Original Article

Streptococcus Agalactiae Carriage, Serotype Distribution and Associated Antibiogram among Pregnant and Non-pregnant Women in Jos City, Nigeria

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Abstract

Background: *Streptococcus agalactiae (S.agalactiae*) largely colonizes the gastrointestinal and genitourinary tissues and poses an increased risk for early-onset and late-onset sepsis in neonates delivered by colonized mothers. This comparative cross-sectional study aimed to determine the *S. agalactiae* carriage rate, serotypes, as well as antimicrobial resistance (AMR) of the isolates among pregnant and non-pregnant women in Jos metropolis, Nigeria.

Methodology: High vagina and anorectal swab samples were collected from 200 pregnant women and 100 non-pregnant women. Isolates were identified and characterized biochemically and serologically. Epsilometer test was used to determine the antimicrobial susceptibility of isolates. Sociodemographic variables of participants were collated through the structured questionnaire.

Results: Of all the participants, a prevalence of 3.0% *S. agalactiae carriage was obtained (3.5% and 2.0% in pregnant and non-pregnant women, respectively)*. The isolation rate of GBS from the high vagina was higher (2.0%) compared to the anorectum (1.0%). There was no significant association between the isolation rate with the age of subjects, sample type, and trimester of pregnant women (P>0.05). The serotypes distributions of the *S. agalactiae* isolates were; Ia (22.2%), II (33.3%), III (33.3%) and IX (11.1%). The highest antimicrobial susceptibility of the isolates was to benzylpenicillin (88.9%), clindamycin (88.9%) and least to ampicillin(66.7%).

Conclusion: These findings revealed a moderate level of *S. agalactiae* colonization, low AMR and varied serotypes. Most of the isolates were susceptible to benzylpenicillin, clindamycin, ampicillin, and erythromycin. The screening of pregnant women during antenatal visits should be encouraged to detect infections and minimize potential transmissions to neonates.

Keywords: Group-B streptococci; Neonatal sepsis; Vaginal streptococci; Anorectal Streptococci; Bacterial colonisation; *Streptococcus agalactiae*.

Introduction

Streptococcus agalactiae or Group B Streptococci (GBS) is a leading bacterial pathogen in perinatal infections. In the USA, GBSaccount for the most common aetiology of neonatalsepticaemia and results in considerable morbidity and mortality. [1]

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GBS has been one of the important pathogens in obstetric patients and can cause urinary tract infections, amnionitis, post-partum endometritis, wound infection, and intrapartum and/or postpartum bacteremia.[2] GBS infection may also lead to premature rupture of membranes and preterm delivery. [2]GBS is a natural flora of the gastrointestinal tract, which is the likely source of vaginal and rectal colonization.[1. 2] Group B streptococci are Gram-positive cocci, occurring characteristically in short chains but also in pairs and singly. The organisms are non-motile, and some strains are capsulated. [3]

Worldwide, it is recognized that the colonization rate of GBS in pregnant women is 1 in 4 women. [4] GBS is transmitted from mother to baby vertically during labour and delivery. The current approach for the prevention of GBS neonatal disease involves screening for GBS during pregnancy and administering intrapartum antimicrobial prophylaxis (IAP) to colonized women. [5]In Nigeria, the anorectal and vaginal carriage rates of GBS vary depending on the geographic location.[6, 7, 8, 9] Rates as high as 11.3% have been reported from Ile-lfe, 9% in Zaria and Calabar. [6, 7, 8] In Jos, a previous study by Nsagha *et al*[9] showed a carriage rate of 7.0%.

Obtaining specimens from the anorectum and vagina increases the likelihood of isolation of GBS over obtaining specimens from the vagina alone.[9]The gold standard method for detecting colonized mothers is the culture of vagi-perirectal swabs after overnight enrichment in a selective broth medium followed by subculture onto blood agar to isolate GBS.[10]

Based on their capsular polysaccharides, GBS isolates can be classified into ten different serotypes (Ia, Ib, and II to IX), with serotypes Ia, II, III, and V being the predominant causes of human GBS diseases.[11, 12]Serotype IX is a relatively new serotype that was discovered in 2007 by Slotved *et al*.[13]It is important to note that the prevalence of a given GBS serotype varies according to geographical location. Thus, epidemiological studies of seroprevalence are important in assessing periodic, national and regional changes in GBS distribution.

It is important to remark thatGBS is universally sensitive to penicillin.[13] On the other hand, resistance to erythromycin and clindamycin, the previous second-line agents for GBS prevention, has been increasing and they are no longer recommended for use without antimicrobialsusceptibility testing. Pregnant women are not routinely screened in Nigeria for GBS during antenatal clinics and the chances of mother-to-childtransmission during delivery are high when the mother is a carrier. [12,14]

S. agalactiae vulvo-anal colonization and infection are clinical problems that are not routinely screened during antenatal clinics in Nigeria, perhaps due to low levels of awareness among healthcare providers. Hence, it is necessary to screen pregnant women and identify *S. agalactiae* colonized mothers and associated antimicrobial susceptibility profiles to provide rational direction on how to adequately control or prevent possible early infant onset of disease. Also, the detection of antimicrobial-resistant strains could indicate the relevance of AMR surveillance. Thus, there is a need to determine the current carriage rate, serotype distribution, and antimicrobial susceptibility of GBS isolates in pregnancy in Jos, Nigeria.

Material and Methods

Study Area

The study was carried out in Jos South and Jos North Local Government areas of Plateau State. Plateau State is one of the 36 states of Nigeria. The study area is 510km^2 and has a population of 306,716 at the 2006 census. It is the second most populated Local Government Area in the State after Jos North. Jos North is a Local Government Area in Plateau State. It has an area of 291km^2 and a population of 429,300 at the 2006 census. A total of three (3) hospitals, Bingham University Teaching Hospital and Vom Christian which are missionary based and Plateau State Specialist Hospital which is a government hospital served as sampling areas for the study.

Study population

The study population consisted of pregnant women between the ages of fifteen to forty-five years attending antenatal clinic (ANC) in Vom Christian Hospital (VCH), Bingham University teaching hospital (BUTH) and Plateau State Specialist Hospital (PSSH) while the non-pregnant were attending gynaecological clinics. Written informed consent was obtained from all the women. The women were educated on the research work.

Subjects Selection Criteria

Inclusion criteria: Pregnant and non-pregnant women between the ages of 15 to 45 years and above; Pregnant women attending antenatal clinic in VCH,

BUTH and PSSH; Non-pregnant women attending gynaecology clinicsof VCH, BUTH and PSSH; Pregnant and non-pregnant women who have not received antibiotic therapy for the past four weeks and Pregnant and non-pregnant women without apparent symptoms of bacterial infection.

Exclusion Criteria: Pregnant and non-pregnant women that were below 15years and above 45years; Pregnant and non-pregnant women that were on antibiotics treatment within the last two weeks before recruitment; Pregnant and non-pregnant women with apparent symptoms of bacterial infection and Pregnant and non-pregnant women who do not consent to participate in the study.

Sample Size

A total number of three hundred (300) women were investigated in this study. This was derived from the previous prevalence data of 7.0% reported by Nsagha *et al.* [9]

Ethical Consideration

The study was approved by the research ethics committee of Vom Christian Hospital (VCH), Vom, Bingham University Teaching Hospital (BUTH) and Plateau State Specialists Hospital (PSSH), Jos, Nigeria.

Sampling Method

Simple random sampling techniques were adopted in choosing the area of this study. Out of a total of 9 major hospitals in Jos, 3 were selected randomly which were VCH, BUTH and PSSH, for the study. The authorities of each antenatal clinic involved were presented with an introductory letter from the various research ethical committee and an explanation was given to the nurses in charge of the study and the involvement of the pregnant women. The women were selected by the use of folded tossed papers that had "yes" or "no" written on them. Those that picked "yes" were selected for the studies while those that picked "no" were not selected. This method was used to avoid bias and other conflicts of interest.

Data Collection

The structured questionnaire which captured basic information on *S. agalactiae* carriage among the subjects was designed. A total of two hundred pregnant women and one hundred non-pregnant women (control) between the ages of 15 to 45 years from the participating hospitals were recruited for the study. After giving their due consent by filling out a consent form, each woman carefully filled out the questionnaire and returned them.

Analytical Laboratory protocol

Sample collection

The samples were collected by gynaecologists and nurses. Anorectal and high Vagina swabs were collected from each study subject for whom a questionnaire has been correctly filled. The swab samples were put in a cold box with ice packs. The samples with the filled questionnaire and consent forms were immediately transported to the Central Diagnostic Laboratory of the National Veterinary Research Institute and were processed in the Microbiology Laboratory.

Test for Pregnancy

Human Chorionic Gonadotropin (HCG) is a glycoprotein hormone produced by placental cells after the fertilized ovum is implanted in the uterine wall. The urine is applied to an absorbent pad. The antibody-dye conjugate binds to the HCG in the specimen forming an HCG antibody-antigen complex. This complex migrates by capillary action to the reaction zone where it binds to the anti-HCG antibody, producing a coloured band. In a negative test, no coloured band is produced. This investigation was carried out on all non-pregnant women.

Bacteriological Isolation

Immediately on arrival at the laboratory, the Todd-Hewitt broth supplemented with antibiotics was brought out of the refrigerator and allowed to come down to room temperature. Each high vaginal and rectal swab was aseptically incubated into 0.5ml of Todd-Hewitt broth supplemented with Colistin (10µg) and Nalidixic acid (15µg). They have incubated at 37°C aerobically for 18-24 hours. The Petri-dishes containing solid culture media whose surfaces had been dried in the incubator at 37°C were labelled with the identities on the collected samples. The samples were cultured in duplicates; each sample was inoculated onto 10% sheep blood agar and chromatic Strepto B medium.

Inoculation was done by gently rubbing the swab on the surfaces of the respective culture medium. It was streaked across the medium aseptically. The culture in 10% sheep blood agar was incubated aerobically (using Oxoid aerogen) with 5% CO2 at 37°C for 18-24 hours. While the culture on Chromatic strepto-B was incubated aerobically at 37°C for 18-24 hours (Dunne, 1999). A control strain of *Streptococcus agalactiae*

was also inoculated and incubated alongside the cultured samples.

Reference Bacterial Strain

Staphylococcus aureus subspecies aureus p-1 ATCC 6538 was obtained from National Veterinary Research Institute (NVRI) Vom. Positive local controls of *Streptococcus agalactiae* and *Enterococcus species* were obtained from National Hospital Abuja.

Identification of *Streptococcus agalactiae* Isolates

After the 18-24hours incubation period, the inoculated plates were examined for bacterial growth. All isolates obtained from the primary culture were identified macroscopically, using, modified gram stain, catalase test**,** **c**AMP test, sero-grouping and serotyping.

Sero-grouping of Isolates using a Polyvalent Kit

It has been shown that most pathogenic streptococci possess a specific carbohydrate antigen, which permits the classification of streptococci into groups. These streptococcal group antigens can be extracted from the cells and their presence is demonstrated with latex particles coated with group-specific antibodies. These Latex particles will agglutinate in the presence of homologous antigen but will remain in smooth suspension in the absence of antigen. The latex agglutination test kit identifies streptococcal groups A, B, C, D, F and G. the isolates obtained were serogrouped following the manufacturer's instructions provided in the products leaflet of the streptococcal grouping kit used (Oxoid Ltd, Wade Road, Basing- Stoke Hants RG24 8PW, UK).

Serotyping of Isolates

The Immulex™ strep – B is a rapid latex agglutination test for serotyping of Group B streptococci. It consists of ten (10) bottles (1a to 1x) containing latex particles coated with GBS types antiserum raised in rabbits containing 0.0975% sodium azide as a preservative (SSI Diagnostica, 2 Herredsvejen, DK-3400 Hillerod, Denmark). Three colonies were picked from 10% sheep blood agar, inoculated into 0.5ml of Todd-Hewitt broth and incubated at 37°C for 18-24hours. The Latex coated type antisera: 1a-1b-II-III-IV-VI-VII-VIII-IX were allowed to reach room temperature and the bottles were shaken before use. One drop (approximately 10µl) of latex reagent each was placed in the circle on the reaction card. One drop (approximately 10 µl) of Todd-Hewitt broth culture (bacterial suspension) was placed next to the latex suspension. The two drops were mixed using a mixing stick. A separate stick was used for each reaction. It was spread to cover the area of the circle. The card was rocked slowly and observed for agglutination within 30seconds. The appearance of agglutination within 30 seconds in one of the circles in the card signifies a positive serotype.

Determination of Minimum Inhibitory Concentration (M.I.C)

The minimum inhibitory concentration (MIC) of penicillin G (0.016-256µg/ml), ampicillin (0.016-256µg/ml), erythromycin (0.016-256µg/ml) and clindamycin (0.016-256µg/ml) against all the obtained isolates was performed using the Epsilometer test (E – test) method. The E test is a commercially available antimicrobial testing product that consists of a plastic strip calibrated with MIC scale in µg/ml and code (s) to identify the antimicrobial agent. A predefined concentration gradient of antibiotics across 15 two-fold dilutions of a conventional MIC method, is immobilized on the other surface of the carrier. The procedure is as provided in the product leaflet of the manufacturer (AB BioMerieux, Dalvagen 10, 169 56 Solna, Sweden).The clinical and laboratory standards institute (CLSI) MIC interpretation standard was to determine the antimicrobial susceptibility pattern of the isolates.

Statistical Analysis

The data were analysed using Statistical Package for Social Science (SPSS) version 23 (IBM, New York, USA). The Chi-squared test at 95% confidence interval was used to determine the association between the prevalence of *S. agalactiae* carriage and sociodemographic variables. P values less than 0.05 were considered statically significant.

Results

A total number of 300 women between the ages of 15 to 45 years from three different hospitals participated in the study. The study comprised 200 pregnant women and 100 non-pregnant women. High vaginal and rectal swabs were collected from each of the women, making a total number of 600 samples.

The carriage rate of *S. agalactiae* among pregnant women and non-pregnant women showed some variation as *S. agalactiae* was isolated from 7 (3.5%) of pregnant women and 2 (2.0%) from non-pregnant women. The total carriage rate was 9 (3.0%). Subjects attending PSSH had 80 pregnant women tested of which 5(6.3%) were carriers, while in non-pregnant

women, 40 subjects were tested and had a carriage rate of 1 (2.5%). Subjects attending BUTH, had 80 pregnant women tested and none of the subjects had *S. agalactiae*. In the same hospital, 40 non-pregnant women were also tested, and none was a carrier of *S. agalactiae*.

Forty pregnant women were tested from VCH and the occurrence rate of *S. agalactiae* was 2 (5.0 %) while the non-pregnant women tested, had a carriage rate of 1 (5.0 %). There was no significant difference between *S. agalactiae* carriage rates among pregnant and non-pregnant women in Jos in relation to Hospital attended ($p > 0.05$) (Table 1).

Among the 200 pregnant women tested, *S. agalactiae* was isolated from 7 subjects while from the 100 non-pregnant women tested; *S. agalactiae* was isolated from 2 subjects. *S. agalactiae* carriage varied according to the different age groups. One (25%) was obtained among pregnant women> 40 years, 2 (20%), 2 (7.7%), 1(2.0%) and 1 (1.3%) for age groups 36-40 years, 31-35 years, 26-30 years and 21-25 years, respectively. In the non-pregnant women, *S. agalactiae* carriage among the age groups of 26-30 years and 31-35 years were 1 (3.7%) and 1(5.9%) respectively. None was isolated from the age groups of 16-20, 21-25, 36-40 and >40 years. There was a significant association between the *S. agalactiae carriage,* age and pregnancy status of subjects. results were not statistically significant ($p=0.042$). The highest prevalence of *S. agalactiae* for both pregnant and non-pregnant women was found in the age group >40 years (11.1%) followed by 36-40 (8.0%), 31-35 (7.0%), 26-30 (2.6%) and 21-25 (1.0%) years, respectively (Table 1).

Based on colonization sites, the carriage of *S. agalactiae* among the study subjects showed some variations. The carriage of *S. agalactiae* for the high vaginal site from 200 pregnant women was 4 (2.0%) while from 100 non-pregnant women was 2 (2.0%) which made up for a total carriage rate of 6 (2.0%). The carriage of *S. agalactiae* from the anorectal site of 200 pregnant women was 3(1.5 %) however no *S. agalactiae* isolates were obtained from 100 anorectal samples of non-pregnant women. There was no significant difference between *S. agalactiae* carriage rates among pregnant and non-pregnant women in relation to colonization sites ($p > 0.05$) (Tables 1).

It was observed that the total carriage rate of *S. agalactiae* among pregnant and non-pregnant women varied according to high vaginal swab and anorectal sites. The total carriage rate for the high vaginal site was 6 (2.0%) out of the total of 300 samples including pregnant and non-pregnant women while that of the anorectal site was 3(1.0%) out of the total of 300 samples among pregnant and non-pregnant women (Table 1).

The 200 pregnant women tested for *S. agalactiae* were categorized into first, second and third trimesters based on gestational period. The carriage of *S. agalactiae* was highest in third-trimester subjects who had a carriage rate of 6 (4.7%) followed by second-trimester subjects with 1 (1.9%) and there was no carriage among first trimester subjects. These results however were not statistically significant ($p>0.05$) (Table 2).

Out of the nine *S. agalactiae* isolated, all were serotypable. The serotypes obtained from the 9 isolates of *S. agalactiae* were serotype Ia which was 2(22.2%), serotype II and III which were 3(33.3%) each as well as serotype IX which was 1(11.1%). None of the isolates was serotypes Ib, IV, V, VI, VII and VIII. The distribution of the different serotypes in relation to the colonization site showed no statistical significance ($P>0.05$). The high vagina and anorectum of pregnant women were found to be colonized with serotype Ia,1 (11.1%) each for the high vagina and anorectum. While none was isolated from the non-pregnant women. One (11.1%) of serotype II was isolated from the high vagina and anorectum of pregnant women. While only 1(11.1%) was isolated from the high vagina of non-pregnant women, making a total of 3(33.3%) serotype II isolated. For serotype III, 2(22.2%) came from the high vagina of pregnant women and 1(11.1%) from that site also for non-pregnant women giving a total of 3(33.3%) all from the high vagina. The only serotype IX isolated came from the anorectum of a pregnant woman giving an isolation rate of 1(11.1%).

Of the 9 *S. agalactiae* isolated 4(44.4%) came from the high vagina of pregnant women and 3(33.3%) came from the anorectum. For the non-pregnant women, all the isolates 2(22.2%) came from the high vagina. There was no significant difference in the distribution of serotypes of *S. agalactiae* isolates from pregnant and non-pregnant women in Jos at ($p > 0.05$) (Table 3). From the antibiotics susceptibility tests carried out, the highest susceptibility rate was observed for benzylpenicillin and clindamycin, the isolates showed 89.9% sensitivity for each of the 2 antibiotics.

Of the 2 serotypes, Ia isolates all were 100% sensitive to benzylpenicillin, clindamycin, ampicillin and erythromycin. Out of the 3 serotypes, II isolates 3(100%) were each sensitive to benzylpenicillin and clindamycin while 2(66.7%) were each sensitive to ampicillin and erythromycin. The 3 serotypes III isolates were 100% sensitive to benzylpenicillin and clindamycin while 2(66.7%) were sensitive to ampicillin and erythromycin. Serotype IX was 100% resistant to the 4 antibiotics tested. On the whole, the sensitivity rates for the 9 isolates were as follows in terms of decreasing sensitivity benzylpenicillin and clindamycin 88.9%, ampicillin and erythromycin 66.7%. The overall resistance of the isolates to benzylpenicillin, erythromycin and clindamycin was 11.1% for each while that for ampicillin was 22.2 %. A few of the 9 isolates showed intermediate sensitivity to erythromycin, which was found to be 2(22.2%) (Table 4).

Based on epidemiological characteristics that captured educational levels (uneducated, primary, secondary, and tertiary), marital status (monogamy, polygamy, and single), history of miscarriage, number of children and occupation (civil servant, housewife and traders) the occurrence of *S. agalactiae* carriage among pregnant women were analyzed (Table 5). On the educational level, *S. agalactiae* carriage was observed in uneducated 1 (2.5%), primary 4(3.3%), secondary 1(4.0%) and tertiary 1(10.0%). for pregnant women while in non-pregnant women the occurrence of *S. agalactiae* was 1(2.9%) for uneducated and 1(3.7%) for secondary (Table 5).

In terms of marital status, the occurrence of *S.agalactiae* was 2(2.1%) for monogamy and 5(7.6%) for polygamy pregnant women while in non-pregnant women the occurrence rate was 1(1.9%) in monogamy and 1(2. 8%) in polygamy (Table 5). Based on the history of miscarriage, the occurrence of *S. agalactiae* was 2(1.2%) among women who never had a miscarriage and 5(13.2%) in pregnant women who had one or more miscarriages while non-pregnant women, with no history of miscarriage, had 1(1.1%) while those with one or more miscarriage had 1(7.7%). For women with one or more children, the occurrence of *S. agalactiae* was 6(3.6%) while those with no children had 1(2.9%) in pregnant women. In non-pregnant women, those with one or more children havean occurrence rate of 2(2.2%). Based on occupation, the carriage of *S. agalactiae* was 2(3.0%), 2(3.8%), 3(3.8%) among civil servants, housewives and traders among pregnant women respectively and 1(5.0%), 1(1.7%) from housewife, traders among non-pregnant women respectively (Table 5).

Table 1: Distribution of Group B Streptococci among pregnant and non- pregnant women in Jos in relation to their ages, colonization sites and hospitals.

Variables	Pregnant		Non- Pregnant		Total	X^2	*p* value
	No. Tested	No. (%) positive	No. Tested	No. (%) positive	No. (%) positive		
Age group (years)							
15-20	34	0 (0.0)	12	0 (0.0)	0 (0.0)		
21-25	76	1 (1.3)	24	0 (0.0)	1(1.0)		
26-30	50	1 (2.0)	27	1 (3.7)	2 (2.6)		
31-35	26	2 (7.7)	17	1 (5.9)	3 (7.0)		
36-40	10	2 (20.0)	15	0 (0.0)	2 (8.0)		
>40	4	1(25.0)	5	0 (0.0)	1 (11.1)	2.712	0.042
Colonization site							
High vagina	200	4 (2.0)	100	2 (2.0)	6 (2.0)		
Anorectum	200	3 (1.5)	100	0 (0.0)	3 (1.0)	5.00	0.126
Hospital							
PSSH	80	5 (6.3)	40	1 (2.5)	6 (5.0)		
VCH	40	2 (5.0)	20	1 (5.0)	3 (5.0)		
BUTH	80	0 (0.0)	40	0 (0.0)	0 (0.0)	1.387	0.300

Table 2: Carriage of Group B streptococci among pregnant women in Jos according to gestation period

Gestation period (Trimesters)	No. of patient Tested	No. positive	% positive
First	19	0	(0.0)
Second	52	1	(1.9)
Third	129	6	(4.7)
Total	200	7	(3.5)

$p = 0.336$, $X^2 = 1.257$, df = 2

Table 3: Distribution of the various serotypes of *Streptococcus agalactiae* isolated among pregnant and non- pregnant women in Jos in relation to colonization sites

Serotypes isolated	Pregnant		Non-pregnant		Total No. (%)
	High Vagina	Anorectum	High Vagina	Anorectum	
	No. (%) isolated		No. (%) isolated		
Ia	1(11.1)	1(11.1)	0(0.0)	0(0.0)	2(22.2)
II	1(11.1)	1(11,1)	1(11.1)	0(0.0)	3(33.3)
III	2(22.2)	0(0.0)	1(11.1)	0(0.0)	3(33.3)
IX	0(0.0)	1(11.1)	0(0.0)	0(0.0)	1(11.1)
Total	4(44.4)	3(33.3)	2(22.2)	0(0.0)	9(100.0)

P = 0.058, $X^2 = 3.00$, df =3

Table 4: Antibiotics susceptibility profiles of *Streptococcus agalactiae* serotypes isolated from pregnant and non-pregnant women in Jos

Serotypes	Number Tested	No. (%)											
		Benzyl Penicillin			Ampicillin			Erythromycin			Clindamycin		
		S	I	R	S	I	R	S	I	R	S	I	R
Ia	2	2 (100)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)
II	3	3 (100)	0 (0.0)	0 (0.0)	2 (66.7)	0 (0.0)	1 (33.3)	2 (66.7)	1 (33.3)	0 (0.0)	3 (100)	0 (0.0)	0 (0.0)
III	3	3 (100)	0 (0.0)	0 (0.0)	2 (66.7)	0 (0.0)	1 (33.3)	2 (66.7)	1 (33.7)	0 (0.0)	3 (100)	0 (0.0)	0 (0.0)
IX	1	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (100)
Total	9	8 (88.9)	0 (0.0)	1 (11.1)	6 (66.7)	0 (0.0)	3 (33.7)	6 (66.7)	2 (22.2)	1 (11.1)	8 (88.9)	0 (0.0)	1 (11.1)

Key

Antibiotics	Sensitive	Intermediate	Resistant
Benzyl Penicillin	⩽0.12µg/ml	---	⩾0.12µg/ml
Erythromycin	⩽0.25µg/ml	0.5µg/ml	⩾1µg/ml
Ampicillin	⩽0.25µg/ml	---	⩾0.25µg/ml
Clindamycin	⩽0.25µg/ml	0.5µg/ml	⩾1µg/ml

Concentration of all antibiotics (0.016 - 256µg/ml

Table 5: Group *B* streptococcicarriage rates among pregnant and non- pregnant women in Jos in relation to epidemiological characteristics

Epidemiological characteristics	Pregnant women n=200	No. (%) positive	Non pregnant women n=100	No. (%) positive	Total No. (%) positive
Education level					
Uneducated	10	1(2.5)	8	1(2.9)	2(11.1)
Primary	45	4(3.3)	35	0(0.0)	4(5.0)
Secondary	120	1(4.0)	30	1(3.7)	2(1.3)
Tertiary	25	1(10.0)	27	0(0.0)	1(1.9)
Marital status					
Monogamy	96	2(2.1)	52	1(1.9)	3(2.0)
Polygamy	66	5(7.6)	36	1(2.8)	6(5.9)
Single	38	0(0.0)	12	0(0.0)	0(0.0)
History of miscarriage					
None	162	2(1.2)	87	1(1.1)	3(1.2)
Yes	38	5(13.2)	13	1(7.7)	6(11.8)
No. of children					
One or more	165	6(3.6)	91	2(2.2)	8(3.1)
None	35	1(2.9)	9	0(0.0)	1(2.3)
Occupation					
Civil servant	67	2(3.0)	21	0(0.0)	2(2.3)
Housewife	53	2(3.8)	20	1(5.0)	3(4.1)
Traders	80	3(3.8)	59	1(1.7)	4(2.9)

Discussion

In this study, 300 women between 15 to 45 years that attended antenatal clinics in three hospitals in Jos (Plateau State Specialist Hospital, Vom Christian Hospital and Bingham University Teaching Hospital) were screened for GBS colonization. In this current study, the overall carriage rate in both pregnant and non-pregnant women was 3.0%. However, the carriage rate was 3.5% in pregnant women and 2.0% in non-pregnant women. The vaginal introitus is assumed to be the origin of *S. agalactiae*colonization. The ecological conditions of the pregnant cervix and the acidic milieu created by the lactobacilli and a glycogen rich mucosa seem conducive to the growth of GBS.[9] The absence of these conditions in non-pregnant women may be responsible for the lower colonization rates in non-pregnant women.

The 3.0% carriage rate obtained in this study was lower compared to several studies conducted in other parts of Nigeria, Africa and some developed countries. In Nigeria, Onipede *et al* [6]reported a carriage rate of (11.3%) in IIe-Ife[7] obtained a carriage rate of (9.0%) in Calabar, Nsagha *et al*[9]reported a carriage rate of (7.0%) in Jos. Shabayek *et al*[8]obtained a 9.0% carriage rate in Zaria.

In Africa, Suara *et al*[15] reported a carriage rate of (22.0%) in The Gambia. Moyo *et al*[16] also reported a carriage rate of 20-32% in Zimbabwe. In developed countries, Clouse*et al* [17]reported a carriage rate of (19.5%) in Jordan and Tsui *et al*[18] reported a carriage of (10.4%) in Hong Kong. The differences could be due to geographic, ethnic, and socio-economic factors as well as differences in sampling and culturing techniques.

Although the isolation rate in this study is similar to that of Nsagha *et al*[9] in being low compared with others, there was, however, a decrease in carriage rate between Nsagha *et al*[9]study (7.0%) and this study (3.0%). Considering that both studies were carried out within the same area and that there was an improvement in culturing technique with the use of chromatic Strepto B selective and differential medium which can detect even rare non-haemolytic GBS strains yet there was a reduction in carriage rate in this study. This finding may probably represent a decreased burden and improvement in maternal hygiene as it relates to*S. agalactiae* carriage. Alternatively, in this study, samples were not collected from women attending Jos University Teaching Hospital (JUTH), whereas, in Nsagha *et al*[9] study samples were collected from there. JUTH is a referral hospital handling a large number of complicated cases. Therefore, because it was not one of the hospitals included in this study; this could be a reason for the lower carriage rate encountered when compared with Nsagha's study. [9]To have an idea of the potential prevalence difference of GBS carriage between a tertiary (government) healthcare facility, secondary (government) and faith-based specialist hospitals, JUTH was excluded. Moreover, a previous study had already provided data about GBS carriage from JUTH. [9]

Subjects were drawn from three different hospitals, Plateau State Specialist Hospital, Vom Christian Hospital and Bingham University Teaching Hospital. The carriage of *S. agalactiae* appeared not to be affected by the hospitals the subjects attended as the distribution showed no statistical significance.

Although increasing age appeared to be an important influencing factor in the carriage of *S. agalactiae* with the highest occurrence rate (11.1%) being found among women >40 years, this result is not statistically significant. The high occurrence rate within this age range could probably be a result of the small sample size.

The carriage has previously been reported to increase in age and number of parities. [19]However, Cools *et al* [20] reported that the distribution of isolates from asymptomatic colonized pregnant women was irrespective of age. In this study, non-pregnant women between the ages of 26-30 years and 31-35 years were colonized. In a study conducted by Donbraye-Emmanuel [21] in Ibadan, it was reported that, in non-pregnant women, GBS colonization was highest in women between the ages of 21-25 years. This is also in concordance with a study conducted by Ezeonu *et al*[22] in Enugu which reported that *S. agalactiae* colonization was highest in women between ages 21-25 years.

This may be attributed to the fact that young women in this age group are more sexually active than older women. Meyn *et al*[23] reported that GBS is more common in sexually active women including both frequent intercourse and multiple sex partners. The isolation rate of GBS from the high vagina (2.0%) was higher compared to the anorectum (1.0 %). This is in concordance with the report of Joachim *et al*[24]who observed the distribution of isolated GBS to be higher in the vagina (12.3%) as compared to the rectum (5%) in Tanzania.

Findings from this study indicated that women in their 3rd trimester were more colonized with GBS compared to those in 2nd trimester. This agrees with findings from studies conducted by Nsagha *et al*[9] in Jos and another study by Okon *et al*[25] in Maiduguri. This finding is in contrast to that observed by Donbraye *et al*[21] in Ibadan, where women in their 2nd and 3rd trimesters were more colonized. The current recommendation by CDC is to screen pregnant women at 35th to 37th weeks (3rd trimester) of gestation using a culture of vaginal and anal swabs. [26] Raj *et al*[27]observed that the vaginal colonization of GBS varies during the period of gestation and that screening earlier than six weeks before delivery may not be a true reflection at delivery and may not accurately predict the vaginal colonization at delivery.

All the *S. agalactiae* isolates obtained in this study fell within serotype Ia (22.22%), II (33.33%) III (33.33%) and IX (11.11%). The finding from this study is different from the report of Eren *et al*[28]who reported serotypes Ia, II and IV in Turkey. Simoes *et al*[29] reported serotypes Ia, Ib and II in Brazil. Ippolito *et al*[30], Zhang *et al*[31] and Barcaite *et al*[32] reported serotypes Ia, II, III and V from pregnant women and

neonates with early-onset disease (EOD) in the USA and some European countries. In a study by Charlene-Africa *et al* [33], serotypes Ia, II and IV were reported in South African pregnant women. It was also observed in our study that serotypes II and III were predominant, followed by serotype Ia and the newly discovered serotype IX was the least. Limited population seroprevalence data are available for serotype IX given its recent emergence in the general population.

The antibiotic susceptibility patterns of *S. agalactiae* isolates in this study showed a higher susceptibility to benzylpenicillin and clindamycin (88.9% each) followed by ampicillin and erythromycin (66.7% each). The resistance rate to benzylpenicillin, erythromycin and clindamycin in this study was 11.1% each, while ampicillin was33.7%.

The isolates showed an intermediate susceptibility rate of 11.1% to erythromycin. Castellano-Filho *et al*[34] reported that 22.7% and 50% of GBS isolates were resistant to erythromycin and clindamycin respectively. Simeos *et al*[29]reported (39.9%) of GBS isolates were resistant to erythromycin as a result of the acquisition of an erythromycin ribosomal methylase *(erm)* gene which encodes a methylase enzyme that modifies the binding site on ribosomal RNA or through the constitutive expression of an erm-encoded methylase which results in resistance to erythromycin and clindamycin.These genes may be responsible for the observations in this study. From this study benzylpenicillin and clindamycin appears to be the drugs of choice with 89.9% sensitivity.

However, for women with severe penicillin allergy who are colonized with GBS erythromycin and clindamycin are commonly used as alternatives. It should be noted that none of the antibiotics tested was 100% effective against all isolates. This, therefore, makes antibiotic susceptibility testing mandatory.

In regard to epidemiological characteristics, the assessment of the educational level of the women revealed that those that were uneducated had the highest carriage rate of 11.1% followed by primary 5.0%, tertiary 1.9% and those with secondary had the least with (1.3%). Based on marital status, the carriage rate of *S. agalactiae* was highest among polygamous families (5.9%), followed by monogamous with (2.0%). The higher carriage rate among polygamous families could be due to multiple sex partners.

The occupation of the pregnant and non-pregnant showed that housewives had 4.1%, traders, 2.9% and civil servants, 2.3%. This is similar to a study conducted by Okon *et al*[25] which showed a colonization rate of 5.3% among housewives, 3.0% among civil servants and 1.5% among traders. The poor socio-economic status of housewives may be responsible for the higher carriage rate. Results from this study showed that 44.4% of the isolates were CAMP positive. This agrees with a study carried out by Nsagha *et al*[9]which showed that the CAMP test had a positivity of 30%.

Conclusion

These findings revealed a moderate level of *S. agalactiae* colonization, low AMR and varied serotypes. Most of the isolates were susceptible to benzylpenicillin, clindamycin, ampicillin, and erythromycin. The screening of pregnant women during antenatal visits should be encouraged to detect infections and minimizepotential transmissions to neonates.

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