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Evaluation of Colistin and Nalidixic Acid in Todd-Hewitt Broth for Selective Isolation of Group B Streptococci

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Todd-Hewitt broth (THB) containing colistin and nalidixic acid was compared with four other media with respect to efficacy of isolation of group B streptococci. It was as effective as plain THB, THB with gentamicin and nalidixic acid and blood, and THB with colistin, nalidixic acid, and blood. THB with gentamicin and nalidixic acid, but without blood, was inhibitory to group B streptococci. The value of THB with colistin and nalidixic acid lies in its ability to successfully inhibit gram-negative organisms and still promote group B streptococcal growth without the addition of blood. This greatly reduces the time and expense of media preparation and permits early determination of bacterial growth, so that other means of rapidly identifying group B streptococci can be applied at the earliest possible time.

The high mortality and morbidity associated with group B streptococcal infection in newborns (2) have made the rapid and accurate identification of this organism a high-priority item in most clinical laboratories. A selective medium has been found desirable because of the need to recover group B streptococci (GBS) from areas such as the vagina and rectum, which are highly colonized with gram-negative and gram-positive organisms. Baker et al. developed a medium containing nalidixic acid and gentamicin (4) which was quite successful (3, 8) but required the addition of blood to assure good streptococcal growth. The presence of blood in the initial isolate not only increases the time and expense of preparing such a medium but also may preclude early visual assessment of bacterial growth. Although colistin and nalidixic acid have long been used as selective agents in an agar medium (5), their value in broth has not been studied. This study is an evaluation of the comparative efficacy of Todd-Hewitt broth (THB) containing colistin and nalidixic acid, with and without blood; Baker medium, with and without blood; and plain THB.

MATERIALS AND METHODS

A total of 2,024 mothers were routinely cultured either immediately prior to delivery or during their first prenatal clinic visit. All cultures were done in accordance with guidelines from the Human Subjects Committee of the University of Arizona Health Sciences Center. Samples were obtained from the vagina, periurethral area, and rectum by using sterile, cotton-tipped applicators packaged two per package. The two swabs were used as one to obtain the specimen. Both were then placed in one silica gel packet (Dri-Pax; Davison Chemical, Baltimore, Md.). Packets were collected once every 24 h.

Upon arrival in the laboratory, one swab from each pair was placed in THB containing colistin (Burroughs-Wellcome; 10 μg/ml) and nalidixic acid (Winthrop; 15 μg/ml). The other swab was placed in one of four other media for the purposes of comparison. A total of 254 mothers (12.5%) were ultimately positive for GBS at at least one culture site. From these 254 mothers, there were 480 positive culture sites; i.e., at least one swab of each pair grew GBS. Inasmuch as THB with colistin and nalidixic acid was always one of the two media being compared, there was a total of 480 comparisons in which this medium was matched against one of four other media. Thus, there was a total of 112 positive cultures in which THB with colistin and nalidixic acid was compared with THB alone; 139 comparisons were made with THB plus gentamicin (Schering) (8 μg/ml) and nalidixic acid (15 μg/ml); 121 comparisons were made with THB plus gentamicin and nalidixic acid (same concentration) with 5% defibrinated sheep blood; 108 comparisons were made with THB plus colistin and nalidixic acid with 5% defribrinated sheep blood.

All cultures were incubated in 3 ml of the various media at 37°C for 18 h. If the tubes were turbid after that time, they were plated on Trypticase soy agar with 5% defibrinated sheep blood and incubated anaerobically (GasPak, BBL) overnight. Tubes were checked for growth at 48 and 72 h. If there was no growth at 72 h, the specimen was discarded. Nonhemolytic and beta-hemolytic streptococci were isolated from the Trypticase soy agar plates. All isolates that

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were bile esculin negative and sodium hippurate positive were definitively identified using the Lancefield hot-HCl extraction method (7) followed by capillary precipitin techniques (9, 10).

RESULTS

The THB containing colistin and nalidixic acid was compared with the four other media with respect to (i) percent recovery of GBS among the positive cultures; (ii) length of time required for initial growth in the broth to recover the organism; and (iii) the purity of the initial isolate (Table 1). Chi-square analysis was performed for each parameter, comparing THB containing colistin and nalidixic acid with each of the other media. Colistin and nalidixic acid addition had no statistical advantage over plain THB but was significantly better than gentamicin and nalidixic acid without blood in terms of overall percentage of positive cultures and rapidity of recovery ($P < 0.001$). Ninety percent of positive cultures were identified with colistin and nalidixic acid, only 85% with gentamicin and nalidixic acid and sheep blood, and 94% with colistin, nalidixic acid, and sheep blood. The 9% higher rate of recovery of colistin, nalidixic acid, and sheep blood over gentamicin, nalidixic acid, and sheep blood was significant ($\chi^2 = 5.24$, $P < 0.025$).

Plain THB recovered significantly more organisms after 18 h of incubation than THB with colistin and nalidixic acid (98% versus 89%, $P < 0.01$); gentamicin and nalidixic acid alone only permitted a 43% recovery at 18 h of incubation, which was significantly lower than any other medium. Gentamicin and nalidixic acid with sheep blood had a significantly higher percentage of organisms recovered in pure culture than colistin and nalidixic acid ($P < 0.005$).

The most frequent (and almost the only) contaminants in those media containing antibiotics were alpha-hemolytic streptococci, whereas gram-negative organisms were the most frequent contaminants in plain THB.

There was no correlation between the serotype of GBS and recoverability in any of the media.

DISCUSSION

The data indicate that the addition of colistin and nalidixic acid to THB has no real advantage in terms of the ultimate outcome of the broth culture. This finding is supported by the fact that, although there is a large variation in the reported incidence of maternal colonization, very high colonization rates have been reported with both selective (3) and nonselective media (1). However, the nature of the contaminant in the nonselective media is usually gram-negative, and isolation may be much more difficult and time consuming in the laboratory because of the rapidity of spread of some gram-negatives. Gentamicin, in the final concentration of 8 μg/ml as recommended by Baker et al. (4), seems to be quite inhibitory, but the addition of 5% defibrinated sheep blood apparently overcomes this effect. Baker medium had no advantage over colistin and nalidixic acid without blood except for a 10% higher rate of pure cultures. The similarity of ultimate recovery and the lack of necessity of adding blood to the broth more than makes up for the slight difference in purity of recovery. Although the addition of blood to colistin and nalidixic acid provided a significant improvement over gentamicin, nalidixic acid, and blood, the advantage over colistin and nalidixic acid without blood was not significant.

In summary, the addition of colistin and nalidixic acid to THB creates an effective selective medium. It is as good as gentamicin, nalidixic acid, and blood in terms of ultimate recovery and length of time to isolation. The fact that

TABLE 1. *Comparison of five media*[a]

Medium	Total comparisons in which at least one medium yielded positive culture	Overall % positive cultures	% Positive cultures recovered at 18 h incubation	% Positive cultures recovered in pure culture
Colistin and nalidixic acid in THB	480	90	89	26
Plain THB	112	85	98* ($P < 0.01$)	28
Gentamicin and nalidixic acid in THB	139	54* ($P < 0.001$)	43* ($P < 0.001$)	31
Gentamicin and nalidixic acid with 5% sheep blood in THB	121	85	87	42* ($P < 0.005$)
Colistin and nalidixic acid with 5% sheep blood in THB	108	94	94	32

[a] Comparison of five media, using colistin and nalidixic acid in THB as the standard against which each of the other four media were matched. Asterisks mark those results that are significantly (chi-square) different from the colistin and nalidixic acid in THB medium.

blood is not necessary makes the early recognition of turbidity possible so that counterimmunoelectrophoresis or other methods of rapid identification can be applied at the earliest possible time. In addition, the time and cost of media preparation are greatly reduced. An ongoing evaluation of other selective agents for the isolation of GBS is required. For example, sulfamethoxazole and trimethoprim, which have been shown to be of value in agar, have not been evaluated in a broth (6). Although colistin and nalidixic acid in THB seems to be an excellent medium, none of the media produced better than a 94% recovery of GBS. This indicates that a great deal more work needs to be done with regard to isolation of this important pathogen.

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