Antibiogram of Group B Streptococci Isolated from the Vagina of Pregnant Women in Third Trimester of Pregnancy

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Abstract:
Group B streptococcal (GBS) prevalence varies from place to place; this organism is responsible for serious infections in newborns, such as septicemia and meningitis. The present study was aimed to find out the antibiogram of Group B Streptococci isolated from the vagina of pregnant women who were in the third trimester of pregnancy and to identify the risk factors for its colonization in vagina. The study was carried out in the Department of Microbiology, M. R. Medical College & Hospital, Gulbarga during a period from January 2007 to December 2007. Two hundred pregnant women who were in the third trimester of pregnancy and attending the antenatal clinics were included in the study. Two low vaginal swabs were taken from each women included in the study and were immediately transported to the laboratory for processing. Direct gram staining was done from one swab and the other swab was inoculated into sheep blood agar plate and incubated at 37ºC for 24-48 hours. Identification of organism was based on gram staining, colony morphology, catalase reaction, Christie Atkins Munch Petersen (CAMP) and Hippurate hydrolysis test.

Of the 200 pregnant women screened, 7.5% were colonized by GBS. Incidence of GBS colonization was higher among pregnant women in the third trimester who were 25 years of age and primigravida. All the isolates were sensitive to Ampicillin, Erythromycin and Penicillin but were resistant to Gentamicin and Kanamycin.

Key Words: Group B streptococcus; Colonization; Drug resistance, Drug sensitivity.

Introduction:
Group B streptococci (GBS), which is a beta-hemolytic streptococci of Lancefield Group B, has been causally linked to human diseases since 1938 (Lancefield, 1938). Group B streptococci has become the leading pathogen causing serious neonatal infections like sepsis and meningitis (Eickhoff et al, 1964; Hood et al 1961). The GBS is present in 15-20% of pregnant women in the lower vaginal tract (Hoogkamp-Korstanje et al, 1982). Colonization of GBS in the neonate usually occurs during its passage through the birth canal (Baker et al, 1973; Baker et al, 1977). The mother’s birth canal is the principal reservoir of this infectious agent for infants (Franciosi et al, 1973 ). In India, very few studies have been carried out, mainly to study the prevalence of GBS infections during pregnancy. The incidence of GBS colonization in the vaginal flora of pregnant women varied from 0.47 to 23.3% as reported by various workers (Baker et al, 1977; Beachler et al, 1979; Yow et al, 1980; Kishore et al, 1986; Arora et al, 1994; Lakshmi et al, 1998).

Hence, the present study was planned to know the magnitude of GBS colonization, and antibiogram of isolates from the vagina with a view to determine the pattern of antibiotic resistance among pregnant women in the third trimester of pregnancy.

Material and Methods:
The present hospital based cross sectional study was carried out in the antenatal clinic & Department of Microbiology, M.R. Medical College, Gulbarga, from January 2007 to December 2007. A recent study, carried out by Valkenburg-van den Berg et al (2006) showed the GBS carriage rate in late pregnancy to be 21%. Considering this prevalence of GBS carriage rate, sample size was calculated.

A total of 200 pregnant women attending Antenatal clinics (ANC) were included in the study. The inclusion criteria for study were the presence of signs & symptoms of pregnancy & willingness to participate in the study. These women were in the age group of 19 to 37 years. Each week, 4-5 pregnant women who were in the third trimester of pregnancy (29-40 weeks) were selected during the study period of one year. Detailed history of each case was taken...
during their clinical examination. Group B streptococcal positive women were divided in three groups: Group I: ≤ 20 years; Group II: 21 to 25 years and Group III: women above 25 years.

Two low vaginal swabs were taken aseptically prior to first pelvic examination. The swabs were immediately transported to the Microbiology laboratory. Modified Stuarts transport media was used in case of delay. One swab was used for direct gram staining and the other swab was inoculated on to sheep blood agar containing 5% sheep blood; it was incubated at 37°C for 24-48 hours. Identification was done based on gram stain, colony morphology, catalase reaction, CAMP test, hippurate hydrolysis test and Lancefield grouping.

The presumptive diagnosis of GBS was based on the following criteria:
(i) Direct gram staining showing gram positive cocci arranged in pairs and short chains. (ii) The colony appearance of GBS on sheep blood agar at 24 hours is usually grey, smooth, shiny, convex, moist, regular, soft and mucoid in appearance and about 1 mm in diameter, often surrounded by a small hazy zone of beta-hemolysis.

The confirmation of GBS was made by subculturing colony from the blood agar on to chocolate agar to check the catalase activity. A clean (grease free) glass slide was taken, 1 or 2 drops of H₂O₂ (3%) was put on the slide. Using a clean glass rod, a colony was picked and dipped into H₂O₂. Staphylococci produce catalase while streptococci does not. Effervescence is seen in positive catalase test where as no effervescence is seen in negative catalase test. Other confirmatory tests carried out were CAMP and Hippurate hydrolysis test. Grouping of Streptococci was done by the Lancefield’s method.

Antimicrobial sensitivity of the GBS was done by the Kirby-Bauer disc diffusion method (de Lourdes et al, 1981). Fresh sub-cultures of GBS were used after overnight growth (16 hours) on blood agar plate. The inoculum was prepared by suspending several of the colonies in sterile phosphate buffered saline (pH 7.2) to achieve a turbidity of 0.5 McFarland standard. This resulted in a suspension containing approximately 1-2 x 10⁸ CFU/ml. A sterile cotton swab was dipped into the bacterial suspension, elevated above the liquid and rotated several times against the inside wall of the tube to remove excess of the inoculum. This swab was streaked evenly in three different directions onto the blood agar containing 5% sheep blood.

Six antibiotic discs were employed namely Penicillin (10 µg/disc), Erythromycin (15 µg/disc), Ampicillin (10 µg/disc), Gentamicin (10 µg/disc), Chloramphenicol (30 µg/disc) & Kanamycin (30 µg/disc).

The data was analyzed and interpreted. The fisher’s exact test was used.

Results:
In the present study, out of 200 women, 15 (7.5 %) showed GBS colonization (Fig. I). Among GBS positive women, 53.3% belonged to Group I, 40% to Group II and 6.7% to Group III. The maximum number of GBS positive women were primigravida (46.7%), followed by second gravida (33.3%), third gravida (13.3%) and only 6.7% were multi gravida. No multi gravida women above 20 years of age showed GBS colonization. The relationship between GBS colonization with age & gravida was not statistically significant (Table I).

All the strains were 100% sensitive to ampicillin, erythromycin and penicillin followed by chloramphenicol (66.6%). A marked resistance was observed with Gentamicin (100%) and Kanamycin (80%) as shown in Fig. II.

Discussion:
Group A streptococci had a place of pride as causative agent of important clinical diseases and syndrome till recently; other members of this family are now coming to light as human pathogens and Group B streptococci is topping the list since the last few decades. In the present study the prevalence rate of GBS colonization was 7.5 % in third trimester. The incidence was higher amongst primigravida followed by second gravida. Kishore et al (1986) showed the incidence to be as low as 0.47%. In the study of Baker et al (1977) & Beachler et al (1979), the vaginal colonization of GBS was shown to be 18% & 23.4% respectively. Yow et al (1980) showed that 12% of
pregnant women were colonized by GBS. An epidemiological study carried out by Stoll et al. (1998), showed a prevalence of 12% in Indo-Pakistan region. In the year 2002, Grimwood et al showed the colonization rate of GBS to be 22%. In a recent study by Valkenburg-van den Berg et al. (2006), the GBS carriage rate in late pregnancy was observed to be 21%. Papapetropoulou & Kondakis (1987) and Suara et al. (1994), showed incidence of 12% and 22% respectively. In comparison to the above studies, the present study showed the incidence of colonization rate to be much lower (7.5%).

In contrast, study of deLourdes et al. (1981) and Dillon et al. (1982), showed the colonization rate of only 4%. The colonization rate of the present study (7.5%), was found to be at par with that of Lewin & Amstey, 1981 (7.8%). The reasons for variation in incidence in different studies may be attributed to the fact that maternal colonization with GBS varies from place to place. Other factors that may have contributed to this variation include: socio-economic factors; variation in sample collection and the technique used for sampling. Ethnic and genetic factors might play a role in variation of the incidence of colonization with GBS.

In the present study, the antibiotic sensitivity testing of GBS isolates showed that all the Group B streptococci were 100% sensitive to ampicillin, penicillin and erythromycin followed by chloramphenicol (66.6%). A high resistance was seen with gentamicin (100%).

Olanisebe & Adetosoye (1986) reported that
all the 8 GBS isolates were sensitive to erythromycin, methicillin, penicillin G, ampicillin and chloramphenicol in descending order. They were resistant to tetracycline, sulphamethoxazole and streptomycin. However, in contrast to the present study, Lakshmi et al (1988) showed penicillin and erythromycin sensitivity against GBS to be 81.8% & 72.7% respectively. Arora et al (1994) also found all the GBS strains to be sensitive to penicillin and ampicillin. However, they observed resistance to gentamycin, chloramphenicol and erythromycin.

**Conclusion & Recommendation:**
Since genital colonization of parturient women continues to be the single most significant factor in GBS colonization of newborns, two methods of chemoprophylaxis are being suggested to prevent vertical transmission. One such method of chemoprophylaxis against GBS is treating pregnant women colonized with GBS prior to delivery and second approach is to treat all neonates born to such women with penicillin shortly after birth.

**Bibliography:**


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