



Vaginal Colonization of Group-B *Streptococcal Agalactiae* in Antenatal Mothers from Central Nepal

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ABSTRACT

Group-B *Streptococcus agalactiae* is a significant cause of neonatal morbidity and mortality for over last two decades. It causes two types of GBS infection in early neonates onset disease and late onset disease, characterized by pneumonia, Septicaemia and meningitis and among adults it causes UTI, wound infections, endocarditis and intra- uterine asphyxia. This study was done to determine the prevalence of vaginal colonization of Group-B *Streptococcus agalactiae*, its risk factor and antibiotic susceptibility patterns among the isolates. Four hundred and twenty two samples were collected from antenatal mothers, between 7th week to 40th week of gestation were screened for Group-B *Streptococci* colonization from 1st Jan 2010 to 25th Dec 2013. The subjects selected were between 17 to 38 years of age group. Group-B *Streptococci* were isolated only 136 cases from pregnant women which is about 8.5%. Among the risk factor, low maternal age, no good nutrition, unhygienic condition and those who have animal, in their house is the major factor for *Streptococcus* colonization among the mothers with positive culture. Neonatal infection was only present in 33 cases of neonates born from culture positive mothers. All isolates were sensitive to Ampicillin, Erythromycin and Vancomycin but moderately sensitive to Chloramphenical and Clindamycin. All isolates were resistant to Gentamycin and Tetracycline. Study done in Central Nepal showed low colonization rate of GBS in the vagina of antenatal mothers so neonatal infection was not evident. This finding is also supported by the fact that the fatal neonates colonization with GBS only 1-2% develops clinically apparent disease. Intrapartum antibiotic therapy of women colonized with Group-B *Streptococcus* can decrease neonatal infection. Effective strategies to detect maternal colonization with GBS infection needs to be studied properly in different populations of the world.

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Introduction

Streptococci species has important position in clinical medicine as human pathogens. In clinical medicine as human pathogens. Among them Group-A *Streptococci* were considered to have a special place as a causative agent of perinatal sepsis. Prior to the introduction of the penicillin, this organisms was a major cause of puerperal sepsis and 75% of maternal motility due to this infection.¹ Whereas, Group-B *Streptococci agalactiae* have been recognized in veterinary medicine for many years as a cause of *Bovine mastitis*, they were virtually ignored as human pathogens until 1964¹ when Eickhoff and Alnated the role of GBS in perinatal infections.²

Since then it is emergence in 1964, GBS disease has been the leading cause of bacterial infections associated with the illness and death among newborns.^{3,4} In Belgium 150-300 newborn babies present each year with a serious infection due to GBS and more than 10% die due to it.⁵ Neonatal infection shows two types of patterns, early onset (0.5-3.7 per 100 live births and late onset, 0.5-1.8 per 100 live birth disease).⁶ Early onset infection is usually evident during the first few hours of life and is caused by any of five GBS serotypes (Ia, Ib, Ic, II and III). Late onset disease usually appears 5 or more days after birth. About 90% of the late onset disease are caused by typeIII.⁷

Transmission of GBS from mother to the child can occur during birth in the case of vaginal and rectal colonization.

Higher neonatal transmission rates occur when women are persistently culture positive carriers or when women are heavily colonized with GBS as demonstrated by semi qualitative vaginal culture.⁸ Early onset disease is acquired by variable transmission from mother to fetus, generally after the rupture of the membrane. But the newborn baby can also acquire the infection by contrast or inhalation at the time of the passage in the genital tract or by haematogenous route.^{9,10,11} Early onset of the infection is characterized by the fast development of severe respiratory distress, fever, septicemia, shock, disseminated intravascular coagulation, pneumonias, meningitis and a failure of vital organs. More than 30% of the children suffering from meningitis show neurological effects like blindness, deafness and delayed development.⁵

Late onset, disease is either nosocomial (spread in the nursery from colonized person or other colonized neonates) or it can be acquired from community sources.^{12,13} Late onset, infection is characterized by lethargy, fever, bacteremia, septic arthritis, osteomyelitis, cellulitis and otitis media. The attack rate is only a fraction of the colonization rate and is directly related to the severity of the colonization of GBS in the vagina of antenatal mothers.⁷ overall, 3%-12% of all neonatal are colonized with GBS in the first week of life.⁸ Whereas, the majority of the colonized babies remains asymptomatic, 1-4% develops clinically apparent infection.⁵ The gastro-intestinal

tract is the most likely human reservoir of GBS, with the genitourinary tract, the most common site of secondary spread.² The vaginal and cervical contamination and colonization occurs from Gastro-intestinal tract (GIT) source.⁵ In the most population studied, 10-30% of the pregnant women were found to be colonized with GBS in the vaginal or rectal areas.⁷ Colonization rate differs among ethnic groups, geographical location, age, gravidity, duration of gestation, the location and the number of sites cultured.

Vaginal carriage has been found to be the higher in the earlier part of the menstrual cycle, in teenagers compared to older women, in sexually active women and in women with a history of three or fewer pregnancy.^{14,15} GBS also infects old person, diabetic person, cancer person and Human Immunodeficient Virus (HIV) infected persons.¹⁶ Colonization, in general, is asymptomatic only isolation can recognize the carriers of GBS.⁵ The choice of culture medium is a crucial determinants of the prevalence of GBS. The isolation rate has been found to be higher when selective broth rather than agar plates are used as culture media and when samples are taken from lower third region of vaginal and the rectum.¹⁷

Penicillin remains the drug of choice for symptomatic and asymptomatic carriers. But now-a-days Ampicillin is more frequently used and effective drug which provides adequate treatment for GBS. It also response good against Erythromycin and Clindamycin but shows resistant to Aminoglycosides, addition of Gentamicin or Tobramycin to one of the Penicillin results in a synergistic action against GBS.¹

Materials and Methods

Two hundred antenatal mothers in between 7th weeks to 40th weeks of gestation will be screened for GBS colonization from 1st Jan 2010 to 25th Dec 2013 period. The subject selected will be aged between 17 to 38 years.

Sample collection

Two swabs from lower third region of the vagina will be collected from antenatal mothers. Sterilized nontoxic cotton swabs will be used to collect the sample.

Transport of the sample

The sample collected will be transported immediately to the laboratory without delay. But when we suspect for delay that time two samples will be collected and one transported to the laboratory in Selective Broth Medium (SBM) another without transport medium. SBM is a selective transport medium for GBS.¹⁴ To prepare this medium a solution of 15 microgram of Halidixic acid and 8 microgram of Gentamycin will be mixed with 0.5 ml of water and 0.25ml of sterile defibrinated sheep blood will be added to 4.75 ml of Todd-Hewitt Broth.¹⁸

Processing of the sample

Among the two samples of the vaginal swab collected one sample will be used for microscopy and another for culture. Culture of the sample will be done in two ways, 1st vaginal swab without SBM, dull directly inoculated on 5% sheep blood Agar with a Bacitracin disc (0.04units) and incubated at 37°C in Candle jar for 24 hours. Another sample with transport medium. Initially, incubated at 37°C for 24 hours. If there will not be growth that plates will be further incubated for 24 hours.

Presumptive Identification of GBS

After 24-48 hours of incubation, the colonies from 5% sheep blood agar will appear as small, round, convex, soft, opaque, moist and gray. Some colonies will may show a zone of beta-hemolysis and some may not. These colonies will be further conformed by Gram's stain, catalase resistance to Bacitracin, CAMP test and finally by hippurate hydrolysis test.

Antibiotic susceptibility test²³

Antibiotic sensitivity test will be done on 5% sheep blood agar using Kirby-Bauer method.²³ The antibiotic discs used will be Amoxycillin (30 mcg/disc), Erythromycin (15 mcg/disc), Penicillin (10 units/disc), Gentamycin (10 mcg/disc), Chloramphenicol (30 mcg/disc), Tetracycline (30 mcg/disc) and Clindamycin (2 mcg/disc).

Data analysis

Data were entered into, verified in, and calculations performed with Microsoft Office Excel 2003, with SPSS-16.

Result

Among 422 samples collected and processed where 247 (58.5%) were culture positive, Group-B *Streptococci* isolates from antenatal mother was 36 (14.5%) and also other organisms were high as compared to Group-B *Streptococci*. Mostly *Staphylococcus aureus* 74 (29.9%) *Candida* species 66 (26.7%), Group-B *Streptococci agalactiae* 36 (14.5%), Gram negative bacilli (GNB) 32 (12.9%), Gram negative coccobacilli (GNCB) 28 (11.3%) and *Enterococcus* species 11 (4.5%). There was no growth in 175 (41.5%), among the sample cultured. In this study GBS was found to be highest in the age group between 17-27 years with the colonization rate of 83.33% as given in Table-1.

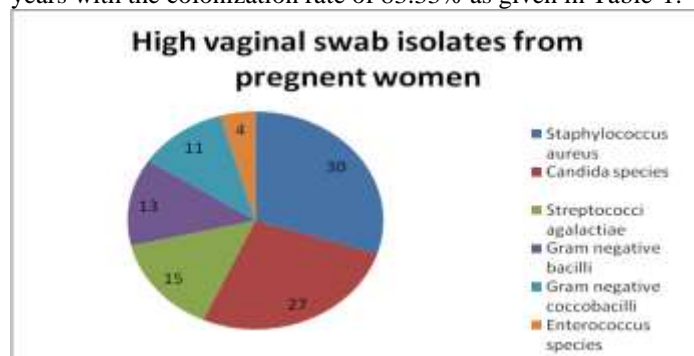


Table-1: Shows age wise distribution of GBS.

Age group	Positive	Percentage (%)
13-25	24	66.6
26-35	10	36.0
>36	2	5.4

The prevalence rate of GBS was found to be highest in the latter stage of pregnancy (between 29 to 36 weeks) with the percentage rate of (66.6%). The colonization rate of GBS in different period of gestation is shown in Table-2.

Table-2: Shows prevalence of Group- B *Streptococcus agalactiae* isolates in the 1st, 2nd and 3rd trimester.

Trimester	Positive	Percentage (%)
1-12 weeks	4	11.1
13-28 weeks	11	30.6
29-36 weeks	21	58.3

Table-3: Prevalence rate of GBS in primi and multigravida females.

Gravida	Positive	Percentage (%)
Primi gravid	14	38.8
Multi gravid	22	61.1

Table-4: Antibiotic susceptibility patterns of *S. agalactiae* isolates.

Sl. No.	Antibiotics	Sensitive	Moderate sensitive	Resistant
1.	Penicillin	36	-	-
2.	Ampicillin	36	-	-
3.	Erythromycin	36	-	-
4.	Chloramphenicol	-	36	-
5.	Clindamycin	-	36	-
6.	Gentamycin	-	-	36
7.	Tetracycline	-	-	36

All isolates of GBS were sensitive to Ampicillin, Erythromycin and Penicillin. Three strains were moderately sensitive to Chloramphenicol and Clindamycin. All strains were resistant to Gentamycin and Tetracycline.

Discussion

In all developed and developing countries Group-B Streptococci has become more prevalent day by day, which is an important etiological agent for neonatal sepsis and also responsible to cause many complication among pregnant women. In Nepal the problem is being studied in few tertiary care centers but not yet published in any index journal so magnitude of infection rate in general population is not yet clear.

The study provided information on the colonization rate of GBS in antenatal mothers in Central Nepal. It was found that the percentage rate of vaginal colonization by Group-B Streptococci in antenatal mother was 36(8.5%). This value is low when compared to other reports as in India (5.8%) and quite different from those found in Saudi Arabia (13.9%), Nigeria (19.5%), USA (20.4%) and Gambia (22.0%).

However, the prevalence of 8.5% in the present study is similar to the findings of Kulkarni et al. They screened a total of 317 pregnant women in the Department of Microbiology of Government Medical College, Miroj, Maharashtra and reported the colonization rate of GBS to be 2.52%. The low rate of vaginal colonization in Central Nepal to other part of the world may be associated with differences in variation in the strain, their distributions, genetic constitution of the people and environmental factors.

Studies done elsewhere have estimated colonization rate by Vaginal and rectal culture. In contrast, single vaginal culture was done in the study. Besides this, less sample size and the less number of samples per pregnant women could also be the reason of the low prevalence rate of GBS colonization in the antenatal mother in Nepal.

Others studies have shown that early to mid-gestation screening cultures do not correctly identify all women, who are positive for GBS at delivery. The cultures performed in later pregnancy have shown better. This statement is supported by the finding that 66.6% of antenatal mothers in this study were GBS carriers in the later week of their pregnancy. One of the study estimated that a single positive GBS culture at 26-28 weeks of the gestation predicted carriage at delivery with sensitivity of 70% and specificity of 90% while 26 women, whose prenatal cultures were obtained within 5 weeks of delivery showed results of 100%.

In the present study, among the risk factors, it was found that higher percentage of GBS positive mothers (83.33%) were in between the age group 17-27 years. This finding is alarming because of the fact that the colonization rate is highest in pregnant women less than 20 years of age. Apart from this most of the positive mothers were of low socio-economic status. In this study, the yield of GBS was highest with the use of selective medium. SBM (Todd-Hewitt broth with sheep blood, Nalidixic acid and Gentamycin). Out of six isolates, four of them were isolated upon use of the selective medium.

Our findings seem to suggest that a quarter of pregnant women attending ANC clinic at MNH and approximately 10% of their newborns are colonized with GBS. All isolates of GBS were sensitive to Ampicillin, Erythromycin and Penicillin. Three strains were moderately sensitive to Chloramphenicol and Clindamycin. All strains were resistant to Gentamycin and Tetracycline. However there is a need for continuous antibiotics surveillance of GBS to monitor trend of resistance. The high

isolation frequency of GBS among pregnant women suggests routine antenatal screening at 35 to 37 weeks of gestation in order to provide antibiotic prophylaxis to GBS carrier.

Conclusion

Overall study in Central Nepal showed low colonization rate of GBS in the vagina of antenatal mothers. Neonatal infection was not evident because of the low colonization of GBS in the mothers vagina. This finding is also supported by the fact that the fetal neonates colonization with GBS only 1-2% develops clinically apparent disease. Intrapartum antibiotic therapy of women colonized with Group-B Streptococcus can decrease neonatal infection. Effective strategies to detect maternal colonization with GBS infection needs to be studied properly in different populations of the world.

Author's contribution

BJ himself received sample processed and identified the organism. KP did all microscopy and culture where as SM is expert in data maintenance and statistical analysis by SPSS.

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This research work was carried out as a routine examination from antenatal mothers in central Nepal, College of Medical Sciences, Bharatpur. There is no objection to publish this article and none of conflict of interest for research.

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