ABSTRACT
Background: Early-onset Group B Streptococcal (GBS) infection is an important cause of perinatal morbidity and mortality. Policy of active prevention by antepartum screening and treatment is not a popular practice in resource-constrained settings.
Objectives: This study determined the prevalence of asymptomatic GBS infection and antimicrobial sensitivity pattern. It also determined the outcome of Intrapartum antimicrobial prophylaxis (IAP).
Methodology: It was a prospective and longitudinal study done in Ahmadu Bello University Teaching Hospital, Zaria, between June 2014 and April 2015. Two hundred and twenty consenting pregnant women with gestational ages between 35 and 37 completed weeks were participated in the study. Vagina and rectum were swabbed using different rayon swab sticks. Swabs were placed in Amies, nonnutritive transport medium. Bacteriological procedures to culture GBS and confirmation with biochemical tests and serological test were done. Antibiotic sensitivity pattern was determined. Participants who had GBS rectovaginal colonization had intrapartum antibiotic prophylaxis with penicillin G. All participants were followed up till to a week after birth.
Results: Out of the 220 pregnant participants, 19 (8.6%) had GBS rectovaginal colonization. Antibiotic sensitivity pattern revealed that GBS isolates were all sensitive to penicillin, ampicillin, and cefazolin, while 4 (21.1%) were resistant to ceftriaxone and 6 (31.6%) were resistant to both erythromycin and clindamycin. None of the isolates were resistant to erythromycin and sensitive to clindamycin. Of the 19 participants with GBS rectovaginal colonization, 2 (10.5%) delivered low birth weight baby, but there was no incidence of early-onset GBS disease.
Conclusion: The prevalence of GBS rectovaginal colonization in this study is similar to figures from other parts of the country. The GBS sensitivity pattern to penicillin was similar to those reported elsewhere. Fetal outcome following intrapartum antibiotic prophylaxis was good.
Key words: Group B streptococcal infection; pregnancy; prevalence; sensitivity.

Introduction
Group B streptococcus (GBS) is also known as Streptococcus agalactiae. GBS is a known cause of morbidity and mortality among neonates, infants, the geriatric age group, and immunosuppressed persons. GBS is a prominent veterinary pathogen because it can cause bovine mastitis in dairy cows. The species name “agalactiae” which means “no milk” alludes to this. GBS is a Gram-positive coccus,\(^1\) an encapsulated

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How to cite this article: Akinniyi AM, Adesiyun AG, Kolawole A, Giwa F, Randawa A. The prevalence of asymptomatic group B streptococcal infection and antimicrobial sensitivity pattern among parturients at Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. Trop J Obstet Gynaecol 2017;34:182-7.
organism capable of producing severe disease. GBS is a cause of neonatal sepsis which can be classified as “early-onset” neonatal sepsis if it occurs within the 1st week of life and as “late-onset” neonatal sepsis if it occurs after the 1st week till the end of the neonatal period. Maternal infection with GBS constitutes one of the leading causes of both early- and late-onset neonatal sepsis. The major human reservoir of GBS is the vagina and the perianal regions. The bacteria are normally found in the vagina and lower intestine of 15%–40% of all healthy adult women. Other sites frequently colonized are the oropharynx and the external auditory meatus of neonates. An association has been shown between GBS colonization of the vagina in pregnancy and a subsequent adverse outcome of the pregnancy. The carrier rate in early pregnancy is less than at term. Studies have observed higher GBS colonization in women toward the end of the gestational period.

Early-onset perinatal GBS infection is a cause of early neonatal morbidity and mortality. Some series have reported case-fatality ratios as high as 50%. Estimates of the incidence of neonatal sepsis are mostly from single-facility studies and vary in their findings. In Nigeria, 65 cases of neonatal sepsis per 1000 live births occurring in a referral hospital have been recorded. A study in Malawi showed that early-onset neonatal sepsis caused by GBS alone was reported as 92 cases per 1000 live births. Estimates of incidence of early-onset neonatal bacterial sepsis vary widely.

Clinical trials have demonstrated that administering intravenous antibiotics during labor to women at risk of vertically transmitting GBS to their newborns could prevent perinatal early-onset GBS disease in the 1st week of life. A striking decline in perinatal early-onset GBS disease was observed to have coincided with increased prevention activities. A further reduction occurred following the issuance of the recommendation for universal screening of pregnant women in 2002 by the center for disease control and prevention.

Early-onset GBS disease remains a leading cause of illness and death among newborns. Perinatal morbidity and mortality is a challenge in most African settings and infection is a foremost contributor. Routine screening and appropriate treatment of pregnant women for GBS infection would help decrease the rate of infection as a cause of perinatal morbidity and mortality. In the United States, before-active prevention was initiated an estimated 7500 cases of neonatal GBS occurred annually. The purpose of this study is to determine the prevalence of asymptomatic GBS infection and antimicrobial sensitivity pattern among parturients at ABUTH, Zaria, Nigeria.

**Methodology**

The study was carried out in Ahmadu Bello University Teaching Hospital, Zaria, Kaduna state. It was a prospective and longitudinal study among pregnant women attending the antenatal clinic of ABUTH, Zaria.

It was carried out among pregnant women at a gestational age of 35–37 completed weeks that presented to the antenatal clinic and the delivery suite. Two hundred and twenty consenting pregnant women, fulfilling the inclusion and exclusion criteria as stated below, were enrolled for the study. The participants underwent microbiology evaluation for GBS rectovaginal colonization. Participants with positive GBS colonization had intrapartum treatment with penicillin G. The babies were followed up to a week postdelivery.

**Inclusion criteria**

Consenting pregnant women at the gestational age of 35–37 completed weeks were included in the study.

**Exclusion criteria**

Pregnant women with abnormal vaginal discharge, women with symptoms and signs of urinary tract infection, pregnant women who received antibiotics within the past 1 month, and all pregnant women with risk factors for abnormal vaginal discharge and urinary tract infection such as immunocompromised women were excluded from the study.

**Sample collection**

Samples were collected in the outpatient setting by the researcher in the presence of a female chaperone. Pregnant women at 35–37 completed weeks’ gestation had the lower vagina, followed by the rectum swabbed with 2 different rayon swabs using aseptic technique. The swabs were placed in a nonnutritive transport medium (Amies transport medium) to maintain viability of GBS; this was labelled for client identification. None of the participants had a history suggestive of penicillin or cephalosporin allergy.

**Sample processing**

At the Medical Microbiology Laboratory of Ahmadu Bello University Teaching Hospital, the swabs were removed from the transport medium and inoculated into a selective broth medium, LIM broth (Todd-Hewitt broth supplemented with colistin 10 µg/ml and nalidixic acid 15 µg/ml). The inoculated selective broth was incubated for 24 h at 37°C in ambient air. The inoculated selective broth was subcultured onto sheep blood agar plate for 24 h, and the sheep blood agar plate
was then inspected to identify organisms suggestive of GBS by a narrow zone of beta-hemolysis.

Identification
If GBS was not identified after incubation for 24 h, the sheep blood agar plate was re-incubated and inspected at 48 h to identify suspected organisms. Suspected organisms were subjected to biochemical tests consisting of the catalase test and Christie–Atkins–Munch-Petersen (CAMP) test. *Streptococcus* grouping latex agglutination tests for GBS antigen detection were used for specific identification.

The catalase test was used to identify GBS. The catalase test was done by placing a drop of hydrogen peroxide on a microscope slide; an applicator stick was then used to obtain a sample of the growth from the agar plate; the obtained sample was mixed into the hydrogen peroxide drop on the microscope slide. If the mixture produces bubbles or froth, the isolated organism was ascribed to be catalase positive. If no bubbles or froth is formed, the organism will be said to be catalase negative. GBS is catalase-negative.

The CAMP test is an acronym for the three researchers “Christie–Atkins–Munch-Petersen” who discovered the lytic phenomenon between *Staphylococcus aureus* and GBS. The CAMP test is based on the principle that colonies of *Streptococcus* were only surrounded by zones of complete hemolysis when they were growing on blood agar plate in proximity to colonies of β-hemolytic *Staphylococcus*, due to the formation of CAMP factor by GBS which enlarges the area of hemolysis formed by β-hemolysin from *S. aureus*. Other organisms apart from GBS fail to exhibit this enhanced hemolysis when grown near colonies of β-hemolytic *Staphylococcus*.

*Streptococcus* grouping latex agglutination tests for GBS antigen detection were used for serological identification. A *Streptococcus* latex group kit was used for serotyping. The kit has the capability to identify groups A, B, C, D, F, and G streptococci which are the most prevalent streptococci. The kit consists of latex particles covered with streptococcal group antiserum for groups A, B, C, D, F, and G streptococci. Accompanying this antiserum is extraction reagents, reaction cards, and mixing sticks. The test was conducted by mixing on the reaction card, the group antiserum with a specimen obtained from the selective broth which had been inoculated and incubated for 24 h at 37°C in ambient air. If there was no reaction or the reaction is indistinct, the extraction reagent was added. Agglutination was observed within 30 s for a positive reaction, while for a negative reaction, there was no agglutination within 30 s of the test.

**Antibiotic susceptibility testing**

The modified Kirby-Bauer standardized disc diffusion testing was done. It entails using a sterile cotton swab to make a suspension from a 24-h growth of the organism in saline to match a 0.5 McFarland turbidity standard. Within 15 min of adjusting the turbidity, a sterile cotton swab was dipped into the adjusted suspension. The swab was then be used to inoculate the entire surface of a Mueller-Hinton sheep blood agar (MHA) plate while rotating the plate at an angle of 60° three times. After 10 mins of inoculating, the plate sterile forceps were used to place penicillin G (10 µg), ampicillin, (10 µg), cefazolin (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), clindamycin (2 µg), and erythromycin (15 µg). The MHA plate was then incubated at 35°C in 5% CO₂-enriched atmosphere for 24 h, after which zones of growth inhibition around the disks are identified. The presence of zones of growth inhibition is synonymous with susceptibility, and the absence of zones of growth inhibition is synonymous with resistance.

For isolates showing resistance to erythromycin but susceptibility to clindamycin, a double-disk diffusion test (D-test) was conducted on them because of the risk of inducible clindamycin resistance. Such strains were tested (D-test) for inducible clindamycin resistance by placing an erythromycin, and clindamycin disks 20 mm apart on a MHA plate inoculated with the strain in question. After overnight incubation, the plates were observed for a blunted zone of growth inhibition around the clindamycin disk. If the zone was not blunted, the isolate would be reported as susceptible to clindamycin. If there was a blunted zone of growth or a D-shape is formed around the clindamycin disk, the isolate was reported either as resistant or as susceptible with a comment that resistance may develop during clindamycin therapy. The diameter of the zone of inhibition was measured using a caliper and interpreted according to Clinical and Laboratory Standards Institute 2011 guidelines for *Streptococcus* species other than *Streptococcus pneumoniae* (breakpoints: clindamycin: ≥19 mm = susceptible, 16–18 = intermediate, and ≤15 = resistant; erythromycin: ≥21 mm = susceptible, 16–20 = intermediate, and ≤15 = resistant).

**Results**

A total of two hundred and twenty pregnant women who fulfilled the inclusion criteria between the gestational ages of 35–37 completed weeks and who consented to participate in the study were recruited into the study. There was a 100% participation.
The sociodemographic characteristics of the participants showed that they were aged between 18 and 42 years with a mean of 27.4 ± 5.3 years. The age distribution of the participants showed that 1.4% were less 20 years, 28.6% were 20–24 years, 44.5% were 25–29 years, 12.7% were 30–34 years, 9.1% were 35–39 years, and 3.6% were ≥40 years. Twelve participants (5.5%) were nulliparous, 37 (16.7%) were primiparous, 4 (1.8%) were grand multiparous, and 167 (76%) had parity ranging from 2 to 4.

Majority, i.e., 42.7%, had pregnancies at a gestational age of 37 weeks, 39.5% of the participants were at a gestational age of 36–37 weeks, and 17.7% had pregnancies at a gestational age of 35–36 weeks.

All the participants were married, 9.1% were in the upper socioeconomic class, 31.8% were in the middle socioeconomic class, while 59.1% were in the lower socioeconomic class.

All the two hundred and twenty rectovaginal cultures yielded a growth of microorganisms. Rectovaginal cultures isolated GBS in 19 (8.6%) of the specimens obtained while 201 (91.4%) rectovaginal cultures did not isolate GBS. Serological/antigen testing of the cultures was positive for GBS in 19 (8.6%) of the specimens and negative for GBS in the remaining 201 (91.4%). All the 19 (8.6%) rectovaginal cultures in which GBS was isolated had a positive serological test for GBS [Table 1].

In 19 participants with positive GBS isolates, ages 20–24 years constituted 36.8% while ages 25–29 years constituted 57.9% and ages ≥40 years constituted 5.3%. There was no statistically significant association between maternal age and GBS rectovaginal colonization (Fisher’s exact = 6.224, \( P = 0.229 \)) [Table 1].

Of the 19 (8.6%) with GBS rectovaginal colonization, 52.6% were nulliparous, 26.3% were primiparous, 15.9% were multiparous, while 5.3% were grand multiparous. There was a statistically significant association between parity and GBS rectovaginal colonization (Fisher’s exact = 53.289, \( P = 0.001 \)) [Table 1].

Gestational age of the 19 participants with GBS colonization showed that 57.8% were at a gestational age of 37 weeks while 21.1% were at a gestational age of 35–36 weeks’ gestation and another 21.1% were at a gestational age of 36–37 weeks. There was no statistically significant association between gestational age and GBS rectovaginal colonization (\( \chi^2 = 3.048, P = 0.218 \)) [Table 1].

In relation to social class, 10.0% of the women who were in the upper socioeconomic class had GBS rectovaginal colonization, 15.6% were in the upper middle class, 5.3% in the lower middle class, while 14.3% were in the lower socioeconomic class. There was no statistically significant association between socioeconomic class and GBS rectovaginal colonization (Fisher’s exact = 4.720, \( P = 0.299 \)) [Table 1].

Among the 201 participants who were negative for GBS isolates, 7.9% of babies born were low birth weight as against 10.5% of low birth weight babies born to 19 participants with GBS rectovaginal colonization [Table 2].
All 19 participants who had GBS rectovaginal colonization in pregnancy had intrapartum antibiotic prophylaxis with intravenous penicillin G. All babies born to these women did not show clinical features of sepsis during the 24-h mandatory hospital stay. These babies were followed up during the 1st week of life; during this period, none of the babies had fever, difficulty with sucking, or breathing difficulties.

The antibiotic sensitivity pattern revealed that the GBS isolates were all sensitive to penicillin, ampicillin, and cefazolin. Six (31.6%) of GBS isolates were resistant to both erythromycin and clindamycin. None of the isolates were resistant to erythromycin and sensitive to clindamycin or vice versa.

Comment
There was a statistically significant association between parity and presence of GBS while age group, gestational age, and socioeconomic class showed no statistically significant association with GBS.

Discussion
GBS is a leading cause of morbidity and mortality among neonates. Screening for rectovaginal colonization among pregnant women is not a routine practice at ABUTH, Zaria. This study showed the prevalence of GBS rectovaginal colonization among pregnant women attending ANC at ABUTH to be 8.6%. A study in Nigeria by Nwachukwu[17] on genital colonization with GBS at 36–40 weeks' gestation done in Calabar reported a prevalence rate of 9.0%, which is similar to the prevalence obtained in this study. In contrast, a high prevalence of 11.3% was obtained from the study by Onipede et al.[18] at Ile-Ife. The variation between the Ile-Ife study and this study could possibly be due to variation in maternal colonization from place to place, and the wider gestation age ranges of 35–40 weeks different to 35 weeks to 37 completed weeks used in this study. Other factors that may have contributed to this variation include ethnic and genetic factors.

In this study, there was no significant statistical association between GBS rectovaginal colonization and the age of the participants. However, a study in Ibadan[19] documented an increase in GBS rectovaginal colonization with increasing maternal age so also did the study in Calabar[17] and Ile-Ife[18] however, a study from Malawi[20] reported a decrease in GBS rectovaginal colonization with increasing maternal age.

This study showed that there was a statistically significant association between parity and rectovaginal colonization among pregnant women attending ANC at ABUTH, $P = 0.001$. The prevalence of rectovaginal colonization decreased with increasing parity. This finding is in corroboration with the statement that some women with GBS colonization during pregnancy will be colonized during subsequent pregnancy; however, a substantial proportion will not.[21,22]

This study showed no statistically significant association between GBS rectovaginal colonization and the socioeconomic status of the participant similar to finding by Zusman et al. in Brazil.[23] The Multicenter Vaginal Infection and Prematurity Study group in the United States also reported a weak association between GBS colonization and socioeconomic standing[24]

In this study, all GBS isolates showed a susceptibility to penicillin which is the common agent used for intrapartum antibiotic prophylaxis. The study done in Calabar[17] showed that GBS organisms isolated were all susceptible *in vitro* to penicillin; this was consistent with observations by other investigators.[25] However, in Ile-Ife, there was a high level of resistance of GBS isolates to ampicillin and penicillin. Factors attributed to this high level of resistance of GBS isolates to ampicillin and penicillin included the ease of procurement of over-the-counter antibiotics in developing countries resulting in inappropriate use and abuse of these antibiotics consequently resulting into drug-resistant strains of GBS. In this study, 6 (31.6%) of the GBS isolates were resistant to both erythromycin and clindamycin.

Intrapartum antibiotic prophylaxis (IAP) using intravenous penicillin G was administered to participants who had GBS rectovaginal colonization, and none of the babies had fever, difficulty in breathing, or difficulty in the 1st week of life. Intrapartum antibiotic prophylaxis (IAP) has been shown to reduce vertical transmission of GBS, as measured by infant colonization[11] or by protection against early-onset disease.[12,13] With the use of intrapartum antibiotic prophylaxis, observational studies found an effectiveness of 86%–89% in the prevention of early-onset disease among infants born to women with GBS colonization.[26,27] In conclusion, this study demonstrated that the prevalence of GBS rectovaginal colonization of 8.6%. Parity was found to have a significant statistical association with GBS rectovaginal colonization among participants in this study; there was an inversely proportionate relationship between parity and GBS rectovaginal colonization in this study. This study showed that GBS was susceptible *in vitro* to penicillin, thus making penicillin the first-line choice in women who are not allergic to penicillin.

This study showed a high prevalence of GBS rectovaginal colonization. Public enlightenment of the general population
on screening for GBS rectovaginal colonization in pregnant women and its relationship with prevention of early-onset neonatal sepsis is suggested. Detection of GBS rectovaginal colonization through routine screening of all antenatal patients at 35–37 weeks is advocated. Administration of intrapartum antibiotics prophylaxis during labor is recommended for all pregnant women who screened positive for GBS rectovaginal colonization. The aforementioned suggestions may be appropriate in resource-constrained medical settings in most African countries. However, in some developed countries like the United Kingdom, routine bacteriological screening is not advocated in pregnancy except the woman has an increased risk of having a baby with early-onset GBS disease.\[28\]

Financial support and sponsorship Nil.

Conflicts of interest There are no conflicts of interest.

References