PREVALENCE OF VAGINAL GROUP-B-STREPTOCOCCUS, ANTIBIOTIC AND ANTIGEN SENSITIVITY AMONGST PARTURIENTS AT THE FEDERAL MEDICAL CENTER OWERRI, NIGERIA

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ABSTRACT

BACKGROUND: Group B streptococcus (GBS) is one of the principal agents of early onset neonatal sepsis, pneumonia and meningitis with significant morbidity for newborns and parturients.

AIM: This study was done to determine the prevalence of vaginal group B streptococcus amongst parturients in FMC Owerri. It also aimed to elucidate the antibiotic sensitivity of the strains of GBS isolated while comparing the sensitivity of antigen detection tests of GBS to culture.

PATIENTS AND METHODS: This cross-sectional study was done at the labour ward and lying-in units of the Federal Medical Center, Owerri,from February 2015 to October, 2015. It involved one hundred and eighty (180) women recruited systematically. Two swab samples, high vaginal and rectal were collected from the parturients intrapartum and cultured. The babies were examined and weighed immediately after delivery, seen on the second and tenth days after delivery for features of fever or any other complaints.

RESULTS: The overall prevalence of GBS amongst parturients was found to be 6.1%. Prevalence of vaginal colonization was 3.3% and that of the rectum was 2.8%, (2c=0.1282, df=1,p=0.502). Neonates with colonized mothers all had complaints of fever postpartum which was statistically significant (=55.86, df=1, p<0.001). All the microbes showed 100% sensitivity to penicillin G, erythromycin, gentamycin and ceftriaxone. Antibiotic resistance was discovered in augmentin (67%), Cloxacillin (55.6%) and ofloxacin (33.3%). Antigen detection tests gave Sensitivity=100%, specificity=96.4%, positive predictive value (PPV) = 64.7%, negative predictive value (NPV) =100%. Detection in both vaginal (=60.290,p=0.000) and rectal (=50.799,p=0.000) samples were statistically significant.

CONCLUSION: The prevalence of GBS in parturients in the Centre is low. Strains of GBS isolated amongst the sampled population in FMC Owerri had a high sensitivity to penicillin G, erythromycin, gentamycin and 3rd generation cephalosporins. Antigen detection method for screening of GBS though very sensitive is however not specific with a significant false positive detection rate due to antigen cross reaction. It is however recommended that antigen detection be employed as intrapartum measure in high risk cases to reduce turnover time whilst supported by culture results later. There was associated significant neonatal sequaelae and further research to establish causal relationship will be advised.

KEYWORDS: Prevalence, Group B streptococcus, colonization, antibiotic, antigen sensitivity.

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INTRODUCTION

Group B *Streptococcus* (*Streptococcus agalactiae*) is an important cause of neonatal and maternal infection. It is also an important cause of disease in immunocompromised adults and the elderly.[1] Maternal GBS colonization rates in developing countries were estimated to be about 12.7%.[2] Individual studies in Nigeria gave prevalence rates of 7.5% to 20%.[3,4,5] Prenatal cultures have been found not to accurately predict GBS carriage during labor. At least 4.0 to 11.6% of prenatally GBS-negative women had positive GBS cultures during labor. [3]

Although the epidemiology of GBS in the developed world is well documented, its contribution to the burden of neonatal infection in the developing world has proved more difficult to assess.[5] GBS incidence estimates from developing countries are confounded by case under-ascertainment due to barriers to accessing healthcare and challenges in equipping and maintaining diagnostic laboratories.[3,4,6] Only Zimbabwe and Malawi have an active research programme on GBS colonization and burden of disease.[5,7]

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Several factors increase the susceptibility of the parturients and their neonates to disease. These include; maternal age, occurrence of previous spontaneous abortion, presence of candidiasis and vaginitis, positive prenatal GBS culture, GBS bacteriuria during the current pregnancy, previous infant with invasive GBS disease, prolonged labour, low birth weight and black race.[3,7,8] Others are; maternal fever in labour, gestational age more than 42 weeks, GBS strain (type III) and gestational diabetes.[3,7] Other less frequent modes of transmission include lactational, nosocomial and community acquired.[3,7,9]

GBS is the most prevalent infection in the first week of life, presenting in two forms: early-onset and late-onset. The newborns present with symptoms such as breathing problems, blood pressure and heart rate instability, gastro-intestinal and urinary system disturbances, sepsis, pneumonia, meningitis, cellulitis, osteomyelitis, and septic arthritis. A late-onset infection usually presents with meningitis and septicemia in weeks or months after birth.[8,9]

Intra-partum Antibiotic Prophylaxis (IAP) is usually offered to women who had the afore mentioned risk factors. Beta-lactam prophylaxis given 4 or more hours before delivery is highly effective and prevented 95% of EOD.[10] Studies in Africa and Nigeria have demonstrated good sensitivity to penicillins.[5,7,9,11] The threat of antibiotic resistance has however emerged worldwide.[3,9]

This has potential for public health concern especially in Nigeria, underlining the need for regional antibiotic surveillance and targeted treatment. Antigen testing and immunological tests have also been developed for GBS detection during labor in an effort to reduce turnover time and optimize specific intra-partum antibiotic prophylaxis and avoid antibiotic resistance.[9,12] This may allow the screening of pregnant women from remote areas where there are no laboratory facilities.[9,12]

PATIENTS AND METHODS

Study population and sampling

This cross-sectional study was carried out in the labor and lying-in wards of the Federal Medical Center, Owerri, between February and October 2015. After ethical approval, 180 parturients of different ages and parities that fulfilled the criteria were recruited at the delivery unit. Inclusion criteria included parturients in latent phase of labour at 37 completed weeks of gestation and above who have given informed consent for the study.

Exclusion criteria included parturients currently on antibiotics or those who had used it in the last two weeks, signs of erosion on the cervix or vagina, those using feminine hygiene products and those who did not give consent. A questionnaire was administered by the researchers to get the biodata and past obstetric history and other relevant information. Two swab samples were taken per participant by the researcher, using a sterile speculum from the vagina and another swab from the rectum under aseptic conditions.

Isolation and identification of organism

A commercially available collection and transport system for aerobes and anaerobes (Charcoal Swabs Amies Plastic Applicator with Rayon Tipped Black Cap, Stone, Staffs, UK) was employed.

All swab specimen were transported to the Medical Microbiology Laboratory of The Federal Medical Center, Owerri. Modified Stuarts transport media was used in case of delay. The swab samples were inoculated into selective enrichment broth medium (Todd-Hewitt broth supplemented with 10G/ml colistin and 15G/ml nalidixic acid, Oxoid England) and incubated aerobically at 37^{O}C for 24 hours. After 24 hours incubation, broth cultures were observed for growth (Turbidity). This was then sub-cultured onto 5% Sheep Blood Agar (Oxoid England) and incubated overnight as above.

The colony appearance of GBS at 24 hours is usually grey, smooth, shiny, convex, moist, regular, soft and mucoid in appearance and about 1 mm in diameter, often surrounded by a small hazy zone of beta- hemolysis. Suspected GBS isolates were identified based on colony morphology, catalase reaction, CAMP (Christy, Atkins, Munch, Peterson) test, hippurate hydrolysis test and Lancefield grouping.

Antigen testing

GBS antigen was determined by serological grouping using Streptococcal group B reagent kit (Prolex™) testing of selective broth. Fresh (18-24) hour colony samples were used. GBS colonies were picked from areas with the lowest probability of contamination with other organisms. A test tube was labeled for each isolate and a drop of extraction reagent 1 was added to each tube.

A disposable needle was used to suspend the colonies in the test tubes till there is noticeable turbidity after which a drop of extraction reagent 2 was added to each of the test tubes. Five drops of extraction reagent 3 were then added and each test tube was mixed by gentle tapping for 5-10 seconds. A drop of the antigen reagent was dispensed on separate labeled test cards.

A Pasteur pipette was used to dispense a drop of colony mixture beside each drop of reagent and both mixed with sticks provided. A new stick was used for each test circle. The cards were rocked gently allowing the mixture to flow slowly over the entire test ring area. The mixture was observed for agglutination over one minute and recorded.

Antimicrobial susceptibility test

A small number of colonies obtained from each 24 hours old 5% Sheep blood agar plate culture were 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 G, ceftriaxone, cefotaxime, clindamycin, gentamycin and erythromycin using microdilution panels (MicroScan, Dade Behring Inc, USA).

The isolates were considered susceptible or resistant according to the MIC (minimal inhibitory concentration) breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI).[13] High-level resistance to gentamicin was defined as a MIC of ≥500 µg/ml. Resistance to erythromycin was determined by the double-disk diffusion method with disks containing erythromycin (15 µg) and clindamycin (2 µg) onto sheep blood agar plates. Streptococcus *pneumoniae* ATCC 6305 was used as control organism and drug free plate was included as negative control.

RESULTS

One hundred and eighty (180) women were recruited and each had a vaginal/ rectal swab pair taken.

Table 1: Prevalence of Group B Streptococcus amongst parturients

GBS	Frequency		Total n (%)	χ^2	FE; p-value
	Yes n(%)	No n(%)			
Vaginal	6(3.3)	174(96.7)	180(100)		
Rectal	5(2.8)	175(97.2)	180(100)	0.1282	0.502
Total	11(6.1)	349(93.9)	360(100)	df=1	

FE= Fisher's Exact; P value

Prevalence of vaginal GBS 6(3.3%), was slightly higher than rectal GBS, 5(2.8%) amongst the participants. This difference however was not significant (2c=0.1282,df=1,p=0.502).None of the respondents had concomittant vaginal and rectal colonization.

Table 2: Socio-demographic and obstetric characteristics of the mothers, birth weight and sex variations of neonates

Variable	Frequency (n=180)	%
Age (years)		
20-24	17	9.4
25-29	73	40.6
30-35	63	35.0
>35	27	15.0
Mean age= 28.2± **4.2yrs**		
Booking status		
Booked	175	97.2
Unbooked	5	2.8
Parity		
0-1	114	63.3
2-3	62	34.5
≥4	4	2.2
Religion		
Christianity	180	100
Islam	0	0
Others	0	0
HIV status		
Negative	161	89.4
Positive	19	10.6
Highest Educational Attainment		
Secondary	65	36.1
Tertiary	115	63.9
Birth weight (kg)		
<2.5	6	3.3
2.5-4	154	85.6
>4	20	11.1
Average weight of babies= 3.42 ± 0.40kg		
Gender		
Male	104	57.8
Female	76	42.2

All the pregnancies were term with an average gestational age of 39 weeks and 5 days ± 11 days. Seventy three (40.6%) of the participants were within the 25-29 year age group with mean age of 28.2±4.2years. Most of the participants were booked, 175(97.2%), had a low parity of 0-1, 114(63.3%) were HIV negative, 161(89.4%), and had tertiary education, 115 (63.9%). Most of the neonates weighed between 2.5-4.0kg (85.6%) with an average weight of 3.42±0.40kg. 6(3.4%) of them were of low birth weight. One hundred and four(57.8%) of the births were male, with a male to female ratio of 1.4:1. (Table 2)

Table 3: Neonatal parameters and vaginal GBS

Neonates	Vaginal GBS		Total(n=180)	x^2	FE;
	Yes(n=6) n(100%)	N0(n=174) n(100%)	n(%)		P-value
Birth weight					
<2.5kg	0(0.0)	6(3.3)	6(3.3)	0.630	0.526
2.5-4kg	6(3.3)	150(83.4)	156(86.7)	df=1	
>4kg	0(0.0)	18(10.0)	18(10.0)		
Total	**6(3.3)**	**174(96.7)**	**180(100)**		
Day 2 complaints					
Fever	6(3.3)	12(6.7)	18(10.0)	55.86	<0.001*
Eye discharge	0(0.0)	24(13.3)	24(13.3)	df=1	
Admission	0(0.0)	6(3.3)	6(3.3)		
None	0(0.0)	132(73.3)	132(73.3)		
Total	**6(3.3)**	**174(96.7)**	**180(100)**		
Day 10 complaints					
Fever	3(1.6)	12(6.7)	15(8.3)	14.12	<0.001*
Eye discharge	0(0.0)	3(1.7)	3(1.7)	df=1	
Admission	3(1.7)	3(1.6)	6(3.3)		
None	0(0.0)	156(86.7)	153(86.7)		
Total	**6(3.3)**	**174(96.7)**	**180(100)**		

***significant**
FE=Fisher's Exact, P-value

None of the neonates born to mothers with vaginal colonization was of low birth weight. All neonates of mothers with vaginal GBS colonization had complaints on the 2nd and 10th day. This difference was statistically significant (p=<0.05).

Figure 1: Profile of cultured micro-organisms in vaginal swabs

One hundred and thirty four (74.4%) microbial growth in vaginal swabs was recorded. 57 (31.7%) of the participants had mixed bacterial growth while 46(25.6%) had no growth. The values for *S agalactiae* and *C albicans* were 6.1% and 3.3% respectively while *C albicans* and Coliform bacilli were found to be 20(11.1%) and 40(22.2%) respectively.

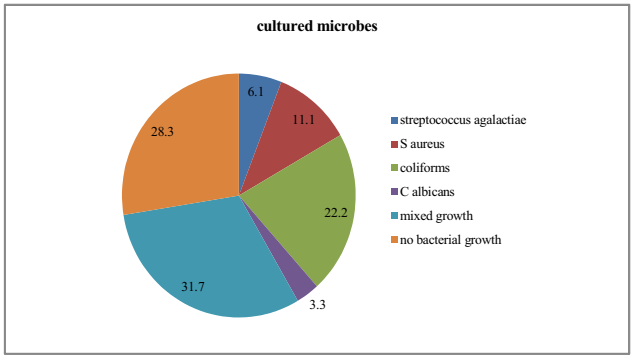
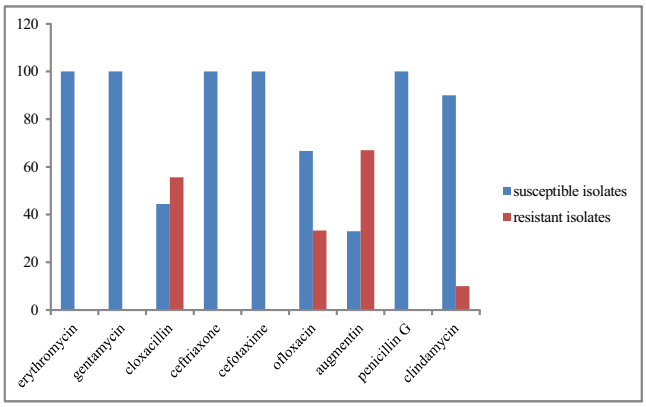


Figure 2: Antibiotic sensitivity/resistance profile of GBS isolates

Erythromycin, penicillin G, gentamycin, ceftriaxone and cefuroxime were found to have a high sensitivity in all cultured samples (100%). Clindamycin had 90% sensitivity. Sixty seven percent (66.7%) of the isolates were sensitive to Ofloxacin while 33.3% were resistant. For Cloxacillin, 44.4% were sensitive while 55.6% were resistant. Augmentin showed only 33% sensitivity. One (11.1%) strain exhibited a multiple antibiotic resistance pattern.



X-axis represents frequency in percentage

Figure 3:Antibiotic sensitivity of the strains of GBS isolated from the affected respondents

Antimicrobial susceptibility pattern of the 11 GBS isolates is shown in Figure 3. The X-axis represents the Zone of Growth Inhibition of the antibiotic disc in millimeters. Zones of 6 and below are considered not sensitive.

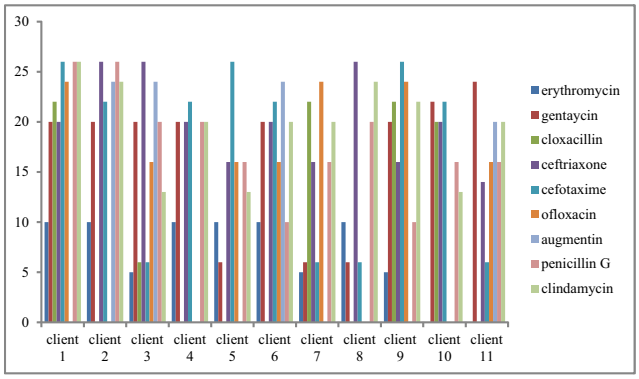


Table 4: Antigen detection in culture positive and culture negative plates

Antigen test results	Culture positive	Culture negative	Total
Antigen positive	11	6	17
Antigen negative	0	163	163
Total	**11**	**169**	**180**

Sensitivity=100%, specificity=96.4%, positive predictive value (PPV)=64.7%, negative predictive value (NPV)=100%

DISCUSSION

The prevalence of vaginal group B streptococcus (GBS) in this study was 3.3 % (table 1). When combined with values of 2.8% obtained from rectal samples this gives an overall GBS rate of 6.1%. This value is comparable to values of 2.5% by Konikkara *et al*[14] but much lower than that found in local studies; 8.3% in Ibadan, 9.8% in Maiduguri,11.3% in Ile-Ife and 18% in Enugu.[9,7,3,4] Studies in other localities also gave higher values of 7.5% in India[11], 12% in the Indo-Pakistan, 20% in Gambia[2], 16.5% in Malawi[5], 22.76% in Iran[6] and 25.4% by Rochetti *et al*.[8]

The difference in values may be attributed to the fact that GBS maternal colonization varies from place to place. Local studies by Onipede *et al* and Okon *et al* used specimen from the introitus and lower vagina respectively.[3,7] Also, the study by Hajare *et al* was done with lower vaginal swabs.[11] This may suggest a higher yield due to proximity

to the surface skin and anus. Racial and genetic factors are contributory factors as documented in some studies suggesting innate ability to clear colonization.[11]

The clients most affected in this study were in the 25-29 year age group with a mean age of 28.2 ± 4.2years (table 2). This was comparable to findings by Okon *et al* and Javanmanesh *et al* where the mean ages were 28.7 years and 26.7 respectively.[6,7] In other studies, the most affected ages ranged between 31-45 years, suggesting no particular association with age.[4,8,11] Women with lower parities of 0-1 were more affected in this study although this was not statistically significant. This was also observed in the study by Hajare *et al* where 46.7% isolation rate was seen in primigravidae, while 25.5% prevalence was seen in primiparae by Ezeonu *et al*.[11,4] Women with tertiary education only were affected in this study. This was a similar finding in the study done in Maiduguri.[7] The reason for this is unclear. No definite pattern was found with parity and educational level in several other studies.[6,9] None of the affected women was HIV positive. This is supported by Stoll *et al* that showed no overall difference in GBS carriage rate in HIV positive women[2].

This differed from a finding in Malawi that gave a prevalence of 44%.[5] This finding may probably reflect the overall HIV positivity rate amongst their parturients. HIV positive women in this center are managed with Cotrimoxazole as prophylaxis all through their pregnancy and this may be protective. Streptococcus agalactiae occurred in all samples found positive for GBS. The ratio of vaginal to rectal colonization was found to be 1.2:1. This was comparable to findings by Ezeonu *et al* who found an equal ratio[4] but differed from Konnikara *et al* that suggested that anal swabs gave a better yield.[14] None of the clients in this study showed concomitant infection of both the vagina and the rectum.

The sampling for GBS carriage among pregnant women is recommended to be done at the third trimester which provides the information necessary for antibiotic prophylaxis prior to delivery.[10] The United States policy is more aggressive with routine screening of all women and concordant reduction in early onset disease (EOD). European countries however are more conservative, with screening done for women with high risk factors combined with early treatment of neonatal disease.[10] This has been found to reduce the cost of screening while ensuring patients at risk are taken care of. It may also ensure earlier screening for women who are at high risk to avoid preterm deliveries. In this study however, none of the parturients found colonized with GBS had any known risk factors. This may have been due to their low gravidity, as most affected mothers were primigravidae and primiparae. Also, the possibility exists that women with established risk factors may have been delivered before term as preterm delivery is one of the complications of vaginal GBS colonization.

One of the most important risk factors for EOD in the neonate is exposure to the organism colonizing the mother's genital tract. All the neonates whose mothers screened positive for vaginal GBS had post-partum complaints (table 3) ranging from fever, 6(3.3%) in the 1st 2 days to admission in the special care baby unit 3(1.7%) by the tenth day. This was a statistically significant association ($p<0.001$). Vertical transmission rates have been shown to approach 70% with 2-3 fold higher incidence than blood / cerebro-spinal fluid proven sepsis.[15,16] No neonatal death was recorded in the time frame this study was done although the incidence of late onset GBS infections was not done.

In this study there was 74.4% microbial growth in vaginal swabs while 25.6% of samples showed no bacterial growth (figure 1). This was lower than findings in Ibadan where the microbial isolation rate was 92%.[9] Higher numbers of *S aureus* and Candida species were also isolated (18.3% and 14.2% respectively) against 11.1% and 3.3% each found in this study. Coliformlevels were however higher in this study with values of 22.2% against 15% found in the study by Dombraye *et al*[9].

The relatively higher recovery of coliforms from vaginal swabs may have been due to contamination from rectal contents during the birth process and obstetric procedures the parturients were subjected to. Although intra-partum vaginal examinations are done as aseptically as possible in this Centre, this possibility cannot be ruled out. All the GBS species isolated were sensitive to penicillin G,

erythromycin, gentamycin, ceftriaxone, cefuroxime (100%) and clindamycin (90%) (figures 2,3). This was consistent with findings by Hajare *et al* in 2012 and Dombraye *et al* in 2010 where erythromycin and penicillin were found to have 100% sensitivity[11,9]. There was also 80% sensitivity to ampicillin, vancomycin and augmentin.[11,9] This corroborates the revised recommendation by the CDC (2010) that penicillin G be the first line of treatment for women with GBS colonization in labour.[12] The antibiotics vancomycin or clindamycin may be employed in those that have serious penicillin G allergy.[12] Marked resistance to augmentin (67%) and cloxacillin (55.6%) was noted in this study, and differed from the findings of resistance to gentamycin and kanamycin in the Hajare study (100% and 80% respectively).[11] Resistance to quinolones (perfloxacin and ciprofloxacin) has only recently been described for GBS[3] and also noted in this study with Ofloxacin having a resistance of 33.3%.

Resistance of GBS to erythromycin and penicillin G, though not seen in this study, has been reported by several other studies and also noted by Ezeonu *et al* (8.6%) and Onipede *et al* (40%).[4,3]As penicillin is the first line treatment for intra-partum GBS this may be a cause for worry in Nigeria. The resistance noted with augmentin and cloxacillin in this study may be related to the ease of procurement of over the counter antibiotics in the country for self-medication and its frequent use for therapy, prophylaxis and other socio economic factors[3,7]. This may have led to the emergence of multiple drug resistant strains of GBS to these common antibiotics. The high sensitivity noted with the 3rd generation cephalosprins and gentamycin in this study may have been due to the relatively expensive nature of the drugs and the need for parenteral administration which makes them unattractive for over the counter purchase. As recommended by the CDC, antibiotic susceptibility testing should be performed if therapy is needed to avoid neonatal GBS infection[3,12].If antibiotic resistance continues to be identified and found to be on the increase, surveillance of antibiotic resistance patterns among several antimicrobial classes will be important in determining optimal prophylaxis and treatment of GBS infections. GBS colonization was confirmed by antigen detection from enrichment broth culture and direct culture isolation of the swab samples (Table 4).

The antigen detection test in this study gave a sensitivity of 100% and a specificity of 96.4% comparable to values of 100% and 95.6% recorded by Onipede *et al*[3]. There was also a positive predictive value (PPV) of 64.7% and a negative predictive value (NPV) of 100%. This was much lower than the PPV of 80% seen in the study in Ile-Ife.[3] Eleven (11) out of the 17 pregnant women that were positive forantigen detection by latex agglutination test after 18 hours incubation in enrichment broth were also culture positive. In the 6 cases where there was antigen positivity and negative culture, possible explanation could include cross reaction with other microbial antigens as no GBS was cultured even after 72 hours.

In all those cases coliforms was noted as heavy growth and this may have affected the outcome. Though antigen detection kits reliably reduced the waiting time from 24-48hours to 18hours thereby allowing evidence based antibiotic therapy earlier on in labour, this finding suggests that culture is still the gold standard for isolation. Antigen detection kits may be used for screening and field studies due to its higher sensitivity, lower cost and easy assay.[16] This can isolate women who may then be followed up by culture to avoid development of antibiotic resistance.

CONCLUSION

The prevalence of GBS in parturients in the Centre is low. Strains of GBS isolated amongst the sampled population in FMC Owerri had a high sensitivity to penicillin G, erythromycin, gentamycin and 3rd generation cephalosporins. Antigen detection method for screening of GBS though very sensitive is however not specific with a significant false positive detection rate due to antigen cross reaction. It is however recommended that antigen detection be employed as intrapartum measure in high risk cases to reduce turnover time whilst supported by culture results later. There was associated significant neonatal sequaelae and further research to establish causal relationship will be advised.

CONSENT

All authors declare that written informed consent was obtained from the patient for publication of this paper.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 2013 Declaration of Helsinki as amended.

REFERENCES

1. Kessous R, Weintraub AY, Sergienko R, Lazer T, Wiznitzer A, Sheiner E. Bacteraemia with Group B Streptococcus: Is it a risk factor for adverse pregnancy outcomes? *J Mat-Fetal Neonat Med* 2012;25(10):1983-86.
2. Stoll BJ, Schuchat A. Maternal carriage of group B streptococci in developing countries. *Pediat Infect Dis J* 1998;17(6):499–503.
3. Onipede A, Adefusi O, Adeyemi A, Adejuyigbe E, Oyelese A, Ogunniyi T. Group B Streptococcus Carriage in Late Pregnancy in Ile-Ife, Nigeria. *Afr. J. Cln. Exper. Microbiol* 2012;13(3):135-43.
4. Ezeonu IM, Agbo MC. Incidence and antimicrobial resistance profile of Group B Streptococcus (GBS) infection in pregnant women in Nsukka, Enugu State, Nigeria. *Afr J Microbiol Res* 2014;8:91-95.
5. Dzowela T, Komolafe OO, Igbigbi A. Prevalence of Group B Streptococcus colonization in antenatal women at the Queen Elizabeth Central Hospital, Blantyre—A preliminary study. *Mal Med J. 2005*. 17 (3): 97-99.
6. Javanmanesh F, Eshraghi N. Prevalence of positive recto-vaginal culture for Group B streptococcus in pregnant women at 35-37 weeks of gestation.*Med J Islam Repub Iran.* 2013; 27(1): 7–11.
7. Okon KO, Usman H, Umar Z, Balogun ST. Prevalence of group B streptococcus (GBS) colonization among pregnant women attending antenatal clinic of a tertiary hospital in northeastern, Nigeria. *Am J Res Com* 2013; 1(6): 54-66.
8. Rocchetti TT, Marconi C, Rall VLM,Borges VTM, Corrente JE, da Silva MG. Group B streptococci colonization in pregnant women: risk factors and evaluation of the vaginal flora. *Archives Gynaecol Obstet,* 2011;283(4):717-21.
9. Donbraye-Emmanuel OOB, Okonko ID, Donbraye E, Fadeyi A, Abubakar MJ, Adebiyi OE, Fashina NA. Isolation and characterization of Group B Steptococci and other pathogens among pregnant women in Ibadan Southwest Nigeria. *J Appl Biosci*. 2010; 5902. (29): 1781-1792.
10. Royal College of Obstetricians and Gynaecologists. Prevention of early onset neonatal group B streptococcaldisease. http:// www.rcog.org. uk/ resources/Public/pdf/GroupB_strep_no36.pdf (accessed Sept 2013)
11. Hajare V, Madhari LH, Singh HKG. Antibiogram of Group B Streptococci isolated from the vagina of pregnant women in third trimester of pregnancy. *Peop J SciRes* 2012;5(2):22-26.
12. Centers for Disease Control and Prevention (CDC). Prevention of Perinatal Group B Streptococcal disease: Revised guidelines from CDC-United States, 2010. *MMWR Morb Mortal Wkly Rep*. 2010;59:1-32.
13. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; eighteenth informational supplement.2010; 30(1): M100-S20.
14. Konikkara KP, Baliga S, Shenoy SM, Bharati B. Comparison of Various Culture Methods for Isolation of *Group B Streptococcus* from Intrapartum Vaginal Colonization. *J Lab Physicians*. 2013; 5(1): 42–45.
15. Verani JR, Spina NL, Lynfield R, Schaffner W, Harrison LH, Holst A et al. Early- onset group B streptococcal disease in the United States: potential for further reduction. *Obstet Gynaecol* 2014;123(4):828-837.
16. West BA, Peterside O. Sensitivity pattern among bacterial isolates in neonatal septicaemia in Port-Harcourt. *Ann Clin Microbiol Antimicrob* 2012;11:7.