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Original Article

Comparison of Urine Specimen with Recto-Vaginal Swab to establish carriage of Group B *Streptococcus* Colonization in Pregnant Females

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ABSTRACT

Objective: To determine maternal vaginal colonization with Group B *Streptococcus* using both recto-vaginal swab and mid-stream urine in order to determine which specimen type can give better results.
Study Design: Cross-sectional study.
Place and Duration of Study: Armed Forces Institute of Pathology, Rawalpindi Pakistan, Department of Gynecology and Obstetrics, Combined Military Hospital and Pak Emirates Military Hospital, Rawalpindi Pakistan, from Jan to Jun 2022.
Methodology: Recto-vaginal swabs and mid-stream urine were taken from 194 pregnant women between 36th and 37th weeks of pregnancy. Swabs were inoculated on blood and MacConkey agar while urine specimen was inoculated on blood and CLED agar. All plates were incubated at 37°C in either ambient air or CO_2 incubator. Identification of Group B *Streptococcus* was made on the basis of colony morphology (β-haemolytic colonies on blood agar), Gram stain, catalase test and confirmation were done by means of latex agglutination tests.
Results: In comparison to urine, a Group B *Streptococcus* carriage rate of 8.2% was detected in recto-vaginal swab. Urine specimen of only 3 patients yielded growth of Group B *Streptococcus* giving a urine carriage rate of 1.5%.
Conclusion: Recto-vaginal swab gives a better insight to the presence of Group B *Streptococcus* as compared to mid-stream urine.

Keywords: Group B Streptococcus, Recto-vaginal swab, Streptococcus agalactiae, urine.

How to Cite This Article: *Chaudary NN, Mirza IA, Khurshid U, Muhammad AI, Gullalai S, Naqvi SH. Comparison of Urine Specimen with Recto-Vaginal Swab to establish carriage of Group B Streptococcus Colonization in Pregnant Females. Pak Armed Forces Med J 2024; 74(5): 1355-1359.*
DOI: https://doi.org/10.51253/pafmj.v74i5.10382

INTRODUCTION

Streptococcus agalactiae, also known as group B *Streptococcus* (GBS), a Gram positive, catalase negative cocci was first differentiated from other Streptococci by Rebecca Lancefield in the 1930s.[1] Lancefield also described the presence of GBS as a colonizer of the vaginal tract of asymptomatic women in child bearing age.[2]

GBS can be found in about 15-40% of healthy women's colon and vagina and may not cause any symptoms in a majority of the cases.[3] During pregnancy, GBS can be the causative agent of various maternal infections such as chorioamnionitis, maternal sepsis, cystitis or endometritis. GBS may also lead to cesarean delivery and postoperative wound infections in some patients.[4] Some cases have also reported about GBS contributing to preterm delivery.[5] Transmission to the newborns may occur in colonized pregnant women during passage through the vaginal tract. Ascending infections have also been reported during pregnancy or in settings of premature rupture of membranes in colonized females leading to fetal and maternal complications.[6,7] Some reports on the various aspects of neonatal infections caused by this organism from the USA as well as other developed countries have demonstrated it to have replaced staphylococcus as the leading Gram-positive organism in neonatal nurseries.[8] GBS can be responsible for severe infection in infants younger than 3 months of age, presenting with various clinical manifestations such as sepsis, pneumonia and meningitis, and early onset disease is acquired from GBS colonized mother.[9,10]

Recommendations from various institutes such as American college of obstetrics and gynecology (ACOG) and the CDC include GBS screening of pregnant females as well as intrapartum prophylactic antibiotics to prevent early-onset neonatal infection. Paucity of local data on the topic forms the rationale for our study.

METHODOLOGY

This Cross-sectional study was performed at the Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi for a period of six

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Received: 20 Mar 2023; revision received: 05 Jul 2023; accepted: 10 Jul 2023

months after taking approval from Institutional Review Board (vide reference no. FC-MIC19-5/READ-IRB/23/1792).

Inclusion criteria: Pregnant females aged 18 to 40 years, in their 36th–37th week of pregnancy were included.

Exclusion criteria: Females with history of vaginal bleeding, ruptured membrane, recent intake of antibiotics or chronic illness were not included in the study. Patients with positive GBS bacteriuria anytime during current pregnancy or having a previous GBS infected newborn were not excluded.

We calculated a sample size of 194 via the WHO calculator, keeping the previous prevalence at 14%,[11] which came to 194. Data was collected using non-probability consecutive sampling. Two samples were taken from each patient after obtaining written informed consent, a combined recto-vaginal swab and a fresh mid-stream urine. Combined recto-vaginal swab were taken using a single swab to obtain culture specimen first from lower vagina, near the introitus, without using any speculum and then from the lower rectum, through the anal sphincter. Amies Agar Gel Transport Swab manufactured by Thermo Fisher were used. The swabs were made of rayon. Patients were instructed on how to collect fresh urine sample themselves. Both samples were transported to microbiology department of Armed Forces institute of pathology, Rawalpindi at room temperature as early as possible with a maximum transport time of 2 hours. Recto-vaginal swabs were inoculated on 5% sheep blood agar and MacConkey agar (Oxoid, UK) plates using 4-quadrant streaking technique. Urine samples were inoculated on 5% sheep blood agar and CLED agar (Oxoid, UK) plates using fish-bone streaking technique. Blood agar plates were incubated in CO_2 incubator while MacConkey and CLED agar plates were incubated in ambient air at 35+/-2°C for 24 hours. Plates not showing any growth were further incubated for another 24 hours before being reported as no growth. Any growth encountered on each of the plates was noted. Any colony resembling *Streptococcus* spp. with a zone of beta hemolysis on BAP was gram stained. Catalase test was performed. Confirmation of identification was done via latex agglutination test using Streptococcal Grouping Kit manufactured by Thermo Fisher.

Patients testing positive for colonization of GBS on either recto-vaginal swab or urine sample were informed about carriage of GBS and given guidance about intrapartum GBS prophylaxis.

Data was analyzed by using Statistical Package for the social sciences (SPSS) version 23. Mean±SD was calculated for continuous variables. Frequency and percentage was calculated for categorical variables. Fisher's exact test was for the comparison of GBS. The *p*-value less than or equal to 0.05 was considered statistically significant.

RESULTS

A total of 194 pregnant females were sampled, mean age of the patients was 28.87±4.29 years range from 19 to 39 Years. The mean gestational age was 36.49±0.51 weeks range from 36+0/7 to 37+6/7 weeks.

Out of 194 Recto-vaginal swabs, 16(8.2%) samples yielded positive culture for GBS, other organisms isolated included *Escherichia coli*(n=6, 3.1%), *Klebsiella pneumoniae* (n=10, 5.2%), *Enterococcus* spp (n=1, 0.5%), *Proteus mirabilis* (n=4, 2.1%), *Pseudomonas aeruginosa* (n=3, 1.5%), Mixed growth of Enteric GNRs was seen in 99(51.0%) samples and 55(28.4%) samples yielded no growth (Figure-1).

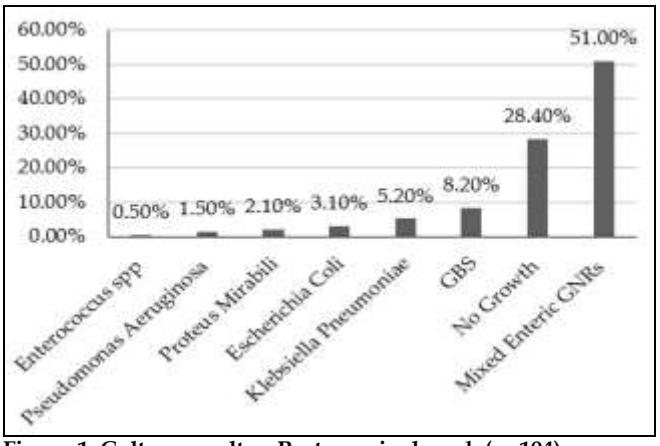

Figure-1: Culture results – Recto-vaginal swab (n=194)

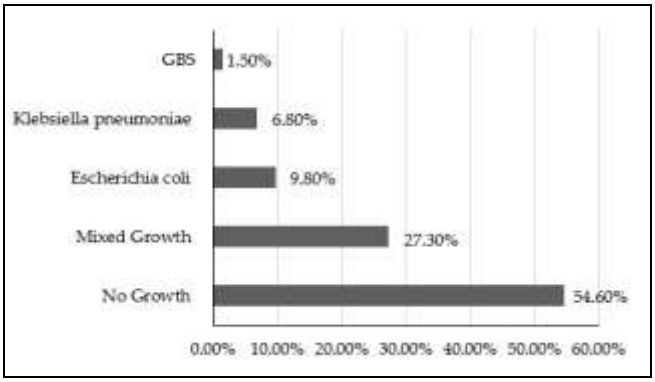

Figure-2: Culture - Urine (n=194)

Out of 194 urine cultures, 3(1.5%) samples yielded growth of GBS. All of these 3 patients yielded growth of GBS in their recto-vaginal swabs as well. Other organisms isolated included *Escherichia coli*(n=19, 9.8%), *Klebsiella pneumoniae* (n=13, 6.8%), Mixed growth of Gram-negative rods (n=53, 27.3%) while 106(54.6%) samples did not yield any growth (Figure-2).

Overall, 16(8.2%) recto-vaginal swab cultures were positive for growth of GBS. Out of these 16, urine culture of 3(1.5%) patients also yielded growth of GBS. Recto-vaginal swabs detected GBS more frequently as compared to urine cultures as *p*-value <0.001 shown in Table-I.

Table-I: Group-B *Streptococcus* (Recto-vaginal swab in comparison with Urine Culture) (n=194)

Urine	Recto-vaginal swab		*p*-value
	Group-B Streptococcus	Non Group-B Streptococcus	
Group-B Streptococcus	3(18.8%)	0%	<0.001
Non Group-B Streptococcus	13(81.3%)	178(100.0%)	

Keeping both specimen in consideration, 3(1.5%) cultures of both recto-vaginal swab and urine yielded growth of GBS, followed by *Klebsiella pneumoniae* (n=2; 1.0%), mixed growth (n=28; 14.4%) and no growth (n=38: 19.6%) with significant *p*-value <0.001 shown in Table-II.

We wanted to compare results of recto-vaginal swab and urine cultures to determine which sample can serve as a better indicator of GBS colonization when they´re taken at the same time.

All isolated GBS gave small β hemolytic colonies on blood agar plates. All were Gram positive cocci in chains, catalase negative and group B Lancefield positive by serological testing. Different methods to isolate and detect GBS have been recommended in studies which include use of GBS agar and GBS broth, rapid testing methods such as PCR and GeneXpert have also been employed.[14-15]

We found a GBS carriage rate of 8.2% in the recto-vaginal swab. While urine specimen gave a GBS carriage rate of only 1.5%. All the patients with positive GBS growth in their urine cultures also yielded growth of GBS in their recto-vaginal swab. Self-collection of urine sample may contribute as a factor leading to low recovery of the organism. Although submitting a sample that can be self-collected seems more acceptable to patients in general and giving a sample such as recto-vaginal swab may come with certain apprehensions but our study has indicated that taking a recto-vaginal swab to determine colonization of GBS is better than relying on urine cultures only.

Carriage rate of GBS in our study was consistent with a study conducted in Rawalpindi, Pakistan.[17] A study conducted in Karachi in 2007 detected GBS by taking high vaginal swabs of pregnant women in 35-37 weeks of pregnancy and found a prevalence of 17%.[18] It is worth mentioning at this stage that very few studies have been conducted on this topic in our population and limited recent data is available.

Table-II: Comparison of Organisms Isolated in Recto-Vaginal Swabs and Urine Cultures (n=194)

Culture – Urine	Culture - Recto-vaginal swab								*p*-value
	E. coli	*Enterococcus spp*	GBS	KP	Mixed enteric GNRs	No growth	Proteus mirabilis	*P. Aeruginosa*	
E. coli	0%	0%	2(1.0%)	0%	13(6.7%)	3(1.5%)	0%	1(0.5%)	<0.001
GBS	0%	0%	3(1.5%)	0%	0%	0%	0%	0%	
KP	0%	1(0.5%)	0%	2(1.0%)	6(3.1%)	3(1.5%)	1(0.5%)	0%	
Mixed Growth	3(1.5%)	0%	6(3.1%)	3(1.5%)	28(14.4%)	11(5.7%)	1(0.5%)	1(0.5%)	
No Growth	6(3.1%)	0%	5(2.6%)	5(2.6%)	52(26.8%)	38(19.6%)	2(1.0%)	1(0.5%)	

DISCUSSION

In our study, recto-vaginal swabs were collected along with urine specimen from women in their 36th – 37th week of pregnancy. Only patients willing to give both specimens were included in the study. Samples were collected in recommendations of the most recent ACOG guidelines from lower rectum and lower vagina near the introitus without using any speculum with the same swab.[13] According to guidelines, patients with positive GBS colonization either on recto-vaginal swab or on urine cultures are regarded as candidates to receive intrapartum GBS prophylaxis.

GBS colonization rate was found to be 27.6% amongst full term pregnant women in a study conducted in Kingdom of Saudi Arabia indicating a very high risk of transmission to their newborns resulting in early neonatal meningitis or sepsis.[19] Another study studied 245 pregnant women and found a colonization rate of 9.7% GBS in rectovaginal swab by culture.[20] Another study conducted in Turkey

isolated GBS in 8.7% of pregnant females. This study included rectal, vaginal and cervical swabs of 114 pregnant women.[21] The sampling techniques as well as results of these studies are in agreement to the outcomes of our study, indicating an accuracy of our sampling technique as well as our lab practices

Our study focused on the carriage rate of GBS in pregnant females. We did not screen these female´s newborns to see how many of them were born with GBS colonization but the patients were given complete guidance about intrapartum GBS prophylaxis. Various studies have determined GBS carriage rate of mothers as well as their newborns to determine how many neonates born to GBS colonized mothers are also colonized by the pathogen.[17] It is said that neonates are colonized only briefly after rupture of membranes and lower bacterial load makes isolation by culture more difficult.[22]

The most important risk factor in early onset neonatal disease due to GBS is maternal colonization at the time of delivery.[23] A study conducted on neonatal sepsis caused by GBS identified GBS in 14.1% positive blood cultures with majority causing early onset neonatal disease.[24] Implementation of screening programs is vital in timely identifying GBS carriage in mothers so that intra partum prophylaxis may be administered to all these women.[25]

CONCLUSION

Recto-vaginal swab can serve as a better specimen to indicate GBS colonization as compared to urine culture. GBS screening programs need to carried out at national level to diagnose and prevent neonatal early onset GBS disease.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

NNC & IAM: Data analysis, drafting the manuscript, critical review, approval of the final version to be published.
UK & AIM: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.
SG & SHN: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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