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A Comparison of Two Enrichment Broth Medium for the Isolation and Identification of *Streptococcus agalactiae* from Vaginal Swabs

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Abstract

Aim: To evaluate the performance of brain heart infusion (BHI) versus Todd-Hewitt (TH) media for the culture and identification of Group B *Streptococcus* (GBS) in vaginal swabs from pregnant women in last trimester. Two enrichment broth media were compared in terms of sensitivity, accuracy and cost.

Methodology: 242 vaginal samples collected during March and May 2018 from Tam Anh hospital were included in this study. Each sample was collected in duplicated swabs, each swab was then cultured in BHI and TH broth and following the same method in accordance with the manufacturers' guidelines.

Results: BHI had excellent diagnostic performance compared to TH, with sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 91.30%, 100%, 100%, 98.00% and 98.35%, respectively. BHI decreased material and supply costs 88.31%.

Conclusion: BHI was chosen for introduction into routine use, due to its better sensitivity and accuracy, meanwhile lower cost than TH.

Keywords: GBS; Group B *Streptococcus*; Brain heart infusion; BHI; Todd-Hewitt; TH; Perinatal screening

Introduction

Streptococcus agalactiae (Group B Streptococcus [GBS]) is a leading cause of invasive disease in neonates such as septicemia, meningitis and pneumonia, with resulting high morbidity and mortality rates [1]. Neonates are infected by perinatal transmission and can become ill within hours of becoming infected, or take up to 5 days before showing symptoms [2]. Screening GBS in pregnant women in last trimester is therefore the most effective method in GBS prevention and treatment [1]. However, there are some common problems with the laboratory detection method of GBS such as low colony forming units of GBS in some samples, overgrowth of normal vaginal flora [3]. To detect low numbers of organisms, swabs should be pre-enriched in either Todd-Hewitt (TH) broth or brain heart infusion (BHI) base with added antibiotics to inhibit the growth of bacteria, followed by subculture on blood agar or a selective medium [4]. Commercially available Todd-Hewitt Broth supplemented with antibiotics and BioMérieux ChromID was recommended due to its excellent sensitivity and easier differentiation of false positive growth [3]. However, TH broth results in a increasing cost of GBS culture. In terms of storage condition TH media requires refrigerated transport and storage whilst BHI broth powder can be stored easily at room temperature. Due to the need of screening cultures on vaginal swabs in the context of low- and middle-income countries, it is of interest to further improve the diagnostic performance and efficiency of this method. The aim of this study is therefore to evaluate the performance of two enrichment broth for the detection of GBS in vaginal swabs from pregnant women. The two-broth media were compared and evaluated in terms of sensitivity, accuracy, and cost.

Materials and Methods

242 vaginal samples, collected from 242 pregnant women during March and May 2018, were included in the study. Near-term pregnant women (35 to 37 weeks of gestation) were enrolled with consent that received routine obstetrical care at a single large maternity clinic in the Tam Anh hospital (Clinic number 08, Obstetrics and Gynecological department, Tam Anh hospital, 108 Hoang Nhu Tiep street, Long Bien district, Hanoi, Vietnam). Exclusion criteria were concurrent antibiotic use, an acute illness, a symptomatic vaginal discharge, and a known or suspected condition in which clinical vaginal examinations were contraindicated.

Vaginal samples in duplicate swabs were collected using Copan swabs and immediately inoculated into BHI (prepared in our laboratory as described behind) and Todd Hewitt Broth with antibiotic supplement (BioMérieux SA, 69280 Marcy l'Etoile-France) and transported within 1 h to the laboratory. Cultures were incubated for 18 to 24 h at 36°C. BHI and TH were then subcultured to a chromID® Strepto B (BioMérieux SA, 69280 Marcy l'Etoile-France) with a 10 µL loop, incubated for another 18 to 24 h at 36°C. GBS colonies growing on chromID® Strepto B were identified by the presence of typical colony morphology and biochemical reactions, i.e., red colonies based on production of phosphatase, gram positive coccus on gram strain, catalase negative and the group B carbohydrate latex (Liofilchem s.r.l; Via Scozia-Zona industriale, 64026, Roseto degli Abruzzi (Te), Italia) typing is positive (Figures 1 and 2). All TH and BHI cultures were read as positive if red from colorless occurred on chromID® Strepto B and positive with Strepto B latex kit.

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	BHI		TH		BHI or/and TH		Both method	
	n	(%)	n	(%)	n	(%)	n	(%)
GBS - Positive result	42	17.36	41	16.94	46	19.01	37	15.29
GBS - Negative result	200	82.64	201	83.06	196	80.99	205	84.71

Table 1: Detection of Streptococcus agalactiae (GBS) from 242 vaginal swabs from pregnant women by methods.

	BHI method		TH method	
		(%)		(%)
Sensitivity[a]	42/(42+4)	91.30	41/(41+5)	89.13
Specificity[a]	196/(196+0)	100.00	196/(196+0)	100.00
Positive Predictive Value[a]	42/(42+0)	100.00	41/(41+0)	100.00
Negative Predictive Value[a]	196/(196+4)	98.00	196/(196+5)	97.51
Accuracy[b]	(42+196)/ (42+0+4+196)	98.35	(41+196)/ (41+0+5+196)	97.93

[a] Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value were calculated in comparison to composite scores of all methods.
[b] Accuracy was calculated as the number of correctly classified samples/total number of samples tested.

Table 2: Performance characteristics (sensitivity, specificity, positive predictive value, negative predictive value, and accuracy) of BHI and TH estimated from the 242 vaginal swab tested.

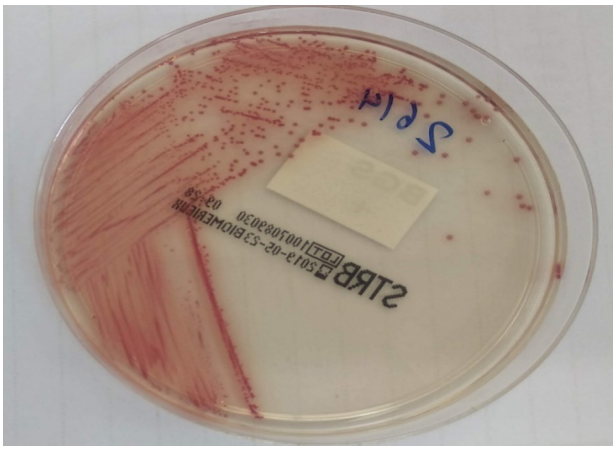

Figure 1: bioMérieux ChromID, with GBS on as pink pigmented colonies.

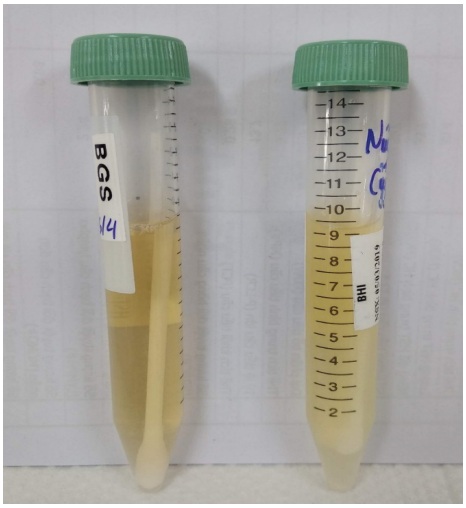

Figure 2: BHI broth with turbidity (right) and without turbidity (left).

Discrepant results between TH and BHI methods may arise due to many factors, given our laboratory's context without "gold standard" nor reference laboratory to provide results that could be useful in resolving the discrepancy. In case of discrepant result occurred, inoculated BHI and TH were repeatedly subcultured to a chromID® Strepto B, the patient's clinical diagnosis and other clinical information (exclusion criteria) for the specimens are reviewed. If there is a predominant clinical condition among the specimens with discrepant results and repeated subculture are not same with first subculture, these sample were also be excluded from study. In this study, there were 3 samples excluded due to ongoing bacterial vaginitis (two samples) and results of first and second subculture without being identical (one sample).

Resource utilization costs from the study period were extrapolated to estimate the projected costs of routine utilization of either mediums in single test. Monthly and annual cost of either mediums were also estimated from single test cost multiplied by number of tests per month and year. Supply and media costs were calculated in Vietnam dong and services tax.

Data were entered and analyzed by MS Excel 2013 Spreadsheet (Microsoft Corporation, Seattle, WA). Descriptive statistics included frequency with percentages, means ± standard deviations (SDs). As recommended by CDC, universal screening for GBS for all pregnant women at 35-37 weeks' gestation, vaginal and rectal GBS culture test should have a high sensitivity to ensure that true-positive results are detected and lower specificity can be tolerated [1]. The isolation of GBS from any of the media used methods (TH or BHI) was a positive sample, and failure to detect GBS on any of the methods was a false negative.

BHI antibiotics broth is prepared as follow 17.25 g of Brain Heart Infusion Broth powder (Mast Group Ltd., Mast House, Derby Road, Bootle, Merseyside, L20 1EA, United Kingdom) in 500 mL of deionized water, mix well with 5 ml reconstitute the content one vial of CNA (Staph/Strep) *Supplement* (Liofilchem s.r.l; Via Scozia-Zona industriale, 64026, Roseto degli Abruzzi (Te), Italia). After complete dissolution, BHI antibiotics broth is dispensed to into tube with volume of 9 ml. Tube is autoclave at 121°C (15 psi) for 15 minutes.

Results

The mean age (± SD) of the women at swabbing was 29.98 years (± 4.647 years). After testing all samples, there were 46 positive GBS samples (they were recovered from either BHI or/and TH broth) (Figure 3). Among that, 37 samples were positive with GBS by both methods, TH and BHI, 5 samples were positive with GBS by BHI methods but negative by TH, and 4 samples were negative with GBS by BHI but positive by TH (Table 1). The prevalence of GBS in our study population during the study period was found to be 19.01% (46/242) among pregnant women at 35-37 weeks of gestation.

Overall, 233 samples did obtained agreement in result by both methods (negative or positive by both methods) and 9 samples didn't obtained agreement in result by both methods (Discrepant Results). These discrepant result samples were repeatedly subcultured from TH and BHI to a chromID® Strepto B, and the discrepant result were not resolved. The sensitivities, specificity, positive predictive value, negative predictive value, and accuracy of BHI and TH shown in Table 2 demonstrated that BHI had excellent diagnostic performance compared to TH.

Table 3 lists the estimated component costs (media and supply)

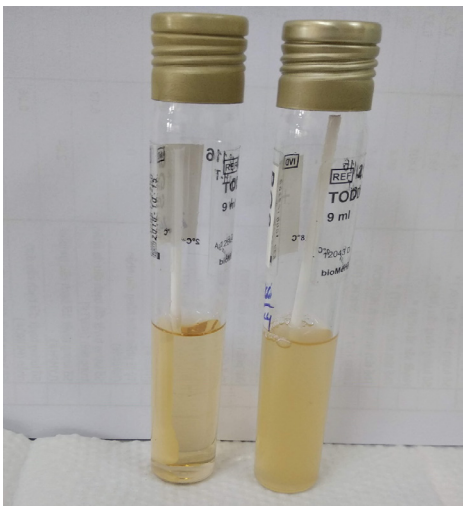

Figure 3: TH broth with turbidity (right) and without turbidity (left).

Component of analysis	Cost[a] for		Effect on cost of change to BHI[b]
	BHI	TH	
Cost per test	4.123 VNĐ	35.255 VNĐ	31.132 VNĐ
Total monthly cost (equivalent to 80 tests)	329.840 VNĐ	2.820.400 VNĐ	2.490.560 VNĐ
Total annual cost (equivalent to 960 tests)	3.958.080 VNĐ	33.844.800 VNĐ	29.886720 VNĐ
[a] Total material costs included the cost of media and supply [b] Cost decrease			

Table 3: Comparison of costs for using BHI versus TH for GBS detection.

of performing GBS vaginal cultures using either type of broth as our routine method during a typical month. Routine utilization of BHI would decrease monthly material costs approximately 88.31% since TH is more expensive than BHI.

Discussion

The prevalence of GBS in our study population during the study period was found to be 19.01% among pregnant women at 35-37 weeks of gestation. The prevalence of maternal GBS colonization in our study was similar to that reported in the literature previously as 11.4-26.5% in both developed and developing countries [5]. The prevalence of GBS colonization may vary due to differences in the specimen collection and culture technique, and the role for ethnic or genetic factors. Swabbing both the lower vagina and rectum increases the culture yield substantially compared with sampling the cervix or the vagina without also swabbing the rectum [1]. Different authors report different results related to the use of selective enrichment broths followed by subculture on commercially chromogenic agars compared to direct culture on plate agar [3,6]. A recent review estimated that prevalence of group B streptococcus colonisation was highest in Africa (22.4%) followed by the Americas (19.7%) and Europe (19.0%) [7]. Our study is the first clinical evaluation of the routine detection of GBS from Vietnamese women in a hospital-based laboratory setting. We found only one other study in English literature have evaluated detection of GBS among nearterm pregnant women and the prevalence of group B streptococcal infections was 4% [8]. The specific culture method for detecting GBS was not described in detail. Therefore, the increase in the rate of GBS detection in our study could be attributed to the use of different culture protocols.

Our study shows that BHI yields better results compared to TH in terms of sensitivity and accuracy. Both mediums were supplemented with antibiotic including colistin (10 µg/ml) and nalidixic acid (15 µg/ml). TH medium originally was described by Todd and Hewitt for the production of antigenic streptococcal hemolysis due to fermentation of glucose included as a growth stimulant, would lead to the destruction of hemolysis by the acid produced [9]. Brain Heart Infusion Broth is essentially a buffered infusion broth, originally employed for the cultivation of streptococci, and other aerobes and anaerobes pathogens [10]. In our laboratory context, BHI are broadly used for culturing a variety of samples such as blood, pus as cultivation and buffer in bacterial preservation.

Our study has several limitations. Firstly, the samples were collected from several patients admitted to Tam Anh hospital, Hanoi, therefore the rate of detection GBS may not represent for the whole Vietnamese population. Secondly, we did not include ATCC or reference GBS strains in this study due to the lack of this commercial strain in Vietnam. The analysis was therefore based on the strains collected from the patients only. Finally, the information of underlying disease condition as well as the number of childbirths for each patient were not included in the analysis.

Conclusions

BHI selective broth enrichment showed higher sensitivity and accuracy for the detection of GBS from vaginal swabs. Moreover, BHI has the advantages of lower cost in preparing comparing with TH. For these reasons, it is recommended to use BHI instead of TH for routine works and research studies as well.

Author Statements

Dat Tran Huu performed the laboratory work. Dinh Thi Hien Le was responsible for specimen collection. Dat Tran Huu and Nguyen Thi Ha participated in study design, data analysis and interpretation. Dat Tran Huu and Hoa Le Nguyen Minh participated in writing, review and editing manuscript. All authors read and approved the final manuscript.

Funding Information

All media and supply were purchased as usual materials of the laboratory routine.

Ethical Approval

Any experimental work in this study was approved by Ethical Committee of the Tam Anh hospital. Informed consent from each client was received prior to sample collection.

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