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Research

GROUP B STREPTOCOCCAL COLONISATION IN PREGNANT WOMEN AT A TERTIARY CARE CENTRE IN CENTRAL KERALA– A CROSS-SECTIONAL STUDY

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Abstract

Background: Group B Streptococcus (GBS) is known to cause life-threatening complications i1n mothers and newborns. Higher colonisation rates are reported in many countries except India. Whether the fastidious nature of GBS with inadequate laboratory facilities are responsible for the lower rate of prevalence is not known. Aim of this study is to estimate the prevalence of GBS colonisation in mothers >35 weeks of gestation and to evaluate the risk factors and antibiotic resistance patterns in those isolates. **Materials and Methods:** A cross-sectional study among 250 pregnant women with gestational age 35 weeks and above, admitted to maternity wards of this hospital, was done. Those who were on antibiotics currently were excluded. Vaginal and perianal swabs were taken and transported in Todd-Hewitt broth with nalidixic acid and gentamicin, as recommended by Verani et al., sub-cultured in Sheep blood agar after two days. GBS was identified by biochemical tests and CAMP test, later confirmed by Lancefield grouping sera. Antibiotic sensitivity test was done using sheep blood agar and clindamycin resistance is tested by D test. Statistical tests were used to find the association between risk factors. **Result:** GBS was isolated in 6.4% from either of the two samples. In GBS colonization positive mothers, have male babies more common. Most of the babies are more than 2.5kgs with >4 apgar score. 3 cases needed NICU admissions and one cases needed antibiotics who are with spesis and 12 cases needed NICU stay for >3 days. Two GBS (12.5%) were resistant to erythromycin and one had inducible clindamycin resistance (5.25%). **Conclusion:** High maternal colonization alerts the need for GBS screening in India. The effect of such a policy on the neonate's microbiome also needs to be considered. However, the study depicts an effect of maternal GBS on neonatal sepsis and NICU admissions.

INTRODUCTION

Group B Streptococcus (GBS) is one of the commensals of lower gastrointestinal and genital tracts in adults. About 10 to 40% of women are asymptomatic carriers of GBS in these sites.[1] At birth, 50% of babies born to colonized mothers have GBS on their skin or mucosal surfaces. 98% of the colonized new-born are asymptomatic at birth and later. The rest develop sepsis, pneumonia, or meningitis in the first week (early-onset) or second week up to three months (late-onset).[2]

GBS is known to cause pregnancy-related chorioamnionitis and endometritis.[3] The burden of GBS disease is well studied in developed countries, but the lower prevalence of this infection is yet to be fully understood in India and Gram negative organisms are major cause of sepsis and infant mortality.[4,5,6] The fastidious nature of GBS, inadequate laboratory facilities, infrequent investigation of preterm and stillbirths, or lower colonization rates of maternal genital tracts, are the probable reasons for the reporting of the low prevalence of GBS sepsis in India. Some Indian studies reported a higher prevalence comparable to western literature, when appropriate sampling techniques and culture media are used.[4] National family health survey-5 (NFHS-5) data of India shows neonatal mortality rate (NNMR) at 3.4/1000 live births in 2021, and maternal mortality ratio (MMR) of Kerala to be 29/100,000 live births and Infections in neonates constitute one of the major causes for NNMR.[7,8,9] Whether the lower NNMR is the result of lower prevalence of GBS genital colonisation in mothers, need to be ascertained. Kerala differs from the rest of the Indian states where nearly 100% of delivery takes place in hospitals. A representative

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sample, hence provide a better estimate of prevalence of GBS genital colonisation in pregnant women in the population of Kerala. There are very few studies available on the prevalence of GBS in Kerala. Warrier LM et al, in 2022 reported prevalence of 12.9% from rectovaginal swabs using broth enrichment from Kerala.[5]

This study aimed to estimate the prevalence of colonised GBS in pregnant women in their gastrointestinal and genital tract and to estimate neonatal morbidity in GBS positive mothers. Also, to study the risk factors and drug resistant pattern of GBS isolates obtained.

MATERIALS AND METHODS

A cross-sectional study was conducted in pregnant women at 35 weeks of gestation or above, admitted to a tertiary care centre in central Kerala, South India from December 2012 to November 2013 after IEC clearance. There was no age or parity restriction. Women who were on antibiotics within the last one week, having placenta praevia where specimen collection might pose risk to the patient and those who refused to give consent were excluded. Nonrepetitive samples were collected 3 days a week until the sample size of 250 was reached. History of current pregnancy and outcomes of previous pregnancies were also recorded.

The samples from the lower vaginal wall and perianal area were collected using separate sterile cotton swabs, and transported to the laboratory in separate Todd-Hewitt broth tubes supplemented with gentamicin and nalidixic acid as recommended by Verani JR et al.[2] The tubes were incubated for 18-24 hours at 37ºC, and subcultured on to 5% Sheep blood agar plate incubated in a candle jar for upto 48 hours. The GBS colonies were identified by Gram stain, catalase, CAMP reaction, Hippurate hydrolysis, PYR hydrolysis, cotrimoxazole, and bacitracin with appropriate controls. The isolate is confirmed by Lancefield grouping (GBS) using SLIDEX Strepto plus latex agglutination test kit according to the manufacturer's instructions. An antimicrobial susceptibility test was done by disk diffusion test on sheep blood agar plate using Penicillin (10 U), Ampicillin (10mg), Erythromycin (15mg) Clindamycin (2mg), and Vancomycin (30 mg); are interpreted as sensitive, intermediate, and resistant as per the criteria mentioned.[6] Inducible clindamycin resistance was tested by the D-zone method. GBS positive women were followed up to see sepsis or other infections in their babies in the first week of life.

Data were analysed using the Statistical software EPI INFO 7 version 3. Chi-square test, Fisher's exact test, and odds ratio were used to determine any association between variables; a p-value of less than 0.05 was considered for statistical significance.

RESULTS

According to the hospital registers, a total of 2741 deliveries took place with 2817 babies born in 2013, during the same period of the study. Preterm births constituted (705 cases)25.5% of total deliveries.

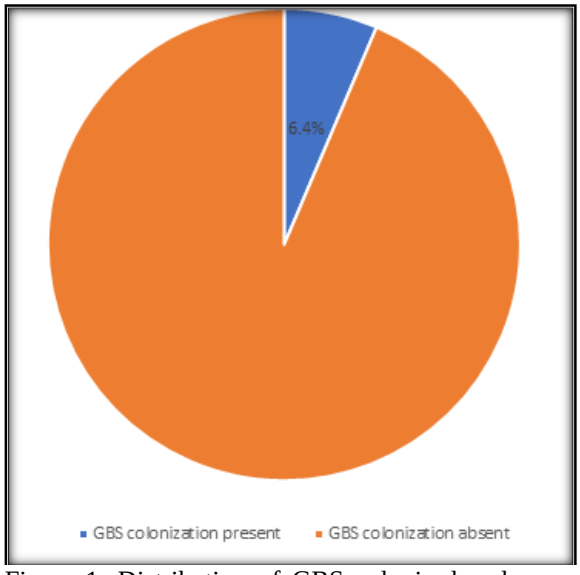

Figure 1: Distribution of GBS colonised and non-colonized women in 250 women

16 (6.4%) cases of 250 cases have been shown positive for GBS colonization. All other 234 cases are negative for GBS colonization (Fig.1)

Table 1: Distribution of GBS colonised and non-colonized women for each characteristic (n=250)

Characteristic	Subgroups	GBS colonization		Total no.of Women (n)	Odds Ratio	p value from Chi-square test/Fisher's exact test
		Present n(%)	Absent n(%)			
Age group	16 – 20	0 (0 %)	19 (100%)	19	6.326	0.176
	21 - 25	12 (9.2%)	118 (90.8%)	130		
	26 – 30	3 (4.3%)	67 (95.7%)	70		
	31- 35	0 (0 %)	25 (100%)	25		
	36 - 40	1 (16.7%)	5 (83.3%)	6		
Gravida	Primigravidae	7 (9.8%)	64 (90.2%)	71	2.0	0.1323
	*Multigravidae	9 (5.00%)	170 (95.0%	179		
Gestational age of mother	<37 weeks	6(37.5%)	96(41%)	102(40.8%)		
	>37 weeks	10(62.5%)	138(60%)	148(59.2%)	1.3	0.1222
Mode of delivery	Vaginal	3 (10%)	27 (90.0%)	29(11.6%)		
	Caesarean	13 (6.0%)	208 (94.0%)	221(88.4%)	OR :1.4	0.47
PROM & Preterm	PROM +	2 (10.0%)	18 (90.0%)	20(8%)	OR :1.7	0.493
	PROM -	14 (6.0%)	216 (94.0%)	230(92%)		

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delivery	Preterm +	2 (12.0%)	15 (88.0%)	17(6.8%)	OR: 2.0	0.349
	Preterm -	14 (87.5%)	219 (93.5%)	233(93.2%)		

* include 2nd, 3rd, 4th and 5th pregnancy. # Baby weight ranged from 1500 -2499 gms, none of them below 1500 gms. PROM+/- premature rupture of membrane present /absent OR: Odds ratio; x2: Chi square

Majority (52.0%) were in the age group 21-25 years. Samples from a total of 250 pregnant women with <37 weeks of gestation were studied of which 102 (40.8%) and 138(60%)of gestation age of >37 weeks. Caesarean section is the mode of delivery in more number of pregnancies with 221(88.4%).

20 cases (8%) are preterm deliveries. With 17 cases(6.8%) are preterm babies.

Table 2: GBS colonization positive Neonates

Neonatal characteristics	Criteria	Number of neonates, N (%)
Gender	Male	9 (56.2)
	Female	7 (43.7)
Birth weight Of Babies	#<2500 gm	3 (18.7%)
	>2500 gm	13 (81.2%)
Apgar 1 min	≤4	6(37.5%)
	>4	11(68.75%)
NICU admission	Yes	3 (18.7%)
	No	13 (81.2%)
Antibiotics required	Yes	2 (12.5%)
	No	14 (87.5%)
Duration of	≤3	4(25%)
NICU Stay	>3	12(75%)
Sepsis	Early onset +	2 (12.5%)
	Early onset -	14 (87.5%)

In GBS colonization positive cases males are more common. Most of the babies are more than 2.5kgs with >4 apgar score. 3 cases needed NICU admissions and one cases needed antibiotics who are with spesis and 12 cases needed NICU stay for >3 days.

Table 3: Antibiotic susceptibility pattern of GBS isolates. (total GBS isolates: 16)

Antibiotic	Susceptible	Resistant	Percentage of susceptibility
Penicillin	16	0	100%
Ampicillin	16	0	100%
Erythromycin	14	2	87.5%
Clindamycin	15	1	93.75%
Vancomycin	16	0	100%

GBS was isolated from 16 (6.4%) of the 250 women of which nine (5.2%) from the vagina alone, three from the perianal area alone, and four (2.8%) cases from both sites.

Antibiotic susceptibility tests were carried out for all 16 GBS isolates, of which two were resistant to Erythromycin and one to Clindamycin.

On follow up, the two babies born to GBS positive mothers showed features suggestive of sepsis and blood cultures were performed. One culture was sterile and the other yielded Staphylococcus aureus. There were no GBS sepsis cases in newborns in this study.

DISCUSSION

Group B Streptococci colonise the genitourinary tract of 10-35% of pregnant women. The mothers can transmit the organisms to their babies making them at risk of developing GBS sepsis. This study included 250 pregnant women at >35 completed weeks of gestation from whom low vaginal and perianal swabs were obtained for detection of colonization with GBS.

In the present study, 16 women of the total 250 subjects had Group B Streptococci isolated from the lower vagina or perianal area, which corresponds to a prevalence of 6.4%. Of these, 13 had vaginal colonization alone (prevalence of 5.2%), while seven had perianal colonization alone (prevalence of 2.8%). Three had GBS isolated from both sites (prevalence of 1.2%). This is in agreement with the low colonization rates 2.3%,[7] 7.8%,[8] 15%,[9] reported in India. The yield of GBS increased when enrichment media were used, similar to this study. One recent study in Kerala, showed prevalence of 12.9%.[5] Higher GBS colonization rates (18.6 to 26%) have been reported in other developed nations and African countries.[10,11,12,13] This variation could be due to the mere geographic, ethnic difference, due to lifestyle or, higher use of enriched media and selective media with high sensitivity used in these countries for their culture and isolation.[2] The low GBS colonisation rate in this study, may be attributed to usage of Indian type toilet against western type toilet, and use of water instead of wipes in toilets which significantly reduce the GBS colonisatin rates (p value 0.017).[14] Another probable reason could be due to better antenatal care received and better health-seeking behaviour with higher antibiotic consumption of women for infections in Kerala, compared to other Indian states.

In the present study, GBS were isolated more frequently in women of age group 21 to 25. (52% of the study population) and 75% of the carriers belonged to this group. But, there were no carriers

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among a younger age group 16 to 20. Multi-gravida had a higher risk of colonization rate than primigravida (O.R:2). In a Turkish study by Eren A et al a statistically significant higher carriage rate was noted among women in the 21-30 years age group, as in this study but no correlation was found with order of pregnancy.[15] Dechen TC et al in Trinidad, reported GBS colonization rates significantly greater among multi-gravid women,[16] Goel N et al in 2020, also reported increased parity associated with higher GBS colonisation (p=0.026)and GBS urinary tract infection (p=0.002) and higher age groups.[14] Anthony BF et al, found colonization was significantly lesser among women 20 years or older, or in pregnancies of order 4 or more.[17] Thus, these studies are with conflicting results of association between age and colonisation rates. Higher colonisation rate in this study may be due to higher proportion of women in this age group, increased sexual activity of this group compared to older group.[18] Further studies are required in this direction to establish the reasons.

In the present study, a higher proportion of of premature rupture of membranes (PROM) (OR 1.7) and preterm deliveries among colonized women (OR 2.0),. Yan JJ et al, reported association between maternal GBS colonisation and premature rupture of membranes (PROM) (p value <0.01), 19.6% and 5.3% in PROM positive and PROM negative respectively.[18] Other studies also shown similar results. The association was more significant in women from whom GBS were also isolated in cervical culture. McDonald H et al, and another systematic review and meta-analysis showed the relationship of GBS colonisation and preterm labour statistically significant.[19,20] These studies showing an association between PROM and colonisation have a higher sample size compared to those without it. In this study, low birth weight, early-onset sepsis among babies of colonized women (OR 3.0) were noted. In a tertiary care centre in Vellore, the incidence of GBS bacteraemia was 0.17 per 1000 live births. GBS accounted for 1% of bacterial isolates causing neonatal sepsis, if a larger number of women were followed up for a longer duration the association could have been observed.[21]

All isolates were sensitive to Penicillin and Ampicillin. Two isolates were resistant to Erythromycin (12.5%), and one of these showed inducible Clindamycin resistance (6.25%). Group B streptococci remain susceptible to Penicillin, Ampicillin, and Cefazolin. Erythromycin and Clindamycin are used as alternative drugs in penicillin-allergic individuals, but increasing resistance to these drugs is being demonstrated. 52.9% of GBS isolates were resistant to Penicillin from a Nicaraguan study,[22] Hsueh et al, reported resistance 6% for penicillin, 46% for erythromycin.[23] Khademi et al in 2020 meta-analysis of GBS drug resistance in pregnant women in Iran, reported 21% for erythromycin, 26.8% for clindamycin, 4.2% for penicillin, 2.7% for ampicillin.[24] The inducible clindamycin resistance of 8.6% was reported by Capanna F et al,[25] Drug resistance of GBS vary different geographic regions. In this study, all the GBS positive mothers had intrapartum antibiotic prophylaxis (IAP) and no early onset GBS sepsis was observed.

Limitations

The study population included pregnant women admitted to the Obstetrics ward, who had one or more comorbidities diagnosed at admission, and thus cannot be said to be a true representative sample of the general population. Analysis of adverse outcomes of pregnancy also becomes difficult in such a population. This study could not include the collection of cervical swabs and rectal swabs. Perianal swabs are inferior for assessing real gastrointestinal colonisation. Intrapartum colonisation status could not be studied in this population.

CONCLUSION

The prevalence of group B Streptococcal colonisation in pregnant women at ≥ 35 weeks of gestation was 6.4%. The vaginal and perianal colonisation rates were 5.2% and 2.8% respectively. The distribution of group B Streptococci was found to be independent of age and order of pregnancy. All isolates were uniformly sensitive to penicillin and ampicillin. Resistance to erythromycin was seen in 12.5% and inducible clindamycin resistance in 6.25%. We could not demonstrate a statistically significant association between GBS colonization and order of pregnancy, PROM, prematurity, lower birth weight of babies, and sepsis. Although a higher Odds Ratio (O R) could be demonstrated in all of them, probably due to smaller sample size.

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