MATERNAL GENITAL TRACT COLONIZATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF STREPTOCOCCUS AGALACTIAE: - A MODALITY FOR INTRAPARTUM PROPHYLACTIC TREATMENT IN JOS

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ABSTRACT

BACKGROUND: Group B *Streptococcus agalactiae* (GBS) has been established as a normal flora of the gastrointestinal tract from where it continually colonizes the vagina and serves as a potential cause of neonatal infections. This necessitated this study to determine the carriage rate among pregnant women.

AIM: The aim of this study was to determine the anogenital colonization and antibiotic susceptibility of Streptococcus agalactiae isolates from women receiving health care at the Jos University Teaching Hospital (JUTH).

MATERIALS AND METHODOLOGY: This was a hospital based descriptive cross-sectional study of 200 pregnant women and 100 non-pregnant women attending antenatal clinic (ANC) and Gynaecology clinic at the Jos University Teaching Hospital respectively, between July 2017 and November 2017. High vaginal and anorectal swabs were collected from the subjects. The specimens were cultured and antibiotic susceptibility testing of the GBS isolates determined. The results obtained were analyzed using SPSS version 21.

RESULTS: The age range (standard deviation) of the pregnant women was between19-48(±7.2) years with an average age of 31.2 years. The overall prevalence rate of GBS among the study participants was 6.3%. Pregnant and non-pregnant women were positive in 6.5% and 6.0% respectively.

The highest colonization rate was found in the maternal age-group 16-20years (11.1%), followed by age-group>40years (10.0%). Low colonization rate of 2.2% was observed among maternal age group 36-40years.Of the 100 non-pregnant women recruited as control for this study, they had age range of 16 years to 48 years with a mean age of 33.4 years (SD ± 6.1). Approximately, 6.0% of the 100 non-pregnant women enrolled were cultured positive for GBS colonization. There was no statistically significance between GBS colonization between the pregnant and non-pregnant women. All the Isolates were sensitivity to penicillin, erythromycin, and clindamycin while 5.3% were resistant to ampicillin, 10.5% to ceftriaxone and 21.1% to vancomycin.

CONCLUSION: This study showed that GBS colonization rate among the study population was 6.3%. Approximately, 6.5% and 6.0% prevalence rate was found among pregnant and non-pregnant women respectively. All the isolates were sensitive to penicillin, erythromycin and clindamycin. A total of 21.5% of the isolates were resistant to vancomycin. Ceftriaxone and ampicillin resistant was demonstrated in 10.5% and 5.3% respectively.

KEYWORDS: Streptococcus agalactiae, colonization rate, pregnant women, antibiotic susceptibility.

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INTRODUCTION

Streptococcus agalactiae also called Group B *Streptococcus* (GBS) is Gram-positive coccus that is currently the leading infectious agent responsible for neonatal morbidity and mortality in the United States[1]. Maternal vaginal colonization by GBS from the gastrointestinal tract is the primary risk factor for GBS newborn diseases[2]. Vertical transmission of GBS from a vagina-colonized mother to her newborn upon rupture of membrane or after the onset of labour can cause life-threatening infections such as neonatal sepsis and meningitis[3].

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The colonization of the vagina by GBS have been shown to be associated with frequency of sexual intercourse and multiple sexual partners[4,5]. This colonization can be transient, chronic, or intermittent and may affect 10% to 30% of pregnant women[6], and usually occurs in late adolescence rather than childhood. Women of childbearing age carry GBS at variable frequencies with similar figures in both developing and developed countries[7].

In 1996, the Centres for Disease Control and Prevention (CDC) in collaboration with the American College of Obstetricians and Gynaecologists (ACOG) and the American Academy of Paediatrics (AAP) published a consensus guideline on the prevention of early-onset neonatal GBS disease and the use of

intrapartum antibiotics in order to prevent vertical transmission of GBS. Two preventive strategies were described, a risk-based approach (based on maternal factors with or without known GBS colonization) and a screening-based approach (based on a combination of risk factors and GBS colonization)[8]. The policy statement strategies involved universal screening of pregnant women for GBS colonization at 35-37 weeks of gestation, or the risk-based approach base on fever ≥38^{0}C (104.5^{0}F), PROM ≥ 18 hours, ˂37 weeks of gestation, established GBS colonization were acceptable alternatives. Subsequent surveillance studies revealed that almost 50% of neonatal GBS EOS infections were not identified using the risk-based approach[9].

In 2002, CDC, ACOG and AAP reissued their most current guidelines to specifically promote universal screening for GBS at 35-37 weeks and treatment of colonized women with intrapartum antibiotic prophylaxis (IAP)[3,8]. To help narrow the use of IAP, the guidelines did not recommend that women with GBS-negative culture within 5 weeks of delivery receive GBS prophylaxis in the presence of intrapartum risk factors. In addition, new algorithms were also included regarding GBS prophylaxis for threatened preterm delivery and the management of neonates exposed to intrapartum prophylaxis. This refocused approach led to an additional decline in GBS-related EOS to a reported incidence of 0.3 per thousand in term neonates, and one which has surpassed the established goals of Healthy People 2010 of achieving an incidence of 0.5 per 1000 for EOS[8,10]. Concurrently, mortality associated with GBS EOS in term infants also dropped dramatically. Although the initial dramatic decrease in the incidence of GBS EOS was reflective of declines among African-American neonates, analysis of more recent data continues to reveal a several fold higher incidence of GBS EOS in African-American *versus* white infants[9,11,12].

Chemoprophylaxis remains the most effective means to prevent maternal and neonatal infections. Penicillin, ampicillin, erythromycin and clindamycin are drugs of choice for antibiotic prophylaxis against GBS. However, GBS resistant to erythromycin and clindamycin have been reported for many years though these antibiotics are used as alternatives for penicillin allergic women who need intrapartum antibiotics prophylaxis[13]. Resistance to erythromycin is frequently associated with concomitant clindamycin resistance in what is called macrolides-inducible resistance to clindamycin produced by an inducible methylase that alters the common ribosomal binding site for macrolides, clindamycin and the group B streptogrammins (quinupristin)[14].

Monitoring for new resistance mechanism in GBS is important as resistance genes can be spread among closely related species such as *Enterococcus species* and *Streptococcus pneumonia.* Group B *Streptococcus* isolates that were susceptible to erythromycin but resistant to clindamycin were recently shown to have the linB gene, which was previously identified only in *Enterococcus faecium*[15].

Considering the above documented resistant pattern, it is advisable to perform antimicrobial susceptibility testing in order to guide the selection of appropriate intrapartum antibiotics prophylaxis.

This study was aimed at determining the anogenital colonization and antibiotic susceptibility of Streptococcus agalactiae isolates from women receiving health care at the Jos University Teaching Hospital (JUTH).

MATERIALS AND METHODS

Study Area

The study was carried out in Jos University Teaching Hospital (JUTH). Jos University Teaching Hospital is located in Jos the Plateau State capital. The hospital is a tertiary health institution with a 600 beds capacity serving Plateau State and majority of the states in the North-central and part of North-east geopolitical zones of Nigeria. Jos University Teaching Hospital is also a centre for AIDS Prevention Initiative in Nigeria (APIN) that cater for most people leaving with HIV (PLHIV) from within and the bordering states. The main occupation of the people is farming with majority of them in the city being civil servants and businessmen and women.

Study Population

The study population included pregnant and non-pregnant women of childbearing age attending ante-natal and gynaecology clinics at Jos University Teaching Hospital between July 2017 and November 2017.

Study Design
The study was a hospital based descriptive, cross-sectional study that recruited 300 consenting pregnant and non-pregnant women attending antenatal and gynaecology clinics at the Jos University teaching Hospital.

Ethical Consideration
This study was approved by the research ethical committee of Jos University Teaching Hospital with reference number JUTH/DCS/ADM/127/XIX/6583. Written informed consents were also signed by all subjects before enrollment in the study.

Sample Collection
Anorectal and vaginal swab samples were carefully and aseptically collected from consenting 200 pregnant and 100 non-pregnant women using sterile swab sticks by the attending physicians after given them appropriate instructions on how the sample should be collected[16].

Specimen Transport
The collected specimens were immediately inoculated into a selective enrichment broth, Todd - Hewitt broth (Oxoid LTD) supplemented with gentamycin (8µg/ml), nalidixic acid (15µg/ml) and 5% sheep blood to increase the recovery rate of GBS[16,17]. These were transported to the laboratory within three hours of inoculation.

Culture and Incubation
The inoculated Todd-Hewitt broths were incubated aerobically at 37^{O}C for 18 to 24 hours. After an overnight incubation, the broths were subcultured onto 10% sheep blood agar and chromatic Strepto B agar (Liofilchem, Italy), a selective medium for GBS and incubated aerobically in 5-10% CO2 (candle extinction jar) at 37^{O}C for 18 to 24 hours, while the inoculated chromatic Strepto B agar plates were incubated aerobically at 37^{O}C for 18 to 24 hours[18]. The *Streptococcus agalactiae* control strain was also inoculated onto 10% sheep blood agar and chromatic Strepto B agar and incubated at the same conditions stated above.

Identification of GBS Isolates
Group B *Streptococcus* isolates were identified by their beta haemolytic pattern on 5% sheep blood agar and blue-green colour on chromatic Strepto B agar. The isolates were further subjected to Gram staining, catalase test, and serogrouping using streptococcal grouping kit (DR0585A OXOID) from Oxoid.

Antibiotics Susceptibility Testing
The GBS isolates were tested for susceptibility to penicillin G (10units), ampicillin (10µg), erythromycin (15µg), vancomycin (30µg), clindamycin (2µg), and ceftriaxone (30µg) (Oxoid Nig. Ltd) using the modified Kirby-Bauer disk diffusion method[19,20].

Data Analysis
The data obtained from the study were analyzed using Statistical Package for Social Sciences (SPSS) version 21 (IBM SPSS Inc, USA). Proportions were compared using Chi-square with confidence limit (p-value) of < 0.05 considered significant.

Consent for publication
All the authors reviewed and gave their approval for this article to be submitted for publication.

Competing of interest
There are no conflicts of interests among the authors.

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RESULTS
Of the overall 300 respondents, 6.3% were culture positive for group B *Streptococcus* (GBS). Out of the 200 pregnant women recruited for this study, GBS was isolated in 6.5% of the participants while 6.0% of the 100 non-pregnant women enrolled were cultured positive for GBS colonization. However, the difference in colonization rate between the pregnant and non-pregnant women was not statistically significant (X^2= 0.028, p = 0.867) (Table 1).

The association between GBS colonization rate and gestational age is summarized in Table 2. None of the respondents was found to be colonized by GBS in first trimester. The rate of colonization in second trimester was 8.9%. In third trimester, the colonization rate dropped slightly to 5.2%. The colonization rate based on gestational age was not statistically significant (X^2 = 1.453, p = 0.484) (Table 2).

In addition, GBS colonization did not appear to be influenced by maternal age. The study revealed a higher colonization rate of 11.1% in pregnant women among the age group 16-20 years and lower rate of 2.2% in age group 36-40 years. For the non-pregnant women, the colonization rate was observed to be highest in the age group 21-25 years (18.2%) followed by the age group 36-40 years (11.8%). GBS was not isolated from the age group 16-20 years and from those ≥40 years among non-pregnant women.

In the overall population, GBS colonization was highest among the age group 21-25 years (11.9%) with lowest rate of 3.6% among age group ≥40 years. The age groups 16-20 years and 26-30 years had colonization rate of 6.3% and 6.8% respectively. Nevertheless, the colonization rate between the age groups was not statistically significant (X^2= 2.780, p = 0.734) (Table 3).

Table 4 summarized the prevalence of GBS colonization in relation to social demographic factors. On the basis of marital status, pregnant women in a polygamy setting had the highest colonization rate of 7.4% followed by those that were single 7.1%, while monogamy had carriage rate of 6.3%. In non-pregnant women, polygamy carriage rate was 7.1%. Rate of 6.7% and 5.6% were recorded for singles and monogamy respectively. The rate was not influence by marital status (X^2 = 0.377, p = 0.828).

Educational level seemed to have significant influence on the occurrence of GBS. Among the pregnant group, those that had non-formal education had the highest carriage rate of 33.3% while 28.6% were obtained from the non-pregnant population. Those with tertiary education recorded a carriage rate of 6.6% among pregnant women while secondary level had the least 3.3%. In non-pregnant women, the second highest rate of occurrence of 9.5% was observed among those with primary education. These values were statistically significant (X^2= 22.427, p = 0.0001).

Religion had no statistical significant on the carriage rate of GBS. The Christian population among pregnant women had a colonization rate of 5.1% while the Muslims recorded 9.7%. In non-pregnant women, the rate was 4.8% and 8.1% for Christians and Muslims respectively.

Another social demographic factor that had significant statistical difference on the colonization rate of GBS was the use of antibiotics. It was observed during this study that pregnant women on antibiotics had a carriage rate of 12.5% and a lower rate of 3.7% among pregnant women not on antibiotics. The colonization rate was also higher among antibiotics exposed non-pregnant participants (33.3%).The influence of antibiotics on GBS colonization rate among the study population was statistically significant (X^2 = 3.889, p = 0.049).

On the basis of co-morbidity, it was observed that diabetic patients had colonization rate of 33.3% among pregnant and non-pregnant women. Among pregnant women, those without diabetic recorded a colonization rate of 6.1% while 5.2% was observed among non-pregnant population. These values were statistically significant (X^2 = 7.524, p = 0.006).

Antibiotic susceptibility testing revealed that all the isolates were susceptible to penicillin, erythromycin and clindamycin. 94.7% of the isolates were sensitive to ampicillin while 5.3% where resistant. Ceftriaxone had sensitivity of 89.5% with 10.5% of the isolates resistant to ceftriaxone. 21.1% of the isolates demonstrated resistant to vancomycin.

This sensitivity pattern shows that penicillin is the drug of choice for intrapartum prophylaxis while, erythromycin and clindamycin can be used in the case of penicillin allergy (Table 5).

Table 1: Group B Streptococcal carriage rates among pregnant and non-pregnant women in Jos University Teaching Hospital

Category of women	No. Tested	No. Positive	% Positive
Pregnant	200	13	6.5
Non-Pregnant	100	6	6.0
Total	300	19	6.3

p >0.05 p = 0.867χ2 = 0.028df = 1

Table 2: GBS colonization rate in relation to gestational age in HIV positive and HIV negative pregnant women in Jos University Teaching Hospital

Gestational age	HIV+ Pregnant women		HIV- Pregnant women		Total No. Positive (%)
	No. Tested	No. Positive (%)	No. Tested	No. Positive (%)	
First trimester	5	0(0.0)	1	0(0.0)	0(0.0%)
Second trimester	39	5(12.8)	40	2(5.0)	7(8.9%)
Third trimester	56	3(5.4)	59	3(5.1)	6(5.2)
Total	100	8(8.0)	100	5(5.0)	13(6.5%)

P = 0.484 $\chi 2 = 1.453$ df = 2

Table 3: Carriage of *Streptococcus agalactiae* among pregnant and non-pregnant women in Jos University teaching Hospital according to maternal age

Age Group (years)	Pregnant		**Non-Pregnant**		Total
	No. Tested	No. Positive(%)	No. Tested	No. Positive(%)	No. Positive(%)
16-20	9	1(11.1)	7	0(0.0)	1(6.3)
21-25	31	3(9.7)	11	2(18.2)	5(11.9)
26-30	59	4(6.8)	15	1(6.7)	5(6.8)
31-35	45	3(6.7)	32	1(3.1)	4(5.2)
36-40	46	1(2.2)	17	2(11.8)	3(4.8)
=40	10	1(10.0)	18	0(0.0)	1(3.6)
Total	200	13(6.5)	100	6(6.0)	19(6.3)

P = 0.734 $\chi 2 = 2.780$ df = 5

Table 4: Group B streptococci carriage rates among pregnant and non-pregnant women in Jos University Teaching Hospital in relation to social demographic factors

Social demographic factors	**Pregnant**		**Non-Pregnant**		**Total (% Positive)**
	No. Tested	**No. Positive (%)**	**No. Tested**	**No. Positive (%)**	
Marital status					
Single	14	1(7.1)	15	1(6.7)	2(6.9)
Monogamy	159	10(6.3)	71	4(5.6)	14(6.1)
Polygamy	27	2(7.4)	14	1(7.1)	3(7.3)
$\chi 2$ =0.377, p = 0.828 df = 2					
Educational level					
Non-formal	12	4(33.3)	7	2(28.6)	6(31.6)
Primary	37	1(2.7)	21	2(9.5)	3(5.2)
Secondary	60	2(3.3)	38	1(2.6)	3(3.1)
Tertiary	91	6(6.6)	34	1(2.9)	7(5.6)
$\chi 2 = 22.427$, p = 0.0001, df =3					
Religion					
Christianity	138	7(5.1)	63	3(4.8)	10(5.0)
Islam	62	6(9.7)	37	3(8.1)	9(9.1)
$\chi 2 = 1.894$ p = 0.169 df = 1					
Occupation					
Civil servant	53	3(5.7)	35	1(2.9)	4(4.5)
Housewife	78	5(6.4)	37	2(5.4)	7(6.1)
Business	69	5(7.2)	28	3(10.7)	8(8.2)
$\chi 2 = 1.085$ p = 1.581 df= 2					
Miscarriage					
None	131	6(4.6)	62	3(4.8)	9(4.7)
Yes	69	7(10.1)	38	3(7.9)	10(9.3)
$\chi 2 = 2.5333$ p = 0.1107 df = 1					
No. Sexual Partners					
One partner	189	12(6.3)	94	6(6.4)	18(6.4)
Multiple	11	1(9.1)	6	0(0.0)	1(5.9)
$\chi 2 = 0.006$ p = 0.937 df=1					
Douching					
Yes	74	5(6.8)	30	2(6.7)	7(6.7)

Table 5: Antibiotics sensitivity profiles of *Streptococcus agalactiae* isolates from HIV-positive and HIV-negative women

Antibiotic	% Sensitive	% Resistant
Penicillin G	100.0	0.0
Ampicillin	94.7	5.3
Vancomycin	78.9	21.1
Erythromycin	100.0	0.0
Clindamycin	100.0	0.0
Ceftriaxone	89.5	10.5

DISCUSSION

An overall GBS colonization rate of 6.3% was observed in this study population. The carriage rate was 6.5% in pregnant women and 6.0% in non-pregnant women. This result is lower than the 7.0% previously reported by Nsagha *et al* (1997) in Jos[21]. The slight decrease in the colonization rate may be attributed to improve health awareness among the general public and also improvement in culturing technique as Todd-Hewitt broth and chromatic Strepto B agar which are selective media for *Streptococcus agalactiae* were used in this study making identification and differentiation easy rather than just blood agar.

In other parts of Nigeria, 19.0% was reported in Ibadan by Onile (1990)[22] and Uhiara (1993)[23] reported a carriage rate of 9.0% in Calaber. Onipede and his colleagues in 2012 reported a higher prevalence rate of 11.3% in Ile-Ife while 9.8% was reported by Okon *et al* 2013 in North-eastern Nigeria[24,25].The colonization rate observed in this study was also lower than reported rates from several other African countries. Approximately 31.6% was reported in Zimbabwe by Moyo*et al* (2000)[26], 25.3% in Egypt by Shabayek *et al* (2014)[27] and 23% in Tanzania[28].

When compared with studies conducted in some developed countries, it was observed that the result of this study was lower to what was obtained in United States of America[29]. Tor-Udom *et al* (2006)[30] reported a carriage rate of 16.0% in Thailand while 24.0% was reported in Belgium[31]. A similar study conducted in Poland by Brzychczy-Wloch *et al* (2013)[32] reported 29.5% carriage rate. These results are higher to 5.7% obtained in Israel[33].

The variations between countries could possibly be due to differences in sampling and culturing techniques, types of media used as well as the population studied[34]. For instance, in this study, samples were collected from pregnant women regardless of gestational age unlike most other studies that recruited pregnant women at 35-37weeks of gestation.

Majority of the subjects recruited in this study were between the ages of 26-30 years. This age range corresponds to the reproductive age period of most women in developing countries[35,4]. This study revealed that the age group 21-25 years was associated with the highest colonization rate of GBS and decreases with advanced age, though, not statistically significant. This is a sexually active group and it has been stated that vaginal colonization by GBS is associated with sexual intercourse[4]. These findings correlated with the report of Ezeonu *et al* (2012)[36] in Enugu that GBS carriage rate was highest in women between age group 21-25 years. This is also similar to the report of Dzowela *et al* (2005)[37] and Lekala *et al* (2015)[38] but in contrast with some other reports that stated that colonization rate increases with advanced maternal age[39,24]

Generally, GBS colonization did not appeared to be influenced by gestational age since there was no statistical association between the gestational age and colonization rate. This study indicated that women were more colonized in the second trimester than the third trimester. This is consistent with the report of Donbraye *et al* (2010)[40] in Ibadan and in contrast with the observation of Joachim *et al* (2009), and Okon *et al* (2013) in North-eastern Nigeria that colonization rate is more in third trimester[28,25]. Group B *Streptococcus* was not isolated from women in their first trimester which correlated with the report of Raj et al (2009)[41] that colonization varied significantly with gestational age and that screening of women six weeks before delivery may not predict vaginal colonization and outcome at birth.

There was a varied association between social demographic factors and GBS colonization. It was observed that GBS colonization was significantly influenced by educational level, antibiotics used, and diabetes. Those with non-formal education were noted to have the highest colonization rate of 6(31.6%). This finding is similar to the report of

Regan *et al* (1996)[5] that colonization rate was higher in women with low social status and education level but contrary to Okon *et al* (2013)[25] report that women with non-formal education had lower carriage rate. Participants with tertiary had the second highest carriage rate but this may be due to the fact that majority of the recruited participants were from this group. These finding were statistically significant ($p < 0.05$).

Another important social demographic factor that influenced the carriage rate of GBS in pregnancy was co-morbidity with diabetes. It was observed that those who were diabetic had a carriage rate of 33.3% and the value was statistically significant as p-value was <0.05. This may be due to the fact that diabetes is an immunosuppressive disease resulting in increased risk of infection even by normal flora.

The use of antibiotics in pregnancy and GBS colonization was found to be statistically significant. This is probably significant because only few subjects recruited were on antibiotics. The 2(17.6%) isolates that were resistant to ceftriaxone were isolated from patients on antibiotics. This may possibly be due to indiscriminate used of the antibiotic in pregnancy to treat other infection making the organism to develop resistant.

In this study, maternal colonization rate was seen more among the primigravida (8.1%) and grandmultigravida (7.6%) and lower in nulliparous women. This observation is similar to the report of Yow *et al* (1979)[42] but in contrast with the findings of Baker *et al* (1976)[43]. It is also suggested that this greater GBS colonization often observed among primigravida and grandmultiparous has epidemiological implications in term of maternal complications and neonatal infections and policy issues in introducing preventive interventions[34].

Antibiotic susceptibility testing was performed on all the isolates to determine the sensitivity pattern. The isolates showed 100% sensitivity to penicillin G, erythromycin and clindamycin. Though ampicillin has been preferred by numerous investigators as a drug of choice because it is safe and has a broader spectrum than penicillin G, 5.3% of the isolates were resistant to ampicillin. About 21.1% and 10.5% demonstrated resistant to vancomycin and ceftriaxone respectively. Erythromycin and clindamycin is usually the preferred antibiotic in penicillin allergy but resistant to this antibiotic has been reported in some countries. In Canada, erythromycin and clindamycin resistant rates were found to be 17 and 8% respectively[44]. This is similar to a Tanzanian study that reported a GBS resistance rate of 17.4 and 13% for erythromycin and clindamycin respectively and 21% erythromycin resistant in Malawi[39,28]. This suggests that antibiotic resistance in GBS may be similar despite different geographic locations in sub-Saharan Africa. However, phylogenetic studies are necessary to verify this.

CONCLUSION

This study showed that GBS colonization rate among the study population was 6.3%. Approximately, 6.5% and 6.0% prevalence rate was found among pregnant and non-pregnant women respectively. All the isolates were sensitive to penicillin, erythromycin and clindamycin. A total of 21.5% of the isolates were resistant to vancomycin. Ceftriaxone and ampicillin resistant was demonstrated in 10.5% and 5.3% respectively.

RECOMMENDATIONS

The high colonization rate of GBS in pregnancy is a potential risk for preterm delivery, low-birth weight, still birth and neonatal infections. There is need for a national policy and guideline for screening of pregnant women at 35-37weeks of gestation as recommended by the CDC. Antibiotics susceptibility testing should also be done on all GBS isolates to determine their sensitivity pattern in order to guide the choice of intrapartum prophylaxis. Penicillin could be a drug of choice for intrapartum empirical prophylaxis in JUTH while erythromycin and clindamycin could be alternative options in pregnant women who are allergic to penicillin.

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