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GROUP B STREPTOCOCCUS CARRIAGE DURING LATE PREGNANCY IN ILE-IFE, NIGERIA

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ABSTRACT

This study determined the prevalence of Group B Streptococcus (GBS) in late pregnancy and the antimicrobial susceptibility of isolated GBS as well as the impact of GBS infections on pregnancy related clinical outcome with a view of providing an epidemiological baseline data for policy formulation in the teaching hospital. It is an observational and cross-sectional hospital based study. One hundred and fifty pregnant women from 35-40 weeks of gestation were purposively selected and included in the study from May to December 2010. Vaginal swab samples were aseptically collected from the subjects after informed consent. The samples were assayed for presence of GBS. The susceptibility pattern of the isolated GBS to different antibiotics were assessed using disc diffusion and agar dilution techniques based on the Clinical and Laboratory Standards institute(CLSI) standards. The result showed prevalence of 11.3% GBS vaginal colonization which increased with age. There was no significant association between GBS colonization status and age (p >0.05)), gestational age (p >0.05)), gravidity (p >0.05) and obstetric risk factors (p >0.05)). There was no incidence of GBS infection observed. Although, all (17) the GBS isolates were 100% resistance to penicillin, ampicillin, cefoxitin and clindamycin. Resistance to cefotaxime (11.8%), erythromycin (64.7%) and vancomycin 70.6% were observed. Group B Streptococcus colonization in vagina in late pregnancy has been established in the antenatal clinic of the teaching hospital with the attendant risk to the fetus in the population of those affected. There were high and multiple resistance patterns of the GBS isolates to different antibiotics in this study. This calls for a review of the present hospital policy to include the routine screening of GBS during antenatal visits and surveillance.

Keywords: *GBS, Carriage, Pregnancy and antibiotics resistance.*

INTRODUCTION:

Maternal infections of Group B Streptococcus (GBS) constitute one of the leading pathogens associated with both early and late-onset neonatal sepsis (1). The bacteria are normally found in the vagina and/or lower intestine of 15% to 40% of all healthy.adult women (2). Early onset neonatal sepsis is normally related to vaginal carriage in the motherand subsequent colonization during birth in approximately 70-75% of infants (3). Intrapartum prophylaxis has been established to lead to a 70% decline in the incidence of GBS disease, however, early-onset GBS disease (in infants <7 days old) remains a leading cause of illness and death among newborns (3, 4).

Most data on GBS epidemiology are from Europe and North America and a few cases of GBS infections (5-7) and carriage as a public health problem have been reported from Nigeria (5, 8, 9, 10, 11) and other African countries (12, 13, 14).

This study was designed to determine the prevalence of GBS in pregnancy and the incidence of GBS infections in intrapartum in a tertiary hospital in Nigeria. It also determined the antimicrobial susceptibility of isolated GBS and assessed the impact of GBS infections on the pregnancy related clinical outcome with a view of providing an epidemiological baseline data for policy formulation in the teaching hospital.

MATERIALS AND METHODS

Study Area: The study was carried out at the Antenatal Clinic of Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) and the Department of Medical Microbiology and Parasitology, Obafemi Awolowo University (OAU), Ile-Ife Osun, Nigeria from May to December 2010. It was a cross-sectional observational study involving pregnant women between 35 and 40 weeks who attended the antenatal clinic. Pregnant women who

had had antibiotic treatment within the last two weeks prior to recruitment were excluded from the study. The Ethical Committee of the hospital approved the study.

Study Population

One hundred and fifty pregnant women were sampled following the ethical approval of the study. The patients' consents were sought and gained by explaining to them the objectives of the study and the benefits there in. Profomas questionnaires were filled for the volunteers to acquire demographic and other relevant obstetric history.

Specimen Collection

Two swab samples were taken per participant and using a speculum from the vaginal introitus under aseptic condition with a commercially available collection and transport system for aerobes and anaerobes (Charcoal Swabs Amies Plastic Applicator with Rayon Tipped Black Cap, Stone, Staffs, UK). All swab specimens were transported to the Medical Microbiology Laboratory of OAU.

Specimen Culture Isolation and Identification, Microscopy and Antigen Detection

The swab samples were inoculated into selective enrichment broth medium (Todd-Hewitt broth supplemented with 10µ/ml colistin and 15µ/ml nalidixic acid, Oxoid England) and were incubated aerobically at 37^{O}C for 24 hours. After 24 hours incubation, broth cultures were observed for growth (Turbidity) and then sub cultured onto 5% Sheep Blood Agar (Oxoid England) and incubated overnight as above. Suspected GBS isolates were identified appropriately as earlier described (9). GBS antigen was confirmed definitively by serological grouping using Streptococcal group B reagent kit (Oxoid England) testing of selective broth (15).

The results were analyzed using the SPSS software (version 11.0) and evaluated statistically by Chi square, Pearson Yate's correction test. Level of significance was taken to be P - values <0.05. The sensitivity, specificity, positive and negative predictive values and efficiency of the antigen detection test were evaluated against the culture isolation. A pregnant woman was deemed to have been positive for GBS colonization when either or both culture or antigen detection is positive.

Antibiotic Susceptibility Testing

A small number of colonies obtained from each 24 hours old 5% Sheep blood agar plate culture was incubated in Todd-Hewitt broth for 2 hours at 37^{O}C to obtain a McFarland level of turbidity of 10^5 and logarithmic-growth phase culture. These were tested against different antibiotic discs which included penicillin, ampicillin, cefotaxime, clindamycin and

erythromycin and the Minimum inhibitory concentrations were determined based on recommended standards (16, 17) and Streptococcus *pneumoniae* ATCC 6305 was used as control organism and free drug plate was included as negative control.

RESULTS

The study participants reflected the composition of the low risk antenatal group at our hospital in their age, parity, gravidity and gestational age. One hundred and fifty (150) pregnant women participated in the study over a period of six months (May to December 2010). Their age range was 18-42 years with a mean of 30.25 (±4.65) and the age group in years was categorized as younger (18-29) and older (≥30) pregnant women, which were 64 (42.7%) and 86 (57.3%) respectively. Table 1 shows parity and gestational age among other parameters as indicated.

In this study, GBS colonization was confirmed by antigen detection from enrichment broth culture and direct culture isolation of the vaginal swab samples (Table 2). Seventeen (17) out of the 20 pregnant women that were positive for antigen detection by latex agglutination test after 18 hours incubation in enrichment broth were also culture positive. In the remaining 3 antigen positive women, no GBS could be isolated in spite of prolonged incubation (>72hours).

Among the subjects that were colonized with GBS, GBS colonization was higher among older women with age ≥30 years (12.79%) against 9.38% in younger (18-29) women (Table 3). The difference was not statistically significant (X^2= 0.426; P= 0.514). The age distribution of the GBS colonized and non GBS colonized population is shown in Figure 1. The GBS colonization increases as the age increases and a higher colonization was observed in the age range 34-37 years. The age range of GBS positive women was 26-36 years with mean of 30.31(±4.346) while GBS negative women had age ranging from 18-42 years with an average age of 30.17 (±4.741).

Out of the 17 GBS positive pregnant women, 10 pregnant women had their gestational age to be ≤37 weeks but ≥35 weeks while 7 women had their gestational age >37 weeks but ≤40 weeks, the difference was not statistically significant (X^2 = 1.297; P = 0.225)(Table 4). The prevalence of GBS colonization was observed to be higher at 35 weeks gestational age. Among the GBS positive cases, the

prevalence of GBS colonization was higher among the mtltigravida (64.71%) than the primigravida (35.29%) (Table 5). GBS vaginal colonization was not statistically associated with gravidity ($X^2 = 0.375$; P = 0.54).

TABLE 1: CHARACTERISTICS OF THE STUDY PARTICIPANTS

Characteristics	Frequency (%)
Age range	18-42
Age mean (±sd)	30.25(±4.65)
Age group in years	
Younger (18-29)	64(42.7)
Older (≥30)	86(57.3)
Parity range	0-6
Mean (±sd)	1.07(±1.221)
Gravidity	
Primigravida	42(28)
Multigravida	108(72)
Gestational age in weeks	
Range	35-40
Mean (±sd)	36.81(±1.526)

The statistical analysis of GBS carriage in relation to obstetric characteristics is shown in Table 6. Out of the one hundred and fifty studied population, only forty-two (38%) women came to deliver in the hospital eventually. Four (4) out of the forty-two (9.52%) were GBS positive women while thirty-eight (90.48%) were negative cases. Among the 4 GBS positive women, 2 women had gestational age at delivery to be <40 weeks whereas, the other 2 delivered at >40 weeks. The duration of the rupture of membrane (ROM) of the 4 positive pregnant women was less than 10 hours and only one had spontaneous vaginal delivery, the other three delivered through emergency cesarean section due to failure to progress to secondary labour. Twenty seven (27) (96.43%) out of the GBS negative subjects delivered through vagina while the remaining 11 (78.57%) subjects had cesarean type of delivery. However, GBS colonization was not statistically associated with gestational age at delivery, duration of rupture of membrane and mode of delivery (P = 1) (Table 6).

TABLE 2: ANTIGEN DETECTION IN CULTURE POSITIVE AND CULTURE NEGATIVE CASES

Test Result	Culture Positive	Culture Negative	Total
Ag Positive	17 (True Positive)	3 (False Positive)	20
Ag Negative	0 (False Negative)	130 (True Negative)	130
Total	17	133	150

Sensitivity = 100%, Specificity = 95.6%, Positive predictive value = 85%, Negative predictive value = 100%

TABLE 3: PREVALENCE OF GBS VAGINAL COLONIZATION IN RELATION TO AGE

Age (Years)	GBS-positive	GBS-negative	Total
Younger (18-29)	6(9.38)	58(90.62)	64(100)
Older (≥30)	11(12.79)	75(87.21)	86(100)

X^2=0.426; P= 0.514

Out of the 42 subjects that delivered in the hospital, 2 subjects had intrapartum fever; one was GBS positive while the second was GBS negative. Four subjects had vaginal related infection (Vaginitis) out of which one was GBS positive. None of the 4 GBS cases that delivered in the hospital had chorioamnionitis, on the other hand, only one subject out of the GBS negative cases had the infection. One GBS positive subject had fetal distress for an unrelated reason whereas five of the GBS negative also had fetal distress. One subject each from GBS positive and GBS negative gave birth to a neonate with low birth weight. Babies born to 3 GBS positive subjects had normal .Activity, Pulse, Grimace, Appearance and Respiration (APGAR) score. While the baby born to the fourth GBS positive subject had a critically low APGAR score. On the other hand, 35 GBS negative subjects had babies with normal APGAR score while the remaining 3 subjects delivered babies with low APGAR. For the total population that delivered in the hospital, none of the following factors that might contribute to colonization were found to be significantly associated with GBS colonization status (P>0.05) (Table 7).

Antibiotic resistant pattern of GBS isolates by Disc diffusion and agar dilution techniques

Antimicrobial susceptibility pattern of the 17 GBS isolates is shown in Figure 2. All the GBS isolates showed uniform resistance to penicillin, ampicillin, clindamycin and cefoxitin. However, 82.4% of the isolates were sensitive to cefotaxime while 17.6% were resistant. For erythromycin, 35.3% of the GBS isolates displayed both intermediate and total resistance whereas, 29.4% of the isolates were sensitive to the antibiotic. 70.6% were sensitive to vancomycin. Two (11.76%) strains exhibited a multiple antibiotic resistance pattern.

Minimum inhibitory concentrations (MICs) were determined by testing all the 17 GBS isolates against penicillin, ampicillin, cefotaxime, erythromycin and clindamycin by agar dilution method (Figure 3). All the isolates tested had uniform resistance (100%) to penicillin, ampicillin and clindamycin with MIC ≥1milligram per litre (mg/l). Of the 17 GBS isolates tested for cefotaxime susceptibility, 88.2% (15) were susceptible with MICs ≤ 0.5mg/l and 11.8 % (2) were resistant with MICs = 8mg/l. For erythromycin susceptibility, 35.3% (6) were susceptible with MICs ≤ 0.25mg/l and 64.7% (11) GBS isolates had MICs ≥ 1mg/l which were regarded as resistant.

TABLE 4: PREVALENCE OF GBS COLONIZATION IN RELATION TO GESTATIONAL AGE

Gestational age	GBS-positive	GBS-negative	Total
≥35≤37	10(9.43)	96(90.57)	106(100)
>37≤40	7(15.91)	37(84.09)	44(100)

X^2= 1.297; P=0.225

TABLE 5: PREVALENCE OF GBS COLONIZATION IN RELATION TO GRAVIDITY

GBS colonization	Gravidae		Total
	Primi- (%)	Multi- (%)	
Yes	6(35.29)	11(64.71)	17(100)
No	38(28.15)	97(71.85)	135(100)

X^2 = 0.375; P = 0.540

TABLE 6: GBS CARRIAGE RATE AND OBSTETRIC CHARACTERISTICS

Obstetric characteristics	GBS-positive Number (%)	GBS-negative Number (%)	Total Number (%)	P-value
Gestational age at delivery				
<40	**2(9.09)**	**20(90.91)**	**22(100)**	**1**
>40	**2(10.00)**	**18(90.00)**	**20(100)**	
Membrane rupture duration				
<10hours	**4(9.76)**	**4(9.76)**	**41(100)**	**1**
10-18hours	**0(0)**	**1(100)**	**1(100)**	
Type of delivery				
Vaginal	**1(3.57)**	**27(96.43)**	**28(100)**	**1**
Cesarean	**3(21.43)**	**11(78.57)**	**14(100)**	

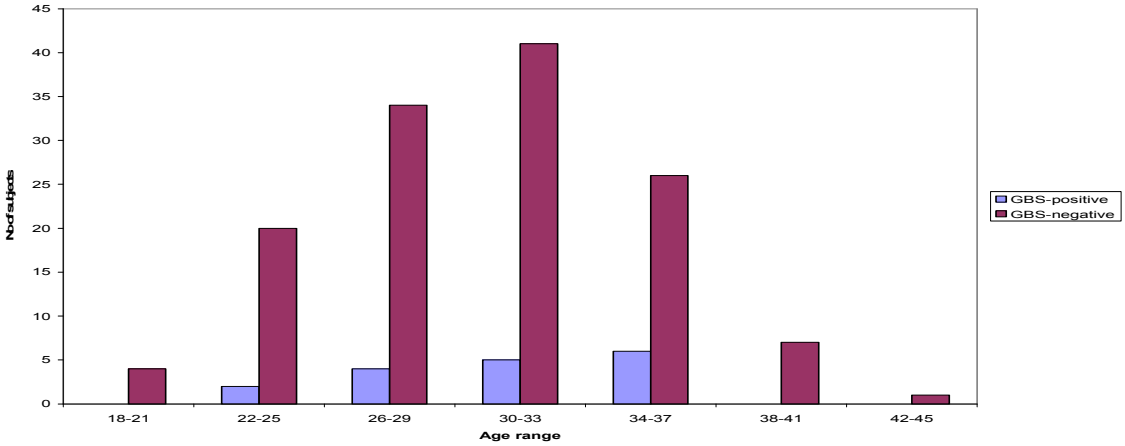

Fig 1: Age distribution and GBS colonization **KEY**: Age range in years

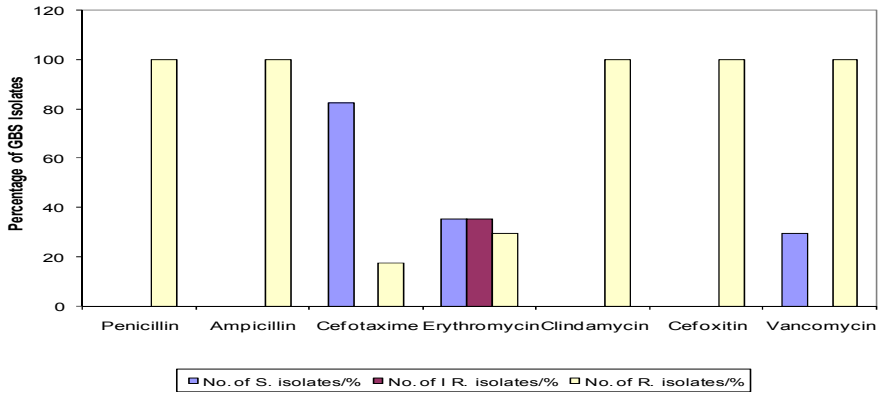

Fig 2: Antibiotic resistant profile of GBS isolates by agar diffusion technique
KEYS: S = Susceptible, IR = Intermediate Resistant, R = Resistant

TABLE 7: GBS CARRIAGE RATE AND REPRODUCTIVE HISTORIES

Reproductive Histories	GBS positive Number (%)	GBS Negative Number (%)	Total Number (%)	P-value
Fever				
Yes	1(50.00)	1(50.00)	2(100)	0.184
No	3(7.50)	37(92.50)	40(100)	
Vaginitis				
Yes	1(25.00)	3(75.00)	4(100)	0.341
No	3(7.89)	35(92.11)	38(100)	
Chorioamnionitis				
Yes	0(0)	1(100)	1(100)	1
No	4(9.76)	37(90.24)	41(100)	
Fetal distress				
Yes	1(16.67)	5(83.33)	6(100)	0.474
No	3(8.33)	33(91.67)	36(100)	
Low birth weight				
Yes	1(50.00)	1(50.00)	2(100)	0.184
No	3(7.50)	37(92.50)	40(100)	
Normal APGAR score				
Yes	3(7.89)	35(92.11)	38(100)	1
No	1(25.00)	3(75.00)	4(100)	

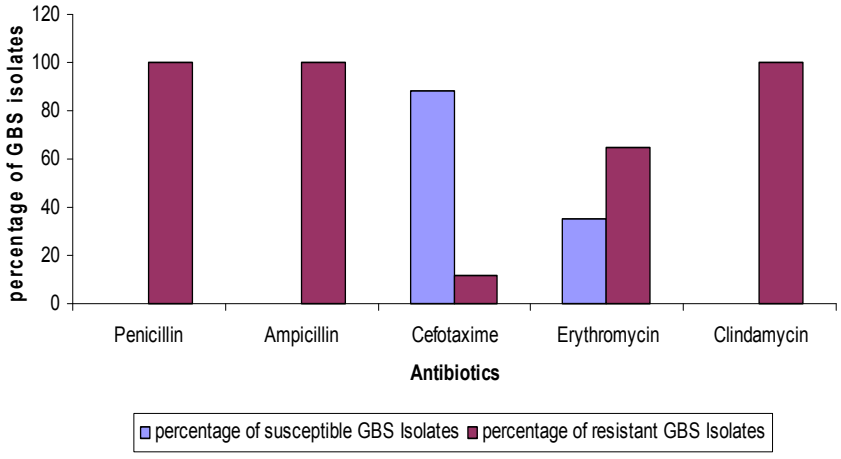

Fig 3: Antibiotic resistance profile of GBS isolates by agar dilution technique

DISCUSSION

This present study has shown the prevalence of GBS (*Streptococcus agalactiae*) colonization in Ile-Ife to be 11.3%. The rapid antigen detection had a sensitivity of 100 percent and a specificity of 95.6 percent in our center which is comparable to 100% sensitivity and 98.37% specificity reported by several other authors (15, 18). The possibility of a higher sensitivity for latex particle agglutination test than the recommended culture isolation cannot be ruled out since agglutination test is independent of the viability of the organism. In this study, there were 3 likely false positive and no false negative cases showing that antigen detection had a sensitivity of 100% and a

specificity of 95.6% with positive predictive value of 85% and negative predictive value of 100%.

In this study, we recorded a higher prevalence of 11.3% GBS vaginal colonization than that reported in Jos (6.6%) (9) and Calabar(9%) (19) but lower than that reported in Ibadan (17.6%) (5) and Zaria (14%) (8). The prevalence rate recorded is comparable to 11% prevalence obtained in Abeokuta (20) and the recent study carried out in Ibadan (10%) (21). Therefore, our study further confirms the presence of GBS colonization of the vagina in late pregnancy in Ile-Ife and corroborated reports from other geopolitical zones of the country establishing carriage status in pregnancy and the consequent risk to maternal and neonatal life. However, there is an urgent need to establish the disease burden arising from this colonization as all studies reported so far failed to address this.

However, in studies carried out in other African countries, higher prevalence rates were obtained, such as: Malawi (16.5%) (12), Gambia (22.0%) (13), Zimbabwe (20-32%), (14) and) Ivory Coast (19.0%) (23). Other countries where a similar rate was reported include; Korea (9.5%) (24), USA (12.2%) (25), Iran (9.1%) (26) and Hong Kong (10.4%) (27). Single vaginal culture and lack of rectal culture can partly explain the low prevalence in our study, although; this may not be a limitation of this study as a higher proportion of GBS have been documented to be isolated from the vagina (12.3%) as compared to the rectum (5%) in Tanzania (28). Higher prevalence rates have been reported in studies that involved rectovaginal sample collections and also women with ruptured membranes (26). The reason for the varying results may be attributed to the fact that GBS maternal colonization varies from place to place. Other factors that may have contributed to this variation include socioeconomic factors, ethnic and genetic factors, variation in clinical practices of samples collected and the techniques used for the sampling. Differences in environmental factors such as hygiene and nutrition may also play a role.

In this study, GBS colonization increases with age. An observation also documented by a study in Ibadan(22) where they reported increase in GBS positivity as age increases. However, this observation was not corroborated by another study reported from Malawi (12) which reported a decrease in GBS colonization as age increases. GBS vaginal colonization rate in this present study does not statistically relate with age nor gestational age ($P > 0.05$). The culture positivity among mothers ≤37 weeks in gestational age was far less than that among mothers >37 weeks which is in agreement with the observation of Raj, *et. al.* (31) 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. Other investigators have documented that cultures obtained between one and five weeks before delivery have sensitivity of 87% and specificity of 97% or higher (32).

Although very few GBS isolates were evaluated in this study in a limited population of pregnant women. We are nonetheless alarmed by the high level of resistance observed to penicillin and ampicillin antibiotics which are the first choice of drugs for intrapartum prophylaxis and the challenges which this implies. However, many factors may be responsible for this observation in this environment such as: the ease of procurement of antibiotics in the developing country, the frequent use of antibiotics for therapy and prophylaxis, and other socio-economic factors as documented by Okeke *et al.* (33). The expanded use of beta-lactam antimicrobials in the treatment of several infective clinical syndromes and the easy of purchase over the counter might be the cause of emergence of GBS resistance strains in this environment. A study in Tanzania on GBS in pregnant women documented resistance to clindamycin, erythromycin and penicillin G was found to be 17.6%, 13% and 9.4%, respectively (28). The high rate of erythromycin and clindamycin resistance in GBS in this study is consistent with earlier reported observations (24, 25, and 29) on the increasing resistant trend of GBS to this drugs which underscores the current CDC recommendations that antibiotic susceptibility testing be performed if erythromycin or clindamycin therapy is needed to prevent neonatal GBS infection. GBS erythromycin and clindamycin resistance is 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 via the constitutive expression of an *erm*-encoded methylase which results in resistance to erythromycin, clindamycin and streptogramin B drugs ($cMLS_B$ phenotype) (34, 35), we believe that these genes may be responsible for the observations in this study. However, the high susceptibility of 88.2% to cefotaxime, a third generation cephalosporin which was observed in this study, may possibly be due to the limited exposure of subjects to this antibiotics and the relatively expensive nature of the drug which makes it un-attractive for purchase over the counter.

This study demonstrated that detection of GBS vaginal colonization by lance field antigen grouping following enrichment broth culture is more sensitive than direct culture isolation; also it reduces the turnaround time to 18-24 hours for availability of result, unlike the standard culture method that takes about 48-72 hours before result is out.

The high percentage and multiple resistance patterns of GBS to beta-lactam drugs and to clindamycin and erythromycin in this environment need to be further verified through an expanded study with many more women and at several sites with the same study protocol. However, the establishment of carriage status in pregnant women for GBS calls for a review of the present hospital policy on antenatal care to include routine screening and reporting of GBS prevalence as well as monitoring the level of antibiotic resistance in GBS among pregnant women during antenatal visits.

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