Prevalence, characterization, antimicrobial susceptibility pattern and factors associated with group B streptococci (*Streptococcus agalactiae*) from clinical and non- clinical sources in South-East Nigeria

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Abstract

Many years ago most human infections caused by aerobic streptococci, were attributed to Lancefield groups A and D or to the so called non-groupable viridans streptococci. The first human isolates of the Group B Streptococci (GBS) were isolated from the vagina of post-partum women. This study is aimed at assessing GBS colonization of some clinical and common non-clinical surfaces. This study utilized a total of 615 samples (300 clinicals and 315 non-clinicals). Samples were from neonates and women who were respectively seen at the Institute of Child Health, of the University of Nigeria Teaching Hospital and Amblim Reference Laboratory in Enugu. The 300 clinical samples were cultured from 119 pregnant and 79 non-pregnant women, 52 neonates and 25 (7 primary and 18 secondary infertility) infertile couples (50 individuals). All samples were cultured on a modified Islam medium and identification carried out using standard bacteriological methods. Characterizations of the GBS isolates were carried out using sodium hippurate reactions, aesculin reactions, Christie, Atkin Muchin–Patterson (CAMP) test and definite hydrolysis on blood agar. The overall GBS isolation of 15.8% was observed in the clinical and non-clinical surfaces assessed. For the clinical subjects, GBS colonization of 11.9%, 7.6%, 9.6% and 17.0% were observed in the pregnant women, non-pregnant women, neonates and couples admitted for infertility. Prevalence of GBS isolation on inflamed cattle udder and milk products were 4.0% and 83.3%, respectively. Gestational period was associated with GBS colonization in pregnant women. All the GBS isolates were susceptible to penicillin and methicillin while all were resistant to streptomycin, tetracycline and sulphafurazole. GBS colonization of vagina was found and this poses a risk for neonatal sepsis.

Introduction

Many years ago most human infections caused by aerobic streptococci were attributed to Lancefield groups A and D or to

the so called non-groupable viridans streptococci. The first human isolates of the Group B Streptococci (GBS) were isolated from the vagina of post-partum women.[1] In 1938, Fry found GBS present in the vaginal cultures of both symptomatic and asymptomatic women at the time of delivery.[2] Some of the symptomatic patients had the organism both in the vaginal swabs and blood cultures.

Despite these early associations with human infections, the GBS continued for many years to be considered as primarily an agent responsible for mastitis in cattle, goats and sheep with little human relevance and was therefore named *Streptococcus agalactiae*.[1] Clinically, microbiological characteristics of the GBS are definitively differentiated from other streptococci by Lancefield typing or by other serologic methods such as counter immunoelectrophoresis. These techniques however, are not available in all clinical laboratories. Hence, in this study, presumptive identification of GBS was made by a number of other properties of these organisms as indicated below.

Morphologically, these human GBS strains usually appear pigmented. They show a narrow but definite zone of weak beta haemolysis unlike group A *Streptococci*,[3] though, about 5% of the human strains are not/slightly beta haemolytic and these are normally missed.[1] About one-third of GBS strains are Bacitracin sensitive by disk testing and are thus often confused with group A *Streptococci*. The fluorescent antibody method available in most Laboratories for identifying group A beta-haemolytic *Streptococci* will differentiate these strains.[4]

Further studies suggest that by the use of haemolytic reactions on blood agar, sensitivity by the same bacitracin disk, bile aesculin reactions and hydrolysis of sodium hippurate, most GBS can reliably be detected in the clinical Laboratory.[3] These organisms fail to produce a bile aesculin reaction but nearly always hydrolyze sodium hippurate. CAMP (Christie Atkins Munch-Peterson) test, which is an abbreviation named after the initials of the three original authors, now served as a confirmatory test of this organism (GBS).[4,5] The CAMP reaction was based on the observation by Christie *et al.*,[5] that a lytic phenomenon occurs when group B, but not other streptococci, are grown in a zone of *Staphylococcus* β toxin activity on a sheep's blood agar plate.

From the previous studies done in some parts of the world, it had been observed that numerous reports in the past decade have established the association of GBS with man. Here, the organism is carried asymptomatically in various sites of the body such as the vagina of pregnant women, urethra of males and the Gastrointestinal Tract (GIT).[6,7]

Patients in all age groups may be infected with GBS, but the vast majority of infections caused by this pathogen occur among the very young, especially in infants, with a greater incidence among the neonates aged ≤5 days.[8] There had been a microbiologic scientific adage that says 'when thinking of GBS, think of group B for baby'.[8] Two distinct syndromes have been described to be associated with this GBS in infants, namely: i) Early-onset or acute onset; these are normally observed in neonates of ≤5 days old. This is often seen in premature neonates/or neonates born in the setting of serious maternal complications like in prolonged rupture of membranes;[9] ii) Late-onset; this shows non-specific findings of neonatal sepsis, bacteraemia and meningitis. These are very common while pulmonary symptoms are infrequent.[5] In addition to symptomatic bacteraemia, pneumonia and meningitis, other clinical infections in infants have also been noted. These include otitis media, ethmoiditis, conjunctivitis, omphalitis, cellulitis, impetigo, emphysema, pericarditis, peritonitis, oesteomyelitis, arthritis and endocarditis.[10]

Some studies have shown that about 25% of women carry these 'bugs' vaginally, and a baby can acquire these bacteria during delivery.[11] In the study done by Baker,[12] about 1% of children born to mothers infected with GBS develop bacteraemia and pneumonia within the first five days of life in the United States. In spite of intensive antibiotic therapy, such infections carry a mortality rate of 50–70%. The carrier rates of this organism especially during pregnancy vary with localities and socio-economic backgrounds.[12] It is generally believed that more than 10% of pregnant women are carriers of the organisms and are believed to transmit it to newborns; up to forty five thousand cases per year of post partum endometriosis in women following childbirth occur in different parts of the world.[13] Hence, neonates from these women develop bacteraemia in less than five days after birth which may result to neonatal meningitis or pneumonia.[14] Whether this organism can indeed play a role in abortions of pregnancies and infertility has been a subject of intense research. Some studies have shown its significance especially in secondary infertility. There is however, scarcity and uncoordinated information in this part of the country on GBS. Consequently, this study evaluates GBS colonization on non clinical and clinical surface against their demographic background in the studied area.

Materials and Methods

Study areas

The studied areas were randomly selected from the two geopolitical zones of Enugu State of Nigeria, namely Enugu zone and Nsukka zone. These zones comprise of ten and eight Local Government Areas (LGAs) respectively, from which four LGAs namely Enugu North, Enugu East, Nkanu West and Nsukka LGAs were selected (Figure 1). Clinical samples were collected from different hospitals and laboratories namely: Institute of Child Health (ICH) University of Nigeria Teaching Hospital, Enugu (UNTH), gynaecology clinics of UNTH, Christian Miracle Hospital, Amblim Reference Laboratories, all in Enugu zone; Bishop Shanahan Hospital, All Saints Hospital and Maternity, both in Nsukka zone from aforementioned latter LGAs, according to their environmental and socioeconomic status e.g. occupation. The private-owned hospitals were selected based on the physician's consultation charges. Also the non-clinical samples were randomly selected from major areas of Fulani tribe settlement in Enugu and Nsukka zones, abbatoir benches in the meat markets and milk products, e.g ìFura-da-nunuî in Enugu (Enugu North and Enugu East) and Igbo-eze South LGAs in Enugu and Nsukka zones respectively. The Enugu zone comprised the core urban areas while the Nsukka zone comprised the sub-urban areas.

Types of samples used

Samples were divided into two major groups namely clinical and non clinical together with their different sub-groups.

Clinical sources

High Vaginal swabs (HVS) were collected from 119 pregnant women with signs and symptoms previously suggestive of early rupture of membranes before labour and other antenatal complaints (like pelvic inflammatory diseases) as deduced by the gynaecologist while some of the women reported for their normal ante-natal visits. These consisted of 25 pregnant cases from Nsukka zone and 94 pregnant cases in Enugu zone. These were based on questionnaires distributions to the antenatal women dur-

ing antenatal clinic. These were then confirmed from their past medical history in their folders (case note).

HVS were also collected from 79 non-pregnant women who attended the clinics during the period of the study with past clinical history of premature babies during previous delivery (ies), complaints of itchy vaginal discharges, while some just came for check up. Out of these 79 non-pregnant women, 48 were from Enugu zone while 31were from Nsukka zone. Biodata were collected from the gynaecologist and directly from the patients (age, gestational age, parity and their clinical conditions) using a well structured questionnaires.

Criteria for selection

The subjects were selected based on gynaecologist's diagnosis and the patient's complaints, which include: itchy discharge, infertility, and intrauterine devices (I.U.D).

The second batch of the clinical samples were 52 neonates aged ≤5 days old. These neonates were brought to the clinic for immunization against tuberculosis at the Institute of Child Health (ICH) unit of University of Nigeria Teaching Hospital (UNTH) Enugu (n=32) and Children's Welfare clinic of Bishop Shanahan Hospital Nsukka (n=20).

The selection of neonates were based on the refusal of immunization by the nurses on the ground that the neonate had fever after taking the vital signs. This refers to neonates presenting with signs and symptoms suggestive of neonatal sepsis as a result of bacteraemia (characterized by persistent fever, irritation with constant crying, foul smelling undetached/detached umbilical cord).

The last batch of the clinical sample collection were fifty samples from 25 couples (7 primary and 18 secondary infertility cases) who reported for infertility investigations in the hospitals (with laboratory observations suggestive of azospermia, oligospermia etc. for the men and menorrhagia with past medical history of pelvic inflammatory diseases for the women), and sent to the Amblim Reference Laboratories Enugu for tests (semen analysis and culture of their urethral swabs (U/S) of males as well as culture of HVS and the Endocervical Swabs (ECS) of their female counterparts by the gynaecologist. The duration of this study lasted for 15 months (from June 2018 to September 2019).

Candidates were seen both as in-patients and out-patients at UNTH Enugu, Christian Miracle Hospital and Maternity Enugu, Bishop Shanahan Hospital Nsukka, All Saints Hospital and Maternity Nsukka and Amblim Reference Laboratories Enugu.

The last criteria were based on the written information by the gynaecologist in their laboratory forms.

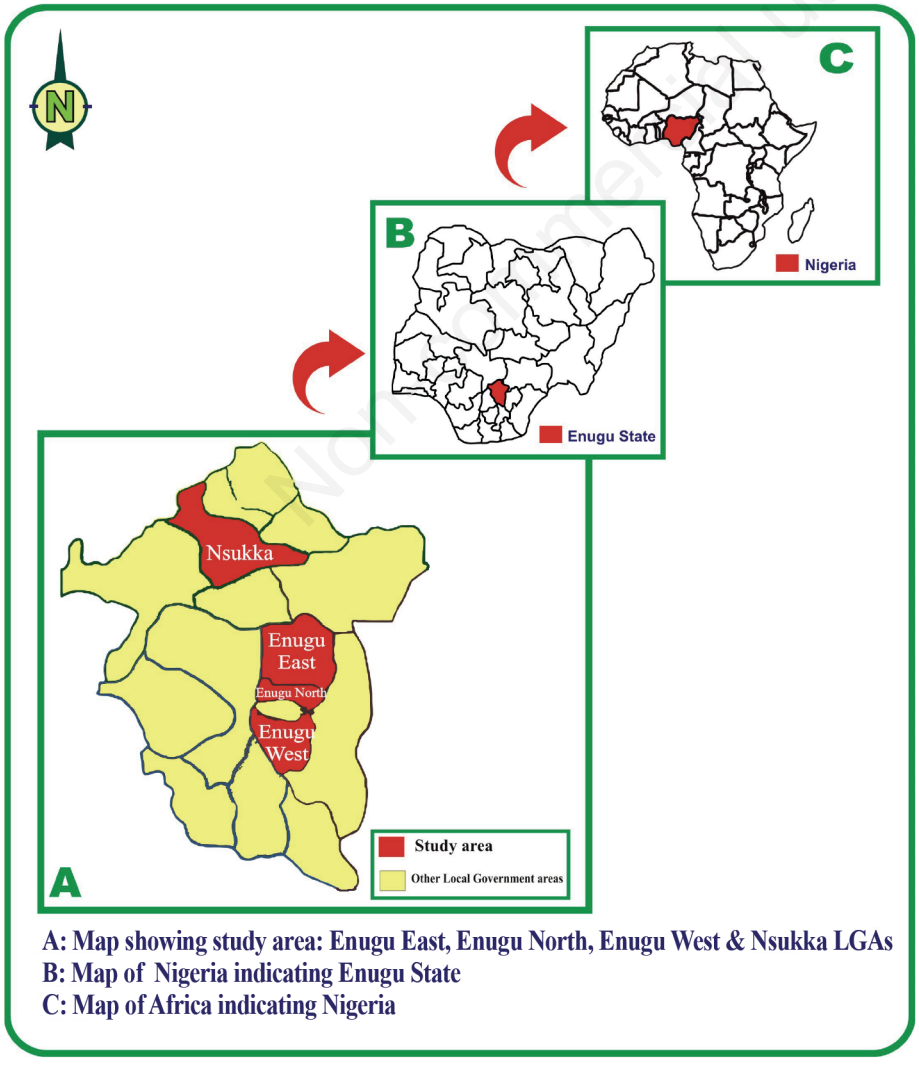

Figure 1. Map of study area.

Non-clinical samples

Udder samples from cattle from two major areas of Fulani tribe settlemen; Onyema mine area in Enugu North L.G.A (130 cattle) and Ekpoto Enugu-Ezike in Igbo-eze L.G.A (145 cattle) were collected. The inflamed udders of the cattle were swabbed using pre-moistened sterile cotton swabs and sent in for laboratory processing.

Criteria for selection

The criteria were based upon inflamed udder as a result of mastitis. (These were observed when the cows resisted the calf to suck the breast).

Cattle milk/milk products: The second stage included culturing 30 samples of freshly prepared milk products popularly known as ìFura-da-nunuî hawked by Fulani milk maidens in their Nike settlement, Enugu East LGA. The various samples were carried to the Department of Medical Laboratory Science, Teaching Laboratory for immediate processing within 2 hours of collection. The above criteria were based on the assumption of the Fulani milk maiden that after extraction of the raw milk that the cattle normally develop mastitis.

Laboratory investigations

The following steps were used for isolation and identification of GBS as described in Figure 2. All analyses were performed in duplicates.

Cultural identification using modified ISLAM AGAR

An inoculum of each specimen was made on a freshly prepared ISLAM AGAR (Oxoid, UK, code CM0755) using the following Compositions (Reagents from (Scharlau Laboratories Ltd, Spain): Peptone Water (23 g), Soluble Starch (5 g), Disodium hydrogen Phosphate (5.75 g), Agar No. 2 (13.5 g), Distilled Water (1L). The various components were mixed, free-steamed for 5 min, the pH adjusted to 7.4; it was then autoclaved at 121°C for 15 min (Sterilization process). After cooling, 30 ml of sterile sheep's blood, Neomycin at a final concentration of 30 mg/L, Nalidixic Acid at a final concentration of 15 mg/L, and Metronidazole at a final concentration of 50 mg/L, were added (as selective and differential agents) and the medium were poured into culture plates. All beta-haemolytic colonies on the blood agar plates; and all isolates from the Islam's medium were Gram-stained. The presence of colonies with orange red or yellow-green pigments was noted.

Characterization

The following steps were used for the characterization process. This is represented in Figure 2.

Gram's staining and Catalase Activity

Pure discrete colonies were Gram stained using Gram's staining technique, catalase test was carried out on a loop-full of gram positive cocci colony procedure as described by Cheesbrough.[15] Screening for catalase activity was performed on all pigmented and non pigmented isolates which were gram positive. *Staphylococcus aureus* NCTC 6571 and *Streptococcus pyogenes* NCTC 8198 (type 1, group A) from United Kingdom stocked at the Microbiology culture stock unit University of Ibadan, Nigeria, were used as positive and negative controls.

Growth on Gential Violet Blood Agar plates (GVBA)

All catalase negative and pigmented strains on Islam's media were each streaked on 1:500,000 GVBA and incubated at 37°C for 24 h. *Streptococcus agalactiae*, mastitis streptococci grew and gave pale-gray colonies on GVBA. This method was according to Collins and Lyne.[16] A reference strain of *Streptococcus agalactiae* was used as positive control.

Growth on Mackonkey Medium

All Catalase negative and pigmented strains on Islam's medium were each streaked on a freshly prepared Mackonkey (Biotech, UK) plates and incubated at 37°C for 24 h. *Streptococcus agalactiae*, mastitis streptococci grew and gave orange pigmented colonies on Mackonkey medium. This method was according to Merritt and Jacobs.[17]

A distinguishing test between GBS and group A streptococci (GAS) using: Bacitracin sensitivity disc testing

Bacitracin sensitivity disc test was performed according to Wessels *et al*.[18] A loop full colony of GBS was streaked on a nutrient agar plate after which bacitracin disks were placed on it. This was incubated for 24 h. GAS streaked and incubated the same way on a different nutrient agar plate was used as a control test. GBS was found resistant to bacitracin antibiotics while GAS was found sensitive to bacitracin antibiotics.

A presumptive identification test for GBS: Hippurate hydrolysis test

Hippurate hydrolysis test was performed according to Wessels *et al.,*[18] A sodium hippurate (Scharlau Laboratories Ltd, Spain) substrate was defrosted and heavily inoculated in two test tubes with a pure culture of the GBS organisms; one extra test tube was

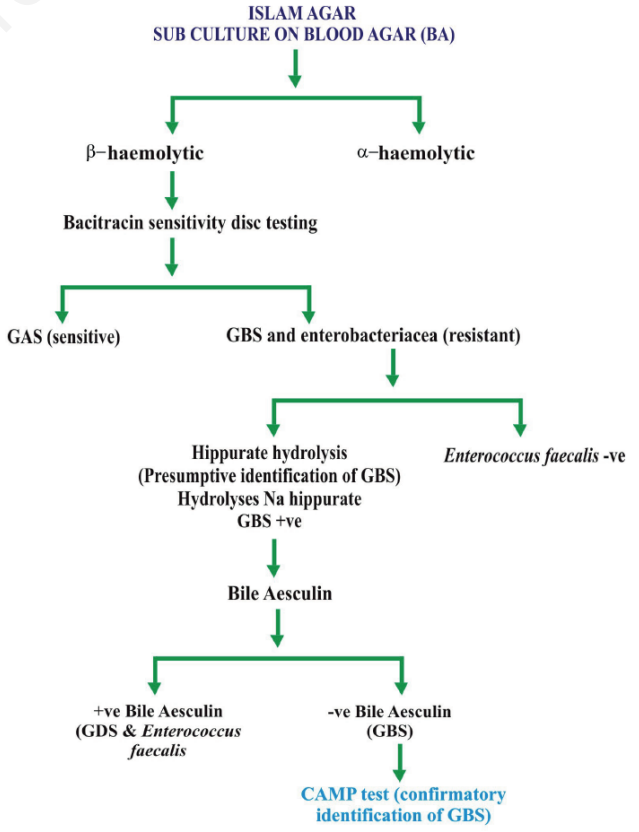

Figure 2. Steps used for isolation and identification of group B streptococci (GBS).

inoculated with a pure culture of *Streptococcus faecalis* ATCC 19433. This served as a control test. These test tubes were then incubated for 2 h in a water bath at 35ºC after which 0.2 ml of the prepared ninhydrin reagent was added and reincubated for an additional 15 min. The two test tubes inoculated with GBS turned deep purple. This was an indication that hippurate had been hydrolyzed; while the test tube inoculated with *Streptococcus feacalis* remained colourless which was an indication that hippurate was not hydrolyzed.

A distinguishing tests of GBS from GDS (group D streptococci) and Enterococcus sp.: Aesculin hydrolysis test

Aesculin hydrolysis test was performed according to modified method of Edwards *et al.,*[19] using reagent from Scharlau Laboratories Ltd, Spain (Ref 265).

GDS and *Enterococcus species* grow in the presence of bile and also hydrolyze aesculin to aesculetin and glucose. Aesculin diffuses into the agar and combines with ferric citrate in the medium to give a black complex.

On a freshly grown colony of GBS blood agar plate, a ìRoscoî bile aesculin tablet was placed on top of the inoculated organism and the same was also done on a control test in which the organism used was *Streptococcus faecalis* ATCC 19433 (*Enterococcus faecalis*). These plates were incubated aerobically at 37ºC for 24 h.

The plate containing GBS colony remained colourless which was an indication that GBS did not hydrolyze aesculin while the control test plate turned black which was an indication that *Streptococcus faecalis* hydrolyzed aesculin.

A distinguishing test between GBS from other Streptococcus species: CAMP test

This served as a confirmatory test in the characterization processes. Briefly, as the inoculums of GBS were streaked on a blood agar plate perpendicular to *Staphylococcus aureus* streaking on a nutrient agar plate, an accentuated zone of haemolysis was seen. This was the positive CAMP reaction. It is only GBS that produces a positive CAMP reaction amongst the other *Streptococcus* species.

Antibiotic sensitivity

Each isolate was evenly streaked onto a human blood agar plate. The plates were left at room temperature for ten min. The Oxoid multidisc consisting of PN 1.5 units, E_{10}µg, S_{10}µg, Te_{10}µg, CF_{10}µg, SF_2µg, MT_{20} µg and AM_2 µg, (Oxoid, UK, No.S3) were aseptically placed on each inoculated plate surface. The plates were left on the bench for 10 min and later incubated at 37ºC aerobically for 18 h. Zones of inhibition were measured manually with a slide rule and the ìOxoidî zone reader.

Micro-organism used (test substance)

The organism were grown in Brain Heart Infusion (BHI) broth and incubated at 37ºC aerobically for 48 h. Cells were harvested by centrifugation at 3000 rpm for 10 min and washed three times with phosphate buffered saline (PBS) pH 7.4 and re-suspended in PBS to give a final concentration of $5x10^8$ CFU/mL, determined by OD550 nm using a spectrophotometer (DME-21 spectrophotometer, Digimed SP, Brazil). The purity of the culture was further determined by plating and gram staining.

Ethics approval and consent to participate

The research ethical approval was obtained from the University of Nigeria Teaching Hospital, Enugu (UNTH) Ethics Committee (UNTH/CSA.329/VOL. 5). Enrollment into the study was voluntary. Informed consent was written and only consenting adults were recruited for the study. Written informed consent was obtained from parents in the case of children.

Statistical analysis

All generated data were subjected to statistical analysis using Statistical Package for Social Science (SPSS version 20.0, California Inc.). Categorical variables were represented as frequencies and percentages. Associations between variables were assessed using Fisher exact test. Alpha value was set at 0.05 (95% significant rate).

Results

Table 1 shows the distribution of the clinical and non-clinical sources used for the study. Among the clinical sources, neonates, pregnant women, non-pregnant women and couples for infertility tests composed 18.9% (n=52), 43.3% (n=119), 28.7% (n=79) and 14.4% (n=25) of the sources, respectively. While among the non-clinical sources, inflamed udder, milk products and abattoir bench constituted 87.3% (n=275), 9.5% (n=30) and 3.0% (n=10), respectively.

Demographic characteristics of the pregnant women showed age brackets of 15-20, 21-26, 27-32, 33-38, 39-44 and 45-50 years comprising 18.5% (n=22), 28.6% (n=34), 11.8% (n=14), 26.9% (n=32), 12.6% (n=15) and 1.7% (n=2), respectively of the studied pregnant women. Most (43.7%; n=52) of the pregnant women were in their 21-23 weeks of gestation. The majority of the non-pregnant women enrolled were in the age bracket of 21-26 years and 39-44 years (29.1%, n=23 and 21.5%, n=17 respectively). Neonates of 0-1, 2-3 and 4-5 days consisted 19.2% (n=10), 40.4 (n=21) and 40.4 (n=21), respectively (Table 2).

Table 3 shows the protocol of the identified streptococci isolates. All GBS isolates encountered in the study showed typical cultural and biochemical characteristics suggestive of *Streptococcus agalactiae.* The GBS isolates showed the typical central pilling-up of cells within each colony (bull's eye appearance). Each colony was semi-opaque, medium sized with a narrow zone of β-haemolysis on blood agar plates. On the Islam-agar plates, the moderately sized colonies showed orange to pinkish pigmentation. The isolates were all not sensitive to the drug Bacitracin (2 micrograms) disk diffusion test; all showed a positive sodium hippurate test, positive CAMP test and a negative bile aesculin test.

The total prevalence of GBS in the clinical and non-clinical

Table 1. Distribution of clinical and non-clinical source used in the study'.

Sample source	No. sampled from each location		
	Enugu (%)	Nsukka (%)	Total
Clinical sources			
Neonates < 5 days	32 (61.5)	20 (38.5)	52 (18.9)
Pregnant women	94 (79.0)	25 (21.0)	119 (43.3)
Non pregnant women	48 (60.8)	31 (39.2)	79 (28.7)
Couples for infertility test	50 (100.0)	-	50 (14.4)
Non-clinical sources			
Inflamed udder	130 (47.2)	145 (52.8)	275 (87.3)
Milk products	30 (100.0)	-	30 (9.5)
Abattoir bench	10 (100.0)	-	10 (3.0)

sources in this study was 15.8%. The highest prevalence of GBS was observed in the milk products (83.3%), followed by couples with infertility problems (17.0%) and pregnant women (11.8%). The least sources of isolation were infected udder of beef (4.0%) and non-pregnant women (7.6%). Milk products were the highest source of non-clinical contamination while pregnant women consisted majority of the clinical sources (Table 4).

Table 5 shows the distribution of GBS in the studied population based on clinical diagnosis and samples assessed. Among the pregnant women assessed, 37.5% (n=9) of those with clinical diagnosis of threatened abortion were infected with GBS while 6.8% (n=5) of those who came for normal cyesis were infected with GBS. On the other hand, 2.0%, 6.7%, 0.0% and 40.0% of the non-pregnant women who visited hospital for normal routine checkup, IUD insert checkup, heavy menstrual flow and secondary infertility, respectively had GBS colonization. The difference in the distribution of GBS based on clinical diagnosis in pregnant and non-pregnant women was significant (P<0.05). Approximately 9.6% (n=5) of the neonates diagnosed with bacteremia were infected with GBS. Majority (72.0%; n=36) of the patients diagnosed with secondary infertility were infected with GBS. For the non-clinical object, 4.0% (n=11) and 83.3% (n=25) of the inflamed udder and ìfura-da-nunuî milk product were infected with GBS.

Table 6 shows factors associated with presence and absence of GBS infection among pregnant and non-pregnant women. While age was not associated with GBS infection in both pregnant and non-pregnant women (P>0.05), gestational age was associated with GBS infection in the pregnant women (P<0.05).

Table 7 shows the susceptibility pattern to some regular antibiotics of GBS isolates. One hundred percent (100%) of the GBS isolates were resistant to streptomycin, sulphafurazole and tetracycline. On the other hand, 99.0%, 94.8%, 100%, 100% and 97.7% were sensitive to chloramphenicol, erythromycin, methicillin, penicillin and ampicillin respectively.

Table 2. Demographic characteristics of human subjects studied.

Demographic characteristics	Area of study Enugu	Nsukka	Total
Pregnant women	**(n=94)**	**(n=25)**	**(n=119)**
Age (Years)			
15-20	15 (15.9)	7 (28.0)	22 (18.5)
21-26	20 (21.3)	14 (56.0)	34 (28.6)
27-32	12 (12.8)	2 (8.0)	14 (11.8)
33-38	30 (31.9)	2 (8.0)	32 (26.9)
39-44	15 (15.9)	0 (0.0	15 (12.6)
45-50	2 (2.1)	0 (0.0)	2 (1.7)
Gestational age (Weeks)			
18-20	25 (26.6)	3 (12.0)	28 (23.5)
21-23	50 (53.2)	2 (8.0)	52 (43.7)
24-26	5 (5.3)	2 (8.0)	7 (5.9)
27-29	4 (4.3)	4 (16.0)	8 (6.7)
30-32	5 (5.3)	5 (20.0)	10 (8.4)
33-35	5 (5.3)	4 (16.0)	9 (7.6)
36-38	0 (0.0)	4 (16.0)	4 (3.4)
39-40	0 (0.0)	1 (4.0)	1 (0.8)
Non-pregnant women	**(n=48)**	**(n=31)**	**(n=79)**
Age (Years)			
15-20	3 (6.3)	8 (25.8)	11 (13.9)
21-26	8 (16.7)	15 (48.4)	23 (29.1)
27-32	10 (20.8)	4 (12.9)	14 (17.7)
33-38	12 (25.0)	2 (6.5)	14 (17.7)
39-44	15 (31.3)	2 (6.5)	17 (21.5)
Neonates	**(n=32)**	**(n=20)**	**(n=52)**
Age (Days)			
0-1	3 (9.4)	7 (35.0)	10 (19.2)
2-3	12 (37.5)	9 (45.0)	21 (40.4)
4-5	17 (53.1)	4 (20.0)	21 (40.4)
Infertile couples			
Male	25 (100.0)	-	25 (100.0)
Female	25 (100.0)	-	25 (100.0)

Discussion

Overall GBS isolation

The overall GBS isolation prevalence in this study on the clinical and non-clinical surfaces is 15.8%. However, vaginal colonization of 11.8% was found in pregnant women studied. This observation is similar to 11.3% reported at Ile-Ife, Nigeria.[20] However, lower values of 8.6% and 9.0% have been reported in Zaria[21] and Calabar,[22] respectively. In terms of studies outside Nigeria, lower prevalence of 10.0%, 7.1%, 7.2% has been reported in South Africa,[10] China[23] and Ethiopia,[24] respectively, while higher values of 14.3% and 30.0% have been reported among Kenyans[10] and South Africans,[25] respectively. The variation in prevalence of GBS vaginal colonization has been attributed to regional variations, variation in gestational age of study participants, socio-economic factor, differences in clinical practice, differences in culture media used, differences in sample collection technique, ethnic and genetic factors.[21,24]

Table 3. Characterization of streptococci isolates

Streptococcus isolates	Colonial Morphology On Islam Agar	Haemolytic Activity on Blood Agar	Bacitracin Sensitivity Disk (2µg)	Sodium Hippurate Test	Bile Aesculin	CAMP Test
GBS	Bull's eye, medium sized creamy white colonies compared with GAS	Narrow diffuse zone of β-haemolysis	Resistant	+ve	-ve	+ve
GAS	Pin point, brittle &translucent Gray colonies (which may turn brown on continued incubation	Large & deep zone of β-haemolysis in comparism with colony size	Sensitive	-ve	-ve	-ve
Streptococcus faecalis	Pin point, white opaque colonies	Moderate diffuse zone of β-haemolysis	Resistant	-ve	+ve	-ve
GDS	Pin point, creamy to pinkish colonies	Moderate diffuse zone of β-heamolysis	Resistant	-ve	+ve	-ve

Keys: GAS; Group A Streptococci, GBS; Group B Streptococci and GDS; Group D Streptococci -ve; Negative and +ve; positive.

Table 4. Distribution of studied subjects and objects yielding positive isolates of GBS.

Sampled population	No. sampled		Total	No. positive for GBS		Total (%)
	Enugu	Nsukka		Enugu (%)	Nsukka (%)	
Pregnant women	94	25	119	6 (6.4)	8 (32.0)	14 (11.8)
Non-pregnant women	48	31	79	4 (8.3)	2 (6.5)	6 (7.6)
Neonates	32	20	52	2 (6.3)	3 (15.0)	5 (9.6)
Infertile male partners	25	-	25	18	-	18 (17.0)
Infertile female partners	25	-	25	18	-	18 (17.0)
Inflamed udder	130	145	275	5 (3.8)	6 (4.1)	11 (4.0)
Milk product	30	-	30	25	-	25 (83.3)
Abattoir bench	10	-	10	0	0	0 (0.0)
Total	394	221	615	78 (20.3)	19 (8.6)	97 (15.8)

Table 5. Prevalence of GBS based on clinical diagnosis.

Clinical diagnosis	No. analyzed	No. positive for GBS (%)	No. negative for GBS (%)	Test statistics
Pregnant women				
Cyesis after 20 infertility management	21	0 (0.0)	21 (100.0)	
Threatening abortion	24	9 (37.5)	15 (62.5)	Fisher's exact test
Normal cyesis	74	5 (6.8)	69 (93.2)	P=0.001
Non-pregnant women				
Routine check-up	50	1 (2.0)	49 (98.0)	Fisher's exact test
IUD insertion check-up	6	1 (6.7)	5 (83.3)	P=0.002
Heavy menstrual flow	13	0 (0.0)	13 (100.0)	
2° infertility	10	4 (40.0)	6 (60.0)	
Neonates				
Bacteremia	52	5 (9.6)	47 (90.4)	
Infertile couples				
2° infertility	50	36 (72.0)	14 (28.0)	

2°: secondary. No.: Number.

Table 6. Analysis of independent variables of some of the clinical subjects and presence or absence of GBS.

Variable	GBS positive	GBS negative	Test statistics
Pregnant women			
Age (Years)			
15-20	3 (13.6)	19 (86.4)	Fisher's exact test
21-26	5 (14.7)	29 (85.3)	P=0.238
27-32	2 (14.3)	12 (85.7)	
33-38	1 (3.1)	31 (96.9)	
39-44	2 (13.3)	13 (86.7)	
45-50	1 (50.0)	1 (50.0)	
Gestation age (weeks)			
18-20	1 (3.6)	27 (96.4)	Fisher's exact test
21-23	0 (0.0)	52 (100.0)	P<0.01
24-26	1 (14.3)	6 (85.7)	
27-29	4 (50.0)	4 (50.0)	
30-32	5 (50.0)	5 (50.0)	
33-35	3 (33.3)	6 (66.7)	
36-38	0 (0.0)	4 (100.0)	
39-41	0 (0.0)	1 (100.0)	
Non-pregnant women			
Age (Years)			
15-20	1 (9.1)	10 (90.9)	Fisher's exact test
21-26	0 (0.0)	23 (100.0)	P=0.148
27-32	3 (21.4)	11 (78.6)	
33-38	1 (7.1)	13 (92.9)	
39-44	1 (5.9)	16 (94.1)	

GBS colonization among pregnant women and associated factors

In this study, prevalence of GBS colonization among the pregnant women was associated with the gestational age. This finding is in consonance with previous reports.[26,27] Gestational age has been reported as a risk factor for GBS with prevalence being higher among women of higher gestational age. In the present study, there was no association between age and vaginal colonization of GBS among pregnant women. However, studies in Calabar,[22] Ile-Ife[20] and Ibadan,[28] in Nigeria, have reported association of increasing maternal age with vaginal colonization in pregnant women, while the report by Dzowela and colleagues in Malawi[29] reported decrease in vaginal colonization with increasing maternal age.

GBS colonization among non-pregnant women

The result of this study showed GBS colonization rate of 7.6% among non-pregnant women. This observation in non-pregnant women (together with their pregnant counterparts) corroborates the report of the bacteria colonizing the genital and gastrointestinal tract of healthy females.[30] The colonization is often asymptomatic except in invasive cases which often occur in females with underlying disease.

GBS colonization isolation among neonates

We observed 9.6% GBS infection in neonates in this study. This finding is higher than 3.1% and 6.7% reported in Morocco[31] and India,[32] respectively. A meta-analysis performed in China reported 0.55-1.79%.[33] GBS is the leading cause of neonatal morbidity and mortality via sepsis, meningitis and pneumonia.[32] The fetus often acquires GBS perinatally during labor or *in utero* through transmission of bacteria from the maternal vagina or anorectally colonized mucosa for mothers colonized by the bacteria.[27] This represents the vertical mode of transmission and constitutes the Early Onset of the Disease (EOD). The bacteria infect the placenta either by ascending through the cervix into the amniotic cavity or via the cervix into the amniotic cavity or via the urinary tract resulting in urinary tract infection and bacteremia.[34] Mothers who are carriers of GBS have 50% chances of infecting their babies during birth.[35] On the other hand, horizontal transmission has been documented in series of cases by Morinis *et al.*,[36] which are mostly nosocomially acquired and constitute the Late Onset of the Disease (LOD).

GBS isolation in infertility

Prevalence of 17.0% GBS isolation was recorded in the couples for infertility test. The concomitant GBS in both couples and isolation in urethral swab corroborates earlier suggestion that GBS can be sexually transmitted. Ross *et al.*[37] have reported higher colonization rate in STD patients and also isolation in male urethra. More so, Manning *et al.*[38] reported higher colonization rate in sexually active young people in a dormitory compared to the sexually inexperienced participants. GBS isolation has been also reported in vaginal swab and blood sample of women with secondary infertility.[39] This finding is further strengthened by the observation of the highest isolation of GBS in the non-pregnant women who came to hospital on cases secondary infertility.

GBS isolation on inflamed udder and milk product

We observed a colonization rate of 4.0% in inflamed udder of cattle with GBS and 83.3% in milk product called *fura-da-nunu* that were due to bovine mastitis caused by GBS. It is often seen in cattle as one of the economic challenge in the rearing process.[40] On the other hand, the milk product *fura-da-nunu* is a popular milk drink hawked by Fulani maidens in Nigeria in many states of the country. The high prevalence of GBS isolation in the cattle and the milk product puts up the worry about the cross infection to humans. Although previously there has been a suggestion that interspecies transmission of GBS was unlikely.[41] However, a more recent study by Manning *et al.*[42] strongly suggested an interspecies transmission/zoonotic transmission. This poses a big public health question.

Antimicrobial susceptibility pattern of the isolated GBS

The antimicrobial susceptibility pattern of the isolated GBS showed 100% susceptibility to penicillin and methicillin and; 99.0% and 97.9% susceptibility for chloramphenicol and Ampicillin, respectively on the GBS isolates. The absolute susceptibility to penicillin observed in this study is similar to 100% susceptibility reported in Zaria,[21] Calabar,[22] and Ibadan[28] all being studies in Nigeria. Similar penicillin susceptibility on GBS isolates have been reported in, South Africa,[25] However, contrasting result of 100%, and 72.4% penicillin resistance have been reported in Ile-Ife, Nigeria[20] and Kenya,[27] respectively. The resistance to chloramphenicol (1.0%) observed in this study is lower than 33.4% reported in Calabar, Nigeria.[28] More so, the resistance of 2.1% to Ampicillin observed on GBS isolates in this study is higher than 0% (100% susceptibility) reported in Calabar,[28] South Africa,[32] Argentina,[44] Germany,[45] but lower than 54.2%, and 100% resist-

Table 7. Antibiogram pattern of group B streptococci (GBS) isolated from different population samples.

N=97 Drug Tested	Concentration per disk (µg)	No. of GBS screened	No. sensitive susceptibility	% resistance	%
Chloramphenicol	10	97	96	99.0	1
Erythromycin	10	97	92	94.8	5.2
Sulphafurazole	2	97	0	0.0	100.0
Methicillin	20	97	97	100.0	0.0
Penicillin	1.5 units	97	97	100.0	0.0
Ampicillin	2	97	95	97.9	2.1
Streptomycin	10	97	0	0	100.0
Tetracycline	10	97	0	0	100.0

ance reported in Kenya[35] and Ile-Ife, Nigeria,[26] respectively. The GBS isolates in this study showed erythromycin resistance of 5.2%. This value is lower than 9.8%, 16.1%, 22.3%, 41.7%, 60.0% and 100% GBS resistance to erythromycin reported in Argentina, Germany, Calabar Nigeria, Hungary, Ile-Ife Nigeria and Zaria Nigeria, respectively. [28,38,26,27,44,45] All the GBS isolates observed in this study were resistant to streptomycin. The susceptibility pattern mirrors absolute resistance to streptomycin of GBS isolates reported by Nwachukwu and colleagues in Calabar, Nigeria.[28] Also, all the isolates in this study were resistant to tetracycline. However, lower values of 29.0%, 44.4% and 82.3% have been reported in Argentina, Calabar Nigeria and Hungary respectively.[28,38,44] More so, all isolates were resistant to sulphafurazole.

The performance of penicillin in this study and others compared with justifies its use for both prophylaxis and treatment of GBS infection. Generally, antibiotics resistance has been attributed to factors such as ease of procurement of over-the-counter antibiotics and subsequent abuse in developing countries.[21] Tetracycline resistance as was observed in this study has been attributed to mechanisms such as: efflux with 28 distinct classes of eflux pumps (tetA, tetB, tetC, tetD, tetE, tetG, tetH, tetJ, tetK, tetL, tetA(P), tetV, tetY, tetZ, tet30, tet31, tet33, tet35, tet38, tet39, tet40, tet41, tet42, tet45, tetAB(46), ter3, otrC, otrB) that expel tetracycline out of the cell; presence of ribosomal proteins (tetM, tetO, tetQ, tetS, tetT, tetW, tetB(P), tet 32, tet36, tet44, otrA, tet) that binds to ribosome and subsequently remove the drug from the binding site; Genes (tetX and tet37) that modify tetracyclin and subsequently promote the degradation; mutation in 16S rRNA (G1058C, A926T, G927T, A928C and ΔG942 mutations) that reduces the binding affinity of tetracycline to ribosome; and lastly the tetU encoded on the plasmid pKq10 in *E. faecium* that has been reported to cause resistance in tetracycline.[44] On the other hand, streptomycin resistance has been attributed to mutations in 16S rRNA genes (rrs, rpsL, gidB, strA-strB aadA and others);[45] ribosomal proteins S12 and 16S rRNA specific methyltransferase. The resistance to erythromycin has been attributed to ribosomal methylation; expression of erm(B) gene; and use of efflux pump (mef(A).[44,45]

Conclusions

Unlike previous studies, this study examined non-clinical samples and surfaces as well as GBS colonization in view of infertility. This study showed high prevalence of GBS colonization of studied clinical and non-clinical samples. The vaginal colonization of GBS poses the risk of perinatal acquisition of GBS by the neonate during labour. The presence of GBS on cattle udder gives a red flag to ilook deepî on zoonotic GBS transmission. We also found penicillin and methicillin (both β-lactams) suitable for management of GBS infection in the studied setting. Considering the high colonization recorded and the odd of vertical transmission to neonates, we strongly recommend the administration of intrapartum antibiotic prophylaxis using penicillin in the studied area and in other parts of Nigeria based on the antibiogram profile of the area.

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