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Occurrence of Streptococcus agalactiae serogroups in isolates from pregnant women of Peshawar

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ABSTRACT

Introduction: Maternal rectovaginal colonization during pregnancy by Group B *Streptococcus agalactiae* (GBS) is one of the major preventable and easily recognizable causes of neonatal morbidity and mortality but in Pakistan, GBS is inadequately explored.

Objectives: To estimate the frequency of recto-vaginal GBS colonization and its serogroup distribution among pregnant women in their 3rd trimester presenting to antenatal clinics of Hayatabad Medical Complex (HMC), Peshawar.

Materials & Methods: This descriptive cross-sectional study was conducted at HMC from October 18, 2019, to February 05, 2020, through serial sampling of 220 pregnant women. Two swabs, one from vagina and the other from anorectal region were taken. The samples were transported to the laboratory in Amies transport medium, first inoculated in Todd-Hewitt broth and then subcultured on 5% sheep blood. After gram staining and microscopy, gram positive cocci were examined for catalase production and Lancefield latex agglutination to confirm GBS. The confirmed GBS were serogrouped by using Immulex Strep-B kit (54991, SSI DIAGNOSTICA, Denmark). A structured questionnaire was used to record data. Data were analysed using SPSS version 22 for descriptive statistics.

Results: The GBS maternal colonization was found in 22(10%) cases. The serogroups identified among the GBS positive cases were Ia, Ib, II, III, VI and IX. No untypeable GBS strain was isolated. The identified strains were serogroup II (13, 59.09%), Ia (10, 45.45%), Ib (05, 22.72%), III (01, 4.54%), VI (01, 4.54%) and IX (01, 4.54%). In 6 cases the same serogroups were identified, while in one case 3 different serogroups were isolated (both from vagina and rectum).

Conclusion: Favorable conditions exist for the occurrence of invasive GBS disease in pregnant women in Peshawar.

Keywords: Pregnant Women; Rectum; Vagina; Serogroup; *Streptococcus agalactiae*.

The authors declared no conflict of interest. All authors contributed substantially to the planning of research, data collection, data analysis, and write-up of the article, and agreed to be accountable for all aspects of the work.

INTRODUCTION

Streptococcus agalactiae is a Gram positive, catalase negative, β-hemolytic facultative anaerobe. Due to the presence of Lancefield group B antigen, it is also called group B streptococcus (GBS). GBS may be found as part of normal gut microbiota in healthy adults;[1-2] however, it is the most common source of maternal, fetal and newborn GBS disease due to vaginal colonization.[3,5]

Globally, *S. agalactiae* is one of the major preventable causes of neonatal morbidity and mortality.[1,5] The true burden of maternal GBS colonization and serogroup distribution for many countries including Pakistan is unknown.[6] There are significant regional variations globally, rendering the suitable vaccine production difficult.[7,8] There seems to be a true difference in the prevalence of Group B Streptococcus colonization across the world, which can be due to regional variation.[9] A meta-analysis involving 85 countries estimated global maternal GBS colonization rate of 18% with regional variation of 11-35%, the lowest for Eastern Asia (11%), Southern Asia (12.5%), and highest for the Caribbean (35%), 20% for Pakistan, 10% for India, and 11% for both China and Bangladesh.[10] In another hospital based study carried out at Aga Khan Hospital, Karachi, colonization was 17%.[1] Globally, 98% of GBS isolates belong to serogroups I-V, 25% to serogroup III, while serogroups VI-IX are being frequently isolated from Asia.[10] No such study has been carried out in Khyber Pakhtunkhwa (KP), Pakistan.

Pregnancy is associated with a doubled risk of GBS infection, and a 20-fold increased risk of invasive disease in puerperium.[11] The risk of peripartum infection increases after cesarean section, except for GBS meningitis occurring exclusively after vaginal delivery.[11] GBS colonization during pregnancy can lead to maternal urinary tract infection, chorioamnionitis, preterm rupture of membranes (PROM), preterm labor, bacteremia, postpartum endometritis, and perinatal fetal transmission.[11] The intrauterine infection is due to ability of GBS to ascend up the genital tract.[12,13]

In infants, GBS disease can present either as early onset diseases (EOD) that occurs within 6 days of birth or late onset disease (LOD) occurring between 7-90 days of birth. Maternal colonization is prerequisite for early onset disease while a risk for late onset disease. A strong association has been found between intrapartum colonization with GBS and development of early onset disease which can present as infant death, sepsis, pneumonia, meningitis, neonatal encephalopathy with long term disabilities.[14,15]

In order to reduce neonatal GBS associated morbidity and mortality, the Centers for Disease Control and Prevention (CDC) has recommended recto-vaginal screening of all pregnant females at 35-37 weeks of gestation.[16] Group B Streptococcus colonization in the later half of pregnancy is more reliable in predicting Group B streptococcus colonization of vagina during labor, leading to increased risk of invasive disease in the neonates[17] The use of intrapartum antibiotics has reduced early onset disease (EOD) in several countries.[18] Due to regular screening of pregnant mothers for group B streptococcal colonization at 35-37 weeks of pregnancy along with prophylactic use of antibiotics during intrapartum period there has been significant decrease in the occurrence of group B streptococcal disease among the neonates. However, there is no change in rates of maternal *Streptococcus agalactiae* colonization since 1970s and hence the risk of developing early onset GBS disease in the newborns in the absence of prophylactic antibiotics persists during the intrapartum period. Maternal vaccination during the late second or early third trimester of gestation would be effective in prevention of maternal GBS disease.[18,19] The production of suitable vaccine requires a comprehensive study across the continents for better understanding of GBS global variation in colonization and serogroup distribution.

In Pakistan, neonatal mortality, post neonatal mortality, and infant mortality were found to be 42, 20 and 62 per 1000 live births respectively.[20] It is unfortunate that in a country with one of the highest infant & maternal mortality rates no planning has been done to curb an easily identifiable and preventable cause of this mortality. Therefore, this study was carried out to estimate the burden of GBS, determine its antimicrobial sensitivity and identify prevalent serogroups. It is expected to inform evidence-based policy making and clinical management of GBS.

MATERIALS & METHODS

It was a cross-sectional descriptive study conducted from October 18, 2019, to February 5, 2020. The sample size calculated by OpenEpi-Info was 220. Non-probability serial sampling strategy was used to collect data of all pregnant women in third trimester presenting at ante-natal clinic of Hayatabad Medical Complex Peshawar, before the onset of labor. Women with history of antibiotic intake within 2 weeks prior to recruitment and any reported recto-vaginal pathology were excluded.

Vaginal and anorectal swabs were collected from each participant and transported to laboratory in Amies transport medium. Samples were inoculated in Todd-Hewitt for 24 hours at 37ºC in 5% CO_2. Medium showing no turbidity was incubated for another 24 hours. Subculture on 5% Sheep Blood Agar was done to obtain pure GBS colonies with incubation for 24 hours. If there was no growth after 24 hours then culture was incubated for another 24 hours before declaring it negative for growth. If growth was obtained then colonies were noticed for beta hemolysis. Gram staining and conventional microscopy was carried out for Gram positive cocci in chains. A negative catalase test was used to confirm the presence of Gram-positive streptococci. Latex agglutination kit for Lancefield grouping was used to confirm GBS. GBS serogrouping was done for capsular polysaccharide antigens I-IX by using GBS serogrouping kit.

Ethical approval was obtained prior to data collection from the Ethical Committee of Khyber Medical University. Prior approval to collect the specimens from the subjects was taken from the administration of Hayatabad Medical Complex, the heads of all four Gynecology & Obstetrics units & the Microbiology unit of the hospital. Informed written consent was taken from all the subjects. Patients' demographics were entered in a structured Performa to which a specimen number was given for patient's privacy. To assure confidentiality, the data were stored in a password protected computer.

Data were analyzed for descriptive and comparative statistics using SPSS version 22, with $p \leq 0.05$ considered significant.

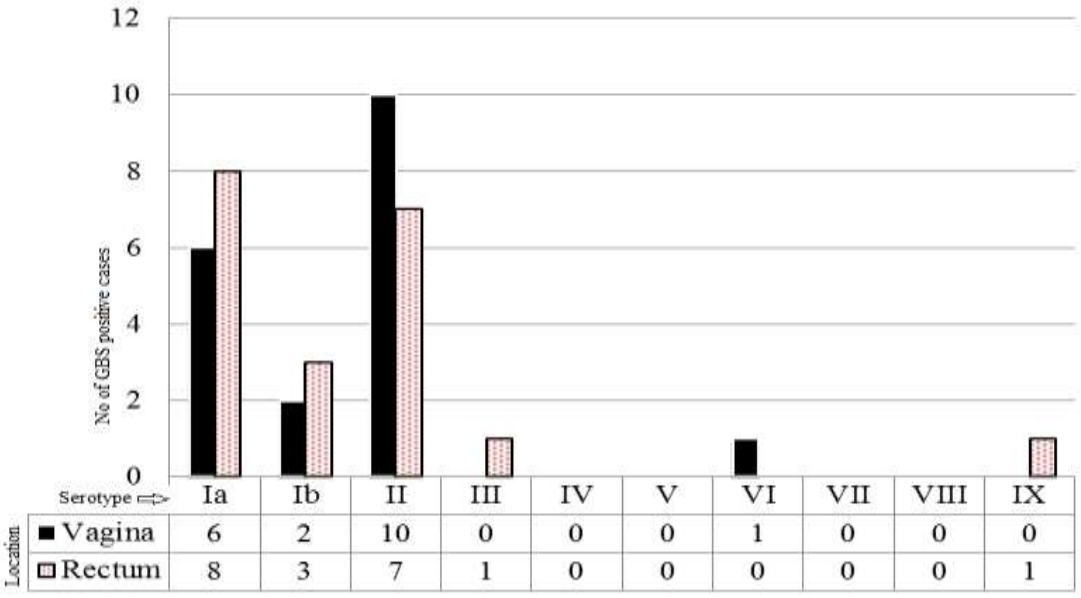
RESULTS

A total of 220 pregnant women who visited the selected hospital were included; 194(88.2%) were Pakistani and 26(11.8%) were Afghani nationals. Out of the total 220 subjects, 144(65.5%) belonged to rural areas, 53(24.1%) to Peshawar and 23(10.4 %) to cities other than Peshawar. The mean age of participants was 28 ± 6.58 years. The women participating in this study were in the range of G1 to G12 with maximum number, 53(24.1%) being primigravida. The GBS maternal colonization was found in 22(10%) subjects. Of the 22 GBS positive cases, 20(90.9%) were Pakistani and 02(11.1%) were Afghans. The mean gestational age of GBS positive cases was 35.95 ± 2.12 weeks. Out of these positive cases 07(31.81%) women carried GBS in both their vagina and rectum; maternal GBS colonization of vagina only was found in 07(31.81%) cases and in 08(36.4%) women the GBS was isolated only from rectum (Table 1). No GBS positive case was found below 20 years.

Table 1: Isolation of GBS from samples (n=220).

Characteristics	Frequency (%)
Total cases containing GBS	22 (10)
Cases with GBS in Vagina & Rectum	07 (31.8)
Cases with GBS in Vagina only	07 (31.8)
Cases with GBS in Rectum only	08 (36.4)

The serogroups identified among the GBS positive cases were Ia, Ib, II, III, VI and IX. No Untypeable GBS strain was isolated. As shown in Figure 1, the frequencies of the identified serogroups were serogroup II, 13(59.09%), Ia, 10(45.45%), Ib, 5(22.72%), and III, VI, and IX 01(4.54%) each. In 6 cases, the same serogroups were identified in both vagina and rectum, these were Ia (3), Ib (1) and II (2). In one case 3 different serogroups were isolated from vagina and rectum (serogroups II and VI were found in vagina and Ia was found in rectum). Out of the 22 GBS positive samples 2(9%) of the *S. agalactiae* cultured on 5% sheep blood agar were found to be non- hemolytic.



Location	Serotype	Ia	Ib	II	III	IV	V	VI	VII	VIII	IX
	Vagina	6	2	10	0	0	0	1	0	0	0
	Rectum	8	3	7	1	0	0	0	0	0	1

Figure 1: GBS serogroups identified in study population

Figure 2 shows the distribution of maternal age in patients with GBS. Most of the GBS positive cases were from the age group of 30-34 years.

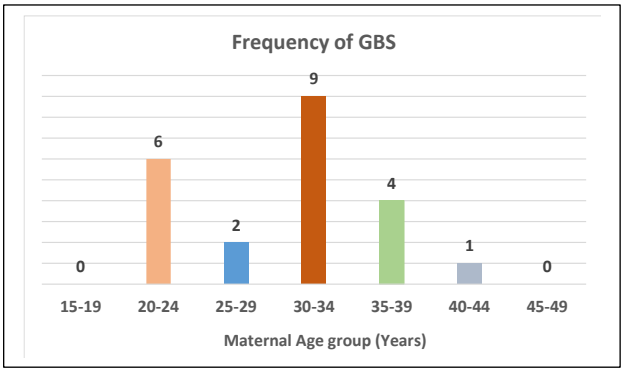


Figure 2: Maternal age wise distribution of GBS cases (n=22).

Figure 3 shows the regional distribution of GBS patients; 12 (54.5%) cases belonged to rural community while 8 (36.4%) to Peshawar and 2 (9.1%) to cities other than Peshawar. The frequency of positive cases was higher i.e. 15% among women belonging to Peshawar when compared to those from other cities (9%) & rural communities (8%). The difference, however, was statistically not significant ($p= 0.643$).

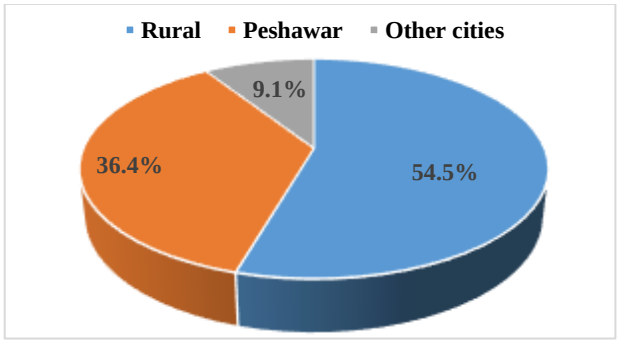


Figure 3: Distribution of GBS cases in rural and urban areas (n=22).

The duration of marriage among the GBS positive cases was between 1-17 years. Thirteen (13) GBS positive women presented up to first 7 years of marriage (Figure 4).

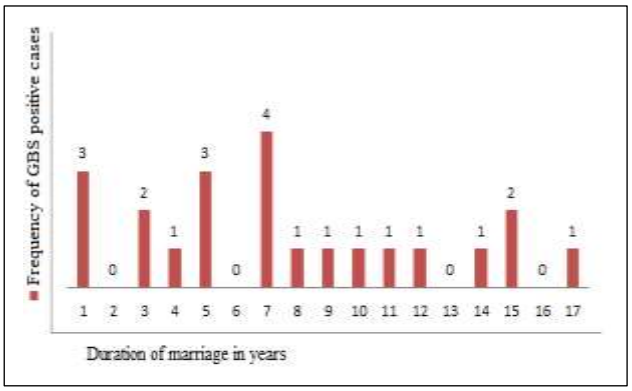


Figure 4: Distribution of GBS by duration of marriage in years (n=22).

History of abortion was more common (32%) among GBS positive women as compared to negative ones (28%).

DISCUSSION

In the current study, maternal GBS carriage rate of 10% was detected. Combined rectovaginal colonization was found in 31.8% of GBS positive women. The findings stress the need of both rectovaginal sampling of every pregnant woman in the third trimester for obtaining a true picture of GBS maternal colonization as is also recommended by CDC in its revised guidelines of 2010.[16] The results of the study correlate well with a study carried out at Rawalpindi, Pakistan stating a GBS maternal carriage rate of 8.5%.[21] The slightly lower carriage rate may be due to omission of rectal sampling in the study. Another study carried out in Karachi reports a higher GBS carriage rate of 17%.[1] The difference in GBS carriage could be due to geographical and ethnic variations. Studies conducted in India, Bangladesh and China have reported similar carriage rates of GBS of 10%, 11%, & 11% respectively.[4] Iran and Japan have reported to have a higher figure of 16% each.[22,23]

There were 6 serogroups identified in this study among the GBS positive pregnant women, these serogroups were Ia, Ib, II, III, VI and IX. Interestingly, no Untypeable GBS serogroup was identified in this study. These findings relate well (with the exception of serogroup V which was not detected in this study) with the findings of a meta-analyses carried out involving 85 countries and a total of 299924 women with pregnancy, declaring serogroups Ia, Ib, II, III and serogroup V to be responsible for making 98% of world serogroup distribution with regional variations in the prevalence of these serogroups.[4] The frequency of the most virulent serogroup III with well-established association with invasive GBS disease in our study was lower (4.54%) than the global prevalence of 25%, it was also lower than that of India and Bangladesh with prevalence of 11% each.[4] A study carried out at Mirzapur, Bangladesh stated the frequency of Ia to be 40%[24] which is comparable to finding of the current study with the Ia frequency of 45.45%. This study shows marked difference from the study carried out in Western Cape region of South Africa[5] reporting the predominant serogroups to be V (66.67%) and III (21.05%). However, the frequency of serogroup IX on the other hand is almost similar in both the studies. These conflicting results of the two studies might be explained on the basis of racial, environmental and geographic differences between the maternal population of Pakistan and South Africa. A systematic review on maternal GBS colonization in European countries reported the serogroups III, II and Ia to be most frequently detected.[10] The current study also shares the same findings except for GBS type III which was found to be among the least frequent (4.54%) serogroups detected. The identification of the existing GBS serogroups among pregnant women of this study is very significant, as with the exception of serogroup V, the serogroups Ia, Ib, II and III have been reported to be associated with GBS disease.[25]

No GBS positive case was found below the age of 20 years and above the age of 40 years, highlighting the occurrence of GBS maternal carriage during the period of active reproductive life, hence stressing upon the need to screen every pregnant woman in her last trimester for GBS carriage.

Furthermore, the study findings regarding rural and urban contexts of maternal GBS carriage correlate well and reinforce the findings of a meta-analysis by Russell et al[10] that documented comparability of maternal GBS colonization among rural and urban community. Interestingly, though the duration of marriage of GBS positive cases ranged between 1 to 17 years, maximum number of women (59%) with GBS carriage was found to be in their initial 7 years of marriage, and 3 women of the total GBS positive colonization mothers presented at their 1st year of marriage; these findings of this study further highlight the importance of GBS screening among the women in their active reproductive life. This study also confirmed the findings of previous studies i.e. association of abortions & stillbirth with GBS carriage among pregnant women.[26] Out of the total 22 GBS positive cases 31.8% had a previous history of abortion, and 4.5% each had a history of stillbirth and PROM.

CONCLUSION

Invasive GBS disease is likely a public health concern in Khyber Pakhtunkhwa and mandates maternal GBS screening for rectovaginal colonization in the third trimester for prophylactic use of antibiotics during labor in positive cases.

LIMITATIONS

The study was conducted at a single tertiary care hospital of Peshawar. Additionally swabs for GBS detection could not be taken from both the mother and baby at the time of birth so as to ascertain the degree of neonatal GBS vertical transmission. Another limitation of this study was not performing antibiotic sensitivity testing of the isolated GBS due to inability to get ATCC *Streptococcus agalactiae* strains and financial constraints.

RECOMMENDATIONS

For better understanding of the local GBS disease dynamics, a multicenter well planned and coordinated, comprehensive, multifaceted research project throughout Pakistan involving microbiologists, immunologists, gynecologists, neonatologists, pediatricians and epidemiologists is recommended. Maternal GBS screening should be mandated in the investigations of maternal bad obstetric history. It is further recommended to launch a public awareness campaign regarding the importance of maternal GBS screening.

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