

Evaluation of Culture, Antigen Detection and Polymerase Chain Reaction for Detection of Vaginal Colonization of *Group B Streptococcus (GBS)* in Pregnant Women

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ABSTRACT

Background and Objective: Group B *Streptococcal* infection is an important cause of neonatal morbidity and mortality. Early detection of perinatal vagino-rectal (VR) carriage of Group B *Streptococcus* (GBS) is important in the management of newborn infections. The objective of the study was to evaluate Culture, antigen detection and Polymerase chain reaction for detection of GBS in Pregnant women.

Settings and Design: Observational descriptive study was done in a tertiary care hospital in Southern India.

Materials and Methods: VR swabs were collected from 50 women at 35 to 37 weeks of gestation. Culture in a selective Lim enrichment broth with subsequent culture on 5% sheep blood agar, Conventional PCR assay and antigen detection method were performed.

The performance of antigen detection and PCR methods were compared with culture.

Statistical analysis: Statistical analysis was performed by Chi-Square test.

Results: GBS cultures were positive for 16% of the specimen (8 out of 50). Considering culture as a gold standard, Sensitivity, Specificity, Positive predictive value and Negative predictive value of antigen detection was 100%, 92.86%, 72.73%, 100% and similarly for that of PCR was 100%, 45.23%, 25.80%, 100%, respectively.

Conclusion: Antigen detection method was the rapid, sensitive and specific test for the detection of GBS colonizers during pregnancy.

Keywords: Group B *Streptococcus*, Pregnancy, Antigen detection

INTRODUCTION

Infections by GBS or *Streptococcus agalactiae* is still a common cause of neonatal diseases, such as pneumonia, septicaemia, meningitis, although the incidence has declined in some countries as a result of active prevention efforts [1]. Newborns with early onset (first week of life) GBS disease acquire the organism intrapartum from their mothers who are colonized with GBS in the genital tract [1-3]. Most early disease results either by ascending spread of the organism into the amniotic fluid or by acquisition during passage through birth canal [1,2]. Centres for Disease Control and Prevention recommends culture based screening at 35 to 37 weeks of gestation which can reduce or eliminate transmission of GBS to the neonate by giving appropriate antibiotics to the pregnant women.

The isolation of GBS depends on the specimen sampling site, the timing of collection and the sensitivity of microbiological methods used [4]. Cultures performed in the early third trimester have a relatively low predictive value in identifying women colonized at term [5]. Collection of samples from both the lower vagina and rectum through the anal sphincter provides significantly higher yield than does collection of vaginal samples alone [1,2].

Currently enrichment in Lim broth followed by culture is considered to be the gold standard method [2,6]. However the culture methods require upto 48hrs to yield results. A rapid, sensitive and specific test for detection of GBS would allow early effective diagnosis and a more efficient prevention program [6]. Hence a study was conducted in a tertiary care hospital to evaluate the sensitivity and specificity of the PCR assay, and antigen detection methods in comparison to standard culture method.

MATERIALS AND METHODS

The study was conducted from January to June 2008 in the Diagnostic Microbiology Laboratory of a tertiary care referral hospital in South Kanara District of Karnataka, India. Fifty pregnant women who had come for antenatal check-up at 35 to 37 weeks of gestation were screened for VR colonization of GBS. Patients who had received antibiotic therapy in the last trimester of pregnancy were not included.

Three VR swabs were collected from each pregnant woman. These swabs were processed as follows:

- First swab was inoculated in Lim enrichment Broth (Todd-Hewitt broth with 15µg nalidixic acid and 10µg colistin) and incubated for 24hrs-48hrs at 35°C in 5% CO₂. The broth was observed for turbidity and sub-cultured on to 5% sheep blood agar plate. The plate was read daily for two days and was examined for haemolytic and non-haemolytic colonies. Suspected colonies were identified as GBS by catalase test, Bile-Esculin test and confirmed as GBS by CAMP test and Latex Agglutination test (Streptex B, Remel. Europe. Ltd. UK) [7-9].
- Second swab was inoculated in Lim enrichment Broth and incubated for 24hrs at 35°C in 5% CO₂. A pronase extraction Latex particle Agglutination method (Streptex B, Remel. Europe. Ltd. UK) was used to detect GBS antigen from the 150µl of enriched Lim Broth. Any strong evidence of agglutination that was visible with Group B Latex reagent was considered to represent a positive result [8,9].
- Third swab was inoculated in Lim enrichment Broth and incubated for 24hrs at 35°C in 5% CO₂. DNA was extracted

from the incubated Lim broth using 500µl of the broth. DNA was prepared from lysed bacterial cells by phenol/chloroform extraction procedure as described by Shabayek et al., [10].

AMPLIFICATION

The cfb gene encoding the Christie – Atkins – Munch – Petersen (CAMP) factor was selected as the target for the assay. This includes the Sag 59 and Sag 190 primers defined by Ke et al., [11] and also by Bergeron et al., [12]. The following was the sequence of the GBS specific primers.

Sag 59: 5' – TTTACCAGCTGTATTAGAAGTA – 3'

Sag 190: 5' – GTTCCTGAACATTATCTTTGAT – 3'

Amplification was performed after initial denaturation for 5 minutes at 94°C followed by 45 cycles of 30 seconds at 94°, 30 seconds at 55°C, and 30 seconds at 72°C. The amplified products were analyzed by electrophoresis on 2% agarose gel (Himedia Pvt Ltd Mumbai) which was stained with 0.5µg of ethidium bromide per ml. The final amplified product was 153bps. Positive control GBS (ATCC 13813), negative controls and molecular weight markers 100bps were run with each batch of sample analyzed.

Sensitivity and Specificity were calculated for each of these assays by comparing to standard Culture method which is considered to be the gold standard.

The study and data accumulation were carried out with approval from the appropriate institutional ethical committee and informed consent was obtained from the subjects. Statistical analysis was performed by Chi-square test.

RESULTS

The performance of three methods (Culture, antigen detection and PCR) was analysed for fifty randomly selected pregnant women. Sensitivity and specificity was calculated by comparing to standard culture method [Table/Fig-1].

Group B Streptococci were isolated in eight pregnant women among fifty patients tested (16%). All specimens that yielded GBS by culture were also positive by antigen detection method and PCR assay.

Considering Culture as a gold standard, Sensitivity, Specificity, Positive predictive value and Negative predictive value of PCR was 100%, 45.23%, 25.80%, 100% respectively. (p=0.04) [Table/Fig-2]. Sensitivity, Specificity, Positive predictive value and Negative predictive value of antigen detection was 100%, 92.86%, 72.73%, 100%. These were statistically significant (p=0.0001) [Table/Fig-3]. The length of time required to obtain results for culture was 48-72 hrs. The GBS antigen detection method and the PCR assay were performed directly on incubated Lim enrichment broth and thus

No. of cases	Method	No. of Colonized Women	Percentage of Colonization
50	Culture	8	16.00%
	Antigen detection	11	22.00%
	PCR	31	62.00%

[Table/Fig-1]: GBS isolated by culture, antigen detection and PCR assay

PCR	Culture		Total
	+	-	
+	8	23	31
-	0	19	19
Total	8	42	50

[Table/Fig-2]: Comparison of the results of PCR and culture for the detection of GBS in pregnancy
Chi-square=4.07, P=0.04 Significant.
The Sensitivity (100%), Specificity (45.23%), Positive predictive value (25.80%) and Negative predictive value (100%).

final results were obtained within 15mts and 2hrs respectively after incubation process [Table/Fig-4].

DISCUSSION

The colonization of female genital tract with GBS is significantly associated with infections in neonates and it should be carefully monitored [3]. A more rapid and sensitive method would be beneficial, especially in dealing with patients who present at term with unknown GBS colonization status and preterm labour conditions [2,13]. GBS colonization rate of culture (16%) and antigen detection method (22%) in a study done by Rallu et al., correlate well with the present study [14]. [Table/Fig-1].

The colonization rate by culture is low when compared with antigen detection method and PCR. Although we cannot rule out the possibility of detection of nonviable GBS by antigen detection and PCR assays, several conditions can explain the false negative culture results. These include antibiotics, feminine hygiene products and scanty colonization which would be difficult to obtain in culture [14,15]. We excluded the patients with prior antibiotic treatment in the third trimester of pregnancy. Besides being time consuming culture requires an experienced technician to identify the suspected colonies, which are not always beta-hemolytic [16].

Among the three methods, antigen detection method is the simplest to implement and subculture for susceptibility testing, could be done only if needed. For resource constrained laboratories in developing world, this could mean saving on time, money and labour on unnecessary subcultures. In our study GBS colonization rate by antigen detection method was 22%. Even though the sensitivity (100%) and specificity (92.86%) were high, the cost of the antigen detection test will impede its access to poor people.

Increased GBS isolation rates by PCR diagnostic tests over standard culture methods have been reported in other studies [10,17]. Although available nucleic acid amplification tests have demonstrated high sensitivity when performed on enriched samples, enrichment is not feasible in the intrapartum setting when results are needed quickly. However, for antenatal testing, the accuracy of results is much more important than timeliness [2]. In the present study, PCR as a rapid method to detect GBS from clinical specimens is unsuitable for replacement of culture method mainly because of their lack of specificity (45.23%). PCR needs sophisticated setup and is not available in all obstetrical centres. Another drawback of PCR is its inability to provide antimicrobial susceptibility testing; cultures would still need to be done, especially in penicillin allergic patients [18]. In the current scenario of antibiotic resistance exhibited by GBS, it becomes imperative to test strains for antimicrobial resistance [19,20].

Antigen detection	Culture		Total
	+	-	
+	8	3	11
-	0	39	39
Total	8	42	50

[Table/Fig-3]: Comparison of the results of antigen detection method and culture for the detection of GBS in pregnancy
Chi-square=28.5, p=0.0001 Significant.
The Sensitivity 100%, Specificity 92.86%, Positive predictive value 72.73%, and Negative predictive value 100%.

	PCR	
	+	-
Antigen Detection	11 (35.5%)	0
	20 (64.5%)	19
Total	31	19

[Table/Fig-4]: Comparison of the results of PCR and Antigen detection
Chi-square=5.97, p=0.01 Significant.
Sensitivity (35.5%) and Specificity (100%) of Antigen detection when compared to PCR.

CONCLUSION

PCR assay from incubated Lim broth is highly sensitive but not specific enough to be accepted as a reliable method. The small sample size may have contributed to the lower specificity of PCR. antigen detection method was a rapid, sensitive and specific method. Routine screening of pregnant women with antigen detection test using Lim enrichment broth is recommended at 35-37 weeks of gestation, and this could aid clinicians to rapidly determine pregnant women colonised with GBS so as to take necessary steps of treatment; but this could increase the cost of the test. The low sensitivity of culture could be improved by repeated cultures. Even in an economically constrained country like ours, antigen detection may prove to be useful as it saves on the number of cultures.

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