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ORIGINAL ARTICLE

The Performance of Strep B Carrot Broth™ Versus Latex Agglutination Test for Rapid Detection of Group B *Streptococcus* Colonization Status and its Prevalence in Near- Term Pregnant Women

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ABSTRACT

Background*: Group B Streptococcus (GBS) has been described as one of the commonest causes of the early onset of sepsis among the newborns, which leads to high rate of mortality and morbidity. Centers for Diseases Control (CDC) (2o16) recommended GBS screening for all pregnant women between 35 and 37 weeks of pregnancy,* ***Objectives:*** *This work aims for evaluation of Strep B carrot broth (SCB) versus Latex agglutination technique (LA) as a screening method for early detection of Group B Streptococci colonizing the genital tract of pregnant females* ***Methodology****: the present study was conducted on 1oo pregnant women attending the antenatal care clinic of Maternity Hospitals, Faculty of Medicine, Ain shams University. Duplicate vaginal swabs were taken, one for detection of GBS by Strep B Carrot Broth (SBC) and the second swab for detection of GBS by latex agglutination test.* ***Results****: The prevalence of GBS was 33% by SCB. SCB had excellent performance compared to LA test, with a sensitivity of 9o.9%, specificity of 1oo%, and negative predictive value (NPV) and positive predictive value (PPV) of 95.7% and 1oo%, respectively.* ***Conclusion:*** *We can use SCB for rapid and reliable GBS screening in pregnancy as it has less false positive results in comparison to other conventional methods. Antepartum GBS screening is highly recommended to reduce the emerging antibiotic resistance among GBS strains*

INTRODUCTION

Group B *Streptococcus* (GBS) has been described as one of the common causes of the early onset of sepsis among the newborns, which leads to high rate of mortality and morbidity [1]. The primary risk factor for neonatal GBS infection is the maternal colonization with the organism. This is also the basis of preventive strategies in western countries [2]. Most women infected by GBS are asymptomatic, and the organism can be isolated from their throat, vagina and rectum[3]. Furthermore, according to a report by World Health Organization (WHO), the prevalence of GBS colonization in pregnant women is about from 5-40% in different countries. Among infected women, 50% showed GBS colonization in their vagina, while the rest revealed infection in their rectum and throat[4]. GBS colonization of the maternal genital tract is considered to be one of the causes of early onset neonatal sepsis, either due to vertical transmission or during labor [5]. The rate of vertical transmission of GBS between mothers and their fetuses is about 29-85%. The transmission depends to some extent on factors including the severity of maternal colonization in birth canal. Moreover, in the presence of other predisposing factors like maternal fever, prematurity, premature rupture of membranes (PRoM) more than 18 hours, low birth weight and multi-parity, the infection rate increases [3]. In the USA, the two major prevention strategies for GBS disease include the screening method and the risk-based approach. Pregnant women carrying GBS are offered to take intrapartum antibiotic prophylaxis[6]. Furthermore, the Centers for Diseases Control (CDC) recommended GBS screening for all pregnant women between 35 and 37 weeks of pregnancy, as well as taking intrapartum antibiotic prophylaxis[7], so pregnant women with unknown GBS status should be treated with antibiotic at the time of delivery [3]. However, in developing countries the problem has not been adequately studied and there are only a few studies available.

The aim of this study was to evaluate Strep B carrot broth versus Latex agglutination technique as a screening method for early detection of Group B *Streptococci* colonizing the genital tract of pregnant females.

METHODOLOGY

Patients:

The present study was conducted on 1oo pregnant women their age range from 16 years to 40 years (mean 27.8 years) above 35 weeks of gestation, attending the

antenatal care clinic of Maternity Hospitals, Faculty of Medicine, Ain shams University from December 2017 till March 2018. An informed oral consent was taken from all participants after discussing the study's aim and benefits. A full history was taken including name, age, obstetric history; as number of pregnancies and normal deliveries, history of previous preterm labor, abortions and peri-natal mortality and medical history as DM and Hypertension.

Methods:

I- Processing of samples:

Duplicate vaginal swabs were collected from each pregnant female (at 35-37 weeks of gestation) using Bio Cult Amies + charcoal sterile swabs by introducing the swab 1-2 inches beyond the vaginal vestibule and rotating it against the vaginal wall and transported within 4 to 6 hours to the laboratory of Medical Microbiology and Immunology Department of Faculty of Medicine of Ain Shams University.

One vaginal swab was cultured on 4 ml Strep B Carrot Broth™ (SCB) (Hardy Diagnostics, Santa Maria, CA) and the other swab was immersed in 4 ml Todd Hewitt broth (THB) (liofelchim, Italy) for cultivation and isolation of GBS. If there was any delay in culturing, swabs were placed into 5 ml of Amies transport medium with charcoal. All SCB cultures were incubated at 35°C, and at 37°C for THB samples for 18 to 24 hours.

II- Identification of GBS colonies:

After incubating the inoculated broth at 35˚C for 24 hours for SCB, and at 37˚C for THB, they were examined for GBS growth. Cultures growing in SCB were positive if a visible change from colourless to red/Orange was observed (Figure 1)

Fig. 1: Positive results of strep B carrot broth: The right tube shows GBS growth (orange colonies), the left tube shows negative result for GBS

- ***Culture on blood agar :***

All SCB cultures were then sub-cultured onto 5% blood agar for confirmation of GBS presence, incubated for another 18 to 24 h at 35°C, and examined for the presence of GBS, GBS colonies growing on 5% blood agar were identified by Colonial morphology, Gram staining, catalase test, CAMPS test according to *Cheesbrough M. (2009)* and latex agglutination test with specific antisera.

Colonies were small about 1 mm in diameter, soft, convex, moist, regular, usually grey white or colourless, usually shiny or dry colonies may be surrounded with narrow hazy zone of beta-hemolysis or no hemolysis.

Furthermore, Bacitracin test was done to differentiate between group A and B *streptococci*.

- ***Gram stain and catalase test:***

Typical colonies were Gram positive cocci arranged in chains. Catalase test was done for all colonies grown on blood agar. Colonies of GBS are catalase negative

Latex agglutination test for GBS using Strepto B Latex Kit (Liofilchem-Italy).

The extraction enzyme was reconstituted with 1o ml of Distilled water and gently mixed to ensure complete reconstitution. 200 µl of extraction enzyme was dispensed into a test tube. 15 µl of THB suspension was added and emulsified gently. The tube was incubated for 10-15 minutes at 37°C. The tube was shaken vigorously after the first 5 minutes of incubation prior to testing to obtain even suspension of antigen. 15 µl of latex reagent was dispensed separately into one of three circles on a reaction card. one drop of well mixed extract was transferred next to the drop of latex reagent. The content mixed using mixing stick to cover the area of the circle, the reaction card rocked and rotated to mix the reagents for a maximum of one minute. The card was observed for agglutination. Fig 2

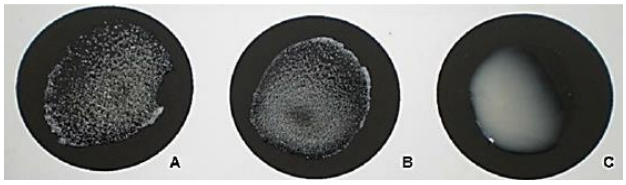

Fig. 1: Agglutination reaction between the latex particles coupled with Strept antibodies and S.agalactiae in A, B spots.

Statistical analysis of the data:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0**.** Qualitative data were described using number and percent. Significance of the obtained results was judged at the 5% level.

RESULTS

The current study was conducted on the outpatient antenatal clinic of Ain-Shams University Maternity Hospital during December 2017 and March 2018. A total of 1oo pregnant women with gestational age ranging from 35 to 37 weeks gestation were included in the study, their age range from 16 years to 40 years (mean 27.8 years). The vaginal swabs were examined for the presence of GBS by inoculation onto Strep B Carrot Broth™ and confirmed by subculture into blood agar and compared to latex agglutination test.

Table 1: Prevalence of GBS among the participants in relation to age

Age group	Culture			
	Positive	%	Negative	%
Years 25 >	10	35%	18	64%
25<	23	32%	49	68%
Total (no. 1oo)	33	33%	67	67%

Table (1) shows that the presence of GBS colonization based on age ranges, where the highest presence of GBS were in females less than 25 years old.

Table 2: Comparison of SCB and Latex agglutination to the gold standard blood agar culture

	Negative		Positive	
	N	%	N	%
Carrot Broth	70	70.0%	30	30.0%
Latex Agglutination	77	77.0%	23	23.0%
Culture	67	67.0%	33	33.0%

Table 2 shows that out of 1oo, 33 (33%) of consecutive specimens were found to be positive for GBS by either the latex agglutination method or the selective carrot broth method, The carrot broth method was more sensitive than the latex method overall, GBS was detected in 30 of 33 (30%) positive specimens by the Carrot Broth method , while the remaining 67 were confirmed to be negative.

Table 3: Comparison between performance of SCB in relation to blood agar culture

		Culture		Total	Agreement			
		positive	negative		%	Kappa	p value	Sig.
Carrot Broth	positive	3o (30%)	0 (0%)	30 (30%)	97.0%	0.931	0.000	S
	negative	3 (3%)	67 (67%)	70 (70%)				
Total		33 (33%)	67 (67%)	100 (100%)				

Table (3) shows a statistically significant difference between culture on blood agar and Strept carrot broth, SCB could detect 3o out of all 33 GBS cases with p value 0.00

Table 4: Sensitivity, specificity, and predictive values of SCB and Latex agglutination for detection of Group B Streptococcus (GBS) in 1oo pregnant women

		Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Accuracy
Carrot Broth	**Value**	90.91%	100.00%	100.00%	95.71 %	97.00%
	95% CI	75.67% to 98.08%	94.64% to 100.00%		88.36% to 98.50%	91.48% to 99.38%
Latex Agglutination	**Value**	69.70%	100.00%	100.00%	87.01 %	90.00%
	95% CI	51.29% to 84.41%	94.64% to 100.00%		79.97% to 91.83%	82.38% to 95.10%

Table 4 shows that SCB had excellent performance compared to LA test, with a sensitivity of 90.9%, specificity of 100%, and negative predictive value(NPV) and positive predictive value (PPV) of 95.7% and 1oo%, respectively. The overall efficiency of SCB was 97%. While LA has sensitivity of 69.7%, specificity of 1oo%, negative predictive value (NPV) and positive predictive value (PPV) of 87 % and 1oo% respectively.

DISCUSSION

Streptococcus agalactiae is recognized as a frequent colonizing agent in pregnant women and is a part of the normal microflora in the vagina in many women. The gastrointestinal tract serves to be the primary reservoir for GBS colonization. The newborn may be infected during delivery causing early-onset infections as pneumonia, sepsis and meningitis[8]. In the absence of a licensed GBS vaccine, intrapartum antibiotic prophylaxis and universal screening continue to be the cornerstones for prevention of early onset neonatal disease [9].

This study was conducted to evaluate Strep B carrot broth versus Latex agglutination technique as a screening method for early detection of Group B *Streptococci* colonizing the genital tract of pregnant females attending Ain Shams University Maternity hospital. Vaginal swabs from 1oo pregnant women in third trimester (≥35 weeks of gestation) were collected. The vaginal swabs were examined for the presence of GBS by inoculation onto StrepB Carrot Broth™. and confirmed by subculture into blood agar and compared to latex agglutination test.

In our study the prevalence of GBS was found to be 33% of the pregnant females by SCB. These results were similar *Kwatra* et al. [10] who showed that the mean prevalence of rectovaginal GBS colonization among pregnant women was 17.9% worldwide.

In the present study, 33 out of the 1oo examined pregnant women (33%) were found to be colonized by GBS in their genital tract. StrepB Carrot Broth™. could detect 3o out of 33 GBS isolates (30%) while latex test could detect 23 (23%). In the present study two screening methods were evaluated for the identification of GBS. Comparison of SCB and latex agglutination methods revealed that SCB had better performance, the sensitivity of SCB was 90.91% and 1oo% specificity while the sensitivity of latex test was 69.7% and 1oo% specificity.

These results were similar to those reported by Church et a.[11] they compared LIM broth as a cultivation method and SCB for detection of GBS from a single vaginal/rectal swab. The authors detected that the performance of SCB was excellent compared to that of LIM with subculture onto blood agar.

A recent Canadian study compared different culture methods for the detection of GBS from several vaginal/rectal swabs collected from near-term pregnant women. Then, culture was done by direct plating onto trimethoprim-sulfamethoxazole and tryptic soy agar with 5% sheep blood then subculture onto blood or Granada media (Hardy Diagnostics, Santa Clara, CA), and SCB (orange color change) were compared. SCB media with an orange color change had the best overall performance, with sensitivity, specificity, PPV, and NPV of 92%, 1oo%, 1oo%, and 98%, respectively[12].

Moreover, other studies demonstrated that a selective broth, such as SCB, is ideal for accuracy at low growth levels of GBS, vaginal swabs were evaluated by three culture methods (sheep blood agar (SBA), selective carrot broth (SCB) and Columbia colistin-nalidixic agar (CAN) followed by analysis using a latex agglutination test. The evaluation of these culture methods for GBS detection had revealed that the sensitivity of SCB (95.2 %, p = 0.004) was significantly higher than that of the SBA medium (57.1%). The sensitivity reported for SCB (95.2%) was higher than CNA (76.0%), although it was not significant, but a direct latex agglutination test could be used as an alternative for the rapid detection of GBS. Therefore, they concluded that the latex method is very likely to be beneficial screening method for pregnant women who are prone to premature labor and require immediate GBS detection[13].

In the current study, we found the latex agglutination test results were different in regard to the hours of incubation of vaginal swabs, 18 to 24 hrs incubation was giving a positive result for GBS cases while decreasing time of incubation was giving false negative results. Hence the test results were dependent on the density of GBS colonization. These result is similar to a comparative study comparing Strept B Select chromogenic agar (SBS) and direct latex agglutination test (DLA) to assure the prevalence of GBS in this population in Kuwait and Lebanon[14]. They found that the sensitivity of the DLA test was found to be dependent on the density of GBS colonization, resulting in 1oo% sensitivity and 1oo% specificity for heavy (>102 c.f.u. per swab) and moderately heavy (5o–1oo c.f.u. per swab) growth of GBS. However, for vaginal specimens yielding <50 c.f.u. per swab, the sensitivity, specificity, positive and negative predictive values of the DLA test were 1oo, 55.5, 63.6 and 1oo%, respectively[14]. Thus, we can conclude that SCB can be more accurate than DLA in having the least false negative results.

CONCLUSION

- We can use SCB for GBS screening in pregnancy as it has less false positive results in comparison to other conventional methods.
- Intrapartum GBS screening is highly recommended or at least risk based screening in order to prevent early onset GBS disease and to reduce the emerging antibiotic resistance among GBS strains.

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