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Correlates of Group B Streptococcal Colonization in Pregnancy at A Tertiary Hospital in South West Nigeria

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ABSTRACT

Background: *Group B Streptococcus (GBS) is a gram-positive bacterium and a major cause of bacterial infections in the newborns delivered by women whose rectum and vagina were colonized by GBS during pregnancy.*

Objectives*: To study the association between maternal GBS recto-vaginal colonization and neonatal colonization and occurrence of early onset neonatal group B Streptococcal sepsis.*

Methodology*: A prospective cohort study involving 28 mother-infant pairs of GBS positive and 28 negative controls.*

Results: *Of the 196 pregnant women screened for GBS recto-vaginal colonization between 35-37weeks, 31 women were positive giving a prevalence rate of 15.8%. Three (3) cases were later excluded for different reasons, thus only 28 infants delivered to GBS-positive mothers and 28 infants delivered to GBS-negative mothers were followed up for signs and symptoms of sepsis. Of the 28 newborns delivered to GBS colonized mother, 12(43.0%) were colonized with GBS at birth compared to 2 (7.1%) colonized newborns delivered to GBS-negative pregnant women. An association was observed between maternal GBS recto-vaginal colonization and neonatal colonization (P=0.0003, RR: 2.25 CI:(1.45 – 3.49)) with a vertical transmission rate of 43% among the GBS colonized mother-infant pairs. A significant difference was also observed between the birth weights of infants delivered to GBS-positive mothers (3100.00 ± 392.8g) and GBS- negative mothers (3338.4 ± 338.4g) (P=0.018). Low social class was associated with higher GBS colonization rate (P = 0.029). A prevalence rate of 17.9/1000 births of GBS early sepsis was found in this study.*

Conclusion: *Low social class increases the risk of maternal colonization by GBS, and maternal colonization in late third trimester is associated with newborn GBS colonization at birth.*

Keywords
Group B Streptococcus, Pregnancy, Maternal colonization, Neonatal sepsis.

Introduction

Group B Streptococcus (also known as *Streptococcus agalactiae)* is a Gram-positive coccus, beta-haemolytic, catalase-negative, and facultative anaerobe which can cause serious infection in newborn, elderly and immune compromised individuals [1]. Infection by this organism may result in varying degrees of neonatal morbidity and mortality, however, in pregnant women, GBS colonization is usually asymptomatic, but may result in upper genital tract infection which can progress to septicaemia and maternal death [2,3]. Women of childbearing age are colonized by GBS with variable frequencies with reported average prevalence rates of 31.3% and 4.6% in developing and developed countries respectively [4,5]. About 50-65% newborn infections by GBS result from ascending infection into the uterus from vagina, foetal aspiration of infected amniotic fluid or during passage through the birth canal [6,7]. Group B Streptococcus may cause severe disease in infants which are classified into two groups; those who become ill in the first seven days of life, referred to as early onset disease (EOD) and late onset disease (LOD), which is infection that occur after seven days of life [6-9]. Colonization with GBS in late pregnancy or during labour is reported as the primary risk factor for the development of GBS-EOD [10-13]. Intrapartum prophylactic intravenous antibiotic decreases risk of vertical transmission of GBS from 47% to 10% and neonatal GBS sepsis by 75% [13-20]. The clear benefits of universal screening and effectiveness of intrapartum chemoprophylaxis for prevention of GBS neonatal disease cannot be underestimated. However, this approach has not translated into maximal benefit to mothers and their newborns due to lack of screening program in Nigeria and other developing countries. This study determined the proportion of pregnant women managed at a rural tertiary institution with GBS recto-vaginal colonization and evaluated the association between maternal GBS recto-vaginal colonization, vertical transmission rate and early-onset neonatal disease.

Materials and Methods

A prospective cohort study among pregnant women who registered for antenatal care at Federal Teaching Hospital, Ido Ekiti, Nigeria, between July 2019 and January 2020. The study was approved by the hospital ethical review committee. It included all consenting pregnant women between gestational ages of 35 and 37 completed weeks, regardless of their planned mode of delivery. Women who had used antibiotics within two weeks of recruitment and those with history of fever, premature rupture of membrane, antepartum heamorrhage, gestational diabetes mellitus, preeclampsia and any other common medical conditions that may affect the neonatal outcome independently were excluded. The calculated minimum sample size was 21, but a delibrate oversampling of 20% was done to allow for attrition, thus giving a total sample size of 26 cases. This gave a statistical power of 80%, a significance level of 5% and 95% confidence interval. In order to obtain this sample size, 196 antenatal attendees between 35 weeks and 37 weeks gestation were recruited through systematic random sampling technique and screened for GBS. Of the total recruited, 31 cases were found to be GBS-positve and for every GBS recto-vaginal colonized case, the next consecutive negative case was recruited as control. Duplication of recruitment was avoided by using the hospital number of the woman as identification on the data collection form. Two women were excluded at the time of delivery having taken antibiotics within the previous two weeks while a participant opted out after delivery. Out of the women who had recto-vaginal culture negative for GBS, three were also excluded intra-partum due to the use of antibiotics as a result of intra-partum fever and prolonged ruptured of foetal membranes. Therefore, a total of 28 women with GBS culture positive and 28 women with GBS negative were followed up in this study.

Culture and further processing of samples

The screening for GBS colonization was done by taking two swab samples from each participant's lower vagina and rectum. One of the samples from each site was suspended in non-nutrient transport medium (Stuart transport medium) and the other directly transported. The swabs were cultured within an hour of collection in the Medical Microbiology Laboratory of the Hospital. The samples were cultured on to Todd-Hewitt broth medium supplemented with 10 ug/ml colistin and 15ug/ml nalidixic acid. Inoculated broth medium was incubated aerobically at 37◦C for 24 h, after which the broth was examined for evidence of bacterial growth (turbidity) and re-incubated for an additional 24 h if necessary. The broth was sub cultured onto a non-selective 5% sheep blood agar (SBA) plates and incubated for 24 h at 37◦C; following which an examination of the plate for beta or non- hemolytic colonies suggestive of group B streptococci was done. Any SBA culture plate that has no growth after 24 hours was re-incubated for an additional 24 h before being reported as negative. Identification of GBS was based on convectional criteria, including colonial morphology, gram stain reaction, negative catalase, positive Christie, Atkins, Munch-Petersen (CAMP) and Hippurate hydrolysis test [21].

Antenatal, Intrapartum and Post -partum Management

Participants were followed up till delivery with routine antenatal care, with emphasis on the need to present early in labour for delivery. Screening results of GBS status of the participants were available prior to or at the delivery, which were reported as either exposed (GBS positive) or unexposed (GBS negative). Conduct of delivery was done in line with departmental delivery protocol. Babies of both GBS exposed and non-exposed mothers had two swab samples taken from the base of umbilical cord immediately after delivery and prior to the cleaning of the babies and two swabs from nostrils and throats were also taken after resuscitation. The samples were treated as described for the maternal samples. The results were reported as GBS positive or negative. All the babies were monitored for early-onset neonatal group B streptococcal sepsis, which is defined as confirmed isolation of GBS from blood and/or cerebro-spinal fluid cultures in neonates who presented with clinical features of sepsis during the first seven days of life.

Data Analysis

All findings were recorded, tabulated and statistically analysed using Statistical Package for Social sciences (SPSS) version 22.0 (IBM Corp., Armonk, New York, USA). The results were expressed in terms of mean, standard deviation and percentages. Continuous variables were compared using the independent t-test while categorical variables were analysed using Pearson's chi-square and/or Fisher's exact test as appropriate. Relative risk (RRs) and 95% confidence intervals (CIs) were calculated to assess the association between infant exposure to maternal recto-vaginal GBS and development of GBS Early-onset neonatal sepsis. A p-value<0.05 was considered to be statistically significant.

Results

A total of 196 pregnant women who satisfied the inclusion criteria were recruited and screened for GBS in this study, out of which of 31 (15.8%) women had GBS recto-vaginal colonization. Three (3) cases were however excluded as earlier stated. Thus 28 cases were followed up and formed the basis for analysis in this study. Table 1 shows the socio-demographic characteristics of the respondents. The mean age of the participants were 32.6 ±4.0 years and 33.2 ±3.2 years for GBS-positive and GBS-negative respectively, with majority being older than 25years in cases (96.4%) and control group (100.0%). Ninety-six percent of the women with GBS colonization had some form of education with majority having secondary level (35.7%) while most of the women without GBS colonization had tertiary level of education (53.6%). Comparable number of women in both arms were married in a monogamous setting (92.3% vs 96.4%). Most women with recto-vaginal colonization with GBS were either trader (35.7%) or unemployed (28.6%) in comparison to women without GBS colonization where 50% of them were civil servants. More than seventy percent of participants with GBS recto-vaginal colonization were in the low social class in contrast to the participants without GBS recto-vaginal colonization where half (50.0%) were in the high social class [22].

Table 1: Socio-demographic characteristics between GBS positive and GBS negative women.

Variable	GBS Colonized N = 28 n (%)	Control N = 28 n (%)	Test	p-value
Age group (in years)				
≤ 25	1 (3.6)	0 (0.0)	1.798	0.705[F]
26 – 30	5 (17.9)	5 (17.9)		
31 – 35	17 (60.7)	15 (53.6)		
36 – 40	5 (17.9)	8 (28.6)		
Mean age ± SD	*32.6 ± 4.0*	*33.2 ± 3.2*	*-0.595[t]*	*0.554*
Educational level				
None	1 (3.6)	0 (0.0)	4.370	0.186[F]
Primary	9 (32.1)	5 (17.9)		
Secondary	10 (35.7)	8 (28.6)		
Tertiary	8 (28.6)	15 (53.6)		
Religion				
Christianity	25 (89.3)	26 (92.9)		1.000[F]
Islam	3 (10.7)	2 (7.1)		
Tribe				
Yoruba	23 (82.1)	26 (92.1)	1.774	0.422[F]
Igbo	4 (14.3)	2 (7.1)		
Hausa	1 (3.6)	0 (0.0)		
Marital Status				
Single	2 (7.1)	0 (0.0)		0.150[F]
Married/ Cohabiting	26 (92.9)	28 (100.0)		
Marriage setting (n=26)				
Monogamous	24 (92.3)	27 (96.4)		0.604[F]
Polygamous	2 (7.7)	1 (3.6)		
Occupation				
Artisan	5 (17.9)	6 (21.4)		**0.032[F]**
Civil servant	5 (17.9)	14 (50.0)		
Trader	10 (35.7)	6 (21.4)		
Housewife/ Unemployed	8 (28.6)	2 (7.1)		
Social Class				
High	5 (17.9)	14 (50.0)	6.613	**0.029[F]**
Medium	3 (10.7)	1 (3.6)		
Low	20 (71.4)	13 (46.4)		

F-Fisher's exact test; t – *Independent t test*

Table 2 shows the pregnancy outcomes in both the GBS colonized and control group. Majority of the respondents with GBS recto-vaginal colonization and control had spontaneous vaginal delivery (82.1% vs 92.9%), while 14.3% of GBS colonized respondents and 7.1% of GBS non colonized respondents had caesarean section. Most of the deliveries (92.9%) in GBS colonised respondents and all deliveries in control group occurred at term with 100% live birth in both groups. Although the birth weights of the newborns were similar in both groups, with 92.9% in both groups having normal birth weights, the mean birth weight in the cases were significantly lower than in the controls (3100.00 ± 392.8g and 3338.4 ± 338.4 g; p_ 0.018). Other foetal outcome indices such as neonatal intensive care admission and Apgar scores were not significantly different in both groups.

Table 2: Comparisons of pregnancy outcomes in GBS colonized and non-colonized participants.

Variable	GBS Colonized N = 28 n (%)	Control N = 28 n (%)	Test	p-value
Mode of delivery				
Spontaneous Vertex Delivery	23 (82.1)	26 (92.9)	1.744	0.422[F]
Caesarean Section	4 (14.3)	2 (7.1)		
Instrumental Vaginal Delivery	1 (3.6)	0 (0.0)		
Delivery Outcome				
Live birth	28 (100.0)	28 (100.0)		
Sex of fetus				
Male	13 (46.4)	14 (50.0)		1.000[F]
Female	15 (53.6)	14 (50.0)		
Birth weight				
< 2500g	2 (7.1)	0 (0.0)	3.256	0.241[F]
2500 – 4000g	26 (92.9)	26 (92.9)		
> 4000g	0 (0.0)	2 (7.1)		
Mean Weight ± SD	*3100.00 ± 392.8*	*3338.4 ± 338.4*	-2.433[t]	**0.018**
Gestational age at delivery				

Preterm (<37 weeks)	2 (7.1)	0 (0.0)		0.491[F]
Term (37 – 42 weeks)	26 (92.9)	28 (100.0)		
Mean GA ± SD	38.0 ± *1.6*	38.3 ± *0.7*	-0.883[t]	0.381
Need for admission in NICU				
Yes	2 (7.1)	0 (0.0)		0.491[F]
No	26 (92.9)	28 (100.0)		
Reason (s) for admission				
Neonatal fever	1 (3.6)	0 (0.0)	-	1.000[F]
Difficulty in breathing	1 (3.6)	0 (0.0)		1.000[F]
Perinatal Asphyxia	1 (3.6)	0 (0.0)		1.000[F]
APGAR Score				
1st minute APGAR 1-3 4-6 7-9	 1 (3.6) 7 (25.0) 20 (71.4)	 0 (0.0) 2 (7.1) 26 (92.9)	4.365	0.078[F]
5th minute APGAR 4-6 7-9	 1 (3.6) 27 (96.4)	 0 (0.0) 28 (100.0)		1.000[F]

t - Independent t test; F-Fisher's exact test

Table 3 shows GBS isolates from cultured specimens in the new-borns at birth. Among the new-borns delivered to mothers with recto-vaginal colonization, 12 (42.9%) of them had their umbilical cord colonized at birth compared to 2 (7.1%) of the new-borns delivered to mothers without recto-vaginal colonization. None of the specimens cultured from the throat and nostrils of the new-borns in both groups grew GBS isolates. One (50.0%) of the two new-borns admitted into NICU with signs and symptoms suspicion of sepsis, had GBS isolated from blood culture specimen indicating a neonatal infection rate of 7.1% among all new-borns colonised with GBS at birth and 3.5% rate among the GBS positive mother-infant pairs. It also gave a prevalence rate of GBS neonatal sepsis in this study as 17.9/1000 births. However, the affected baby recovered fully after treatment, thus there was no case fatality as a result of early onset GBS sepsis.

Table 3: Relationship between maternal GBS status and GBS isolates from culture specimens.

Variable	GBS Colonized N = 28 n (%)	Control N = 28 n (%)	Test	p-value	RR (95% CI)
Newborn GBS status (Umbilical cord swab)					
Positive	12 (42.9)	2 (7.1)		**0.0003[F]**	**2.25 (1.44 – 3.49)**
Negative	16 (57.1)	26 (92.9)			1.00
Newborn GBS status (Throat swab)					
Negative **Newborn GBS status (Nostril swab)** Negative	28 (100.0) 28 (100.0)	28 (100.0) 28 (100.0)			-

F-Fisher's exact test

The sensitivity of the maternal antenatal GBS colonization in predicting newborn colonization at birth in this study was 85.71% with a specificity of 61.9% . The positive predictive value (PPV) and negative predictive value (NPV) of maternal GBS colonization status and neonatal GBS colonization status at birth were 42.86% and 92.86% respectively (**Table 4**).

Table 4: Test performance of Maternal GBS colonization Status between 35 and 37weeks gestation in predicting GBS Neonatal colonization at birth.

Statistic	Value	95% CI
True Positive (TP)	12	
False Positive (FP)	16	
True Negative (TN)	26	
False Negative (FN)	2	
Sensitivity (%)	85.71%	57.19% -98.22%
Specificity (%)	61.90%	45.64% -76.43%
Positive Predictive Value (%)	42.86%	32.55% -53.32%
Negative Predictive Value (%)	92.86%	77.90% - 97.96%

Discussion

A total of 31 participants in this study had GBS positive recto-vagina colonization among 196 screened, with a prevalence of 15.8%. The mean ages were similar in both group (32.6 ± 4.0 Vs 33.2 ± 3.2 years), and there was no statistical association between GBS recto-vaginal colonization status and the age of the participantss (p=0.554). This is similar to the report from Ile-Ife and Uyo, both in Nigeria [23,24]. Although a similar study from Ile-Ife documented an increase in GBS recto-vaginal colonization with increasing maternal age [25]. Also, a study from Accra, Ghana also showed that women below 20 years of age or above 30 years of age have a significantly higher risk of carrying GBS compared to women from the age group between 20 and 30 years [26]. There was association between occupation and GBS colonization in this study (P=0.030). Majority of the respondents with positive GBS recto-vaginal colonization were either trader (35.7%) or housewife/unemployed (28.6%) while respondents with negative GBS recto-vaginal colonization were majorly civil servants (50.0%). Association was also observed in this study between respondents with positive and negative GBS recto-vaginal colonization with respect to their social classes (P=0.029). Majority of respondents with positive GBS recto-vaginal colonization (71.4%) were in the low social class compared to respondents with negative GBS recto-vaginal colonization with majority (50.0%) in high social class. The association between occupation, social class and colonization status from this study was different from reports from Zaria [27], Benin [28] and Netherland [29] where there were no association found between occupation, social class and GBS colonization status. The explanation given for lack of association in the Netherland study was that the population with the higher income group was quite small compared to the other group. All 66 (32.9%) pregnant women with culture positive GBS in a study from Trinida were from low socioeconomic class [30]. In the contrast, maternal GBS colonization was observed to be less common in women with low socio-economic status in Kenya [31]. The absent of association between mode of delivery, delivery outcome, sex of the newborn at birth, mean gestational age at delivery and maternal GBS colonization status were similar to findings recorded in the studies from Iran[3] and Ile-Ife Incidence of still birth of 1.0% and 8.53% in

Benin and in Ethiopia respectively were different from this study where no mortality was recorded [25,28,32]. The high proportion of still births in Ethiopia study may be explained by non-exclusion of hypertension and other medical conditions in respondents recruited for the study as majority of stillbirths occurred prior to the onset of labour in that study. The Kenyan study [31] also reported a comparably high GBS associated stillbirth of 9.1% despite low GBS colonization rate. Respondents with medical conditions or conditions that could affect the fetus independently were also not excluded from the Kenyan study. This may explain the higher stillbirth rate despite low maternal GBS colonization rate in these two studies. The lower mean birth weight among the women whose rectum or vagina were colonised by GBS were similar to findings in the study in Kenya [31] where low birth weight was associated with maternal GBS colonization. However, there were no association between maternal GBS status and birth weight in the studies in Gambia [13], Zaria [27], Benin [28], Ethiopia [32]. The significant association observed between maternal GBS recto-vaginal colonization and neonatal colonization in this study (P=0.0003, RR: 2.25 CI:(1.45 – 3.49)) with a transmission rate of 43% was lower than reported rate in studies from Iran (60%)[3], Gambia (73.5%) [13], Zimbabwe (60.3%) [33] and Benin (100.0%) [28]. However, the transmission rate is comparable with the transmission rate recorded in Abuja (48.5%) [34] and higher than that reported in Lagos (6.8%) [35]. The higher transmission rates in Iran, Gambia and Zimbabwe are likely to be due to the inclusion of preterm deliveries in those studies. Two (7.1%) of the newborn delivered to mother with negative recto-vaginal GBS were found to be colonised at birth. Similar findings have been documented in Iran [3] and Zimbabwe [36] studies but none of the newborns with GBS colonization at birth was delivered to a GBS negative mother in Benin [28] study. This false negative finding in this study may also be due to collection of samples at earlier gestation (35 weeks) with the delivery occurring late in gestation (>41weeks) with time lag of six weeks, during which maternal colonization could have occurred after initial negative screening as occurred in this study. The positive predictive value (PPV) of maternal GBS recto-vaginal status between 35-37weeks gestation and neonatal GBS colonization at birth in this study was 42.86%. This lower positive predictive value compared to other studies may be due to the fact the maternal GBS status was not repeated intra-partum like it was done in Benin study and smaller sample size in this study compared to that of Benin [28]. This finding was also in contrast to the findings of 100% sensitivity, 95.6% specificity and positive and negative predictive values of 86.8% and 100%, respectively in the study from Brazil [36]. This may be due to the use of culture and PCR methods in that study as against conventional culture employed in this study. The finding of 17.9/1000 GBS early onset neonatal sepsis in this study is in contrast to findings from Zaria [27] and Lagos [35] and Iran [37] where no case of GBS sepsis was recorded. The finding is also higher than the reported rate of GBS early onset sepsis in other studies in Nigeria; 2/1000 birth in Abuja [34] and 7.24/1000 births in Ebonyi [38]. It is also higher than reported 2.06/1000 births in South Africa [39]. The findings from Asia and European countries were also lower than the findings from this study; 0.43/1000 births in Netherland [40], 10.7/1000 births in Iran [3] and 14.9/1000 births in Trinidad [30]. Nonetheless a higher rate of 59.7/1000 births of GBS early onset neonatal sepsis were recorded in Benin [28]. The findings of no case of GBS early neonatal sepsis reported in Zaria [27], Lagos [35] and Iran [37] are not surprising as newborns in the Zaria study were not followed up beyond the time of delivery. Study in Iran [37] employed the use of chocolate agar plate as opposed to Todd-Hewitt broth and sheep blood agar which are associated with better yield of GBS as was done in this study. In the other studies where the rates of GBS sepsis were lower in comparison with the findings in this study, the reason may be due to the fact that some were given intra-partum antibiotics or the use of antibiotics prior to the study (Iran [3], Gambia [13] and Abuja [34]). The higher rate of GBS early onset neonatal sepsis reported in Benin [28] which is comparable to this study may be as a result of the timing of maternal recto-vaginal sample collection which was done intra-partum. No case fatality was recorded among the newborn with cultured proven GBS sepsis in this study. This was in contrast to reported case fatality of 15.4% in Benin [28], 47.0% in Kenya [31], 19.8% in South Africa [39] and 33.3% in Trinidad [30]. However, the result from this study is similar to reported fatality for culture proven GBS early onset sepsis in Iran [37] and Ebonyi, Nigeria [38] where no case fatality was reported. Prematurity and low birth weight were added risk factors reported for mortality in newborn with early onset GBS sepsis in South Africa [39], while maternal medical conditions and other risk factors which could independently affect the newborn outcome were not excluded from the study in Kenya [31] and Benin [28].

Conclusion

This study showed that 15 out of every 100 pregnant women in our setting harbored GBS, with a vertical transmission rate of 43% and GBS early onset sepsis prevalence of 17.9/1000 births. These findings underscore the need for antenatal GBS screening and the use of intrapartum antibiotics prophylaxis (IAP) in preventing the early onset newborn GBS disease especially in low socio-economic settings.

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