

Comparison of Various Culture Methods for Isolation of *Group B Streptococcus* from Intrapartum Vaginal Colonization

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ABSTRACT

Aims: *Group B Streptococcus* (GBS) is one of the most common causes of neonatal sepsis throughout the world. Reports of vaginal colonization of GBS in India are few and variable. A study was conducted on pregnant women in a tertiary care hospital to compare various methods for isolation of GBS, to study the prevalence of GBS in pregnant women in third trimester, and to determine risk factors for GBS colonization.

Settings and Design: Observational descriptive study.

Materials and Methods: High vaginal swabs from 150 pregnant women in their third trimester were used to compare three methods for isolation of GBS viz. direct culture on 5% Sheep Blood agar, direct culture on selective Columbia Blood Agar and culture in LIM enrichment broth with subsequent culture on 5% Sheep Blood agar. A history of associated risk factors was also taken.

Statistical Analysis Used: Statistical analysis was performed by Chi-square test.

Results: Isolation was best from LIM enrichment broth with subsequent culture on 5% Sheep Blood Agar. Prevalence of GBS colonization by using culture method was 12.67%. Most frequently associated risk factor was intrapartum fever (42.11%).

Conclusions: Standard Culture Method using LIM enrichment should be adopted as standard practice for isolation of GBS from vaginal swabs.

Key words: *Group B Streptococcus*, LIM enrichment broth, sheep blood agar, vaginal colonization

INTRODUCTION

Group B Streptococcus (GBS) or *Streptococcus agalactiae*, a Gram positive beta hemolytic cocci, is one of the most common causes of neonatal sepsis throughout the world.^[1] Early-onset disease, evident during the first 6 days of life is acquired by neonates through the vertical transmission from colonized mothers either by ascending route or by acquisition during passage through birth canal.^[2] GBS colonizes

genitourinary tract of 10-40% of all pregnant women,^[2] however, there are substantial geographical and racial differences in the incidence of early-onset disease.^[3] Though there are very few reports regarding vaginal colonization of GBS in India, the prevalence is considered very low.^[4,5]

Revised guidelines from the Centers of Disease Control and Prevention (CDC), 2010 for the prevention of perinatal GBS disease recommends that all pregnant women be screened for GBS carriage between 35 and 37 weeks of gestation and intrapartum antibiotic prophylaxis be given to colonized women at the time of labor onset or rupture of membranes.^[6] The incidence of early-onset neonatal infection has declined significantly in association with the implementation of maternal intrapartum chemoprophylaxis.^[7]

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Several investigators have recently reported an increase in incidence of GBS strains resistant to erythromycin and clindamycin.^[8]

A study was conducted in a tertiary care Hospital laboratory to compare three different methods of isolation of GBS, to determine the prevalence of vaginal colonization of GBS in women in the third trimester of pregnancy and to determine the risk factors for colonization of GBS.

MATERIALS AND METHODS

The study was conducted in the Diagnostic Microbiology Laboratory of a tertiary care referral hospital in South Kanara district of Karnataka during March 2007-September 2008. One hundred and fifty pregnant women who had come for antenatal check-up in third trimester, preferably 35-37 weeks and those in preterm labor were screened for vaginal colonization of GBS. Patients who had received antibiotic therapy in the last trimester of pregnancy were not included.

Three high vaginal swabs (Cotton tipped swabs - PW009 Swab, Hi Media Pvt. Ltd., Mumbai, India) were collected from each pregnant woman.

Three different isolation methods were evaluated.^[1,3]

- i. One swab was used for direct culture onto 5% sheep blood agar (SBA) medium (Fi-Tech Chemechtron Pvt. Ltd, Bangalore, India).
- ii. Second swab was used for direct culture on selective Columbia blood agar containing 5% human blood with 15 µg nalidixic acid and 10 µg colistin (Hi Media Pvt. Ltd., Mumbai, India).
- iii. Third swab was inoculated into LIM selective enrichment Broth i.e., Todd-Hewitt broth with 15 µg nalidixic acid and 10 µg colistin (Hi Media Pvt. Ltd., Mumbai, India).

Solid and liquid media were incubated at 35°C with 5% CO₂ for 24-48 hrs. The LIM broth was observed for turbidity and was subcultured onto 5% sheep blood agar plate. The solid media were read daily for 2 days. The agar surface was examined for β-hemolytic and nonhemolytic colonies. Suspected colonies were identified as GBS by catalase test, biochemical tests, and confirmed by Christie, Atkins, Munch, Petersen (CAMP) test and Latex Agglutination (Streptex B, Remel. Europe. Ltd. UK).^[9-11]

All the pregnant women and their newborn babies were followed up. Any event during the antepartum,

intrapartum, and postpartum periods like premature rupture of membranes, maternal fever, intrauterine death, neonatal sepsis were noted. Antibiotic susceptibility test was performed on all the GBS isolated from culture using Disk Diffusion Method^[12] and interpreted using Clinical and Laboratory Standards Institute (CLSI) guidelines.^[13] The following antibiotics were used: Ampicillin (10 µg), penicillin (10 U), clindamycin (2 µg), gentamicin (10 µg), and erythromycin (15 µg) (Hi Media Pvt. Ltd., Mumbai, India). 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

High vaginal swabs from 150 pregnant women were used to evaluate three different isolation techniques for GBS and also to study the prevalence of colonization. The sample size was calculated based on expected proportion vaginal colonization by GBS as 10% is based on previous study done in same setting with an absolute precision of 5% and confidence interval of 95%. This sample size was 138 adding 10% as non response error the sample size came to 150 subjects. Nineteen out of the 150 pregnant women screened were colonized with GBS (12.67%). Out of the three methods of isolation used, GBS was isolated from 19 vaginal swabs screened using LIM broth for isolation, while only three GBS were isolated when inoculated directly on 5% SBA and one GBS was isolated when cultivated from Selective Columbia Blood Agar. Hence, among the different Isolation methods, isolation using LIM Selective Enrichment broth and subsequent subculture on 5% SBA was the best [Table 1].

Risk factors such as Premature Rupture of Membranes (PROM), preterm labor, vaginal discharge and fever were studied [Table 2]. Highest GBS colonization was seen among women with history of fever (42.11%).

Among the 19 pregnant women colonized with GBS, 17 women delivered babies with normal signs (89.47%) and Apgar score of two babies were 47 (10.53%). None of these babies received immediate resuscitation. This was statistically significant ($P = 0.0001$) [Table 3]. None of the babies had any evidence of GBS infection.

Antibiotic sensitivity pattern showed that GBS have increased resistance to erythromycin and clindamycin (42.11%, $n = 8$). All the GBS were sensitive to penicillin [Figure 1].

Table 1: Vaginal colonization of *Group B Streptococcus* using different culture methods

Total no. of cases	Culture method	No. of colonized women	Percentage of colonization
150	Sheep blood agar	3	2
	Selective Columbia blood agar	1	0.67
	LIM enrichment	19	12.67

Table 2: Incidence of risk factors in pregnant women with vaginal colonization of GBS

No. colonized with GBS	Risk factors	No. of colonized women	Percentage of colonization
19	Fever	8	42.11
	Vaginal discharge	5	26.32
	PROM	4	21.05
	Preterm labour	2	10.53

Chi-square=5.26 P=0.15 NS, GBS: *Group B Streptococcus*, PROM: Premature rupture of membranes

Table 3: Apgar score of new born babies of mothers with vaginal colonization of GBS

No. colonized with GBS	Apgar score	No. of colonized women	Percentage
19	Normal 7-10	17	89.47
	Some resuscitative measures 4-7	2	10.53
	Immediate resuscitation 0-3	0	0.00

Chi-square=23.68 P=0.0001 significant, GBS: *Group B Streptococcus*

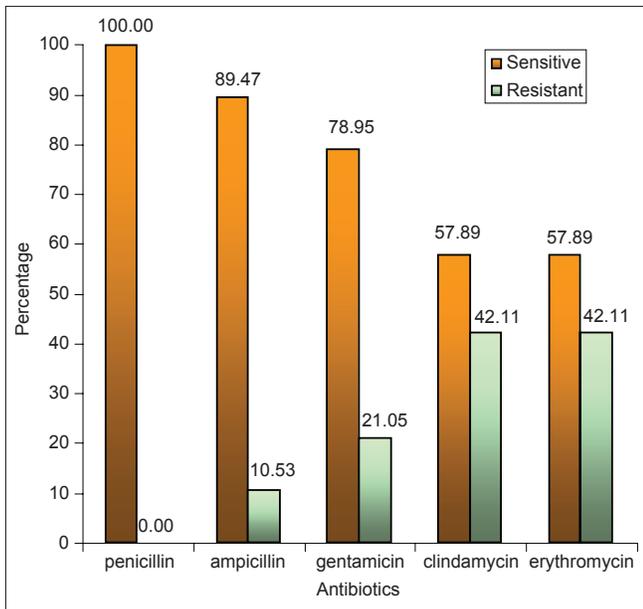


Figure 1: Antibiotic sensitivity pattern of GBS isolated from 150 samples by culture

DISCUSSION

GBS can cause significant morbidity in pregnant women. Manifestations of symptomatic maternal infection include chorioamnionitis, endometritis, cystitis, pyelonephritis and

febrile GBS bacteremia. It is also a common cause of fever in postpartum patients.^[14] GBS has emerged as a major cause of neonatal infectious morbidity over the past several decades.^[15] Newborns are infected while passing through the colonized vagina of the mothers.

The colonization rate in the third trimester of pregnancy was found to be 12.67%. Though this is low when compared to studies done in Europe and Brazil,^[15,16] these are much higher than the colonization rates obtained in Indian studies.^[5] The isolation of GBS from the vaginal swabs depends on the methods used for isolation. Ano-rectal swabs also have greater isolation rates. Most of the Indian studies employ 5% SBA for isolation of GBS, which probably attributes to the low rates of isolation (2-4%). In the study by Kulkarni *et al.*, a selective Broth medium (nalidixic acid 15 µg/ml, gentamicin sulfate 8 µg/ml in Todd- Hewitt broth with 5% sheep blood) was used as a transport medium but the swabs were inoculated on to 5% SBA.^[5] Sharmila V *et al.*, used Todd Hewitt broth for enrichment before plating on to 5% SBA.^[4] Both these studies yielded very low colonization rates i.e., 2.5%^[5] and 2.3%.^[4] In our study too, when only 5% SBA was used, the colonization rate was 2% [Table 2]. When selective Columbia Blood Agar was used, the rate of colonisation was only 0.67%. This could be attributed to the fact that this medium was prepared using human blood which is inhibitory to GBS. But the isolation rate in our study was high after subculture from LIM selective enrichment broth (12.67%). In our study the use of LIM enrichment broth as a transport medium may have further enhanced isolation rates.

In the study done by Yancey *et al.*, specimen collected was anogenital swabs, which therefore may have shown higher carriage rate (26.5%).^[15]

The disparity in isolation rates in various studies may also be due to racial differences, and gestational ages at which the vaginal swabs were collected, inadequate specimen collection and transport to the lab for culture. Treatment with antibiotics and feminine hygiene products also inhibit the isolation of GBS from vagina, but in our study, we had excluded patients with history of prior antibiotic treatment in their third trimester of pregnancy.

Colonization with GBS is significantly associated with prolonged labor, premature rupture of membranes, and preterm delivery.^[14] In our study PROM and preterm labor were associated with 21.5% and 10.53%, respectively, of colonized pregnant women screened. In a study done by Rita *et al.*, (2005) 41.67% of colonized women delivered preterm babies.^[17] Intrapartum fever developed in eight out of 19

colonized women (42.11%). In a study done by Schuchat A *et al.* (1994), PROM and intrapartum fever were observed in 53% and 48%, respectively.^[18] Fever could have been due to chorioamnionitis, which is commonly caused by GBS.

In our study, 42.11% (eight) of isolates were resistant to both erythromycin and clindamycin. The resistance exhibited by GBS to antibiotics was more in our study when compared to other studies.^[8,19] Wide spread use of antibiotics and increased over the counter availability of these may lead to the emergence of antibiotic resistance among GBS. Ongoing surveillance of antibiotic resistance patterns in both pregnant women and their infants will be important in determining optimal prophylaxis and therapy for our patients. The goal of preventive strategies is to reduce or eliminate transmission of GBS to the neonate by giving antibiotics to pregnant women colonized with GBS during delivery and selectively administering antibiotics to newborns after delivery.

Hence, observing the high prevalence rates of vaginal colonization and its detrimental effect on the newborn babies, screening for GBS in pregnant women should be recommended at 35-37 weeks by the Standard Culture method using LIM enrichment broth. This method is highly effective, inexpensive and easy to be used by routine microbiological laboratories.

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