Screening for group B streptococci in antenatal women: Isolation and its antibiogram

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Abstract
Group B streptococci(GBS) is one of those pathogens causing neonatal septicaemia and neonatal meningitis. Objective of this study is to screen antenatal women for Group B Streptococcus colonization and its antibiotic sensitivity pattern by Kirby bauer disk diffusion method. This study was carried out in Department of Microbiology during a period of 4 months.A total of 100 pregnant women attending antenatal clinics in the age group 20-40 years were included in the study. Two vaginal swab were taken aseptically and immediately transported to the laboratory for processing. One swab for direct gram staining and other swab was inoculated into sheep blood agar and incubated at 37 ºC for 24-48 hours. Identification of organism was based on gram staining, colony morphology, catalase reaction, Christie Atkins Munch Peterson(CAMP) and Hippurate hydrolysis test. Results of this study show that prevalence rate for GBS colonization is 8%.Pregnant women less than 20 years of age and primigravida are more commonly colonized with Group B Streptococci. All the isolates were sensitive to Penicillin, Erythromycin, Ampicillin, Gentamicin, Ceftriaxone and Chloramphenicol. Hence all pregnant women should be screened for Group B Streptococci carriage between 35 and 37 weeks of gestation and intrapartum antibiotic prophylaxis be given to colonized women at the time of labour or rupture of membranes.

Keywords: Group B Streptococci, Antenatal pregnant women, Prevalence, Antibiotic susceptibility pattern.

Introduction
Group B streptococci(GBS) also refereed as *Streptococcus agalactiae*, is a Gram positive opportunistic pathogen which is beta hemolytic streptococci of Lancefield group B, that colonizes the gastrointestinal and gastrourinary tract upto 50 % of healthy individuals. However in neonates GBS is one of the most common organisms causing neonatal sepsis and meningitis.[1] The antenatal women GBS colonizes in 15-20% of pregnant women in the lower vaginal tract. During passage of neonate via the mothers birth canal newborn acquires the GBS from the lower vaginal tract since the mother's birth canal is the principal reservoir of this GBS for infants.[2] Very few Indian studies have been carried out, to study the prevalence of GBS colonization and its antibiogram in the vaginal flora of the pregnant women.The incidence of GBS colonization in the vaginal flora of pregnant women varied from 0.48 to 24.3%.[3] Hence the present study was done to know the prevalence of GBS colonization and the antibiotic susceptibility pattern in the antenatal pregnant women. GBS is mostly thought of as a commensal organism that resides in the genitourinary and lower gastrointestinal tract of healthy adults. Approximately 20% to 40% of women are colonized with GBS during pregnancy. While maternal colonization is usually asymptomatic, it places the newborn at immediate risk for vertical transmission.[4] Up to 50% of newborns born to colonized mothers will also become colonized with GBS. Among colonized newborns, 1-2% will develop early onset acquisition from colonized individuals. Guidelines shows all antenatal pregnant women at 35-37 weeks of gestation are universally screened for GBS, and if colonized, intrapartum antibiotic prophylaxis to reduce the risk of transmission from mother to neonates. Antibiotic prophylaxis reduces the incidence of early onset Group B streptococcal disease by 36%, however the incidence of Late onset Group B streptococcal disease has not been affected.[5] Henceforthy GBS remains the leading cause of bacterial disease in newborns with an overall incidence rate of ~0.3 infections per 1,000 live births. Evidence shows that GBS develop resistant to second line antibiotics in recent days and is emerging pathogen among adult and elderly populations which emphasise s the need to make alternative measures for preventing disease in both newborns and the elderly.[6]

Materials and Methods
A cross sectional study was conducted in the department of Microbiology for the period of 4 months. A total of 100 antenatal pregnant women attending antenatal clinics were included in our study. The inclusion criteria were the all antenatal pregnant women in the age group 20-40 years willingness to participate and provide informed consent. The exclusion criteria were those women undergone pelvic examination prior to vaginal swab, already receiving antibiotics and antepartum haemorrhage. Prior to vaginal examination, two sterile vaginal swab were taken aseptically and were immediately transported to the Microbiology department. One swab is used for direct gram staining and the Culture was done with other swab which was inoculated on to 5% sheep blood agar and it was further

incubated at optimum temperature 37ºC for 24-48 hours. Preliminary identification of GBS was done by Direct Gram's staining shows gram positive cocci arranged in short chain and in culture colonies are I mm in diameter smooth, convex, moist, regular, surrounded by a small hazy zone of β hemolysis.

GBS was further tested for catalase activity where 3% hydrogen peroxide was used , presence of effervescence showsa catalase positive and further confirmed by CAMP test, hippurate hydrolysis test and Lancefield grouping. The serogrouping for GBS was done by using latex agglutination test available as commercial detection kit.

The Antibiotic sensitivity test was performed on Muller Hinton agar plates by Kirby-Bauer disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines The following antibiotics (Hi-Media, Mumbai, India) were tested by disk diffusion method Penicillin(10 µg/disc), Erythromycin(15 µg/disc), Ampicillin (10 µg/disc),Gentamicin(10 µg/disc), Chloramphenicol (30 µg/disc)and Ceftriaxone(30 µg/disc).[11] Data was analysed by chisquare test, paired´t' test, and a p-value of <0.05 was taken as statistically significant

Results and Discussion

In our study out of 100 women, 8(08%) were showed GBS colonization. Most of the isolates were from age group ≤20 years (16%) followed by 21-25 years (5.5%) Table 1. The GBS was isolated maximum from the primigravida 4(12.5%) followed by second gravida 2(8%) and 01(4.3%) were multigravida. It was observed GBS was not isolated from multigravida women above 20 years of age .Thenceforth the correlation between GBS colonization with age & gravida was statistically not significant

All most all the strains were 100% sensitive to beta lactam antibiotic(penicillin, ampicillin, cefuroxime and ceftriaxone), Erythromycin, ampicillin, gentamicin, Chloramphenicol,

In newborn and adults Group A Streptococci causes variety of life threatening serious diseases like septicemia, meningitis and pneumonia which has given an alarm in recent years. However during the early onset of this infection lung is the major organ involved for the initiation of infection and for the clinical sequlae, but for the those infants whose death occurred within few hour of birth inflammatory response is minimal or absent. GBS shows very high morbidity and mortality and most of the clinicians are not aware about the prevalence of GBS, so an attempt was made to find out the prevalence of GBS in pregnant women

Present study shows the prevalence rate of GBS colonization was 8% in third trimester. The incidence rate was significantly high in primigravida followed by second gravida. The incidence in our study (8%) which correlates with two Indian studies by Annie Rajaratnam *et al* and Vinay haraze *et al* were colonization rate is 8.3% and 7.5%.[12,13] In contrast by Motlova *et al and Konikkara et al* reported that the prevalence of GBS carriage is 29.3% and 12% was relatively high.[14,15] Many reasons are attributed for GBS colonization and the rate of colonization varies from place to place depending upon varies factors like socioeconomic status, sampling techniques to collect the clinical sample. However genetic polymorphism and Ethnicity might play a very important role in variation of the rates of infection with GBS.

In the present study most of the cases belongs to age group ≤ 20 years(16%) followed by the age group 21-25 years(6.6%), thus most of the GBS colonized pregnant women were from teenage and the younger age group was found to be at par with that of Vinay hazare *et al* and Tsering Chomu *et a.*[13,16] The reason for the predisposition of women younger ≤20 years of age to vaginal colonization with GBS is less apparent.This relationship could be the results of age relared development of local or humoral immunological responses that interfere with mucosal attachment and/or persitance of GBS.

In the present study 9.8% of GBS isolated from third trimester which correlates with Fatemi *et al*, however in contrast Baker *et al* found that the colonization rate almost doubles between the second trimester and delivery.[17,18]

All strains were 100% sensitive penicillin, Erythromycin, ampicillin, gentamicin, Chloramphenicol, Ceftriaxone were Arora *et al* reported that 60 GBS were susceptible to Penicillin, Ampicillin & erythromycin.[19] In 2012 Vinay et al showed all the 15 strains were 100% sensitive Penicillin ,Ampicillin & erythromycin.[13]

Table 1: Age wise distribution of study population in relation to Group B streptococci

Age (Years)	No. of Mothers	Group B streptococci infection			
		Present		Absent	
		No	%	No	%
≤20	24	4	16	20	83
21-25	30	2	6.6	28	93
26-30	19	1	5.2	18	94.7
31-35	14	1	5.2	13	92.8
36-40	9	0	0	9	100
≥40	4	0	0	4	100
Total	100	8	8	92	92

X^2 p> 0.05 insignificant

Table 2: Distribution of Pregnant women according to gravida and Group B Streptococci infection

Gravida	No. of Mothers	Group B streptococci infection			
		Present		Absent	
		No	%	No	%
Primi	32	4	12.5	28	87.5
Second	25	2	8	23	92

Third	20	1	5	19	95
Multi	23	1	4.3	22	95.6
Total	100	8	8	92	92

X^2 p> 0.05 insignificant

Conclusion

Colonization of GBS in the genital tract of the parturient women is the single most important factor for GBS colonizer in the newborn babies. However various strategy are used to prevent the transmission of GBS from mother to newborn, one such method is intravenous intrapartum antibiotic prophylaxis by penicillin or ampicillin to avoid early –onset GBS disease in the infant and to treat all neonates born to such women with penicillin shortly after birth.

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Conflicts of Interest: Nil

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