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A Case study of Group B *Streptococcus* Associated with Women in Antepartum Period and their Neonates, Ile Ife South-western Nigeria

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Abstract

Streptococcus agalactiae colonizes the genital and gastrointestinal tract. In pregnancy, vertical transmission of GBS to the new-born can cause neonatal sepsis, pneumonia and meningitis. The aim of this study was to determine the prevalence of GBS in pregnant women in their third trimester, frequency of neonatal colonization and Antibiotics Susceptibility of the isolates recovered at the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) Ile-Ife, Osun State, Nigeria. Rectum and vaginal swabs were collected from a total number of 24 third trimester pregnant women between 35 - 37 weeks of gestation and their neonate delivered at OAUTHC both at labour ward and labour ward theatre. The samples were cultured in Todd Hewitt Broth and sub cultured on sheep blood agar and Chromogenic Strepto BID agar and incubated at 37ºC for 24 hours. Identification was based on the Gram staining, presence of β-haemolysis and absence of catalase production. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk-diffusion methods. GBS colonization on average was confirmed in 27.8% of pregnant women and their neonate and proportion of GBS isolated from the vagina 6 (30%) as compared to rectum 7(35%), neonates 4 (20%), vaginal and neonate 1(5%), rectum and neonate 1(5%), and both vagina, rectum and neonate1 (5%). All isolates were found susceptible to 40% clindamycin 35% vancomycin, 90% ciprofloxacin 40% erythromycin and 100% resistance to penicillin used. There is need for proper handling of neonates by the health care practitioner and screening of pregnant women attending antenatal care, including known antibiotic Powered by Editorial Manager® and ProduXion Manager® from Aries Systems Corporation susceptibility for an appropriate antepartum antimicrobial prophylaxis can be offered.

Keywords: Antepartum; Neonate; Group B *Streptococcus*; Maternal morbidity; Postpartum; Antibiotics

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Introduction

Group B *Streptococcus* or *Streptococcus agalactiae* is one of the leading preventable causes of mortality and morbidity especially in neonate whose mothers are carrier worldwide [1]. Group B Streptococcus (GBS) is a Gram positive Bacterium regarded mainly as pathogen of pregnant or post-partum women and neonates [2].

It is also a significant cause of mortality and morbidity in non-pregnant adult, particularly those with underlying medical condition and in elderly patient such as stroke, renal failure, breast cancer, Diabetes mellitus, cirrhosis, neurological disorder and other liver disease, but with most incidence occurring among neonates [3].

Group B *Streptococcus* (GBS) is a normal flora or commensal of the genitourinary and gastrointestinal tract of 15%-50% of healthy woman [4]. Neonate can acquire GBS from their mother during the passage through the birth canal or through aspiration

of infected amniotic fluid [5]. Vertical acquisition of GBS involving colonization of the skin or mucus membrane occurs in 15-50% new born to GBS colonized mother [6].

In Adult, Group B *Streptococcus* causes Urinary Tract Infection, osteomyelitis, pneumonia, meningitis, bacteremia, skin and soft tissue infection and Streptococcal Toxic Shock Syndrome [7]. The bacterium also causes high risk of preterm deliveries in pregnant woman [8]. Infections in new-borns occurring within the first week of life are designated Early-onset disease. Late-onset infections occur in infants aged >1 week, with most infections evident during the first 3 months of life (CDC, 2010) **Figure 1.**

GBS transmission rate from mothers to neonates through vaginal delivery is approximately 50%, only 1-2% of colonized neonates develop invasive group B streptococcal disease [9]. The rate of early-onset infection has decreased from 1.7 cases per 1,000 live births in 1993 to 0.28 cases per 1,000 live births in the recent years (CDC, 2010). GBS colonization in women varies among age groups depending on study population, sites sampled and method of detection [10]. About 25% of pregnant women (CDC, 2010) and 35% young, non-pregnant woman [11] carry GBS in the rectum or vagina.

GBS continues to be susceptible to penicillin, ampicillin, and first-generation cephalosporins [12]. Under these circumstances, the alternative antibiotic choices have traditionally been erythromycin or clindamycin. However, resistance to these two antibiotics has been remarkably increasing [13]. Because of possible resistance problems with erythromycin and clindamycin, vancomycin is now the initial recommended treatment of GBS infection in patients who are allergic to penicillin [14].

Fluoroquinolones, especially the later generations, are active against GBS infections. Third and four-generation fluoroquinolones have been recommended by many health authorities and international organizations to treat pneumonia caused by GBS [15].

Maternal morbidity is worrisome, we source to determine the Social demographic factors with relative associated GBS colonization, its prevalence rate among pregnant women between 35-37 weeks of gestation period and their neonates, including the antibiotics susceptibility of the isolates recovered.

fig 4.1: Antibiotic resistance profile of GBS isolates

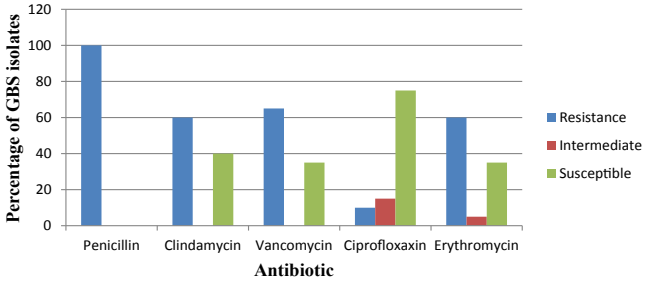

Figure 1 Antibiotics susceptibility profile of GBS isolates recovered in Ile Ife, South werstern Nigeria.

Streptococcus agalactiae was isolated from both the vaginal and rectum of pregnant women between 35-37 weeks of gestation and from the umbilical cord of their newly born neonates delivered at both labour ward and labour ward theatre between June 2016 to February 2017 at the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife, Osun State, Nigeria.

Sample Collection

A total of 24 pregnant women samples were collected from each study site. Two sterile swab sticks were used to take two different samples from each patient. Vaginal sampling was carried out by inserting and rotating the sterile swab stick against the vaginal wall at the mid portion of the vault and the swab was carefully withdrawn to prevent contamination with the microflora of the vulva. Another sterile swab stick was inserted 1.5-2.0 cm beyond the anal sphincter and gently rotated to touch the anal crypts and finally sterile swab was used to swab the umbilical cord of the newly born immediately after delivery.

All samples were collected by Midwifery/Gynaecologist and the swab specimens were placed in Stuart transport media and were transported to Department of Microbiology Laboratory of OAU for Laboratory investigation.

Screening for individual isolates

After samples collection under aseptic conditions, the swab sticks were inoculated into selective enrichment broth medium (Todd-Hewitt broth supplemented with 10 µg/ml Colistinand 15 µg/ml Nalidixicacid, Oxoid England) under aseptic condition and were incubated aerobically at 37°C for 24 hours. After 24 hours incubation, broth cultures were observed for growth (Turbidity) and then sub cultured onto 5% sheep Blood Agar and incubated for 18-24 hours at 37°Cto increase the recovery rate of GBS. The plates that had no growth were re incubated overnight one more time. Where there were growth, the colonies were examined for their characteristics colonial morphology and beta-haemolysis and non beta-haemolysis were also considered. The suspected colonies were Gram stained (Gram positive) and tested for Catalase (catalase negative).

Bacterial isolation and identification

Rectum and vaginal swabs were collected from the third trimester pregnant women between 35-37 weeks of gestation and their neonate both at labour ward and labour ward theatre. The samples were cultured in Todd Hewitt Broth and sub cultured on sheep blood agar and Chromogenic Strepto B ID agar and incubated at 37ºC for 24 hours. Identification was based on the Gram staining, presence of β-haemolysis and absence of catalase production. A known *Staphylococcus aureus* was used as positive control and *Streptococcus pneumonia* was used as a negative control.

Antibiotic susceptibility test

The GBS isolated sensitivity were tested with 5 different classes of antibiotic; vancomycin (5 µg), penicillin (10 µg), erythromycin (15 µg) ciprofloxacin (5 µg), clindamycin (2 µg) Kirby-Bauer disc

diffusion method was employed [12]. An inoculum suspension was prepared from a pure culture in a sterile nutrient broth using an inoculation loop. These were incubated overnight. The turbidity of the inoculums were adjusted and compared with 0.5 McFarland standards so as to obtain a confluent growth. Mueller-Hinton media plates were inoculated with a loop-full of the prepared inoculums and streaked by swabbing the surface of the medium rotating the plate through an angle of 60º at each application. Finally, the swab was passed round the edge of the agar surface. The inoculated plates were left for a few minutes at room temperature with the lid closed. This allowed the moisture on the surface to be absorbed into the medium. The antibiotic discs were placed on the inoculated plates using a pair of sterile forceps. Each disc was gently pressed down to ensure even contact with the medium. A control was set up using *S. pneumonia* which was susceptible to all the antibiotics to be tested. The set up was incubated at 37ºC for 24 hours in 5% CO_2. After overnight incubation, the diameter of each zone inhibition (including the diameter of the disc) of each antibiotic was measured and recorded in millimetres. The results were compared with the zone diameter interpretive standards of the Clinical Laboratory Standards Institute CLS.

Results

A total of 72 swabs were taken from 24 pregnant women at 35-37 weeks of gestation and their neonates at OAUTHC, 20 (27.8%) were cultured positive for GBS based on Bergy's manual Biochemical test and systematic bacteriology. Morphological appearance of all the GBS positive isolates on chromogenic agar and the ability to lyse blood (from 5% sheep blood agar) were observed.

Most isolates were pink or cream in colour, circular in shape, there opacity is either opaque or translucent, entire in edges and have smooth surfaces. Catalase test were carried out on all the isolates to confirm that they were GBS, and all tested catalase negative. The isolates Gram stained and viewed under microscope using immersion oil, also all appeared purple cocci in chains. The isolates were identified based on Bergy's Manual of systematic Bacteriology.

Discussion, Conclusion and Recommendation

A total of 72 rectal and vaginal swabs were collected from 24 third trimester pregnant women and their neonates' umbilical cord. The result of the study showed higher prevalence rate of 27.8% *Streptococcus agalactiae* in this study area therefore confirming GBS colonization among pregnant women and the neonates. The findings agreed with studies of some workers in Tanzania whose reports shown colonization rates among mothers and neonates between 10% - 36% [13]. Consequently, the result is higher than the prevalence rate as described in Nigeria by Onipede in Ile-Ife (11.3%) [14] and Onile in Ibadan (Onile, 1980). The data however, are lower than data reported from Trinidad (32.9%) and Zimbabwe (31.6%) showing country variations [15]. Indicated variations could possibly due to differences in sampling techniques, study sites, populations investigated and the differences in type of culture media and culturing techniques used.

Table 1 summarized the association between GBS status and demographic characteristics of studied participant and the result revealed a higher colonization rate among age group 33 – 36 years (13.89%) which corroborated the report of Onipede where GBS was frequently isolated among older women with age ≥ 30 years (12.79%) against (9.38%) in younger women [16]. These could be due to the fact that pregnant women with age range greater than 30 year were enrolled in the study. Colonization of GBS was higher in pregnant women with purulent vaginal secretion (18.06%) as compare with pregnant women with mild secretion (9.72%).

All isolates were found to be 100% resistant to penicillin this is similar to studies performed by onipede in Ile-Ife [17]. All isolates were also found Susceptible to 40% clindamycin, 35% vancomycin, 90% ciprofloxacin, 40%erythromycin used. High level of resistance to Penicillin G which is a first choice of drug for the treatment of GBS could be as result of drug abuse or

Table 1 Association between GBS status and Demographic Characteristics of Studied participants.

VARIABLE	GBS +VE (%)	GBS –Ve (%)	Total (%)
AGE(YEARS)			
≤ 21	1(1.39)	3(4.17)	4(5.56)
22-25.5	1(1.39)	18(25.00)	19(26.39)
25.6-29.1	2(2.78)	12(16.67)	14(19.44)
29.2-32.7	6(8.33)	11(15.28)	17(23.61)
32.8-36.3	10(13.89)	8(11.11)	18(25.00)
TOTAL	20(27.80)	52(72.23)	72(100)
MARITAL STATUS			
Married	20(100)	52(100)	72(100)
Others (cohabiting)	0(0.00)	0(0.00)	0(0.00)
EXCESSIVE SECRETION			
Mild	7(9.72)	29(55.77)	36(50.00)
Purulent	13(18.06)	23(44.23)	36(50.00)

GBS+VE; Group B Streptococcus Positive
GBS –VE; Group B Streptococcus Negative

Table 2 Distribution Analysis for GBS-Infection.

Sample Code	Trimester	PROM	Antibiotic Prophylaxis	Catalase reaction
IV1	3rd	NO	NO	–
IB1	3rd	NO	NO	–
IR1	3rd	NO	NO	–
IV2	3rd	NO	YES	–
IB2	3rd	NO	YES	–
IV3	3rd	NO	NO	–
IR3	3rd	NO	NO	–
IB4	3rd	NO	YES	–
IR4	3rd	NO	YES	–
IB5	3rd	NO	NO	–
IB6	3rd	NO	YES	–
IB7	3rd	NO	NO	–
IB8	3rd	NO	YES	–
IV9	3rd	NO	NO	–
IV10	3rd	NO	NO	–
IV11	3rd	NO	NO	–
IR12	3rd	NO	YES	–
IR13	3rd	NO	NO	–
IR14	3rd	NO	NO	–
1R15	3rd	NO	NO	–

PROM; Premature/Preterm Rupturing of Membrane IV; Vaginal IR; Rectum IB; Baby
-ve; Catalase Negative+ve; Catalase Postive.

excessive use of the antibiotic in this part of the country and ease procurement of antibiotics in the country [18-23].

Conclusion

Certain degrees of prevalence of GBS infection among the pregnant women and neonates in this study area was observed and all the isolates recovered were found to be 100% resistant to penicillin happened to be the most widely used antibiotics for treatment of Group B *Streptococcal* infection. In this findings, ciprofloxacin remains the antibiotic of choice in treatment of *Streptococcal* infection.

Effective screening for GBS during antenatal care, Proper handling of neonates at postpartum by the health care practitioners with appropriate antepartum antimicrobial prophylaxis should be advocated.

Recommendation

- Women should be educated on GBS infection and the need to improve on personal hygiene.
- All pregnant women at 35 – 37 weeks of gestation should be screened for group B *streptococcus* Infection.
- Aseptic technique should be ensured in all prenatal caring process
- Since GBS has acquired resistance to several of the commonly used antibiotics, such as penicillin, prevention protocols other than antepartum chemoprophylaxis must be utilized to prevent neonatal disease.

Study Design Limitation

Based on our available procedures and constraint on study population at the centre, the findings may not be translated to be exact outcome of antepartum GBS prevalence rate associated with women in the country. However, the data obtained is of useful findings widely applicable as they will assist in control measures and effective therapeutic approach to GBS in pre-and post- natal stages among women in all part of the world.

Table 3 Multiple Antibiotic Resistannce Pattern of GBS Isolates.

Isolate Code	Antibiotic Resisted	Antibiotic intermediate	Antibiotics susceptible
IV1	PEN, ERY	NONE	DA, VA, CIP
IB1	PEN, DA, VA	CIP	ERY
IR1	PEN, ERY	NONE	DA, VA, CIP
IV2	PEN,VA,CIP	NONE	DA, ERY
IB2	PEN, VA	NONE	DA, CIP, ERY
IV3	PEN,DA, VA	NONE	CIP, ERY
IR3	PEN,DA, VA,ERY	NONE	CIP
IB4	PEN, DA, VA	NONE	CIP, ERY
IR4	PEN, VA	ERY	DA, CIP
IB5	PEN, ERY	CIP	DA, VA
IB6	PEN,DA,VA,CIP,ERY,	NONE	NONE
IB7	PEN, DA, VA	NONE	CIP,ERY
IB8	PEN, ERY	NONE	DA, VA, CIP
IV9	PEN, DA, VA	NONE	CIP, ERY
IV10	PEN, DA, ERY	CIP	VA
IV11	PEN,VA	NONE	DA,CIP, ERY
IR12	PEN,DA,ERY	NONE	VA, CIP
IR13	PEN, VA	NONE	DA,CIP, ERY
IR14	PEN,VA,DA,ERY	NONE	CIP
IR15	PEN, DA,ERY	NONE	VA, CIP

CIP: Ciprofloxacin VA Vancomycin DA: Clindamycin; PEN: Penicillin G E: erythromycin

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