Prevalence of Group B Streptococcus Colonisation and Antimicrobial Susceptibility Pattern among Pregnant Women Attending a Tertiary Health Facility in Ogun State, Southwest Nigeria

Abstract
Background: Genital colonisation by group B Streptococcus (GBS) in pregnant women in their third trimester has been shown to be a known risk factor for morbidity and mortality among newborns. Aim: The aim of the study was to determine the prevalence of GBS colonisation among pregnant women in Abeokuta, its associated sociodemographic factors, and the neonatal outcome among exposed babies. Design: Longitudinal cohort study. Setting: Department of Obstetrics and Gynaecology, Federal Medical Centre, Abeokuta, Ogun State. Methodology: One hundred sixty pregnant women presenting for routine antenatal care between 35 and 41 weeks were recruited consecutively. Swabs were taken from the vagina and then the rectum using a single swab. The samples were processed at the hospital’s Medical Microbiology Laboratory using standard microbiological methods. Babies whose mothers were positive had their bodies swabbed and the samples sent for GBS isolates. They were also screened for early-onset neonatal sepsis with C-reactive protein. Results: Prevalence of GBS vaginal colonisation was 4.3%. There was no significant association between GBS colonisation status and age, level of education, or occupation; however, women of parity ≤1 had significantly higher prevalence of GBS colonisation than those of parity ≥2. There was no incidence of GBS infection observed in the babies. The GBS isolates were 100% sensitive to cefuroxime and 83.3% resistant to ampicillin. Conclusion: The prevalence of GBS is low in our environment. The organisms were highly sensitive to cefuroxime, erythromycin, and ceftriaxone. Routine screening of all pregnant women may be unnecessary. However, women at risk of GBS who present in labour without a recent GBS screening should be offered intrapartum prophylactic cefuroxime.

Keywords: Antimicrobial susceptibility, GBS colonisation, neonatal outcome, pregnant women

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**Introduction**

Group B *Streptococcus* (GBS) or *Streptococcus agalactiae* is a constituent of the normal vaginal bacterial microflora and/or lower intestine in about 15–40% of healthy adult women,[1] who often do not demonstrate any clinical symptoms. However, during pregnancy, there are optimal conditions for the multiplication of GBS in the vagina which may have very serious consequences to both the mother and her child. A prevalence of 15.7% and 8.9% GBS colonisation among pregnant women and newborns, respectively, were reported in Ethiopia,[2] while a prevalence of 8.6–64% GBS colonisation of mothers and 19.0–20.6% among newborns were reported in Nigeria depending on the methods of isolation of the organism.[3–6]

GBS is identified as the most common cause of severe early-onset infection in newborn infants. An incidence of 0.57/1000 births (517 cases) early-onset GBS was reported in the UK and Ireland in 2015, a significant increase from the previous surveillance undertaken in 2000 where an incidence of 0.48/1000 was recorded.[7] Two cases of early-onset disease per 1000 live births were reported in Nigeria.[8]

Early diagnosis and proper management of this preventable infection significantly reduce the morbidity and mortality associated with women with vaginal colonisation with GBS. There is a spectrum of maternal and fetal GBS infections ranging from asymptomatic colonisation to sepsis. Perinatal infections are one of the fundamental causes of early puerperal complications in the mother and neonate.

GBS has been implicated as a cause of asymptomatic bacteriuria or urinary tract infection (UTI). It is also a well-known cause of prelabour rupture of membrane (PROM) and preterm labour, preterm delivery with resultant chorioamnionitis and endometritis.[9] Approximately 50% of pregnant women who are GBS carriers will transmit the organism to their newborn infants. Vertical transmission usually occurs during labour or after rupture of membranes.[9]

Neonatal infections associated with vaginal GBS colonisation range from pneumonia, meningitis, and sepsis, and these could lead to neonatal mortality. Amongst newborns with vertical transmission from GBS-positive mothers who did not receive intrapartum antibiotics, up to 1–2% will develop the early neonatal disease (EOD),[10] while 4–6% of babies who acquire the disease suffer mortality.[10] On the other hand, a woman with GBS who had intrapartum antibiotic prophylaxis has the risk to the baby reduced by 80–95%.[11] Term neonates account for 75% of GBS EOD; however, morbidity and mortality related to GBS EOD are much higher among preterm neonates (19.2% vs. 2.1%, respectively).[10] Although the death rate is relatively low, neonates with early GBS infections can have long, expensive stays in the intensive care unit. To avert the resultant maternal and perinatal morbidity and mortality, a screening programme would help to reduce the burden of disease.

Screening for GBS could be by universal approach which entails vaginal/rectal culture of all pregnant women from 35 to 37 weeks’ gestation, or by a risk-based approach, which takes into consideration any of the following intrapartum conditions which include, fever of 38°C and above, PROM longer than 18 h and preterm labour.[9]

The Centre for Disease Control and Prevention recommends screening of all pregnant women at 36 0/7 to 37 6/7 weeks for GBS, unless intrapartum antibiotic prophylaxis for GBS is indicated because of GBS bacteriuria during the pregnancy or because of a history of a previous GBS-infected newborn.[9] The Royal College of Obstetricians and Gynaecologists; however, does not recommend universal screening for all pregnant women, and where a woman meets the criteria for screening it is recommended that it is done between 35 and 37 weeks of gestation or 3 and 5 weeks before the anticipated delivery date, for example, 32–34 weeks of gestation for women with twins.[7]

GBS colonisation status at birth is most accurately predicted by GBS cultures if GBS screening specimens are collected within 5 weeks before delivery. The predictive value reduces significantly if specimens are collected more than 5 weeks before delivery.[2]

Culture is the gold standard of screening of GBS but sensitivity detection is strongly affected by culture enrichment. Another screening method is the nucleic acid amplification test (NAAT). NAAT’s sensitivity from enrichment broth culture varies by specific test, but is typically greater than 96% compared with gold standard culture methods.[11]

It is recommended that patients with positive GBS colonisation should be given intrapartum antibiotics at least 4 h before delivery.[9] Intrapartum antibiotic is also recommended for mothers who have had a previous baby affected by early- or late-onset GBS.[7]

Several studies have been carried out in Nigeria, including Southwest Nigeria, in which GBS colonisation was studied in late pregnancy and a wide range of prevalences has been reported.[3,5,6] Despite the numerous studies, no clear-cut guidelines on screening or management have been developed in Nigeria and presently many centres in Nigeria do not screen for GBS in pregnant women or their newborns, either universally or based on risk. More evidence-based reports may help in designing a guideline and developing recommendations. This study hence becomes relevant to compare its findings with those of similar studies around the world and Nigeria in particular. It will also add to the pool of knowledge on the subject and help relevant organizations/groups in Nigeria in formulating protocols on the screening and management of GBS in pregnancy.

Hence, this study was undertaken to determine the prevalence of GBS colonisation in pregnancy, the socio-economic and obstetric characteristics of affected women, determine the antimicrobial sensitivity pattern of the GBS and assess the perinatal outcome associated with GBS.
Materials and Methods

This was a longitudinal cohort study carried out in the antenatal clinic and labour ward of the Department of Obstetrics and Gynaecology of a tertiary health institution in Abeokuta, Ogun State, Southwest Nigeria between January and June 2016. The institution has an annual average delivery rate of 1300 live births and a caesarean delivery rate of 35%.

The study was carried out among pregnant women presenting for the routine antenatal clinic at our centre.

Inclusion criteria

All pregnant women who booked for routine antenatal care with accurate pregnancy dating from the last normal menstrual period or dating with first-trimester obstetric ultrasound scan and pregnancies with gestational ages between 35 weeks and 41 weeks.

Exclusion criteria

Pregnant women who had had antibiotic treatment within the last 2 weeks before recruitment, women already booked for elective caesarean section and women who declined to give consent.

Patient recruitment and specimen collection

The sample size was calculated using Leslie Kish formula \( N = Z^2 pq/d^2 \) for a single proportion with an absolute error of 5% allowed and prevalence of 11.3% from a study conducted in Ile-Ife Southwest Nigeria.\(^{112}\) The minimum sample size calculated was 154. However, for a small population (1300 deliveries/year in this case), the finite population correction was calculated using the formula: \( n = n_0/(1+\{n_0-1\}/N) \) where \( n_0 \) is the minimum sample size = 154, \( n \) is the finite sample size, and \( N \) is the population size = 1300. Finite sample size \( n = 138 \). Adding 15% for attrition, the total sample size came up to 159. However, 160 pregnant women were recruited. The study participants were recruited consecutively until the sample size was reached. The research was explained to each of the participants and written consent was obtained from those that agreed to participate. The questionnaire was pretested at the antenatal clinic of State Specialist Hospital Ijebu-Ode and administered by trained research assistants. Information obtained included the patient’s biodata (age, parity, level of education, and occupation), date of last menstrual period, and antibiotic use in the index pregnancy. Specimens were collected first from the low vagina (near the introitus), without using a speculum, by inserting a cotton swab about 2 cm into the vagina and then from the rectum by inserting the same swab 1 cm through the anal sphincter. The sample swabs were transported immediately to our centre’s medical microbiology laboratory within few minutes of collection and inoculated immediately.

The specimens were inoculated on selective enrichment broth of Todd-Hewitt broth with gentamicin (8 µg/ml) and nalidixic acid (15 µg/ml) (known as Trans-Vag Broth) and incubated at 35–37°C for 18–24 h in ambient conditions. The broth was observed for the growth of GBS and then subcultured in blood agar. Group B Streptococci grows on blood agar appearing as beta haemolytic organisms. At inoculation, a bacitracin disc was placed centrally. All bacitracin susceptible and Gram-positive cocci isolated were tested for catalase. All catalase-negative Gram-positive cocci were subjected to Christensen Atkins Munch Peterson test to definitively identify GBS. A positive test was seen as an arrowhead haemolysis.

Antibiotic susceptibility was done by preparing the inoculum from a suspension of the organism made by picking two or three colonies of the organism and making an emulsion of it in peptone water. This suspension was then compared against a turbidity standard (0.5 McFarland standard). At this level, growth was expected to be in the logarithmic phase.

Using a sterile swab stick, Mueller-Hinton agar was inoculated with the broth culture. After about 3 min, a multi-antibiotic impregnated disc (containing ceftriaxone 30 µg, ampicillin 5 µg, amoxicillin-clavulanate 10 µg, erythromycin 10 µg, gentamicin 10 µg, cefuroxime 30 µg, ciprofloxacin 10 µg, and ofloxacin 5 µg) was placed on the surface of the Agar and incubated at 35–37°C for 24 h. The results were determined by measuring the diameter of the zones of inhibition with a calibrated meter rule and interpreted with standard interpretative Clinical and Laboratory Standard Institute (CLSI) charts.

Women who tested positive were given intrapartum antibiotics based on sensitivity patterns once in active phase of labour. The interval between the time of administration of the antibiotics and time of delivery was noted.

Swabs were also taken from the newborns whose mothers were positive for GBS by rubbing a sterile swab on their external ears, nasal area, throat, and umbilicus. The swabs were sent to the laboratory and processed immediately as explained to the mother above. Irrespective of the culture outcome, the babies were also observed and screened for evidence of infection by the paediatrician using C-reactive protein kit to detect those with features of early-onset neonatal sepsis and subsequently to have blood culture was done which will help to isolate the causative organism.

All the relevant pieces of information were coded and entered into the computer using IBM SPSS version 25. Data were presented in frequency tables. Continuous variables were summarised using means (with SD). Tests of significance were done using chi-square tests for categorical variables. The level of significance was set at \( P < 0.05 \).

Ethical approval for the study was obtained from the Health Research Ethics Committee of Federal Medical Centre Abeokuta (FMCA/238/HREC/06/2015). All the participants were counselled on the details of the study and written consent was obtained. The study participants were assured of the confidentiality of data obtained from them.
Results

One hundred sixty subjects were consecutively recruited for the study of which 20 were lost to follow up as they did not deliver in the facility. This subset of the participants was not significantly different in characteristics from those that completed the study. A total of 140 participants completed the study and were analysed.

Table 1 shows the sociodemographic and obstetric characteristics of the women. More than half of the women, 77 (54.8%) were within the age group of 30–39 years; the mean age was 30.7 ± 4.2 years. A little above two-thirds of the women, 97 (69.3%) were multipara, the primipara/nullipara and grandmultipara were 25.7% and 5.0%, respectively. The mean gestational age at recruitment of the women was 36.8 ± 1.2 weeks.

Majority 100 (71.4%) of the subjects had had tertiary education; 37 (26.4%) had had up to secondary education while 3 (2.1%) had had only primary education. More than half of the women, 84 (60%), were professionals, whereas 47 (33.6%) and 9 (6.4%) were businesswomen and artisans, respectively.

Table 2 illustrates the GBS status of the women. Of the 140 pregnant women in the study, six were positive for GBS colonisation giving a prevalence of 4.3%. Spontaneous vaginal delivery was reported in 111 (79.3%) of the women while the remaining 29 (20.7%) had caesarean section. Failed vaginal birth after caesarean section, cephalopelvic disproportion, fetal distress, prolonged labour, and a case of intrapartum haemorrhage were the indications for the surgeries. None of them had an instrumental vaginal delivery or assisted vaginal breech delivery.

Table 3 depicts the association between sociodemographic factors and GBS status. All the six positive GBS-positive cases were found among women with tertiary level of education. However, there was no statistically significant association between level of education and GBS status ($\chi^2 = 0.78, P = 0.38$).

Five of the positive cases were found in professionals but there was no statistically significant association between GBS status and economic status ($\chi^2 = 1.42, P = 0.23$). GBS positivity was most common among women of age ≥30 years but the study showed no statistically significant association between GBS status and age group ($\chi^2 = 0.267, P = 0.61$).

Table 4 shows the association between parity and gestational age at sample collection and GBS status. Parity of ≤1 had higher prevalence of GBS positivity compared to parity of ≥2; the difference was statistically significant ($\chi^2 = 5.50, P = 0.02$). Majority of the positive cases had the samples collected at ≤37 weeks’ gestation but no statistically significant association was observed between the gestational age of testing and GBS status.

Among the six women positive for GBS, none of their babies was positive for GBS and C-reactive protein test was negative in all of them. None of the neonates also suffered any sequelae. Regarding antibiotic administration-delivery interval, only one (16.7%) woman each delivered 2 and 3 h, respectively, after administration of antibiotic while the remaining four (66.7%) delivered after 4 h. The mean antibiotics administration-delivery interval was 3.5 ± 0.76 h.

Table 5 illustrates the antibiotic sensitivity pattern of the six GBS-positive cases. Cefuroxime was effective against GBS in all the six patients that had the organism isolated. Ceftriaxone

| Variable                                      | Frequency ($n = 140$) | Percent (%) |
|-----------------------------------------------|-----------------------|-------------|
| Maternal age                                  |                       |             |
| 20–29                                         | 61                    | 43.4        |
| 30–39                                         | 77                    | 54.8        |
| 40–49                                         | 2                     | 1.4         |
| Mean age ± SD                                 | 30.7 ± 4.2            |             |
| Parity                                        |                       |             |
| Para 0–1                                      | 36                    | 25.7        |
| Para 2–4                                      | 97                    | 69.3        |
| Para 5 and above                              | 7                     | 5.0         |
| Gestational age at recruitment                |                       |             |
| 35–37                                         | 83                    | 59.3        |
| >37–39                                        | 51                    | 36.4        |
| >39–40                                        | 6                     | 4.3         |
| Mean G.A ± SD                                 | 36.8 ± 1.2 weeks      |             |
| Level of education                            |                       |             |
| Primary                                       | 3                     | 2.1         |
| Secondary                                     | 37                    | 26.4        |
| Tertiary                                      | 100                   | 71.4        |
| Occupation status                             |                       |             |
| Artisans                                      | 9                     | 6.4         |
| Business women                                | 47                    | 33.6        |
| Professional                                  | 84                    | 60.0        |

Table 1: Sociodemographic and obstetrics characteristics of the women

Table 2 illustrates the GBS status of the women. Of the 140 pregnant women in the study, six were positive for GBS colonisation giving a prevalence of 4.3%. Spontaneous vaginal delivery was reported in 111 (79.3%) of the women while the remaining 29 (20.7%) had caesarean section. Failed vaginal birth after caesarean section, cephalopelvic disproportion, fetal distress, prolonged labour, and a case of intrapartum haemorrhage were the indications for the surgeries. None of them had an instrumental vaginal delivery or assisted vaginal breech delivery.
and erythromycin were effective in 5 (83.3%) of the GBS isolates whereas only one (16.7%) GBS isolate each was sensitive to ofloxacin and ampicillin, respectively. Each of the six women who were GBS positive received a statum dose of intravenous cefuroxime 500mg in labour as prophylaxis based on the sensitivity.

Discussion

This current study recorded a vaginal/rectal GBS colonisation of 4.3% in pregnant women. The prevalence recorded in this current study was lower than that reported in China, Ethiopia, South Africa, and in various regions in Nigeria. A wide range of prevalence rates of 8.6–34.2% have been reported in different studies worldwide. These differences including the one observed in our study probably may be due to the different environments and populations in which the various studies were carried out. The difference in the prevalence may also be due to the different means of inoculation/culture. Although Trans-Vag Broth (Todd-Hewitt broth with gentamicin (8 μg/ml) and nalidixic acid (15 μg/ml) and blood agar were used in inoculating and subculturing the specimen, respectively, in this study, LIM broth (Todd-Hewitt broth supplemented with colistin 10 μg/ml and nalidixic acid 15 μg/ml), and sheep blood agar were used in other studies carried out by Ali et al. in Ethiopia, Akinlolu et al. in Southwest Nigeria, Onipede et al. in Ile-Ife, Southwest Nigeria and Akinniyi et al. in Zaria Northwest Nigeria. Although some studies reported modest differences in GBS detection rate with various media, overall detection of GBS is considered similar.

The highest prevalence of maternal GBS colonisation occurred in the age range of ≥30 years; however, there was no statistically significant association between maternal age and maternal GBS colonisation in this study. This is similar to findings by Akinlolu et al. who reported the highest prevalence rate between 31 and 35 years and Akadri et al. whose study recorded the highest prevalence in women above 30 years of age, although there was no significant association between age and GBS colonisation.

Women of parity 0 or 1 tended to have a significantly higher prevalence of rectovaginal GBS colonisation than women with a parity of ≥2 in this study. This finding was similar to that reported from Zaria and Sagamu in Northwest and Southwest Nigeria, respectively, where nulliparity constituted over half of the cases in both studies, and they also reported a significant association between GBS status and low parity.

From this study, it was noted that the percentage of resistance to ampicillin, a recommended first-line drug of choice for intrapartum prophylaxis for GBS, was high. This was similar to the findings by Onipede et al. in Ile-Ife Southwest Nigeria but completely different from the study by Akinniyi et al. in Zaria where all their GBS isolates were sensitive to penicillin, ampicillin, and cefazolin. The high sensitivity recorded for erythromycin, 83.3%, in our study was similar to the findings by Akadri et al. in Sagamu, Southwest Nigeria and Akinniyi et al. in Zaria, Northwest Nigeria, although with a slightly reduced percentage sensitivities in

### Table 2: GBS status

| Variable          | Frequency | Percent |
|-------------------|-----------|---------|
| GBS status        |           |         |
| Positive          | 6         | 4.3     |
| Negative          | 134       | 95.7    |
| Total             | 140       | 100     |

### Table 3: Association between the sociodemographic characteristics and GBS status

| Variables                  | Positive   | Negative | Total |
|----------------------------|------------|----------|-------|
|                            | n (%)      | n (%)    | n (%) |
| Level of education         |            |          |       |
| Primary/secondary          | 0 (0.0)    | 40 (100.0) | 40 (100.0) |
| Tertiary                   | 6 (6.0)    | 94 (94.0)  | 100 (100.0) |
| Occupational status        |            |          |       |
| Artisan/business women     | 1 (1.8)    | 55 (98.2)  | 56 (100.0) |
| Professionals              | 5 (6.0)    | 79 (94.0)  | 84 (100.0) |
| Maternal age               |            |          |       |
| <30                        | 2 (3.3)    | 59 (96.7)  | 61 (100.0) |
| ≥30                        | 4 (5.1)    | 75 (94.9)  | 79 (100.0) |

### Table 4: Association between Parity/Gestational Age at Sample collection and GBS status

| Obstetrics features | Positive | Negative | Total |
|---------------------|----------|----------|-------|
|                     | n (%)    | n (%)    | n (%) |
| Parity ≤1           | 4 (12.5) | 32 (87.5) | 36 (100.0) |
| ≥2                  | 2 (2.0)  | 102 (98.0) | 104 (100.0) |
| Gestational age ≤37 | 5 (6.0)  | 78 (94.0)  | 83 (100) |
| >37                 | 1 (2.0)  | 56 (98.0)  | 57 (100.0) |
both studies compared to ours. The high resistance of GBS isolates to ampicillin and amoxicillin in this study may not be unconnected with the easy accessibility of the drugs over the counter and their comparatively cheap price leading to their abuse in the community, a situation which might contribute to the emergence of resistant strains. There was high susceptibility of the GBS isolates to cefuroxime (100%) and ceftriaxone (83.3%), (second- and third- generation cephalosporin, respectively) in this study, similar to the report from Sagamu where the GBS isolates also showed high susceptibility to ceftriaxone. Our finding was also similar to that in the study in Ile where the GBS isolates showed relatively high sensitivity to cefotaxin, another third-generation cephalosporin; however, the GBS isolates in that study were 100% resistant to a second-generation cephalosporin, cefoxitin. The high sensitivity of the isolates to third-generation cephalosporin may be due to the less indiscriminate use of the cephalosporins due to their high cost.

The antibiotic administration-delivery interval ranged between 2 and 4 h with a mean of 3.5 h and there was no perinatal transmission of GBS from mothers with positive isolates to their neonates. Also, none of the neonates delivered following antibiotic administration showed signs of early neonatal sepsis. Although American College of Obstetricians and Gynaecologists (ACOG) recommended at least 4 h of antibiotics administration before the expected delivery time, this study suggests that antibiotics administration-delivery interval of less than 4 h may still be effective in the prevention of vertical transmission of GBS in GBS colonised mothers, as none of the newborns delivered to the GBS positive women was infected despite their mothers receiving the prophylactic intrapartum cefuroxime at an average time of 3.5 h to delivery.

For women with no penicillin allergy, intravenous penicillin is the recommended prophylactic antibiotic against GBS colonisation in labour to prevent vertical transmission. This study showed very high resistance of GBS to ampicillin and hence that antibiotic may not be the ideal prophylactic treatment. Cefuroxime proved to be the most effective antibiotic against GBS isolates in this study and was closely followed by erythromycin. Therefore, either of them may be considered for routine empirical use in high-risk patients for GBS where culture is not available.

### Conclusion

The prevalence of GBS is low in our environment. Nulliparity and primiparity were significantly associated with GBS. The organisms were highly sensitive to cefuroxime, erythromycin, and ceftriaxone. There were no adverse perinatal outcomes following intrapartum treatment of GBS-positive pregnant women. We, therefore, recommend that screening for GBS in all pregnant women may not be necessary due to the low prevalence. However, those women at risk of GBS who present in labour without a recent GBS screening should be offered intrapartum prophylactic cefuroxime.

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### Conflicts of interest

There are no conflicts of interest.

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