Group B Streptococcal Colonization Patterns in Mothers and Their Infants

DOUGLAS E. JONES,¹ KEITH S. KANAREK,² AND DANIEL V. LIM^{1*}

Department of Biology, University of South Florida, Tampa, Florida 33620,¹ and Department of Pediatrics, University of South Florida College of Medicine, Tampa, Florida 33612²

Received 17 February 1984/Accepted 30 May 1984

Maternity patients and their newborn infants were cultured for group B streptococci (GBS) at Tampa General Hospital, Tampa, Fla., from September 1982 to May 1983. Culture swabs were placed into Lim Group B Strep Broth (GIBCO Laboratories, Madison, Wis.) and quantitated for GBS. A strong correlation was found between the numbers of GBS in the maternal vagina and the infant rectum. Infants symptomatic for early-onset GBS disease were delivered by mothers heavily colonized ($\geq 3 \times 10^4$ GBS per swab) at the vagina. Such mothers were identified as GBS carriers by slide coagglutination and latex agglutination after their broth cultures had been incubated for 5 h. These data indicate that maternity patients at high risk of delivering infants heavily colonized with GBS and potentially symptomatic for early-onset GBS disease can be rapidly and selectively identified.

Group B streptococci (GBS) are among the most frequent causes of neonatal sepsis, meningitis, and respiratory distress. Approximately 1% of the infants colonized with GBS at surface sites (umbilicus, rectum, and throat) develop early-onset sepsis during the first week of life. Infants often begin showing symptoms of disease several hours after birth. Early-onset GBS disease has a 50% mortality rate after the onset of symptoms, despite antimicrobial therapy (2).

GBS colonize the vaginal tract of 7 to 20% of all pregnant women (4, 7, 8). Infants usually are infected with GBS during birth. Lim et al. (12) showed that heavily colonized infants ($\geq 10^5$ GBS per culture swab at two or more surface sites) are symptomatic for early-onset sepsis. Ancona et al. (1) and Bobitt et al. (5) noted in independent studies that women heavily colonized with GBS deliver infants who also are heavily colonized with the bacteria. Both of these maternal studies, however, were semiquantitative and did not include viable plate count data.

Those maternity patients at high risk of delivering symptomatic infants could be identified during labor if a significant correlation existed between magnitudes of colonization in the mother and her infant. If these women at high risk could be rapidly and accurately identified during labor, such information could be used for immunotherapy or chemoprophylactic therapy. We show in this paper that a significant correlation does exist between GBS colonization magnitudes in mothers and their infants. We furthermore propose the combined use of an enriched, selective broth and slide coagglutination or latex agglutination to rapidly screen for maternity patients at high risk of delivering infants symptomatic for early-onset GBS disease.

MATERIALS AND METHODS

Maternal cultures. Six hundred pregnant women admitted to Tampa General Hospital from September 1982 to May 1983 were cultured for GBS. Vaginal cultures, obtained while the women were prepared for delivery, were collected with Culturette II dual swabs (Marion Scientific Corp., Kansas City, Mo.) and were refrigerated at 4°C until processed.

One swab from each dual culture swab set was inoculated

into 2 ml of enriched, selective Todd-Hewitt broth containing 1% yeast extract, 10 µg of colistin methane sulfonate per ml, and 15 µg of nalidixic acid per ml (Lim Group B Strep Broth; GIBCO Laboratories, Madison, Wis.). The broth cultures were tested for GBS by slide coagglutination (Phadebact Strep B Test; Pharmacia Diagnostics, Piscataway, N.J.) and by latex agglutination (Wellcogen Strep B Test; Wellcome Diagnostics, Dartford, England) after 20 h of incubation at 36°C in 5% CO₂. Those specimens positive for GBS were retested with the second swab from the Culturette set. The second swab was inoculated into 2 ml of Lim Group B Strep Broth. The broth culture was mixed with a Vortex-Genie Mixer (Scientific Industries, Inc., Bohemia, N.Y.), serially diluted in 0.85% saline, and plated onto duplicate plates of Columbia colistin-nalidixic acid agar containing 5% sheep blood (GIBCO). Streptococcal CFU were enumerated from plates incubated for 24 h at 36°C in 5% CO₂. This second broth culture was retested for GBS by slide coagglutination and by latex agglutination after 5 and 20 h of incubation.

Infant cultures. One hundred and eight infants delivered from GBS-colonized mothers were cultured with Culturette swabs at birth before cleansing. All infants were cultured at the rectum, nasopharynx, and umbilicus.

The infant swabs were placed into 2 ml of Lim Group B Strep Broth, thoroughly mixed, and enumerated for GBS in the same manner as maternal swabs. The broth cultures were tested for GBS by slide coagglutination and by latex agglutination after 5 and 20 h of incubation at 36°C in 5% CO₂.

Slide agglutination tests. Slide coagglutination and latex agglutination tests were performed on broth culture cell suspensions, and the results were graded as negative, 1+, 2+, 3+, or 4+ positive. A 4+ reaction was one in which large clumps appeared within 60 s. A 3+ reaction represented the formation of large clumps within 60 to 90 sec. The appearance of medium clumps within 0 to 90 s was graded as 2+, and small clumps appearing within the same time period were graded as 1+. Multiple slide coagglutination reactions caused by blood were eliminated by heating the broth cultures to 90°C before testing (10). Multiple latex agglutination reactions were eliminated by the extraction of bacterial antigen in broth cultures with pronase for 10 min at 37°C.

* Corresponding author.

Serotyping. All positive cultures were serotyped by immunofluorescence to reconfirm them as GBS and to show that the infants were colonized with bacteria of the same serotype as the mothers. Type-specific antiserum was conjugated with fluorescein isothiocyanate by the method of Jones et al. (11). Ethanol-fixed smears of GBS on glass slides were reacted with each fluorescein isothiocyanate-labeled type-specific antiserum for 10 min at 25°C. The slides were then washed in 0.1 M phosphate-buffered saline (pH 7.2), mounted with cover slips, and examined with a fluorescent microscope (Nikon Labophot Microscope, Tokyo, Japan).

Statistical analysis. Statistical analysis of all data was performed on the $\log_{10}$ of viable plate count results. Significant differences between sample populations were determined with the *t* test. Correlation coefficients were calculated by Pearson's product moment correlation coefficient formula. The significance of such correlation coefficients was tested by Fisher and Yates statistical tables.

RESULTS

Colonization rate. Of the 600 maternity patients cultured in this study, 130 (21.7%) were colonized with GBS; 32 (24.6%) of these positive cultures were identified as such by latex agglutination and slide coagglutination after 5 h of incubation. The remaining positive cultures were identified after 20 h of incubation. There were four instances—two involving latex agglutination and two involving slide coagglutination—in which maternal broth cultures, after 20 h of incubation, yielded positive results by either latex agglutination after pronase treatment or slide coagglutination, but not both. No GBS were recovered from plating of these broth cultures. These reactions, therefore, were not considered GBS positive in this study.

Of the 108 infants cultured, 61 (56.5%) were found to be colonized with GBS; 56 (51.9%) of the rectal cultures were positive for GBS, 25 (23.1%) of the umbilical cultures contained GBS, and 34 (31.5%) of the infants were colonized with GBS at the nasopharynx. All GBS-positive infant cultures were positive by both latex agglutination and slide coagglutination.

Correlation of colonization between mothers and infants. The extent of colonization between mothers and their infants was compared by Pearson's product moment correlation coefficient. The number of GBS present in infant umbilical and nasopharyngeal cultures correlated poorly with that found in the mother's vagina. A highly significant ($P < 0.001$) correlation coefficient of 0.75 was found between the

TABLE 1. Magnitude of colonization of symptomatic infants and their mothers

Infant	Clinical condition	Colonization magnitude (GBS per swab) ^a	
		Infant (rectum)	Mother (vagina)
A	Septic	$<10^{1b}$	7.70×10^6
B	Septic	7.02×10^5	6.62×10^7
C	Respiratory distress	5.25×10^6	2.50×10^6

^a Colonization magnitudes were determined by the viable plate count technique on broth cultures before incubation.

^b Infant may have been cleansed before culture.

magnitudes of GBS colonization in the infant rectum and maternal vagina.

The numbers of GBS found in the maternal vagina were plotted against the numbers present in the infant rectal cultures to obtain a linear regression curve (Fig. 1). This curve shows that a vaginal swab must generally contain at least 10^2 GBS before the infant's rectal swab will yield a positive culture. The curve furthermore shows that infants colonized with $\geq 10^5$ GBS per swab at the rectum are delivered by mothers vaginally colonized with $\geq 3 \times 10^4$ GBS per swab.

Symptomatic infants. Three infants in this study developed early-onset GBS disease (Table 1). Two of these infants were septic, as determined by GBS-positive blood cultures. Infant A was lightly colonized at the rectum, but the mother was heavily colonized and was identified as GBS positive within 5 h of broth culture incubation. The low magnitude of colonization in this infant was probably caused by inadvertent culture of the infant immediately after the passage of stool (unpublished data). A repeat culture of infant A two days after birth yielded 6×10^3 GBS per swab at the rectal site.

Infant B was heavily colonized with GBS, and its culture was confirmed as positive after 5 h of broth incubation. The maternal culture also contained a large number of GBS and was identified before broth incubation.

Infant C was observed clinically to have respiratory distress, but not sepsis. The infant was heavily colonized, and its rectal broth culture was identified as GBS positive after an incubation period of 5 h. The vaginal culture from the mother of infant C also contained a large number of GBS, and the broth culture was identified within 5 h of incubation.

Sensitivity and specificity. The sensitivity and specificity of the testing protocol used in this study were determined. The three symptomatic infants were delivered by mothers whose broth cultures were rapidly identified within 5 h of incubation. These three infants were defined as true positives. Seventy-two mothers delivering asymptomatic infants were identified only after their vaginal broth cultures had been incubated for 20 h. These mothers were considered true negatives. This study also yielded what we consider to be 10 false-positive identifications (asymptomatic infants delivered by mothers whose broth cultures were identified within 5 h of incubation). No false-negatives were observed in the study. False-negatives were defined as symptomatic infants delivered by mothers whose broth cultures were not identified within 5 h of incubation. The testing protocol thus was found to be 100% sensitive and 88% specific in identifying maternity patients at high risk of delivering infants with early-onset GBS disease. The predictive value of a negative test in this study was 100%. The predictive value of a positive test was 23%. This low positive predictive value was

FIG. 1. Standard curve comparing maternal cultures to infant rectal cultures taken at birth.

the result of the small number (three) of symptomatic infants included in the study.

DISCUSSION

The maternal colonization patterns observed in this study are consistent with those reported by other investigators using broth media for diagnosis of GBS infections (3, 13). Researchers using solid media report lower colonization rates than those using broth (3, 14). Solid media are less sensitive in isolation of GBS, particularly in instances where such cultures contain low numbers of bacteria. Easmon et al. (6) observed that 44% of infants delivered by mothers colonized with GBS were infected with the bacteria at surface sites. In our study we found that 58% of infants born to colonized mothers carried GBS at surface sites. Ninety percent of the infants not colonized with GBS were delivered by lightly-colonized mothers.

The data from the present study show a correlation between the extent of GBS colonization in mothers and their infants. Heavily colonized maternity patients ($\geq 3 \times 10^4$ GBS per swab) delivered heavily colonized infants ($\geq 10^5$ GBS per swab). Such infants were at increased risk for developing GBS disease than were infants lightly colonized with GBS. The results from a previous study (9) involving GBS colonization patterns in women showed that heavily colonized maternity patients can be identified as such within 5 h of broth culture incubation. These data, in combination with the results presented in this current study that heavily colonized mothers deliver infants also heavily colonized with GBS, provide the basis for a program to screen for these high-risk maternity patients. This program is not intended specifically to predict symptomatic infants, but rather to identify selectively a population of maternity patients that have a high risk of delivering such infants. Rapid and selective identification of such high-risk mothers could reduce nonselective and indiscriminate use of antimicrobial agents, thereby minimizing the risks associated from such administration of these agents.

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