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Rapid Identification of Pregnant Women Heavily Colonized with Group B Streptococci

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Pregnant women admitted to Tampa General Hospital, Tampa, Fla., were cultured for group B streptococci (GBS). Culture swabs were placed into enriched, selective Todd-Hewitt medium and were quantitated for GBS. The broth cultures were tested by slide coagglutination before incubation and after 5 and 20 h of incubation. Fifty-four (27%) of the 201 maternity patients cultured were positive for GBS and were identified as such by slide coagglutination. A strong correlation was found between the magnitudes of colonization and the times required to identify the broth cultures as GBS positive. Cultures from mothers heavily colonized (mean concentrations of 3×10^4 GBS per culture swab or greater) were identified after 5 h or less of incubation. Mothers lightly colonized with GBS (mean concentrations of 2×10^2 GBS per culture swab) were identified only after their broth cultures had been incubated for 20 h.

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 skin sites (umbilicus, rectum, and throat) develop early-onset sepsis during the first week of life. This disease has a 50% mortality rate, despite antimicrobial therapy (3).

GBS have been shown to colonize the vaginal tracts of 7 to 20% of all pregnant women (5, 9, 10). Infants usually become infected with GBS during birth. Data from our laboratory indicate that 70% of the neonates delivered by mothers colonized with GBS are colonized by the organism at external skin sites. Our data show further that heavily colonized infants ($\geq 10^5$ GBS per culture swab at two or more external skin sites) are symptomatic for early-onset sepsis (11). Ancona et al. (1) and Bobitt et al. (6) noted in independent studies that women heavily colonized with GBS delivered infants who were also heavily colonized with the bacteria. If these women at high risk of delivering symptomatic infants could be rapidly and accurately identified in routine prenatal screening, the morbidity and mortality rates of early-onset GBS sepsis would be significantly reduced. We describe in this paper the combined use of an enriched, selective broth medium and slide co-agglutination to screen such maternity patients.

MATERIALS AND METHODS

A total of 201 pregnant women admitted to Tampa General Hospital from September 1982 to January 1983 were cultured for GBS. Vaginal cultures were obtained while the women were prepared for delivery. Bacteriostatic jelly was not used before culturing. Cultures were taken 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 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 (GIBCO Diagnostics, Madison, Wis.). The broth cultures were tested for GBS by slide coagglutination (Phadebact Streptococcus Test, Pharmacia Diagnostics, Piscataway, N.J.) after 20 h of incubation at 36°C in 5% CO_2. All broth cultures were tested with groups A and B streptococcal antisera. Group A antiserum served as a negative control.

Those cultures positive for GBS were retested with the second swab from the dual-culture swab set. The second swab was inoculated into 2 ml of enriched, selective Todd-Hewitt broth. The broth culture was mixed vigorously 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 with 5% sheep blood (GIBCO). The number of CFU was determined by taking the average of duplicate plate counts after incubation of the plates for 24 h at 36°C in 5% CO_2. This second broth culture was retested for GBS by slide coagglutination immediately after inoculation

TABLE 1. Correlation of magnitude of colonization and identification at 0, 5, and 20 h

Time (h)	No. of GBS-positive women colonized with the following concn (GBS per swab):							
	$<1 \times 10^1$	1×10^1–9.99×10^1	1×10^2–9.99×10^2	1×10^3–9.99×10^3	1×10^4–9.99×10^4	1×10^5–9.99×10^5	1×10^6–9.99×10^6	1×10^7–9.99×10^7
0	0	0	0	0	0	0	0	2
5	1	0	3	3	6	2	4	0
20	11	4	7	7	4	0	0	0

(time zero) and after 5 and 20 h of incubation. Negative maternal cultures (one for every two to three positive maternal cultures) were also retested by this procedure and served as negative control cultures for this portion of the study.

Coagglutination test results were graded as negative or 1+, 2+, 3+, or 4+. 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 s. The appearance of medium clumps within 0 to 90 s was graded as 2+, and the appearance of small clumps within the same time period was graded as 1+.

RESULTS

Fifty-four (27%) of the 201 pregnant women cultured in this study were colonized with GBS and were so identified by slide coagglutination. Two (3.7%) of the 54 GBS-positive maternal broth cultures were identified by slide coagglutination before incubation (time zero) (Table 1). Both of these broth cultures were inoculated from culture swabs having 10^7 GBS. Nineteen (35%) of the positive maternal cultures were identified after 5 h of incubation. These cultures had mean concentrations of 3×10^4 GBS per swab. Thirty-three (61%) of the 54 maternity patients were identified as GBS carriers only after their broth cultures had been incubated for 20 h. These cultures contained mean concentrations of 2×10^2 GBS per swab. The differences in magnitudes of colonization among those mothers identified at 5 h were not significant, with the exception of the one patient colonized at only 10^1 GBS per swab. There was no significant difference among those mothers identified at 20 h. Significance was determined by t test analysis, using z scores ($P < 0.01$). Such analysis was performed on the means of the $\log_{10}$ of CFU from the culture swabs.

DISCUSSION

Several investigators have proposed maternal screening programs as a means of identifying infants at high risk of developing symptomatic GBS infections (2, 10, 13). Ryan and Barrett (14) recently reported the use of immunofluorescence to screen mothers and infants for GBS. Selective broth medium is the single most sensitive method of detecting vaginal colonization of GBS (4, 7). In the past, however, broth cultures have been used to identify all colonized mothers, not specifically those at high risk of delivering symptomatic infants.

We describe in this study a screening program that utilizes an enriched, selective broth medium and slide coagglutination to specifically identify high-risk mothers. Coagglutination is a proven technique, shown to be 98% accurate in identifying streptococci (8, 12, 15). The results of this study showed that coagglutination is sensitive in detecting GBS in broth cultures at concentrations of 10^7 GBS per ml or greater. Vaginal cultures containing this concentration of bacteria (10^7 GBS per culture swab or greater, inoculated into 2 ml of broth) could be identified by slide coagglutination immediately upon inoculation (time zero). Cultures containing mean concentrations of 3×10^4 GBS per swab were identified after 5 h of incubation, whereas those with mean concentrations of 2×10^2 GBS per swab took 20 h of incubation for identification. Overlaps in identification times for cultures containing 10^2 to 10^3 GBS per swab were probably due to differences in generation times among strains of GBS.

These results indicate that mothers heavily colonized with GBS can be rapidly (0 to 5 h) identified by inoculating vaginal culture swabs into enriched, selective Todd-Hewitt broth with subsequent identification by slide coagglutination. These data, in combination with those from our previous report (11) that heavily colonized infants develop group B sepsis and the reports of others (1, 6) that heavily colonized infants are delivered from heavily colonized mothers, form the basis of a new screening technique. This technique would allow clinicians to rapidly detect those mothers at high risk of delivering infants symptomatic for early onset group B sepsis. Identification of the high-risk infant before delivery would enable the clinician to initiate chemotherapy immediately after delivery, thereby significantly reducing the morbidity and mortality rates of neonatal group B sepsis.

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