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Selective Broth Medium for Isolation of Group B Streptococci

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A selective medium containing Todd-Hewitt broth, sheep blood, nalidixic acid, and gentamicin sulfate was found to enhance significantly the isolation of group B streptococci from vaginal cultures. Preparation of the medium, which is stable for up to 4 weeks at 4 C, is simple and inexpensive. Use of such a medium should facilitate identification of vaginal colonization with group B streptococci.

Group B streptococci have emerged as frequent pathogens in serious neonatal infections (1-3). Almost all infants who become infected during the first few days of life acquire the organism from the maternal genital tract (1, 3, 4). Because "early-onset-type" neonatal group B streptococcal infections are often fatal (58 to 71% mortality [1, 3]), it has been suggested that routine cervico-vaginal cultures be performed during parturition and that group B streptococcal "carriers" receive antibiotic prophylaxis as a means of preventing neonatal infection (3, 6). Previous surveys of vaginal colonization with group B streptococci among parturients have used nonselective agar (4) or broth media (3). Since higher isolation rates of group A streptococci have been documented by the use of a selective agar medium containing nalidixic acid and neomycin (7), a preliminary study was performed to evaluate a selective broth medium in the isolation of group B streptococci from vaginal cultures as the first part of a comprehensive epidemiological investigation.

MATERIALS AND METHODS

Preliminary studies were performed to determine the effects of varying concentrations of nalidixic acid (Winthrop Laboratories) and gentamicin sulfate (Schering Corporation), alone and in combination, on the growth of group B streptococci. A single strain of *Streptococcus agalactiae* (group B streptococcus, D136C, type III) was innoculated into 10 ml of Todd-Hewitt broth containing 5% defibrinated sheep blood, and incubated for 18 h at 37 C. Colony counts were performed in pour plates containing Casman agar base with 5% sheep blood, and varying concentrations of the two antibiotics. Since no significant inhibition of growth of the group B streptococcal strains was detected (Table 1), a selective broth medium (SBM) was prepared by adding 0.5 ml of antibiotic solution containing nalidixic acid (15 μg/ml) and gentamicin sulfate (8 μg/ml) to a tube containing 4.75 ml of Todd-Hewitt broth and 0.25 ml of defibrinated sheep blood. The effect of SBM on the growth of several organisms which normally inhabit the female genital tract was then tested in the following manner. Appropriate dilutions of 18-h broth cultures of *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus viridans*, and *Streptococcus agalactiae* (D136C) were innoculated into SBM and incubated for 6 h at 37 C. A control experiment was done simultaneously by substituting Todd-Hewitt broth with 5% sheep blood for SBM. Colony counts were performed at 2, 4, and 6 h. SBM inhibited the growth of several gram-positive and gram-negative organisms, normally found in vaginal cultures, without influencing the growth of the two streptococcal strains (Table 2). These studies were repeated after storage of SBM for 2 weeks at 4 C, and similar results were noted. In addition, the above experiments were repeated by substituting strains representing the four other serotypes of group B streptococci (Ia, Ib, Ic, and II) with identical findings.

After these preliminary studies, 166 duplicate vagi-

TABLE 1. *Effect of different concentrations of nalidixic acid and gentamicin on the growth of group B streptococcus (strain D136C)*

Antibiotic concn (μg/ml)	Colony counts at 18 h
Control (TH broth)[a]	3.5×10^8
Gentamicin, 5	1.3×10^8
Gentamicin, 8	1.1×10^8
Gentamicin, 10	1.2×10^8
Nalidixic acid, 5	3.6×10^8
Nalidixic acid, 10	3.4×10^8
Nalidixic acid, 15	4.1×10^8
Gentamicin, 8; and nalidixic acid, 15	1.5×10^8

[a] Todd-Hewitt broth.

TABLE 2. *Effect of selective medium on growth of a variety of bacteria*

Incubation time (h)	Organism	Colony counts	
		Nonselective medium[a]	Selective broth medium (SBM)[b]
2	*Escherichia coli*	1.6×10^3	0
	Klebsiella pneumoniae	1.8×10^4	0
	Staphylococcus aureus	1.2×10^3	0
	Streptococcus viridans	1.1×10^3	1.2×10^3
	Streptococcus agalactiae	1.8×10^3	1.8×10^4
4	*E. coli*	7×10^7	0
	K. pneumoniae	1.4×10^8	0
	S. aureus	1.8×10^6	0
	S. viridans	5×10^6	7×10^3
	S. agalactiae	1.4×10^6	1.7×10^6
6	*E. coli*	1×10^8	0
	K. pneumoniae	1×10^8	0
	S. aureus	3×10^7	0
	S. viridans	5.6×10^5	2.1×10^5
	S. agalactiae	8×10^7	3×10^7

[a] Todd-Hewitt with 5% defibrinated sheep blood.
[b] Todd-Hewitt with 5% sheep blood, nalidixic acid (15 μg/ml), and gentamicin sulfate (8 μg/ml).

nal cultures were obtained from gravid females and nursery personnel. At the bedside, cotton swab cultures were innoculated into tubes containing Todd-Hewitt broth (nonselective medium) and SBM, transported to our laboratory, and incubated overnight at 37 C. The cultures were then streaked onto Casman agar medium with 5% sheep blood, and colonies of beta-hemolytic streptococci were serogrouped by the capillary precipitin method (5) (antisera were supplied through the courtesy of Rebecca Lancefield).

RESULTS

Fifty-six of the 166 vaginal cultures (33.8%) yielded group B streptococcal isolates on SBM, whereas only 23 (13.9%) were positive on the nonselective medium. Most of the difficulty in the isolation and identification of beta-hemolytic streptococci with the nonselective medium was related to overgrowth of gram-negative enteric organisms, especially *Proteus* species. As was expected from the results of the preliminary studies, staphylococci (present in 72% of the specimens) and gram-negative enteric organisms (present in 96% of the specimens) were completely inhibited by SBM.

DISCUSSION

The composition of the selective broth medium described was arbitrarily chosen to facilitate inhibition of organisms which normally inhabit the female genital tract, without influencing the growth of streptococci. No difficulty in isolation of beta-hemolytic streptococci (groups A through G) was found with the use of this medium. A broth rather than solid medium was selected to allow easy transport from bedside to the laboratory. This medium was inexpensive and easy to prepare, and found to be stable after 4 weeks of storage at 4 C.

The reported incidence of cervico-vaginal colonization with group B streptococci, using a single culture on nonselective medium, has been low (4.6% [3], 5.6% [4]). However, higher rates have been recorded (29%) with multiple cultures on nonselective media (H. W. Wilkinson, personal communication, 1973). Duplicate vaginal cultures from Houston women, on both selective and nonselective media, indicated that a significant number of false-negatives (58.9%) occur with the nonselective medium. The group B streptococcal vaginal colonization rate among Houston third trimester parturients was 22.4% with single cultures on SBM (C. J. Baker and F. F. Barrett, J. Pediatr., in press). In contrast, had nonselective medium been used to determine vaginal colonization in the same population, the incidence would have been 10.4%. The use of a selective medium is recommended to facilitate the accurate assessment of vaginal group B streptococcal colonization.

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