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Rapid Isolation and Identification of Group B Streptococci from Selective Broth Medium by Slide Co-Agglutination Test

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Direct identification of group B streptococci from a selective broth medium was performed with the Phadebact[R] streptococcus test to determine the feasibility of this technique for early detection of streptococcal colonization. Of 124 clinical isolates, 122 (98.4%) were correctly identified in less than 24 h from the selective broth medium, whereas standard cultures from blood agar plates identified, after 48 h, only 66 (53.2%). The presence of group B streptococci in mixed cultures was always detected by the Phadebact[R] test, and no false-positive co-agglutination tests were observed in 372 cultures from which organisms other than group B streptococci were isolated.

Streptococci belonging to Lancefield group B have been associated with human disease since 1938. These microorganisms are now considered to be among the most common causes of sepsis and meningitis in the first 2 months of life (3, 8). Data from the Neonatal Meningitis Collaborative Study indicates that nearly one-third of neonatal meningitis is caused by group B streptococci (10). Based on reported attack rates (2, 7) it has been estimated that annually between 12,000 and 15,000 infants in the United States will develop serious group B streptococcus infection, with a mortality rate of 30 to 50% (1). Antimicrobial agents, even when administered at the onset of clinical signs, are frequently unable to arrest the progression of the disease. Laboratory procedure that will identify infants at risk for group B streptococcus infections are thus urgently needed. To that end, we have developed and tested a technique that allows for early and rapid isolation and identification of group B streptococci.

MATERIALS AND METHODS

Collection of specimens. Over a 3-month period 594 consecutive deliveries were studied. Cervical or vaginal cultures were obtained from all mothers on admission to the delivery suite. Umbilical and pharyngeal cultures were obtained on all infants within 1 h after birth, after infants were washed with a neutral soap (Phisoderm; Winthrop Laboratories, New York, N.Y.). A subgroup of 34 infants had additional surface cultures (axilla, groin, ear, and placenta) as part of evaluation for possible neonatal sepsis. Duplicate rayon-tipped swabs of all culture sites were obtained and stored at room temperature in modified Stuart medium (Culturette; Marion Scientific Corp., Kansas City, Mo.) before plating.

Culture media. Specimens from all sites were inoculated in both selective broth medium (SBM) (4) and on blood agar plates (BAP). The SBM consisted of Todd-Hewitt broth (Baltimore Biological Laboratory, Cockeysville, Md.), 5% defibrinated sheep blood, 8 μg of gentamicin sulfate (Schering Laboratories, Bloomfield, N.J.) per ml, and 15 μg of nalidixic acid (Sterling-Winthrop Research Institute, Rensselaer, N.Y.) per ml. BAP utilizing Trypticase soy blood agar base with 5% defibrinated sheep blood (Baltimore Biological Laboratory) were used for both primary plating and replating from the SBM.

Processing of specimen. From each culture site one swab was streaked directly on BAP and incubated at 35°C in 5% CO_2 for 18 to 24 h. A second swab was placed in SBM, incubated aerobically at 35°C overnight, and replated on a BAP which was then incubated in 5% CO_2 at 35°C for 18 to 24 h.

Direct streptococcal grouping from SBM. All SBM which showed evidence of bacterial growth were tested for the presence of group B streptococci by slide co-agglutination of protein A-containing staphylococci coated with A, B, C, and G group-specific antibodies by using the Phadebact[R] streptococcus test (Pharmacia Diagnostics, Piscataway, N.J.) (5, 9). One drop of reconstituted streptococcal reagent was mixed on a glass slide with one drop of broth culture. Within 1 min a visible co-agglutination occurred with the group-specific reagent. The negative results obtained with the other three reagents served as controls.

Streptococcal grouping from BAP. Both BAP (one streaked with the specimen swab, the other replated from SBM) were inspected for the presence of beta-hemolytic and nonhemolytic colonies resembling streptococci. These colonies were gram stained to confirm the presence of gram-positive cocci and subjected to biochemical testing, and their identity as group B

streptococci was presumptively established by positive hippurate hydrolysis and CAMP test, lack of growth in 40% bile, and negative esculin hydrolysis. Definitive serological identification was performed with the Phadebact[R] streptococcus test, as described above, after several colonies were grown in 1 ml of Todd-Hewitt broth for 3 h.

RESULTS

During the 3-month period 1,781 maternal and neonatal specimens were cultured both in the SBM and on BAP. A total of 124 strains of group B streptococci were isolated by both media. Table 1 details the distribution of sites and the recovery rate of streptococci.

Of the 124 clinical isolates of group B streptococci, 122 (98.4%) were detected by the SBM and only 66 (53.2%) were detected by the BAP. Only 2 isolates were recovered by BAP alone, whereas 58 grew only in the SBM. (Tables 2 and 3).

An additional 372 SBM cultures showed evidence of bacterial growth but were negative for group B streptococci by the co-agglutination test. Table 4 details the organisms isolated. Replating these cultures on BAP confirmed the absence of any group B streptococci. All group B streptococci identified by direct co-agglutination of SBM were isolated by replating on BAP. Thus, there was neither false-positive nor false-negative identification of group B streptococci by the co-agglutination test.

TABLE 1. *Distribution of group B streptococci from culture sites*

Specimens cultured		Specimens yielding group B. streptococci	
Site	No.	No.	% of total
Cervical-vaginal[a]	560	81	14.5
Umbilical cord[a]	594	11	1.9
Pharyngeal[a]	593	23	3.9
Other[b]	34	9	26.5

[a] Routine screening.
[b] Placenta, axilla, groin, and ear from infants with suspected sepsis.

TABLE 2. *Comparison of recovery rates on BAP and SBM*

Isolation Medium	Group B Streptococci Isolated	
	No.	% of total
SBM	122	98.4
BAP	66	53.2
SBM and BAP	64	51.6
SBM only	58	46.8
BAP only	2	1.6

TABLE 3. *Number of group B streptococci detected by two methods*

Specimen	No. of strains	No. giving direct grouping from SBM	No. isolated on BAP
Cervical-vaginal	81	81 (100)[a]	37 (45.7)
Umbilical cord	11	10 (90.9)[b]	10 (90.9)[c]
Pharyngeal	23	23 (100)	11 (47.8)
Other[d]	9	8 (88.9)[b]	8 (88.9)[c]

[a] Numbers in parentheses are percentages of the total.
[b] One specimen positive only on BAP.
[c] One specimen positive only in SBM.
[d] Placenta, axilla, groin, and ear.

TABLE 4. *Bacterial isolates other than group B streptococci from SBM*

Organism	No. of specimens
Staphylococcus	118
Alpha-hemolytic streptococcus	109
Nonhemolytic streptococcus	98
Enterococcus	28
Yeasts	23
Lactobacillus	5
Gram-negative bacilli	3
Total	384[a]

[a] Multiple organisms (384) growing from 372 specimens.

All group B streptococci isolated from the SBM were identified within 24 h from the time of inoculation. Identification of group B streptococci from BAP required a minimum of 48 h. Two strains of group B streptococci identified by the co-agglutination test were nonhemolytic.

DISCUSSION

Screening programs that identify maternal carriers and colonized infants will allow clinicians to identify infants with the greatest risk for group B streptococcal disease (8). Because of the early onset of the disease and the high mortality rate, a rapid and accurate method for detecting group B streptococcus is of primary importance. The use of SBM for isolation, combined with direct co-agglutination using the Phadebact[R] streptococcus test for serological grouping of isolates, identified 98.4% of the total positive cultures in less than 24 h. Only 1.6% of the positive cultures were missed by using SBM alone, as compared with 46.8% missed if only BAP were used.

Of interest is that 2% of the group B streptococci were found to be nonhemolytic. This is identical to the incidence reported by Romero & Wilkinson (11) and emphasizes the need to

identify immunologically and not morphologically all suspect streptococci.

The SBM is inhibitory for many organisms indigenous to the cervical-vaginal tract. Only 22.4% of the specimens which did not grow group B streptococci showed bacterial growth. The presence of group B streptococci in a mixed culture was always detected by the co-agglutination test.

Previous studies utilizing BAP have reported maternal colonization rates of less than 10% and newborn colonization rates of approximately 2% (2, 6). Laboratories utilizing selective media base have reported maternal and neonatal colonization rates of 25% (2). The current study is the first to simultaneously compare both techniques in the same population. Utilizing SBM, a maternal colonization rate of 14.5% was noted, compared with 6.6% with BAP. Neonatal colonization rates of 4.8 and 2.9% were found with SBM and BAP, respectively. The significance of different colonization rates by different culture techniques in relation to attack rates of disease remains to be studied. However, when the selectivity of isolation by the SBM is coupled with the speed of identification by the co-agglutination test, the potential clinical use of this technique in identifying high-risk infants is evident. Such a technique could facilitate a national surveillance study documenting and comparing the incidence of group B streptococcal colonization and disease.

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LITERATURE CITED

1. **Baker, C. J.** 1977. From the National Institutes of Health, summary of the workshop on perinatal infections due to group B streptococcus. J. Infect. Dis. **136**:137-152.
2. **Baker, C. J., and F. F. Barrett.** 1973. Transmission of group B streptococci among parturient women and their neonates. J. Pediatr. **83**:919-925.
3. **Baker, C. J., F. F. Barrett, R. C. Gordon, and M. D. Yow.** 1973. Supurative meningitis due to streptococci of Lancefield group B: a study in 33 infants. J. Pediatr. **82**:724-729.
4. **Baker, C. J., D. J. Clark, and F. F. Barrett.** 1973. Selective broth medium for isolation of group B streptococci. Appl. Microbiol. **26**:884-885.
5. **Christensen, P., G. Kahlmeter, S. Jonsson, and G. Kronvall.** 1973. New method for the serological grouping of streptococci with specific antibodies absorbed to protein A-containing staphylococci. Infect. Immun. **7**:881-885.
6. **Eidelman, A. I., and G. Szilagyi.** 1975. Group B beta strep (GBS) colonization of mothers and infants. Pediatr. Res. **9**:296.
7. **Feigin, R. D.** 1976. The perinatal group B streptococcal problem: more questions than answers. N. Engl. J. Med. **294**:106-107.
8. **Franciosi, R. A., J. D. Knostman, and R. A. Zimmerman.** 1973. Group B streptococcal neonatal and infant infections. J. Pediatr. **82**:707-718.
9. **Hahn, G., and J. Nyberg.** 1976. Identification of streptococcal groups A, B, C, and G by slide co-agglutination of antibody-sensitized protein A-containing staphylococci. J. Clin. Microbiol. **4**:99-101.
10. **Howard, J. B., and G. H. McCracken.** 1974. The spectrum of group B streptococcal infections in infancy. Am. J. Dis. Child. **128**:815-818.
11. **Romero, R., and H. W. Wilkinson.** 1974. Identification of group B streptococci by immunofluorescence staining. Appl. Microbiol. **28**:199-204.