Preliminary Evaluation of Hitchens–Pike–Todd–Hewitt Medium (HPTH) for Detection of Group B Streptococci in Pregnant Women

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We aimed to compare Hitchens–Pike–Todd–Hewitt (HPTH) enrichment broth, Todd–Hewitt broth, and direct culture onto blood sheep agar containing selective antimicrobial (ABA) in screening Group B Streptococci (GBS) carriage in pregnant women. From April to June 2007, duplicate lower vaginal and duplicate anorectal specimens of 102 pregnant women at 35 weeks of gestation or more, who were assisted at Santa Casa de Misericórdia Hospital, Maringá, Paraná, Brazil, were screened by GBS using HPTH, Todd–Hewitt broth, and ABA. Twenty-five

(24.5%) pregnant women had one or more positive culture for GBS in those media. The positive rate for each medium was 21.6% (22/102) in the HPTH, 12.8% (13/102) in ABA, and 11.8% (12/102) in Todd–Hewitt broth. HPTH seems to be a high satisfactory medium for screening GBS in vaginal and anorectal samples in pregnant women and additional studies would be interesting in a larger number of pregnant women and in different laboratories. J. Clin. Lab. Anal. 24:403–406, 2010. © 2010 Wiley-Liss, Inc.

Key words: group B streptococci; neonatal infection; culture; enrichment broth; pregnant women

INTRODUCTION

Streptococcus agalactiae (Group B Streptococci, GBS) is a commensal bacterium that colonizes the vagina and the rectum and has major implications for pregnant women in premature birth, urine infection, chorioamnionitis, postpartum endometritis, and others (1). GBS maternal colonization rates range approximately from 10 to 40% and it is the most common cause of the early-onset neonatal sepsis in developed countries (2,3).

The screening for GBS carriage in the vagina and rectum in pregnant women between 35 and 37 weeks of gestation is recommended by Centers for Disease Control and Prevention, and administration of intrapartum chemoprophylaxis in colonized women is the most effective and recommended screening to prevent early neonatal infection by GBS (4).

For culturing GBS from vaginal and anorectal swab samples, it is recommended to use a selective enrichment medium, such as Todd–Hewitt broth supplemented with

antibiotics followed by subculture on sheep blood agar (5). In order to increase the sensitivity and decrease the time to detect GBS carriage in pregnant women, special media have been proposed to be used. One that seems to be faster and more effective for this purpose is Granada medium that shows visible orange colonies of β -hemolytic strains usually within 24 hr. However, variable results on the sensitivity of this medium have been reported (6–8). A modification of Granada medium, the Group B Streptococci differential agar (Becton Dickinson, Sparks, MD), showed to be a satisfactory medium for the rapid detection of most

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GBS in vaginal specimens, but it failed in detecting non-hemolytic and non-pigmented strains (5).

Hitchens–Pike–Todd–Hewitt (HPTH, mod. Solé–Vernin) is a selective beta-hemolytic streptococcal semi solid medium mainly recommended for *Streptococcus pyogenes* detection in pharynx (9) and it has been used in our laboratory for a long time for this purpose. Considering our good experience in recovery β -hemolytic streptococcus in pharynx using HPTH as enrichment medium, our aim was to determine the usefulness of HPTH in screening GBS carriage in pregnant women, compared with the Todd–Hewitt broth and direct plating onto sheep blood agar containing selective antimicrobial (ABA).

MATERIALS AND METHODS

Clinical Specimens

From April to June 2007, a GBS epidemiological study was conducted in pregnant women assisted at Santa Casa Hospital, Maringá, Paraná, South of Brazil (10). Together with this study, we designed a protocol to determine the performance of HPTH in detecting GBS carriage in pregnant women. Lower vagina and anorectal specimens of 102 pregnant women at 35 weeks of gestation or more were collected using duplicate swabs for each clinical specimens (swab 1 and 2 from lower vagina and Swab 3 and 4 from anorectum).

The study was approved by the Ethic Committee of the State University of Maringá, (Protocol n. 013/05). All participants approved the research protocol and signed the informed consent.

Culture

Vaginal (swab 1) and anorectal (swab 3) specimens were firstly inoculated on ABA (Antibiotic Sheep Blood Agar, gentamicin 8 µg/ml and nalidixic acid 15 µg/ml; Inlab, SP, Brazil) and after in the HPTH semi solid medium (9). HPTH formulation was composed by: 1,000 ml of beef heart infusion (BBL, Sparks, MD), 10 g Neopeptone (BBL), 10 g Proteose–Peptone (BBL), 4 g Yeast Extract powder (Himedia, Mumbai, India). The pH was adjusted to 7.0 using 1 N NaOH solution and 2 g NaHCO₃ (VETEC, RJ, Brazil), 2 g D-(+)-Glucose (Sigma, St Louis, MO), 2 g NaCl (F. Maya, Brazil), 0.75 g Na₂HPO₄·7H₂O (Nuclear, SP, Brazil) were added. The medium was boiled for 15 min and filtered. Then 12.5 ml of crystal violet (Sigma, St Louis, MO) aqueous solution (1:25,000), 40 ml of Sodium Azide (Sigma) aqueous solution (1:200) and 1 g agar–agar (Merck, Darmstadt, Germany) were added. The pH was adjusted to 7.6–7.8 using 1 N NaOH solution

and HPTH medium placed in screw caps tubes and sterilized.

Todd–Hewitt broth (Becton Dickinson) added of gentamicin 8 µg/ml and nalidixic acid 15 µg/ml according to the manufacturer's instruction was inoculated with vaginal (swab 2) and anorectal (swab 4) specimens. All inoculated media were immediately sent to the Clinical Bacteriology Laboratory/Laboratório de Ensino e Pesquisa em Análises Clínicas (State University of Maringá) in a thermal box. In the laboratory, three sheep blood drops were aseptically added to the HPTH. Todd–Hewitt broth and HPTH were incubated at 35°C for 18–24 hr and subcultured on sheep blood agar (SBA). ABA and the two subcultures on SBA were incubated at 35°C, in ambient air, for 24–48 hr and then inspected for the presence of suggestive GBS colonies.

Identification

Suggestive GBS colonies were identified by latex agglutination using Streptococcal Grouping Kit (Oxoid, Hampshire, UK), according to the manufacturer's instructions after presumptive identification by biochemical tests.

Statistical Analysis

The data were analyzed by χ^2 test with $P < 0.05$ as significant value using BioEstat 5.0 software (Belém, Pará, Brazil – 2007).

RESULTS

A total of 25 (24.5%) pregnant women showed at least one positive culture for GBS in one of the areas screened and the positive rate for each medium was 21.6% (22/102) in the HPTH, 12.8% (13/102) in ABA, and 11.8% (12/102) in Todd–Hewitt broth (Table 1).

Of the 22 GBS-positive pregnant women in HPTH, 4.9% (5/102) were positive exclusively in vaginal, 1.9% (2/102) exclusively in anorectal, and 14.7% (15/102) in

TABLE 1. GBS Carriage Recovered Rates Only in Vaginal, Anorectal and in Both Specimens from 102 Pregnant Women by Hitchens–Pike–Todd–Hewitt (HPTH), Sheep Blood Agar Containing Selective Antimicrobial (ABA) and Todd–Hewitt Broth

Media	No. (%) of GBS-positive specimens in pregnant women			
	Vaginal	Anorectal	Vagina+Anorectal	Total
HPTH	5 (4.9)	2 (1.9)	15 (14.7)	22 (21.6)
ABA	1 (1.0)	5 (4.9)	7 (6.9)	13 (12.8)
Todd–Hewitt	9 (8.8)	–	3 (2.9)	12 (11.8)

HPTH, Hitchens–Pike–Todd–Hewitt; ABA, sheep blood agar containing selective antimicrobial; GBS, group B Streptococci.

TABLE 2. Sensibility of Hitchens–Pike–Todd–Hewitt (HPTH), Sheep Blood Agar Containing Selective Antimicrobial (ABA) and Todd–Hewitt Broth in Detecting Group B Streptococcus in 25 Pregnant Women

Media	Positive culture— <i>n</i> (%)		GBS carriage detection
	Vaginal	Anorectal	
HPTH (<i>n</i> = 38)	20 (80) (61.10–92.28)	18 (72) (52.30–86.85)	22 (88) (70.72–96.86)
ABA (<i>n</i> = 20)	08 (32) (16.1–51.86)	12 (48) (29.19–67.25)	13 (52) (32.75–70.81)
Todd–Hewitt (<i>n</i> = 15)	12 (48) (29.19–67.25)	3 (12) (3.15–29.28)	12 (48) (29.19–67.25)

HPTH, Hitchens–Pike–Todd–Hewitt; ABA, sheep blood agar containing selective antimicrobial; GBS, Group B Streptococcus. *n*, number of positive culture; 95% confidence interval.

both specimens. ABA showed GBS-positive cultures in 1.0% (1/102) exclusively in vaginal, 4.9% (5/102) exclusively in anorectal, and 6.9 (7/102) in both specimens. The subculture from Todd–Hewitt broth showed GBS detection in 8.8% (9/102) in vaginal specimens exclusively and 2.9 (2/102) in both specimens (Table 1).

The sensitivity of GBS detection in HPTH, ABA, and Todd–Hewitt broth were 80, 32, and 48% in vaginal specimens, respectively. For anorectal specimens, the sensitivity was 72, 48, and 12% in HPTH, ABA, and Todd–Hewitt broth, respectively (Table 2).

Comparing HPTH with ABA and Todd–Hewitt broth, separately for GBS detection, HPTH was more sensitive than the Todd–Hewitt broth ($P = 0.0411$) but not more than ABA ($P = 0.066$).

DISCUSSION

In the last years, some studies refer to the GBS as the main worldwide cause of sepsis and meningitis in newborn babies. The GBS colonization rate of the maternal vagina and/or rectum ranges from 10 to 40% in developed countries (2,3,6–8). In Brazil, the GBS has not been properly valued in the etiology of infectious diseases that affect the newborn and the mothers, despite the severity of infection and the fact it is preventable by prophylactic measure (11).

Using the criteria that it is relevant to look for GBS carriage in vaginal and anorectal areas of women at 35–37 weeks of pregnancy (4), two enrichment broths and a direct plating agar were applied for this purpose in a preliminary study working with 102 pregnant women. Despite the prevalence of GBS carriers varies in relation to the geographical area, some geographical factors and methods for the detection of the GBS, we could reach the result that 24 (5%) of the pregnant women were GBS carrier in vagina and/or anorectum. Our results

corroborate with the percentages found in studies with patients from different geographical areas (3,12).

In our study, we added ABA direct plating method in combination with two selective broths (Todd–Hewitt and HPTH) to detect GBS carriers pregnant women based on the experience of some previous studies (13,14). According to those studies, the clinical sample direct plating particularly increases GBS detection mainly in heavy GBS colonization or by reducing the overgrowth of enterococci (14). Our results showed no significant sensitivity increase in the GBS detection by the use of direct the plating method (ABA) when compared with the subcultures from Todd–Hewitt broth as observed by Guerrero et al. (15). In our experience, no statistical sensitivity increase in the GBS detection by the use of ABA was observed when compared with the subcultures from HPTH medium too. However, considering the time to provide a fast result in cases of higher risk for early-onset disease in pregnant woman with heavy GBS colonization, the use of directing plating yielded identification of GBS, 24 hr sooner when compared with HPTH and Todd–Hewitt broth culture that require subculture onto a blood agar plate in each one.

The sensitivity of the standard method using selective Todd–Hewitt broth in the detection of the vaginal and anorectal GBS carriage was 48 and 12%, respectively. The corresponding values for the HPTH were higher showing values as 80 and 72%, respectively. In our opinion, one explanation for this discrepancy in our results may be due to the resistance of saprophytic flora, especially *Enterococcus faecalis*, to gentamicin and nalidixic acid used in the composition of Todd–Hewitt broth and ABA medium. In this particular case, the recovery of the organism could have been compromised by the competing saprophytic flora. On the other hand, the higher GBS detection in HPTH observed in our study may be because of saprophytic flora sensitivity to the crystal violet and sodium azide added to the selective medium allowing more GBS detection.

In our preliminary study, HPTH medium showed to be statistically more sensitive than Todd–Hewitt broth and ABA direct plating for the GBS detection in vaginal and anorectal specimens in pregnant women. However, it cannot be recommended as the sole medium for screening GBS in pregnant women as it missed up 3 (12%) of the GBS carriage pregnant women enrolled in our study. Two (8%) of these pregnant women were detected only in SBA subculture from Todd–Hewitt broth in vaginal specimen and one (4%) in ABA direct plating in anorectal specimen. It would be interesting to use HPTH in further studies to detect GBS carriers in a larger number of pregnant women and in different laboratories.

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