# Study of Vaginal Group-B Streptococcal Colonization in Pregnant Women Attending a Tertiary Care Centre of Agartala

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## ABSTRACT

## BACKGROUND

Lancefield Group-B *Streptococcus* (GBS) emerged as a significant neonatal pathogen with mortality rates of 15 - 50 % in western countries during 1970s. In India, incidence of invasive neonatal GBS diseases is around 0.17 per 1000 live birth and probably is underestimated. Hence, the study was undertaken to evaluate the vaginal GBS colonization in pregnant women along with the neonatal outcome.

## METHODS

The study was single centered, hospital based observational cross-sectional study done in the dept. of Obstetrics & Gynaecology at Agartala Government Medical College (AGMC), and GBP Hospital for one & half years (Jan' 16 - June' 17). 250 Pregnant mothers with 31 to 40 weeks of gestation with singleton pregnancy, delivering either by Caesarean section & Vaginal delivery, were included in this study. They were recruited from obstetrics OPD and casualty of AGMC & GBP Hospital. During 1<sup>st</sup> pelvic examination, two low vaginal swabs were taken for Gram Staining, and for determination of β-hemolytic colony in blood agar. After identification of GBS from genitalia, outcome of neonates in these positive cases were evaluated for up to 28 days after delivery.

## RESULTS

GBS was found in 8.8 % of total women recruited and was more common in multi-gravida. GBS vaginal colonization was significantly associated with low birth weight, and preterm delivery (p value 0.01) which is significant.

## CONCLUSIONS

Prevalence of vaginal GBS colonization is more in pregnant mothers, more in multigravidas & is associated with low-birth-weight neonates and preterm delivery. So, there is a need to formulate guidelines for incorporation of detection of vaginal colonization in multi gravidas to determine its prevalence.

## **KEYWORDS**

Group-B *Streptococcus* (GBS), Multigravidas, Colonization, Neonates, Pregnant Women

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## BACKGROUND

Lancefield Group-B *Streptococcus* (GBS) or *Streptococcus agalactiae* emerged as a significant neonatal pathogen with reported mortality of 15 - 50 % in western hemisphere during 1970s.<sup>1</sup> About half of the cases of GBS among newborns happen in the 1st week of life (early onset disease), and most of the cases start a few hours after birth leading to sepsis, pneumonia, shock, still-birth and perinatal mortality of 10 - 20 %. Late onset disease occurs from 7 - 90 days after birth and manifest primarily as meningitis.<sup>1</sup>

The recognition that maternal vaginal colonization with the organism is a key factor in the occurrence of GBS associated neonatal morbidity & mortality was milestone in the history of perinatal health. About 10 - 36 % pregnant women in United States were found to have asymptomatic ano-genital carriage of GBS with a vertical transmission rate of 50 - 65 %. Incidence of Early Onset Neonatal Disease (EOND) was around 2-4 cases / 1000 live births with the case fatality rates of 22 - 80 % in 1970s, but was reduced to 0.5 cases / 1000 live births after the inception of intrapartum antibiotic prophylaxis according to the guidelines issued by American college of Obstetricians and Gynaecologists (ACOG) and Centre of Disease control and prevention (CDC). 2 Thus a nationwide change in health practices have helped to diminish morbidity & mortality associated with the disease.

In India, epidemiological surveys have shown lower colonization and infection rates (1.76 % - 16 %) in general, but the vertical transmission rates of (53 - 56 %) are consistent with the rates reported in the other parts of the world.<sup>1,3-8</sup> Despite the significant vaginal GBS colonization rates, reports of invasive neonatal GBS disease in India are infrequent around 0.17 / 1000 live births, though sepsis is the major cause of death in up to 30 - 45 % of neonates.<sup>1</sup>

Studies in India have shown pre-dominant cause of sepsis as gram negative septicemia, it is possible that the role of vaginal GBS has been under estimated due to inadequate culture techniques and micro-biological methods. Hence, the present study was undertaken to find the prevalence of vaginal GBS in pregnant woman and to see the neonatal outcome in GBS positive women.

Currently two alternatives, the Royal College of Obstetricians & Gynecologists (RCOG) risk factor-based protocol and the centres for Disease Control and Prevention, screening-based protocol are in practice, for the prevention of neonatal GBS disease based upon the prevalence of GBS and available resources.<sup>9,2</sup>

Thus, we need to find out the prevalence of vaginal GBS in our pregnant woman & its relation to risk factors (prematurity, intrapartum fever, longer duration of membrane rupture) to device optimum screening protocol for our population which can lead to greatest possible reduction in the early onset neonatal disease.

We wanted to determine vaginal Group B streptococcal colonization among pregnant women during 3<sup>rd</sup> trimester of pregnancy with the following objectives-

- 1. Isolation and identification of GBS from genitalia.
- 2. To study the outcome of neonates in GBS colonized women up to 28 days after delivery.
- 3. To formulate preventive measures for vertical transmission of GBS infection.

#### METHODS

This is a single centred, hospital based observational crosssectional study of 1.5 years (Jan '16 – June '17) conducted in the Department of Obstetrics & Gynaecology at AGMC and GBP Hospital. The patients (age of 18 - 45 years) with pregnancy of  $31^{st}$  to  $40^{th}$  week of gestation were included in this study and sample size was 250.

#### Sample Size Calculation

Taking p = current prevalence at 20 % and allowable error = d  $^{\rm 2}$ 

$$n = \frac{Z^2(1-\alpha/2) \times P (1-P)}{d^2}$$
$$= \frac{(1.96)^2 \times 0.2 (1-0.2)}{0.05^2}$$
$$= \frac{3.84 \times 0.2(1-0.2)}{0.0025}$$
$$= 245.76 \approx 250$$

Where,

 $d^2$  = Allowable error

n = Sample size

p = Current prevalence

After rounding up, 250 cases was included in the present study.

## **Inclusion Criteria**

- All pregnant women with 3<sup>rd</sup> trimester pregnancy (31 -40 weeks).
- Singleton pregnancy.

## **Exclusion Criteria**

- All pregnant women < 31st week period of gestation & > 40th week of period of gestation.
- Twins, hydramnios, major obstetric complications like APH (Ante-Partum Haemorrhage).
- Patients already under antibiotic regimen.
- Known case of pulmonary TB, diabetes mellitus, Rh iso immunization, heart disease & bronchial asthma

## **Ethical Approval**

The protocol of the thesis was submitted to the committee

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for ethical approval; AGMC & GBP Hospital, Agartala for further processing & approval. The study was conducted after the approval from the committee.

## Procedure

The study was done after detailed history and information regarding age & pregnancy period and also with proper informed consent. Under strict aseptic precaution sterile vulsellum and speculum was used for preparation purpose. During the 1<sup>st</sup> pelvic examination of pregnant women, two low vaginal swabs were taken by two sterile cotton tipped swabs, which were inserted simultaneously 2 cm deep into the vagina & were rubbed against vaginal wall. The swabs were immediately transferred to the laboratory for processing without any delay.

Out of the two vaginal swabs, one swab was used for gram staining and another swab was plated on blood agar. It was then incubated for 18 - 24 hours at 37° C and were examined for presence of β-hemolytic colonies. Gram stain & catalase test was performed followed by CAMP (Christie-Atkins-Munch-Peterson) test for phenotypic confirmation. Grouping was done using *Streptococcus* grouping kit (IDI-Strep-B, Infectio Disease, Denher Corps), where it uses PCR (Polymerase Chain Reaction) assay to amplify GBS specific DNA and fluorogenic probe. The entire process was done as per manufacturer's advice using 0.1 ml of study sample with IDI-Strep-B buffer and then added to the supplied lysis tube & vortexed at high speed.

## Statistical Analysis

Analysis of categorical data was done by frequency, percentage and for non-parametric test and for numerical data's, menu, standard and parametric tests using Statistical Package for Social Science (SPSS-16).

## RESULTS

Results are shown in different tables given below. Table 1 shows mean age of study population was 20.6  $\pm$  3.97 and majority 186 (74.4 %) were between 21 - 30 years. Majority of the study participants were (8.8 %) from Hindu religion. 94 % of study population were housewives. 82 % were from the low socioeconomic status. All most all (99 %) of them were having regular previous menstrual cycle. Majority (35.6 %) of the study participants were primigravidas, followed by 2<sup>nd</sup> gravida (34.4 %).

Table 2 shows majority (86 %) of the study population did not give any significant history of past illness. Majority (68.8 %) of the study population belonged to 38 - 39 weeks completed period of gestation. Majority of the study participants had undergone LSCS (Lower Segment Caesarean Section) delivery (55.6 %) compared to vaginal delivery (44.6 %).

Table 3 shows 88 % of the study population were GBS positive vaginal colonization. Muslims were more (10 %) positive GBS as compared to Hindu (8.6 %), 9 % cases

among housewives were GBS positive, 10.2 % of low socioeconomic group was colonized with GBS, where nobody was found positive in high socioeconomic status.

	Ν	%		
Age (in Years)				
15 - 20	15	6		
21 - 25	89	35.6		
26 - 30	97	38.8		
31 - 35	48	19.2		
36 - 40	1	0.4		
Gravida				
1	89	35.6		
2	86	34.4		
3	59	23.6		
4	15	6		
5	1	0.4		
Religion				
Hindu	220	88		
Muslim	30	12		
Parity				
0	114	45.6		
1	120	48		
2	15	6		
3	1	0.4		
Menstrual History				
Regular	248	99		
Irregular	2	1		
Occupation				
Housewife	235	94		
Employed	15	6		
Socioeconomic Status				
Lower	205	82		
Middle	37	14.8		
Upper	8	3.2		
Table 1. Demographic Variables				

of the Study Participants

	N	%		
History of Past Illness				
Anaemia	12	5		
Hypertension	15	6		
Hypothyroidism	6	2		
Pre-eclampsia	2	1		
Nil	215	86		
Period of Gestation (in Weeks)				
31 - 33	5	2		
34 - 35	15	6		
36 - 37	58	23.2		
38 - 39	172	68.8		
Mode of Delivery				
LSCS	139	55.6		
Vaginal delivery	111	44.4		
Table 2. Other Parameters in the Study Participants				

Among the GBS positive cases, majority had no significant past illness.

In this study, it was seen that there were 5.3 % cases of GBS in primigravidas, 10 % cases among primiparas and 20 % in multigravidas which was seen to be statistically significant among these two groups of participants where P value is 0.01. 10.9 % of participants who delivered vaginally were GBS positive, whereas it was 7.2 % in participants who delivered by LSCS.

In table 4, low birth weight was observed in 1 % of the participants, preterm delivery was observed in 3 % neonates, whereas neonatal hyperbilirubinemia was observed among 4 % of the neonates of the study participants.

Table 5 shows 100 % of low-birth-weight neonates were associated with GBS, whereas 7.69 % was not associated

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with low birth weight; statistically significant (p value is 0.01).

		N	%	
	Group B Str	eptococcus		
Positive		22	8.8	
Negative		228	91.2	
Religion	Group B Streptococcus		P Value*	
Religion	Positive (%)	Negative (%)	i value	
Hindu	19 (8.6 %)	201 (91.4 %)	0.42	
Muslim	3 (10 %)	27 (90 %)		
Group B Streptococcus			P Value*	
occupation	Positive (%)	Negative (%)	i value	
Employed	00(0 %)	15(100 %)	0.36	
House wife	22 (9 %)	213 (91 %)		
Socioeconomic	Group B Sti	reptococcus	P Value*	
Status	Positive (%)	Negative (%)	· value	
Lower	21 (10.2 %)	184 (89.8 %)	0.33	
Middle	01 (2.7 %)	36 (97.3 %)	0.55	
Upper	00 (0 %)	8 (100 %)		
Darity	Group B Streptococcus		P Value*	
Failty	Positive (%)	Negative (%)	F value	
0	6 (5.3 %)	108 (94.7 %)		
1	12 (10 %)	108 (90 %)	0.01	
2	3 (20 %)	12 (80 %)		
3	1 (100 %)	00 (0 %)		
History of Past	Group B Sti	reptococcus		
Illness	Positive (%)	Negative (%)		
Anaemia	01 (8.3 %)	11 (91.7 %)		
Hypertension	01 (6.7 %)	14 (93.7 %)		
Hypothyroidism	00 (0 %)	6 (100 %)		
Pre-eclamsia	01 (33.4 %)	2 (66.6 %)		
Nil	20 (9.3 %)	195 (90.7 %)		
Mode of Delivery	Group B Sti	reptococcus	P Value*	
Houe of Delivery	Positive (%)	Negative (%)	P value*	
LSCS	10 (7.2 %)	129 (92.8 %)	0.37	
Vaginal delivery	12 (10.9 %)	99 (89.1 %)		
Table 3. Relation of GBS Positivity				
with Different Parameters				
*Fishers exact test, P	value < 0.05 taken a	as		
significant.				

	Frequency	Percentage	
Low Birth Weight			
No	247	99	
Yes	3	1	
Preterm Delivery			
No	243	97	
Yes	7	3	
Hyperbilirubinemia			
No	239	96	
Yes	11	4	
Table 4. Neonatal Outcome in Terms of Different Conditions			

Low birth Weight	Group B <i>St</i> Positive (%)	<i>reptococcus</i> Negative (%)	P Value*	
Yes	3 (100 %)	00 (0 %)	0.01	
No	19 (7.69 %)	228 (92.31 %)	0.01	
Group B Streptococcus			D.Value*	
Preterm Delivery	Positive (%)	Negative (%)	P value*	
Yes	3 (42.8 %)	4 (57.1 %)	0.02	
No	19 (7.82 %)	224 (92.18 %)	0.03	
Group B Streptococcus			D Value*	
пурегопігиотетта	Positive (%)	Negative (%)	P value*	
Yes	00 (0 %)	11 (100 %)	0.69	
No	22 (9.21 %)	217 (90.79 %)	0.68	
Table 5. Association of GBS Positivity				
in Different Neonatal Condition				
*Fishers exact test, P Va	alue < 0.05 taken as			
significant.				

42.8 % of preterm delivery was associated with GBS, in relation to 7.82 % without association (p value is 0.03).

Hyperbilirubinemia was not associated with GBS colonization.

## DISCUSSION

Group B streptococci are known to cause wide variety of infections in adults and pregnant women but clinical interest in these bacteria mainly relates to their ability to cause serious neonatal illness, especially meningitis and sepsis. In developed countries well formulated antenatal guidelines are available for prenatal screening for anogenital carriage of GBS and intrapartum antibiotic chemoprophylaxis for all GBS colonized women. In India prenatal screening and prevention programme of early onset neonatal disease caused by GBS has been carried out only partially and in a non-standardized way. There are only few reports addressed to the prevalence of the vaginal GBS in pregnant women.

In the present study, it was observed that the mean age of the study population was  $26.6 \pm 3.97$  and majority was from age group 26 - 30 years. Similar results were seen in other studies too as reported in a large cross-sectional study from Texas<sup>10</sup> conducted by Ramos et al.

In this study it was found that majority of study participants were Hindu by religion followed by Muslim. It was not having any statistical difference based on these two religion. Majority of the participants were housewives by profession followed by job holders (both private and government). Majority of the participants were from low socioeconomic status in comparison to high socioeconomic status. No statistical significance were observed in both the above categories. Most of the study population were having regular menstrual cycle.

Here, majority of them were primigravidas followed by  $2^{nd}$  gravida & majority of them were having no significant past illness.

In this study it was seen that the frequency of study participants as per period of gestation and most frequent period of gestation was 38 - 39 weeks (69 %) followed by 36 - 37 weeks (23 %) and 34 - 35 weeks (15 %). It is also seen that the majority of the study participants underwent LSCS (55.6 %) in comparison to vaginal delivery (44.4 %). In this present study it was found that genital carriage of GBS as 8.8 % in parturient women after 31 weeks of gestation. Contrary to this study high prevalence rate have been reported in western literature like 21.3 % in UK, 22.8 % in USA and 14 % in Netherlands<sup>11,12,13</sup> as stated by Stoll BJ et al, Stapleton RD et al and George WD et al. But the findings were in accordance to large metanalysis of 34 studies from developing countries, which documented prevalence in India at 1.76 - 12 %.

In the present study it was observed that 10.2 % of low socioeconomic status group was colonized with vaginal GBS in comparison to 2.7 % in middle socioeconomic status and no colonization in high socioeconomic status. There was no statistical significance (as p value is 0.33).

The present study showed that there were 5.3 % cases of colonization in primigravidas, 10 % among primiparas and

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20 % among multigravidas which was also statistically significant (p value is 0.01) among both groups. A study from Chandigarh found more prevalence in multigravida while a study from Kolkata found higher colonization rates in primigravidas, though none of these were statistically significant<sup>4,6</sup> as shown earlier by A Das et al and Lahiri et al.

In the present study it was also observed that 7.2 % patients were delivered by LSCS and 10.9 % deliver vaginally were associated with vaginal GBS colonization, though no statistical significance was observed in comparison (p value is 0.37).

Another aspect of the present study was to observe neonates of GBS positive women. It was observed significant low birth weight and premature delivery in neonates of GBS positive women. Other significant causes are hyperbilirubinemia and sepsis. That showed vertical transmission of GBS might be a possibility as in other countries. The vertical transmission rate in India were similar as reported in the West<sup>5</sup> as shown by Kulkarni et al.

The prevalence of vaginal GBS in our population is high. So need of screening for antenatal vaginal colonization of GBS in pregnant women is important. Further large prospective studies are required to find the rate of vertical transmission in neonates and strategies for prevention of neonatal GBS infection.

## CONCLUSIONS

Epidemiological spectrum of Group B *Streptococcus* is underestimated in India; hence, the present study was undertaken to determine the prevalence, risk factors and neonatal outcome associated with colonization by GBS in pregnant women. Prevalence of vaginal colonization of GBS was found to be 8.8 % in the pregnant women after 31 weeks of gestation. GBS carriage was significantly associated with multigravidas in comparison to primigravidas. There was significant association of GBS. There was significant association of GBS. There was significant association of preterm delivery with vaginal colonization of GBS.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.

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