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Research Article
Multidrug Resistant Group B *Streptococcus* Isolates from Pregnant Women in Delta State, Nigeria

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Abstract

Background and Objective: Group B *Streptococci* (GBS) are globally recognized as a major risk factor for neonatal infections and various obstetric complications. More so, biofilm formation has been suggested to be important for GBS pathogenesis. The aim of this study was to determine the prevalence and antibiotic susceptibility pattern of GBS among pregnant women and their capacity to form biofilm. **Materials and Methods:** A total of 87 pregnant women at 34 to 37 weeks' gestation aged 17-45 years were recruited from 3 healthcare centres in Delta State, Nigeria. Cultures for the isolation of GBS were carried out using recto-vaginal swabs, according to standard microbiological methods. All strains isolated were used for susceptibility tests to various antibiotics as recommended by CLSI using the disk-diffusion method. **Results:** The overall prevalence of GBS colonization among pregnant women was 43.6% (38/87). The $\leq$30 age group had the highest rate of GBS colonization. Resistance to erythromycin and vancomycin was 48.2 and 66.4%, respectively. The fluoroquinolones had the lowest resistant rates with no isolate showing resistance to ofloxacin. Multidrug resistance (MDR) ($\geq$3 drug classes) was detected in 73.7% (28/38) of the GBS isolates. All GBS isolated in this study were either strong, moderate or weak biofilm producers. However, most 28 (73.7%) were strong biofilm producers. Resistance of GBS isolates to erythromycin and vancomycin, drugs used for treating GBS infection was high. **Conclusion:** This suggested the importance of testing antimicrobial susceptibilities in GBS colonized pregnant women in order to guide antibiotic therapy and minimize newborn infection and co-morbidity.

Key words: Group B *Streptococci*, biofilm formation, multidrug resistance, pregnant women, neonatal sepsis, pathogenesis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Streptococcus agalactiae, also known as Group B *Streptococcus* (GBS), is an opportunistic pathogen that uses numerous apparatus and immune evasion schemes for vaginal colonization. With a weakened host immune system, GBS can progress from a colonization state to an invasive state. It can cause a myriad of clinical infections, with sepsis, pneumonia and meningitis being associated more with neonates who are born from colonized mothers[1,2]. Additionally, it causes various obstetric and gynecological complications as well maternal death in pregnant women and neonatal death.

To mitigate the effects of GBS on pregnant mothers who are carriers, CDC has recommended intrapartum antibiotics prophylaxis (IAP) on colonized mothers with premature membrane rupture, fever, prolong rupture of membrane longer than 18 hrs and on preterm births so as to prevent neonatal GBS disease[3]. Prompt implementation of IAP results in total elimination or drastic reduction in the incidence of GBS disease in US and in most developed countries[4]. While it has not yet been adopted in most sub-Saharan African countries, including Nigeria, the detection of GBS in pregnant women and the use of IAP to prevent perinatal infection by GBS is not a traditional practice.

Persistent colonization and infection of GBS in different host niches is a function of their adherence ability to host cells and tissues. This then expedites the formation of communities of sessile microbial cells known as biofilms, where the bacteria are firmly fixed into a self-produced extracellular polymer matrix. This unique microenvironment formed can provide protection against antibiotics and enhance immune cells which can bring about persistent colonization and chronic infection[5-9]. Biofilm formation in GBS is strain-dependent and various environmental conditions affect it[5,10]. Several studies have documented biofilm formation by different GBS strains globally[6,11]. However, in Nigeria there is paucity of information on biofilm formation.

The prevalence of antimicrobial resistance among GBS has increased, it is a concern because GBS has been recognized as a leading cause of infections among neonates worldwide[12,13] and it is now known to be of significant public health concern[14]. Basically, in Nigeria, over-prescription and non-regulated use of antibiotics has increased the development of antibiotic resistance[15-19] thus resulting in the emergence of bacterial strains that are resistant to the first- and second-line antibiotic treatments, thereby making third-line antibiotics and other alternative drugs as viable options[20]. This has led to the evolution of multidrug resistant bacteria, such as vancomycin and carbapenem resistant bacteria[21,22]. The GBS is increasingly exhibiting resistant to different antibiotics with significant rates of resistance to erythromycin, clindamycin and recently to penicillin and fluoroquinolone[23,24]. There is paucity of information on the biofilm forming capacity and antimicrobial resistance of GBS isolates from Delta State, Nigeria, hence the present study was aimed at determining the recto-vagina carriage of GBS, antimicrobial resistance pattern and biofilm forming capability of GBS associated with colonization of pregnant women from Ethiopia East Local Government Area of Delta State, Nigeria.

MATERIALS AND METHODS

Study design, participants and clinical sampling: A cross-sectional study was conducted on 87 pregnant women attending antenatal clinics from March to May, 2022 at three general hospitals in Ethiope East Local Government Area of Delta State, Nigeria.

Ethical consideration: Informed consent was obtained from all participants and permission to conduct the study was granted by the Hospital Management Board and the University Research Ethics Committee prior to commencement of the study.

Sample collection: A recto-vaginal swab samples were taken with a sterile cotton swab by trained nurses by inserting swabs into the vagina and then the rectum. The cotton swabs were transferred into Amie's transport medium for preservation of bacteria and transported to the laboratory on ice packs for analysis within 2-4 hrs. The swabs were inoculated into Todd-Hewitt broth supplemented with gentamicin 8 µg/mL and nalidixic acid 15 µg/mL, incubated at 37°C for 24 hrs and processed for GBS isolation and identification following the American Society for Microbiology (ASM) guidelines with modifications[25]. Briefly, a 10 µL aliquot of the cultured broth was subcultured onto a blood agar plate and then incubated at 37°C, 5% CO_2, for 24 hrs. After overnight incubation, plates were read by observing the colony morphological characteristics followed by Gram-staining. Biochemical tests like catalase and ability to produce beta-hemolysis on blood agar were to identify the presumptive isolates. Gram-positive cocci that produced beta-hemolysis, catalase-negative were further subjected to Christie-Atkins-Munch-Peterson (CAMP) tests. The test was performed as previously described by Tesfaye *et al.*[26] and results were interpreted accordingly.

Antibiotic resistance testing: The antibiotic resistance profile of the GBS isolates was determined against 12 antibiotics, which include imipenem, cefuroxime, ofloxacin, erythromycin, gentamicin, azithromycin, amoxicillin-clavulanate, cefotaxime, ceftriaxone, cefixime, levofloxacin and ciprofloxacin by the Kirby Bauer disc diffusion method on a Mueller-Hinton agar (MHA) containing 5% blood as described by Gajic *et al.*[27]. The GBS suspensions of 0.5 McFarland turbidity standards were prepared from fresh bacterial cultures, as described by Raabe and Shane[15]. This was done by suspending 3-4 GBS colonies of the same morphology in 5 mL physiological saline and the turbidity was adjusted to 0.5 McFarland standards[28]. The suspension was inoculated on MHA plates with 5% human blood. The antibiotic discs were placed on the MHA plates aseptically by forceps and thereafter incubated overnight at 37°C. The zone of inhibition was manually measured and the results were interpreted as susceptible or resistant according to the CLSI guidelines[26]. Multidrug resistance was defined as resistance to ≥3 drug classes.

Biofilm formation assays: Biofilm formation was assessed by culturing the GBS isolates on Congo red agar (CRA) plates, prepared by adding 0.8 g of Congo red and 36 g of sucrose to 1 L of BHI (Oxoid, Basingstoke, Hampshire, England) and incubated at 37°C for 24 hrs. Results were interpreted based on the observed phenotypic characteristics of the colony as well as color changes. Colonies that appeared black with slime production were regarded as biofilm forming strains[29].

RESULTS

A total of 87 pregnant women from 3 locations were included in the study. Most of the participants were ≤30 years old in the age group of 17-29 years, living in a rural setting, had no formal education and were Christians (Table 1).

Thirty-eight (43.6%) out of 87 pregnant women studied showed GBS-vaginal colonization. Of these, the highest prevalence recorded was 20 (52.6%) from general hospital, Udu. Whilst considering the age group with the highest GBS colonization, it was observed that the ≤30 age group had the highest rate of GBS colonization.

Resistance to the beta lactams, amoxicillin-clavulanic acid and cefotaxime, was the most common 35 (92%), followed by imipenem and cefuroxime 28 (73.7%), then vancomycin (66.4%) and cefixime and gentamicin 14 (36.8%), azithromycin 13 (34.2%) and erythromycin 18 (48.2%). The fluoroquinolones had low resistant rates. No isolate was resistant to ofloxacin while resistance to levofloxacin and ciprofloxacin was 4 (10.5%) (Table 2). Multidrug resistance (MDR) (≥3 drug classes) was detected in 73.7% (28/38) of the

Table 1: Social demographic characteristics of participants

	Frequency	
Demographic	Number of sample collected	GBS colonization
Health centre location		
Udu	40	20 (52.6%)
Abraka	30	10 (26.3%)
Ughelli	17	8 (21.0%)
Age group		
≥30	36	10 (26.3%)
≤30	51	28 (73.7%)
Marital status		
Single	19	8 (21.1%)
Married	64	30 (78.9%)
Not indicated	04	0 (0.0%)
Educational level		
None	2	0 (0.0%)
Primary	12	8 (21.5%)
Secondary	52	24 (63.1%)
Tertiary	21	6 (15.7%)
Not indicated	-	-
Occupation		
None	6	4 (10.5%)
Professional	10	4 (10.5%)
Skilled	21	10 (26.3%)
Unskilled	48	20 (52.6%)
Not indicated	2	0 (0.0%)
Residence		
Urban	20	8 (21.1%)
Rural	67	30 (78.9%)

Table 2: Antibiotic resistance in GBS isolates from pregnant women

Group	Antibiotics	Disk potency (µg)	Resistance N (%)	Sensitive N (%)
Carbapenem	Imipenem	10	28 (73.7)	10 (26.3)
Macrolides	Erythromycin	15	18 (48.2)	20 (51.8)
	Azithromycin	15	13(34.2)	25 (65.8)
Aminoglycosides	Gentamicin	10	14 (36.8)	24 (63.2)
Penicillins and beta-lactamase inhibitors	Amoxicillin-clavunalic acid	30	35 (92.2)	3 (7.2)
Cephalosporins	Cefotaxime	25	33 (86.8)	5 (13.2)
	Ceftriaxone	30	0 (0.0)	38 (100.0)
	Cefixime	5	14 (36.8)	24 (63.2)
	Cefuroxime	30	27 (72.7)	11 (27.3)
Fluoroquinolones	Levofloxacin	30	4 (10.5)	34 (89.5)
	Ofloxacin	5	0 (0.0)	38 (100.0)
	Ciprofloxacin	5	4 (10.5)	34 (89.5)
Glycopeptides	Vancomycin	30	25 (66.4)	13 (33.6)

Table 3: Multidrug resistance (MDR) profile of *Streptococcus* species

Location	Antibiotic combination	Number of antibiotic group	Remark
Udu	IMP, CXM, AUG and ZEM	3	MDR
	VAN, IMP, GN, AZN, AUG, CTX and CIP	7	MDR
	CXM, AUG and CTX	2	-
	VAN, IMP, CXM, ERY, GN, AUG and CTX	6	MDR
	CXM, AUG, CTX and ZEM	2	-
Abraka	IMP, CXM, AUG, CTX and ZEM	3	MDR
	AUG and CTX	2	-
	VAN, IMP, CXM, ERY, AZN and CTX	4	MDR
	IMP, AUG, CTX and ZEM	3	MDR
Ughelli	IMP, CXM, AUG and CTX	3	MDR
	VAN, IMP, CXM, AUG and CTX	4	MDR

IMP: Imipenem/cilastain, CXM: Cefuroxime, OFX: Ofloxacin, ERY: Erythromycin, GN: Gentamicin, AZN: Azithromycin, AUG: Amoxicillin-Clavunate, CTX: Cefotaxime, CRO: Ceftriaxone, ZEM: Cefexime and LFX: Levofloxacin

Table 4: Biofilm forming capacity

Biofilm phenotype	Number
Strong	28
Moderate	7
Weak	3
No biofilm	0

isolates. Resistance to 5 and 6 drugs was found in Udu location. Each GBS isolate had a different resistant pattern, making it a total of 11 different resistant patterns. The MDR GBS isolates having a combination of vancomycin and imipenem in their resistance profile were more prevalent (Table 3).

All GBS isolated in this study were either strong, moderate or weak producers. However, most 28 (73.7%) were strong biofilm producers (Table 4).

DISCUSSION

Maternal colonization in GBS is an issue of increasing concern, especially due to the potential for mother-to-child transmission. It is important to screen for GBS in pregnant women not only to prevent vertical transmission but invasive diseases that can be associated with post-partum infections which may lead to maternal bacteremia. Researchers have shown that the prevalence of GBS colonization varies in different geographical location. In this study, the prevalence of GBS was 43.6%, this is above the carriage rates of 6.6 to 20% reported in previous studies in pregnant Nigerian women[30-32]. Comparable lower rates have also been reported in other countries like 16% in Brazil[31] and 14.6% in Germany[33]. Higher prevalence of maternal colonization with GBS has also been reported in Nigeria[34,35]. Current results on GBS colonization indicated that ≤30 age group had the highest colonization rates. This may be because most of the participants were ≤30 years. However, it may also be related to the higher estrogen levels during pregnancy in these age groups and perhaps their lifestyle, their sexually active life or history of induced abortion. Dai *et al.*[36] had previously reported that GBS colonization was more frequent in pregnant
women with history of abortion. These factors can cause an altered microenvironment within the genital tract and in the bacteria. Therefore, concerned authorities should pay more attention to these age groups. It is important to state that the differences in GBS colonization reported in different studies carried out in Nigeria and elsewhere in the world must be interpreted with caution because of differences in clinical characteristics, personal hygiene practice, body site sampled, mother's age, duration of sample transportation and storage conditions. In addition, differences in study designs, diagnostic techniques and sample size may also contribute to variations in GBS detection among studies.

Screening pregnant women at 34 to 37 weeks of gestation was to identify women who were colonized at the time of delivery and to prevent the infection of neonates through appropriate administration of one of the first lines of antibiotics. In this study, GBS colonization using recto-vaginal swabs was detected using traditional culture method, which is the detection method used in the clinics sampled. This takes time, as the procedure and process involved are relatively difficult and could lead to negative results. There are various other detection methods for GBS, including, automatic systems and PCR methods. While automatic system is limited by high cost the Polymerase Chain Reaction (PCR) methods are rapid, easy and a low-cost detection for GBS, with high sensitivity, specificity and reproducibility[37]. The PCR methods being a more rapid, sensitive and cost-effective approach should be adopted in developing countries. Dai *et al.*[36] and Ge *et al.*[38] reported higher detection of GBS with PCR methods.

The emergence of antimicrobial resistance among GBS isolates has become worrisome and should necessitate the need for prudent prescription and continuous monitoring of antibiotic resistance patterns in order to guide the appropriate health authorities on empirical therapy. Worldwide studies have revealed that erythromycin is recommended for treating infection among pregnant women who are allergic to penicillin while vancomycin is commonly utilized for GBS-colonized mothers with a high risk of anaphylaxis to penicillin and if the isolate poorly responds to clindamycin[3]. Resistance of GBS to erythromycin reported globally ranges from 3 to 16%. In Nigeria, higher levels of 31.6 and 33.3% were reported by Adesiyun *et al.*[39] and Akadri *et al.*[40], respectively. In this study, erythromycin resistance of 48.2% was observed. Comparable resistance profile in Nigeria, as observed in this study has been reported for erythromycin by Medugu *et al.*[35], who reported 19.9%. Globally, most GBS isolates are found to be sensitive or with low levels of resistance to vancomycin. We observed 66.4% resistance (33.6% sensitivity), which is high in resistance level (lower sensitivity) when compared to reports from other African countries. Tesfaye *et al.*[26] in Ethiopia reported 96.6% sensitivity, Sadaka *et al.*[41] in Egypt and Subramaniam *et al.*[42] in Cameroon reported that all isolates of GBS were shown to be sensitive to vancomycin. However, some studies have shown resistance of 21%[43,44] and 17%[45] to vancomycin. These figures are low when compared to the 34.6% reported in this study. The high rate of vancomycin resistance recorded in this study might be attributed to abuse and indiscriminate use of the drug for different clinical cases thus resulting in the emergence of antibiotic-resistance in GBS. The major concern of increased resistance to erythromycin and vancomycin in GBS is that it limits therapeutic choices for patients with severe penicillin anaphylaxis, with an estimated 5-15% of patients carrying a penicillin allergy label globally[46].

Resistances to other classes of antibiotics (fluoroquinolones aminoglycosides, cephalosporins) were determined in this study so that if patients are allergic to penicillin and the second-choice drugs are not effective, these options may serve as antibiotic of last resorts. The rates of resistance to the fluoroquinolones and gentamicin were low. All isolates were susceptible to ofloxacin, which shows that the fluoroquinolones might be the alternative antibiotic for the treatment of GBS infection where first and second choices are not options.

Multidrug resistance (MDR) development in bacteria occurs through the acquisition of numerous resistance genes borne in a plasmid with each of the genes encoding resistance to a single class of antibiotics. When all the genes are expressed within a single cell, the possibility of a multidrug resistant bacteria emerges. In our GBS isolates, MDR was expressed in 8 isolates (72.7%) as presented in Table 3. Misuse of antimicrobial agents might be attributed to the high level of MDR observed in this study as it is very common for anyone to walk into the drug store and purchase antibiotics for any ailment without proper prescription from a clinician neither is any culture and sensitivity performed to aid prescription. In addition to the above, there is compromised quality in the antibiotics sold as importers do bring in drugs that have little amount of the active ingredients, this helps to fuel antimicrobial resistance evolution. Resistance to 5 and 6 antimicrobials tested were observed in Udu and justify further investigations.

Indiscriminate use and abuse of antibiotics contribute significantly to the evolution of drug resistance in bacteria which results in the ultimate development of MDR. It has been reported that biofilm formation is increased in bacteria that have the capability for its production when exposed to antibiotics[47]. Biofilm is an extracellular polymeric substance that is produced by microorganisms that have the genetic ability for its production. These extracellular polymeric substances can protect the bacteria encased within them from harmful chemical substances ranging from disinfectants to antibiotics. Biofilm formation provides protection to the microorganism encrusted within it thereby playing an important role in antibiotic resistance thereby making it harder to treat bacterial infections[48]. Biofilm production assessed in this study and observed that all were biofilm producers (Table 4) and this has huge clinical negative implications.

CONCLUSION

An increased MDR and resistance against erythromycin and vancomycin among other drugs was observed. Also, all isolates formed biofilm. Therefore, prescribing antibiotics without antibacterial susceptibility tests should be avoided because of the high prevalence of resistance.

SIGNIFICANCE STATEMENT

In this study, the prevalence, drug resistance and biofilm forming capacity of Group B *Streptococcus* isolates from pregnant women were assessed. The GBS is known to cause disease in neonates born to colonized pregnant mothers. Antibiotics are needed to treat the infection in newborns but most often the pathogen is resistant to the commonly empiric antibiotics thus making the management of the infection problematic. The prevalence, multidrug resistance and biofilm capacity in GBS isolated from pregnant women was reported.

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