Annals of Microbiology and Infectious Diseases
Volume 4, Issue 1, 2021, PP 01-06
ISSN 2637-5346
https://doi.org/10.22259/2637-5346.0401001

Epidemiology and Capsular Typing of Group B Streptococcus in Pregnant Women in Port Harcourt, Nigeria

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ABSTRACT

Background: *Colonization of the lower vagina and anorectum of pregnant women by group B streptococcus (GBS) in the third trimester is a risk factor for early neonatal disease. This study was carried out to determine the prevalence of rectovaginal colonization and capsular type distribution of GBS among pregnant women in Port Harcourt, Nigeria.*

Methods: *Group B streptococci were isolated and identified from vaginal and anorectal swabs using CHROMagar, CAMP test and latex agglutination while capsular typing was done using multiplex PCR.*

Results: *The prevalence of colonization was 18.9%. The capsular polysaccharide types detected by multiplex PCR were Ia, Ib, II, III, IV and V. Capsular types V and Ia were the most frequently occurring among the isolates at 32.6% and 21.7% respectively. While types IV and Ib were the least in frequency (8.7% and 2.2% respectively). A history of urinary tract infection and douching were identified by multiple logistic regression as risk factors for colonization.*

Conclusion: *Lower vaginal and anorectal colonization by group B streptococcus in late pregnancy is significant in this population. The capsular types Ia, Ib, II, III, IV and V, are similar in their occurrence and distribution to other reports from different parts of the world. Nigeria, therefore, would benefit from existing vaccine efforts, targeting the identified capsular types.*

Keywords: *Group B streptococcus, capsular type, colonization, pregnant women, prevalence*

INTRODUCTION

Group B streptococcus colonizes the vagina and rectum of approximately 18% of pregnant women globally [1]. These colonized women are at risk of vertically transmitting this organism to their babies during late pregnancy and or during delivery [2]. About 1-2% of neonates born to colonized mothers develop GBS early onset neonatal disease [3] which may be fatal or leave the babies with long term disabilities [4]. In the 1970s and 1980s, Group B streptococcus (GBS) was reported to be the leading cause of neonatal sepsis and meningitis in the United States [5]. However, with implementation of intrapartum antibiotic prophylaxis in the early 1990s, the incidence of GBS early onset disease (EOD) declined remarkably [6]. In Nigeria, a few studies performed have reported prevalence rates of maternal rectovaginal GBS colonization of between 11 and 34% in different regions of the country [7–10]. Although the exact burden of EOD in Nigeria is unknown, neonatal sepsis and meningitis remain serious public health issues in Nigeria. Although it is believed that the burden of GBS early onset disease is underreported and may largely be under estimated, there is still the need to explore every preventive means possible to reduce its incidence [11,12]. There is also the need to establish the prevalent GBS capsular types in this region in consideration of the ongoing efforts to develop a vaccine that will be effective in preventing up to 85% of GBS sepsis of both EOD and late onset disease (LOD)[13]. To investigate the epidemiology of GBS maternal colonization, a cross-sectional study was performed on pregnant women in their third trimester to calculate the prevalence of maternal colonization by the organism and to identify the capsular types in this region.

METHODS

Vaginal and rectal swabs were obtained from 185 pregnant women in their third trimester receiving antenatal care at the University of Port Harcourt Teaching Hospital between March and July 2017. Women who declined consent or were on any antibiotic therapy at the time of the study or within the preceding 4 weeks were excluded. Epidemiologic data were obtained using interviewer-administered structured questionnaires.

Specimen collection was based on the standard operating procedures of the Centres for Disease Control and Prevention (CDC)[6], the vaginal introitus was swabbed gently with a cotton swab stick after parting the labia. A second cotton swab stick was gently introduced 2-3cm into the anorectum and gently rotated. Each swab stick was inoculated into a tube containing 5 ml of Lim broth and incubated aerobically at 35-37°C for 18-24 hours. The incubated broth was then subcultured onto CHROMagar StrepB (CHROMagar, Paris, France) chromogenic medium and incubated aerobically at 35-37°C for another 18-24 hours. *S. agalactiae* were presumptively identified as small, mauve-coloured colonies on CHROMagar StrepB and confirmed by a positive CAMP test on 5% sheep blood agar and group B specific latex agglutination using the Remel™ PathoDx™ Strep Grouping kit (ThermoFisher Scientific, Waltham, MA USA). A participant was said to be colonized if *S. agalactiae* was isolated from her vagina, rectum or both.

Capsular typing by multiplex PCR was performed for 46 isolates from the 35 women who had anorectal colonization by *S. agalactiae.* All isolates were stored in tryptic soy broth with 5% glycerol at -70°C until molecular studies were performed. DNA extraction was performed manually according to the protocol by Wilson K[14]. The PCR master mix was prepared according to Imperi *et al*[15]: 1× PCR buffer; 2 mMMgCl2; 200 µM concentrations of dATP, dCTP, dGTP, and dTTP; 250 nM concentrations of all primers with the exception of primers 1 and 16 which were used at a concentration of 400 nM. A final reaction volume of 25 µL reaction mix containing 5 µL of template was used for the PCR. Denaturation, annealing and extension reactions were as follows: 5 min at 95 °C, followed by 15 cycles of 95 °C for 60 s, 54 °C for 60 s, and 72 °C for 2 min and then by additional 25 cycles of 95 °C for 60 s, 56 °C for 60 s, and 72 °C for 2 min and a final cycle of 72 °C for 10 min[15]. The PCR products were visualized and documented using a UVP Bio-Doc-it gel documentation system.

Ethical approval for the study was obtained from the University of Port Harcourt Teaching Hospital Ethical Committee (UPTH/ADM/90/S. II/VOL.XI/369). Data analysis was performed with the software Statistical Package for Social Sciences (SPSS) version 21. Multivariate analysis examined the association between the vaginal colonization with *S. agalactiae* (Group B Streptococcus) and the sociodemographic characteristics and obstetric history using chi-square test. Factors with a p-value of <0.05 were considered as statistically significant.

RESULTS

Thirty-five women (18.9%) were found to be colonized with *S. agalactiae*.

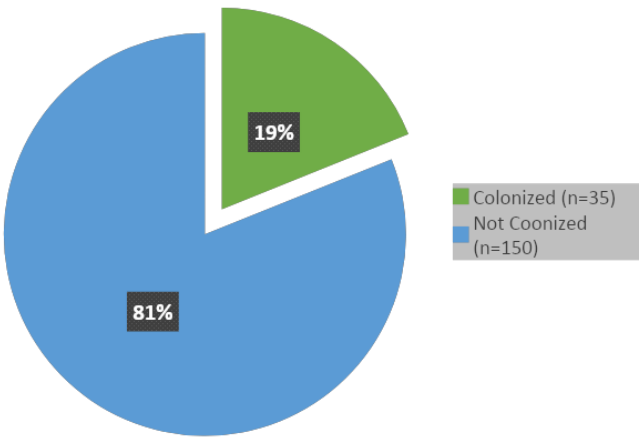

Figure1. *Prevalence of colonization by group B streptococcus*

A total of forty-six (46) *S. agalactiae* isolates were detected from all positive women, and their distribution is as follows: fourteen of the isolates (40%) were of rectal origin, while 12 (34%) of the isolates were present in both vagina and rectum. The vaginal source alone, accounted for 9 (26%) isolates. The chances of being colonized with GBS were higher in participants who practised douching, had a previous miscarriage, previous delivery by caesarean section and urinary tract infection in previous pregnancy, though not significantly associated. (Table 1).

Table1. *Logistic Regression of Selected Factors and Group B Streptococcus Infection*

Variables	Detected (n=35)	Not Detected (n = 150)	OR (95% CI)
Alcohol Consumption			
Yes	2 (5.71)	26 (17.33)	0.2 (0.1 - 1.2)
No	33 (94.29)	124 (82.67)	
Previous Miscarriage			
Yes	9 (25.71)	35 (23.33)	1.1 (0.4 - 2.6)
No	26 (74.29)	115 (76.67)	
Douching			
Yes	20 (57.14)	65 (43.33)	1.7 (0.8 - 3.6)
No	15 (42.86)	85 (56.67)	
C-Section			
Yes	9 (25.71)	21 (14.00)	2.1 (0.8 - 5.1)
No	26 (74.29)	129 (86.00)	
Previous Vaginal Delivery			
Yes	17 (48.57)	77 (51.33)	0.8 (0.4 - 1.8)
No	18 (51.43)	73 (48.67)	
UTI in Previous Delivery			
Yes	4 (11.43)	9 (6.0)	2.0 (0.5 - 6.9)
No	31 (88.57)	141 (94.0)	
Antimicrobial Use			
Yes	7 (20.0)	19 (12.67)	0.5 (0.2 – 1.5)
No	28 (80.0)	131 (87.33)	
Vaginal Discharge			
Yes	8 (22.86)	21 (14.00)	0.5 (0.2 – 1.3)
No	27 (77.14)	129 (86.00)	

OR: Odds Ratio. CI: Confidence Interval. When OR > 1, likelihood of GBS increases

The cps capsular types detected among isolates were Ia, Ib, II, III, IV and V. The capsular type that occurred most frequently was V with 15 (32.6%) isolates, followed by capsular type Ia with 10 (21.7%) isolates, and types III and II with 8 (17.4%) isolates each. There were 4 (8.7%) isolates of capsular type IV and only 1 (2.2%) of type Ib. Capsular types of vaginal and rectal origin were congruent for most participants that had both rectal and vaginal colonization except for two women; one had types Ia and III and the other, types V and II from their vagina and rectum respectively.

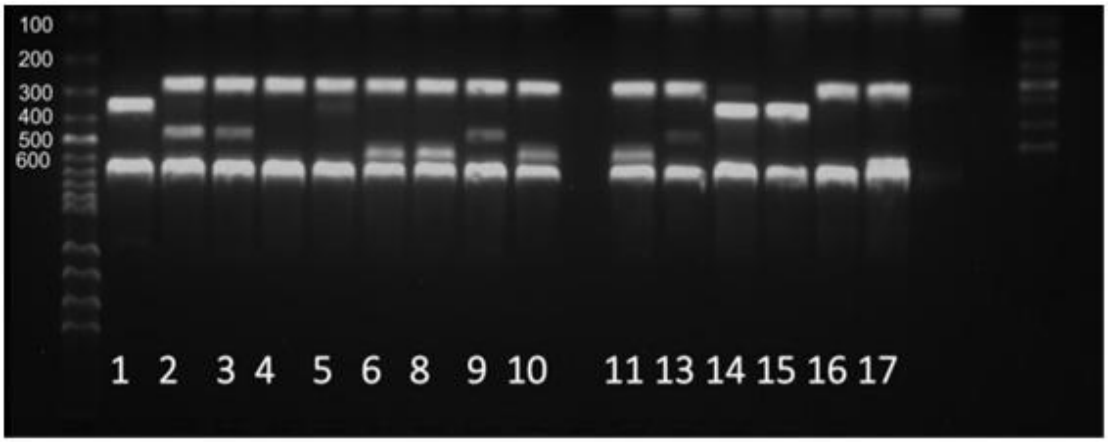

Figure2. *Gel electrophoresis of the products of amplification.*

The amplicon size and band patterns were compared to that of control strains for identification of the capsular types.

DISCUSSION

The prevalence of maternal colonization in this study was 18.9% which is within the range reported in other local studies [8,9,16].

We found a positive association between colonization and a history of douching, a previous miscarriage, previous caesarean section and urinary tract infection in a previous pregnancy. Although it is unclear the relationship between colonization and a previous miscarriage or previous operative delivery, the effect of douching on the normal flora of the vagina may play an important role in GBS colonization. Medugu et al also reported higher risk of colonization in women who douched [9]. The process of douching, may be responsible for the translocation of faecal flora from the rectum to the vaginal, especially in the African context, where the hand-basin washing is commonly practiced [17].

The positive association between GBS colonization and urinary tract infection in pregnancy could be attributed to the fact that GBS is a common cause of asymptomatic bacteriuria in pregnancy [18]. Bacteriuria due to GBS is assumed to be secondary to heavy genital tract colonization by the organism thus supporting guidelines which recommend that women with GBS bacteriuria should be offered intrapartum antibiotic prophylaxis even without rectovaginal screening for GBS, to protect their babies from early neonatal disease [6,19].

The capsular polysaccharide variants determined were Ia, Ib, II, III, IV and V. With the exception of capsular type IV, these capsule types are the most commonly detected globally and responsible for majority of human invasive disease [13] and the distribution of capsular type seems to vary widely across regions globally. The most common type detected in our study was type V. This is consistent with another Nigerian study [9]. The cps types in decreasing order of frequency were: V (32.6%), Ia (21.7%), III (17.4%), II (17.4%), IV (8.7%) and Ib (2.2%) respectively. This is consistent with another Nigerian study reporting capsular type V as the most frequent type detected [9] and studies in Gabon, Gambia, Kuwait and France that reported capsular type V as the most commonly identified capsular type [20–23]. However, a few studies in other parts of Nigeria have reported capsular types Ia and III as most frequently occurring types in their respective study populations [8, 24], suggesting intra-country variations and perhaps a change in the incidence of certain capsular types over time. This is similar to findings in South Africa; a 2011 South African study reported serotypes Ia, Ib and III as accounting for 74.1% of colonizing strains in mothers, with serotype III being the highest[25] while another study in 2018 carried out in another city reported serotype V occurring in 66.6% of the isolates[17]. In other parts of the world, serotype III was the most common capsular type identified in Iran, China and Canada [26–28], type II was most common in Turkey [29]. In a study of up to 1739 women in Oslo, Norway, GBS capsular types III, V and Ia accounted for 57.5% of all the isolates, while types VI, VII and VIII were absent [30].

These variations in the distribution of GBS capsular types further underscores the need for every region to determine the epidemiology for that area, especially as the capsular polysaccharide is the basis for current vaccine trials. These capsular type/serotype distribution variations may change over time, and this is why Breeding et al, recommend that it is important to understand the distribution of vaccine and non-vaccine serotypes in order to observe for capsular switching or serotype replacement [31] as capsular switching between strains may change the distribution of capsular types. Ongoing multivalent vaccine trials however, are targeted at serotypes Ia, Ib, II, III and V[32–34]. It is therefore, reassuring to know that the spectrum of capsular types prevalent in our study area is similar to those being targeted in the current vaccine trials.

ACKNOWLEDGEMENT

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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Citation: *Bob-Manuel Mienye, et al."Epidemiology and Capsular Typing of Group B Streptococcus in Pregnant Women in Port Harcourt, Nigeria", Annals of Microbiology and Infectious Diseases 2021, 4(1), pp. 01-06, https://doi.org/10.22259/2637-5346.0401001.*