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Comparison of Bacteriological Methods for the Isolation of Group of B *Streptococcus* from Vaginal Cultures

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Three bacteriological techniques for the isolation of group B streptococci in vaginal cultures were compared. A selective broth medium (SBM) containing gentamicin and nalidixic acid was more sensitive for the detection of vaginal isolates (28/76, 36.8%) from 76 women enrolled in a venereal disease clinic than was an identical selective plate medium (SPM) (17/76, 25%). Similarly, SBM allowed identification of positive cultures from college women (82/459, 17.9%) significantly more often than direct inoculation of swabs onto nonselective blood agar medium (43/460, 9.4%; $\chi^2 = 42.2$, $P = <0.001$). Failure to isolate group B streptococci detected in SBM occurred in 32.1% of cultures by SPM and 49.4% of cultures by nonselective agar medium. Multiple serotypes were detected in a single vaginal culture from approximately 5% of the patients studied. These data support the routine use of SBM for the most accurate identification of women vaginally colonized with group B *Streptococcus*.

Group B streptococci have become frequent isolates from the blood or cerebrospinal fluid cultures of neonates and young infants (1, 3, 4, 6; E. M. Ayoub, paper presented to the Streptococcal Club, Washington, D. C., 1975). Many of these seriously ill neonates acquire the group B *Streptococcus* intrapartum from the maternal genital tract (1). For this reason, several investigators have sought to determine the rate of group B streptococcal colonization among parturients and their neonates. Reported prevalence rates for vaginal colonization with group B streptococci have varied from 4.6 to 35.9% (3, 6), but a variety of bacteriological methods has been utilized for the isolation of these organisms. Since identification of the parturient with group B streptococcal vaginal colonization is of considerable epidemiological and clinical interest, inexpensive and accurate methods for detection are mandatory. The present study supplements an earlier report (2) comparing the sensitivity of selective broth medium (SBM) versus nonselective broth medium for the isolation of group B streptococci from vaginal cultures. The significantly greater sensitivity of an SBM was established when compared with two previously reported methods for isolation of the group B *Streptococcus* (Ayoub, paper presented to the Streptococcal Club, Washington, D. C., 1975).

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MATERIALS AND METHODS

Study population. Nonpregnant women at a large university health service (460) and women presenting to the venereal disease clinic at Boston City Hospital (210) were invited to participate in this study. Women who agreed to participate and who gave written informed consent had vaginal cultures taken during pelvic examination for the isolation of group B streptococci. Cultures were collected from each patient with sterile cotton-tipped swabs, which were inserted 2 cm into the vagina and rubbed against the vaginal wall.

Bacteriological techniques. The first swab culture from each of the 460 college women was inoculated immediately onto an agar plate containing 5% defibrinated sheep blood, cross-streaked with a wire loop, incubated overnight at 37°C, and examined for colonies of beta- or nonhemolytic streptococci. Seventy-six of the 210 women enrolled in a venereal disease clinic had two swab cultures obtained. The first swab was processed in a manner identical to that described above, but the agar plates contained gentamicin sulfate and nalidixic acid at a final concentration of 8 and 15 μg/ml, respectively. This technique for isolation will be referred to as the selective plate medium (SPM) method. The end of the second swab from both groups of patients was inoculated into a vial containing 5% defibrinated sheep blood in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) and gentamicin and nalidixic

acid at concentrations of 8 and 15 μg/ml, respectively. Additionally, the remaining 134 women in a venereal disease clinic had a single swab culture inoculated into SBM (3). After overnight incubation of this SBM at 37°C, approximately 0.2 ml was streaked onto a blood agar plate, incubated, and examined for colonies of beta- or nonhemolytic streptococci. A single colony identified on blood agar plates by any of the above methods was serogrouped by the capillary precipitin technique (5). Isolates designated group B were serotyped by methods described elsewhere (1). For the SPM, however, five single colonies from each culture plate were selected for serotyping as a means of determining the prevalence of vaginal group B streptococcal colonization with more than one strain serotype. This technique was also used for the SBM cultures inoculated onto blood agar plates for the 210 venereal disease clinic patients.

RESULTS

The rates of vaginal colonization with group B *Streptococcus* among the two groups of patients studied by the three methods of isolation are summarized in Table 1. Eighty-six (18.7%) of the 460 college women and 28 (36.8%) of the 76 women in a venereal disease clinic had group B streptococci isolated from vaginal cultures by any one of the methods used. The SBM detected all strains of group B streptococci isolated from the vaginal cultures of venereal disease clinic patients and 96.5% of those from college women. In contrast, the SPM failed to detect 9 of 28 women who had group B streptococci isolated from SBM, resulting in a false-negative rate of 32.1%. Direct plating of vaginal cultures on nonselective blood agar medium facilitated isolation of group B streptococci from 43 (9.4%) of the 460 college women but failed to detect an additional 39 who had these organisms isolated from SBM (49.4% false negatives). However, use of nonselective agar medium resulted in detection of positive vaginal cultures in three women who failed to have group B streptococci isolated from SBM.

The distribution of serotypes among these 113 strains of group B streptococci isolated from two patient groups was similar (Table 2). Type III strains predominated in both groups, accounting for 44% of isolates from venereal disease clinic patients and 38.4% of those from college women. Types II, Ic, Ia, and Ib, in descending order of incidence, were detected less frequently.

The incidence of more than one serotype of group B *Streptococcus* in a single vaginal culture from women in a venereal disease clinic was 5% (Table 3). SBM allowed identification of two different serotypes in 4 (5.1%) of 79 vaginal cultures containing group B streptococci, and in two of these cultures only a single colony of the five selected was a different serotype. Similarly, when the SPM method was used, one (5.3%) of 19 vaginal cultures that contained group B streptococci had more than one strain serotype detected.

TABLE 1. *Isolation of group B streptococci from vaginal cultures by three culture methods*

Patient group	No. studied	Group B streptococci isolated		Culture method	% False negative
		No.	%		
Nonpregnant college women	459	82	17.9	SBM	3.5
	460	43	9.4	BAP[a]	49.4
Women in a venereal disease clinic	76	28	36.8	SBM	0.0
		19	25.0	SPM	32.1

[a] BAP, Blood agar plate without antibiotics.

TABLE 2. *Frequency of group B streptococcal serotypes among isolates from vaginal cultures*

Serotype	Isolates from venereal disease clinic patients[a]		Isolates from college women	
	No. of strains	% of isolates	No. of strains	% of isolates
Ia	3	3.3	7	8.1
Ib	6	6.6	6	7.0
Ic	12	13.1	18	20.9
II	29	31.9	22	25.6
III	40	44.0	33	38.4
Nontypable	1	1.1		

[a] A total of 210 women had cultures obtained.

TABLE 3. *Occurrence of multiple serotypes of group B streptococci in vaginal cultures from women in a venereal disease clinic*

Total no. of study patients	No. of cultures with group B streptococci	Culture method	Isolation of more than one serotype of GBS[a]	
			No.	%
210	79	SBM	4	5.1
76[a]	19	SPM	1	5.3

[a] These women are included in the 210 who were studied with SBM. GBS, Group B *Streptococcus*.

DISCUSSION

Widely divergent prevalence rates for vaginal colonization with group B streptococci have been reported from several geographic areas (1, 3, 5, 6). Although there are several factors that could account for these variations, the present study suggests that the bacteriological method

selected for the detection of group B streptococci in vaginal cultures significantly influences the number of cultures that will be positive. In a previous study, duplicate vaginal cultures from 166 women were processed in SBM and nonselective broth medium (2). Thirty-three additional cultures were noted to contain group B streptococci with SBM, indicating a false-negative culture rate of 58.9% for the nonselective broth method. Using a nonselective pour plate method and a single vaginal culture, Franciosi et al. (3) reported a prevalence rate of 4.6% among pregnant women, whereas Aber et al. (R. C. Aber, N. Allen, J. T. Howell, H. W. Wilkinson, and R. R. Facklam, Pediatrics, in press) detected group B streptococci in 29% of cultures obtained on multiple occasions with the same method. Direct plating of vaginal swabs onto a nonselective blood agar medium resulted in failure to detect group B streptococci in 66.7% of vaginal cultures positive in SBM (E. O. Mason et al., unpublished data), a failure rate similar to that noted in the present study (49.4%). Addition of the identical antibiotics used in SBM to agar plates similarly resulted in failure to detect a significant number of cultures positive by SBM. Nine of 28 (32.1%) cultures yielding group B streptococci from SBM were negative on selective agar plates.

These data indicate that SBM is the single most sensitive bacteriological technique reported to date for the accurate assessment of vaginal colonization with the group B *Streptococcus*. The sensitivity of this method is probably related to two factors: (i) the ease with which these organisms, if present even in small number, may multiply in a broth medium, and (ii) the inhibition of other vaginal commensals (especially gram-negative enteric organisms) by the antibiotics used. Therefore, the importance of using a medium that is both liquid and "selective" is supported by the present study. SBM is only slightly more expensive to prepare than nonselective broth, can be easily prepared in a clinical microbiology laboratory, and is stable at 4°C for up to 4 weeks before use. Furthermore, this method appears to facilitate the detection of multiple serotypes in a single vaginal culture.

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