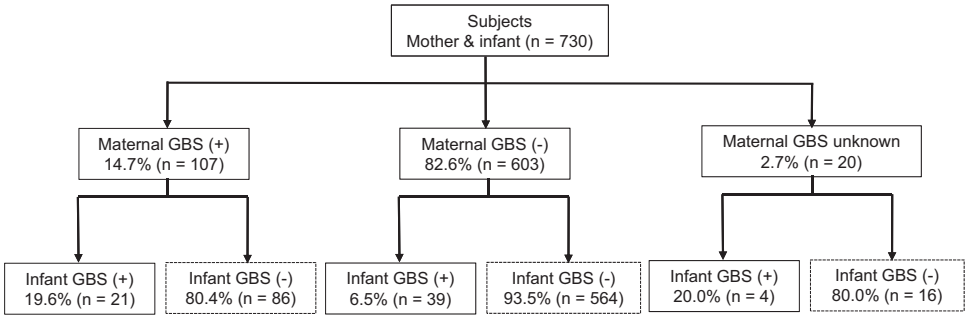

Figure 2. Flowchart showing presence or absence of GBS carriage in mothers and neonates.