

ISSN: 2782-7550 (Print)  
ISSN: 2782-7542 (Online)

# ABMS

ANNALS OF BASIC AND MEDICAL SCIENCES

A Scientific Peer Reviewed Publication of The Faculties of Basic Medical and Basic Clinical Sciences, Usmanu Danfodiyo University Sokoto, Nigeria





# Prevalence of Group B *Streptococcus* Colonization among Pregnant Women and Feto-Maternal Outcomes in a Tertiary Health Institution, North-Western Nigeria

Ibrahim UA<sup>1</sup>, Pantii AA<sup>1</sup>, Mohammed Y<sup>2</sup>, Tunau KA<sup>1</sup>, Adamu A<sup>3</sup>, Adamu NA<sup>4</sup>, Diyo SA<sup>1</sup>

<sup>1</sup>Department of Obstetrics and Gynaecology, Usmanu Danfodiyo University/Teaching Hospital, Sokoto.

<sup>2</sup>Department of Medical Microbiology, Usmanu Danfodiyo University/Teaching Hospital, Sokoto.

<sup>3</sup>Department of Paediatrics Usmanu Danfodiyo University/Teaching Hospital, Sokoto.

<sup>4</sup>Department of Obstetrics and Gynaecology, Federal Medical Centre, Birnin Kebbi.

## Abstract:

**Background:** Group B *Streptococcus* (GBS) infection is a major cause of bacterial infections in the peri-natal period. These include amnionitis, urinary tract infections and endometritis. At birth, 50-60% of the neonates born to colonized mothers have positive cultures taken from mucus membranes and the skin.

**Aim:** The aim of this study is to determine prevalence of GBS colonization and compare the maternal and perinatal outcomes among GBS positive and GBS negative women within 7 days postpartum.

**Methodology:** This was a longitudinal study among pregnant women between 35-37 weeks gestation attending antenatal clinic at Usman Danfodiyo University Teaching Hospital, Sokoto. Vaginal and rectal swabs were taken from the participants and cultured for growth of Group B *Streptococcus* within 24 hours. The participants were followed up to 7 days post-delivery with their newborns to determine the maternal and early neonatal outcomes.

**Results:** One hundred and eighty five (185) women were recruited and 159 (85.9%) participants were available for follow-up to determining feto-maternal outcomes. Among the participants, 3.8% (7) had GBS vaginal colonization. There was no single case of early neonatal infection, intensive neonatal resuscitation nor neonatal mortality among both GBS positive and GBS negative women.

**Conclusion:** It has been found that the prevalence of maternal GBS colonization during pregnancy was low and neither GBS colonization nor GBS non-colonization was associated with poor maternal or poor fetal outcomes.

**Keywords:** Group B *Streptococcus* colonization, prevalence, feto-maternal outcome

## Corresponding author: Introduction

Ibrahim, Umar Augie,

Department of Obstetrics and Gynaecology,  
Usmanu Danfodiyo University  
Teaching Hospital, Sokoto.  
email: umariaugie@gmail.com  
phone: 08034672032

Group B *Streptococcus* is a major cause of bacterial infections in the peri-natal period and these include bacteraemia, amnionitis, endometritis and urinary tract infections in pregnancy (1). It is responsible for meningitis, pneumonia and sepsis in neonates (1). Group B *Streptococcus* can also pass through the cervix without causing cervicitis and cross intact amniotic membranes into the amniotic fluid there by infecting the fetus in-utero (2). It constitutes one of the leading pathogens associated with both early and late neonatal sepsis (3). In early onset disease (age at onset 0-7 days) the neonate is infected by exposure to GBS before or during passage through the birth canal (4). While in late onset disease (7-89 days) the pathogenesis is not yet clear (4).

The transmission is from mother to the child and the gastrointestinal tract is the source of vaginal GBS colonization and many adults are colonized without showing any symptoms (1). Group B *Streptococcus* colonization even when asymptomatic has been associated with adverse pregnancy outcomes such as premature rupture of membranes, pre-term delivery and low birth weight (2,5,6). At birth, 50-65% of neonates born to colonized mothers had positive GBS cultures taken from mucus membranes and skin (external ear canal, oral and naso-pharynx, umbilicus and ano-rectal sites)(4). Approximately 98% of the colonized new-born remained healthy but 1-2% developed invasive GBS disease (1).

It has been observed in our institution that significant number of women presents with premature rupture of membrane (PROM), fever in the advanced gestation and puerperal sepsis. There hasn't been any study to identify the possible causes of such symptoms them. Also some neonates delivered in our maternity unit were noted to be admitted into the special baby care unit with fever, hence managed as 'at risk babies' and discharged home with no identified aetiology neither drugs sensitivity of such aetiological agents. Such babies are usually managed with broad spectrum antibiotics. And where the aetiology has been identified as GBS, there was no established risk factor(s) for the GBS, hence may be difficult to be prevented.

Maternal intra-partum GBS colonization is the primary risk factor for early-onset disease in neonates. A classic prospective cohort study conducted during the 1980s revealed that pregnant women with GBS colonization were >25 times more likely than pregnant women with negative prenatal cultures to deliver a neonate with early-onset GBS disease (7). In the

absence of any intervention, an estimated 1%–2% of infants born to colonized mothers develop early-onset GBS infections (8,9).

There are conflicting data with regards to the fetomaternal outcomes among pregnant women with vaginal GBS colonization. And this data varied from region to region, Centre to Centre and even between ethnic groups. Also there is paucity of data with regards to the fetomaternal outcome among pregnant women with vaginal GBS colonization in North-Western Nigeria. Therefore, the aim of this study was to determine the maternal and neonatal outcomes among GBS positive and GBS negative women within 7 days postnatal and to compare the outcome between the two groups. The study was meant to stimulate research interest in the area which may indirectly impact the high maternal and neonatal morbidity and mortality rate in the country. In Nigeria, no major strategies have been formulated to screen for maternal GBS colonization and subsequent prevention of early neonatal diseases. The reasons are still unclear but one of the possible contributing factors is the lack of local and multi-centre data. The study was meant to generate local data that will inform the development of rational interventions for GBS infection. Also the results of this study may have the potentials to influence local policy with regards to routine antenatal testing of all pregnant women and instituting appropriate preventive measures to reduce maternal and neonatal morbidity and mortality from maternal GBS colonization.

## Materials and Methods

This study was a longitudinal study carried out among pregnant women at Usman Danfodiyo University Teaching Hospital (UDUTH). All pregnant mothers attending antenatal clinic who met the inclusion criteria were approached, counseled and informed consent was obtained. One hundred and eighty-five participants who met the inclusion criteria and willing to participate in the study were recruited. Those included in the study were women with singleton pregnancies between the gestational age of 35 and 37 weeks and their neonates delivered in the delivery room of UDUTH, Sokoto. Those excluded from the study includes all pregnant women who had been on antibiotic treatment in the preceding two weeks prior to recruitment were excluded from the study. Also pregnant women who had been diagnosed and were on management for premature rupture of membranes, antepartum haemorrhage and pre-eclampsia were excluded from the study. Pregnant women who have diabetes and HIV in pregnancy as well as with obvious sexually transmitted infections and genital ulcers on examination were excluded from the study.

The desired sample size was calculated using prevalence formulae for estimating sample size in prevalence studies with a finite population correction as suggested by Daniels (10). The prevalence used to calculate the sample size in this study was that of the study conducted by Onipede *et al* at Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Nigeria on Group B *Streptococcal* Carriage during Late Preg-

nancy and Neonatal Infection (3). A sample size of 154 was calculated with attrition rate of 20% (30.8). A total of 185 women were used for this study.

Women were selected via simple random sampling technique by balloting “YES” or “NO”. Those who picked YES were recruited while those who picked NO were excluded from the study, until the desired sample size was attained.

A vaginal and rectal swab were taken after filling-in the laboratory request forms that bore study numbers corresponding to the respective questionnaires. The questionnaire was adopted from the study conducted by Onipede *et al* (3) with few modifications. The questionnaires bore the client's hospital numbers and the corresponding hospital files had colour coded stickers for easy identification during follow up for outcomes and also to avoid double recruitment during subsequent antenatal visits.

Under aseptic condition, eligible participants were screened in the presence of female chaperone. In dorsal position, pelvis was inspected and sterile disposable cuscus speculum was inserted into the vagina after parting the labia with sterile gloved hands to exclude presence of either genital ulcer or vaginal discharge. Vaginal and rectal swabs were then taken per participant using two separate commercially available collection and transport system for both aerobes and anaerobes (Charcoal Swabs Amies Plastic Applicator with Rayon Tipped Black Cap, Stone, Staffs, UK) (11), the specimen was immediately transported to the Medical Microbiology laboratory of UDUTH for culture.

The swab samples were incubated aerobically at 37°C for 24 hours onto 5% Sheep Blood Agar (Oxoid England) and incubated overnight for evidence of haemolysis which suggest presence of GBS. A quick gram stain smear was done where there was evidence of haemolysis on the blood agar plate. Where the Gram smear showed evidence of Gram positive cocci, it was then sub-cultured and CAMP (Christie, Artkins and Münch-Petersen) test was carried out from the primary plate using the pure colonies. Suspected GBS isolates were identified appropriately (6,7). GBS antigen was confirmed definitively by serological grouping using *Streptococcal* group B reagent kit (Oxoid England) testing of selective broth<sup>12</sup>. Pure growth *beta*-haemolytic colonies were grouped using specific Group B anti-serum by identifying the B antigen extracted from the cell wall of the bacteria. The test uses an enzyme reagent to extract the antigen. Positive group B test indicates that the organism is *B streptococcus* (12).

The women were followed up and data on GBS status, maternal and fetal outcomes and any obstetric outcome prior to delivery, intra-partum and within 7 days post-delivery were collected. Data on maternal history of PROM, fever, urinary tract infection (UTI), endometritis and puerperal sepsis were collected. Any maternal and neonatal outcome for the first 7 days was also collected and analyzed. Women who tested positive for the GBS and had intra-partum antibiotics, had repeat screening on the 7<sup>th</sup> day post-partum to determine whether or not they are

cleared of the bacteria from their vagina.

Maternal outcomes were assessed as follows: fever; axillary body temperature of 37.5<sup>o</sup> C, UTI; as presence GBS bacteria in a clean catch urine sample, PROM; rupture of fetal membranes before onset of labour which is confirmed by sterile speculum examination, PPH; bleeding from genital tract in excess of 500ml after vaginal delivery or 1000ml following Cesaerean section or any amount that caused cardiovascular instability, Endometritis; thickened endometrium 5mm or more with or without lower abdominal pain, tender and sub-involuted uterus, Puerperal sepsis; presence of GBS in the vaginal swab culture in the presence of fever and or malodorous vaginal discharge.

The fetal outcomes were assessed as follows: intensive neonatal resuscitation; a neonatal resuscitation that lasted for 15 minutes or more with or without use of pharmacological agents or endotracheal intubation and neonatal sepsis; presence of GBS in a culture from muco-cutaneous surfaces and umbilical stump of the baby in the presence of fever.

Data analysis was done using Statistical Package for Social Sciences (SPSS) version 20. Descriptive analysis was done and result presented in frequency and percentage. Continuous variables were presented as mean and standard deviation. Feto-maternal outcomes were presented and chi square was used to compare the feto-maternal outcomes between GBS positive and GBS negative. A *p*-value < 0.05 was considered statistically significant.

### Ethical Consideration

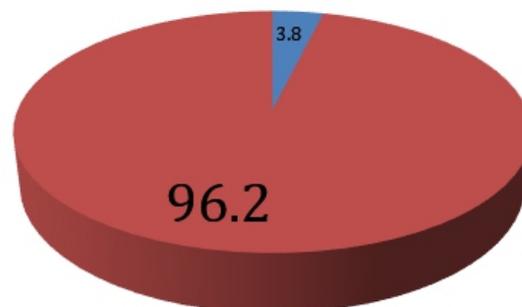
Ethical clearance was sought for from the Human Research and Ethical Committee of Usmanu Danfodiyo University Teaching Hospital, Sokoto with reference number: UDUTH/HREC/2017/No. 621 before embarking on the study. International ethical principles according to the Helsinki declarations were considered during the course of the research. In addition, individual consent was also obtained from the study subjects before participation in the research.

### Results

Among 185 participants recruited into the study, 24 (12.7%) of the study participants were lost to follow-up. Although their vaginal swabs were taken and analyzed for GBS vaginal colonization, none of the lost to follow-up women tested positive for GBS. Seven participants (3.8%) tested positive for GBS as shown in the figure 1 below.

The mean age of the studied population was 28.49 ± 5.6, 73% of them were Hausa/Fulani and majority (85.4%) was Muslims. Mast (57.1%) of the GBS colonized women were between the ages of 30-39 years. None of those below the age of 20 years was GBS positive. However, 51.7% of the GBS negative arms were between the ages of 20-29 years. Table 1 shows the socio-demographic characteristics of the participants.

**GBS positive=3.8%**  
**GBS negative=96.2%**



**Figure 1:** prevalence of GBS among the study participants

**Table 1:** Socio-demographic characteristics of the participants

Characteristics	Frequency(n)	Percentage(%)
<b>Age(years)</b>		
Less than 20	9	49
20 to 29	94	508
30 to 39	78	422
40 and above	4	22
<b>Ethnicity</b>		
Hausa/Fulani	135	730
Igbo	32	173
Yoruba	8	43
Others	10	54
<b>Occupation</b>		
Housewife	97	524
Civil servant	41	222
Business	33	178
Traders	14	76
<b>Religion</b>		
Islam	158	854
Christianity	27	146
Others	0	00
<b>Family setting</b>		
Monogamy	145	784
Polygamy	40	216
<b>Socio -economic class</b>		
Low	37	200
Medium	29	157
High	119	643
<b>Parity</b>		
Primigravida	38	205
Multigravida	118	638
Grand -multiparous	29	157

There were 1 (14.29%) cases each of urinary tract infection (UTI) and pre-mature rupture of membrane (PROM) among GBS positive mothers but none had fever, endometritis, puerperal sepsis or maternal death. However, there were only 5 (3.3%) cases of UTI, 3 (2.0%) cases of PROM and 4 (2.6%) cases of fever among the GBS negative mothers. Though, the rate of UTI and PROM were high among the GBS colonized mothers, but this was not statistically significant relationship (Fisher's

exact test, 8.205,  $p$  value 0.156).

There were 4 (2.6%) cases of fever, 5 (3.3%) cases of UTI, 3 (2.0) cases of PROM and 1 (0.7%) case of PPH among GBS negative women. This is shown in Table 2.

**Table 2:** Maternal Outcomes among GBS Colonized and Non-colonized Mothers

Maternal Outcome	GBS Positive	GBS Negative
Fever	0 (0.0%)	4 (2.6%)
UTI	1 (14.3%)	5 (3.3%)
PROM	1 (14.3%)	3 (2.0%)
PPH	0 (0.0%)	1 (0.7%)
Uneventful	5 (71.4%)	140 (91.4%)
<b>Total</b>	<b>7 (100.0%)</b>	<b>153 (100.0%)</b>

Of the fetal outcomes measured during the study period, though there was a single case of neonatal death among fetuses of GBS positive mother however, this was not related to the neonatal GBS complications. There was no single case of early-neonatal infection with GBS among the neonates of GBS positive mothers. Hence, there was no association between fetal outcome and maternal vaginal GBS colonization. Eight (5.2%) of the neonates delivered to the negative women had intensive neonatal resuscitation and 15 (9.7%) of the neonates of the GBS negative mother were admitted into the NICU. This is shown in Table 3.

**Table 3:** Fetal Outcomes of GBS colonized and Non-colonized Mothers

Outcome	GBS positive	GBS negative	Chi square/ Fischer test
Delivery outcome			
Alive	7(100.0)	154(99.4)	0.045
Still -birth	0(0.0)	1(0.6)	
Intensive resuscitation	1(14.3)	8(5.2)	6.41
SCBU admission	1(14.3)	15(9.7)	
Neonatal sepsis	0(0.0)	0(0.0)	
Neonatal death	1(14.3)	3(1.9)	
Uneventful	4(57.1)	129(83.2)	

## DISCUSSION

The prevalence of GBS among pregnant women attending antenatal care clinic in Usmanu Danfodiyo University Teaching Hospital, Sokoto was 3.8%. This is close to the 2.5% that was reported from Lome, Togo (13). This may be due to the similarity in geographical settings, study design and swab culture technique. This prevalence is however, lower than what was reported by other studies done in Blantyre, Malawi (16.5%) (2), Uganda (28.8%) (14), and Ethiopia (15). Though the studies from Ile-Ife (3) and Enugu (16) were also hospital based cross-sectional studies, however, in these studies the women were screened at 35-40 and 24-37 weeks of gestation respectively. This could have accounted for the higher prevalence in their studies than in the current study. The prevalence in this study is also much lower than the 20-30% prevalence in a multi-centered and multi-ethnic study reported by the Centre for Disease Control (CDC) (7). Probably, this was due to a larger sample size of 1,702 and repeat sample collection at delivery. Our prevalence is also lower than 25.3% and 30% colonization rate reported by Sabayek

et al (17). Though, they used Islam culture media and PCR technic respectively, their technique has sensitivity similar to our method, but in their study, the women were screened between 24 to 40 weeks of gestation which could have accounted for the higher prevalence.

In this study also there was no association between GBS colonization and previous bad pregnancy outcomes (such as previous history of PROM, previous history of pre-term delivery and previous history of stillbirth). These findings are similar to what was reported by Salat et al (13). In their study they found no significant association between GBS colonization and bad pregnancy outcomes such as preterm delivery, premature rupture of membranes as well as early neonatal sepsis, early neonatal death and fever in the previous pregnancy. Similarly, studies from Netherland (18), Canadar (19), Uganda (20), Lome, Togo (13), Enugu, South-Eastern Nigeria (16), and Ile-Ife (3), South-Western Nigeria reported no association between GBS colonization and previous bad pregnancy outcomes.

Though there were 4 (2.6%) cases of fever, 5 (3.3%) cases of UTI, 3 (2.0) cases of PROM and 1 (0.7%) case of PPH among GBS negative women, but this was not statistically significant, this is consistent to what was reported from Netherlands (18) and Lome, Togo (13).

However, a study done in Queen Elizabeth central hospital in Blantyre, Malawi<sup>3</sup> reported significant association (87.5% of the patients who were GBS positive had a history of bad pregnancy) between previous bad pregnancy and GBS colonization, indicating that such episodes could predispose to GBS colonization in subsequent pregnancies (2).

There was no single case of either early neonatal GBS infection detected or neonatal death due to GBS among the neonates delivered to the GBS colonized mothers. This is consistent with what was reported from a similar multi-centre cross-sectional study from USA which reported a prevalence of 1 in 2000 neonates delivered to a GBS colonized mothers (21). Similarly, a retrospective study of all infants with early-onset GBS disease that was done in Manitoba in Winnipeg, Canada (19), revealed a transmission rate of 1.74 per 1000 women and a total of 3449 women would require universal screening to prevent a single case of early-onset neonatal GBS disease that would occur if a universal risk-based approach was used (19) and about 68,966 are required to prevent a single GBS related death (19).

Though there were 8 (5.2%) of the neonates delivered to the negative women had intensive neonatal resuscitation and 15 (9.7%) of the neonates of the GBS negative mother were admitted into the NICU, this was not statistically significant. This is similar to what was reported from Malawi (3), Uganda (14), Netherlands (18) and Canada (19).

Urinary tract infection and pre-mature rupture of membranes were the maternal complication observed in this study. There was single case each of maternal UTI and PROM among GBS colonized women. Although these are

higher in the GBS colonized mothers, but there was no statistical significant relationship between the GBS colonization and developing UTI or PROM. There was no single case of puerperal sepsis or maternal death.

## CONCLUSION AND RECOMMENDATION

This study found that, the prevalence of vaginal GBS colonization among pregnant women attending antenatal care clinic of Usmanu Danfodiyo University Teaching Hospital, Sokoto, was low and does not affect maternal and perinatal outcomes.

Further multi-centres studies with a larger sample size may be required to draw a conclusion on the findings from this study.

## ACKNOWLEDGEMENT

The authors would like to thank Mr Muhammed Abdulqadir, a chief medical laboratory technician with Medical Microbiology Department of Usmanu Danfodiyo University Teaching Hospital, Sokoto. Who assisted in providing both logistic as well as slides preparation.

## CONFLICTS OF INTEREST

There are no conflicts of interest.

## REFERENCES

1. Musa M, Daniel A, Yimtubezinash W. Prevalence of group B streptococcus colonization among pregnant women attending antenatal clinic of Hawassa Health Centre. *Ethiop J Health Dev.* 2012; 26(1):36-42.
2. Dzowela T, Komolafe OO, Igbigbi A. Prevalence of group B streptococcus colonization in ante natal women at the Queen Elizabeth Central Hospital, Blantyre- A preliminary study. *Malawi Medical Journal.* 2005;17(3): 97-99.
3. Onipede A, Adefusi O, Adeyemi A, Adejuyigbe E, Oyelese A, Ogunniyi T. Group B *Streptococcus* carriage during late pregnancy in Ile-Ife, Nigeria. *Afric J Clin and Experimental Biol,* 2012; 13(3):135-143.
4. Rooyen TM, Jan EA, Berit S. Group B *Streptococcus* colonization during pregnancy and maternal-fetal transmission in Zimbabwe. *Acta Obstetrica et Gynecologica Scandinavica,* 2010; 89(2) 250-255.
5. Medical and Surgical Illness Complicating Pregnancy. Hiralal K (Ed). DC Dutta's Textbook of Obstetrics including Perinatology and Contraception. 7<sup>th</sup> (Edn). New Central Book Agency Ltd, London. 2010; 296.
6. Genital Tract Infection in Pregnancy. Frank WL, Patrick D (Ed). Obstetrics and Gynaecology; The Principles of Practice. 1<sup>st</sup> (Edn). McGraw Hill. New York, 2001; 103-4.
7. Centre for Disease Control. Prevention of perinatal group B Streptococcal disease: a public health perspective. *MMWR* 1996; 45(No. RR-7).
8. Lewin EB, Amstey MS. Natural history of group B *Streptococcus* colonization and its therapy during pregnancy. *Am J Obstet Gynecol* 1981;139:512-5.
9. Hoogkamp-Korstanje JA, Gerards LJ, Cats BP. Maternal carriage and neonatal acquisition of group B *Streptococci.* *J Infect Dis.* 1982;145:800-3.
10. Daniel WW. Biostatistics: A foundation for Analysis in the Health Sciences. 7th Edition. New York: John Wiley and Sons ; 1999
11. *Streptococcus agalactiae.* Monica C (Ed). District Laboratory Practice in the Tropics. 2<sup>nd</sup> (Edn). Cambridge University Press. 2006; 162-163
12. Katz V, Bowes WA Jr. Perinatal group B Streptococcal infections across intact amniotic membranes. *J Reprod Med.* 1988;33:445-449.
13. Mounerou S, Dagnra AY, Adama – Hondégla AB, Ekouevi K, Dossim S, Kao K, et al. "Group B Streptococcal Carriage Rate in Vagina of Pregnant Women in Third Trimester in Lomé, Togo." *World J Prevent Med.* 2015; 3(1):7-10.
14. Abdu M, Joel B, Yarina F, Ngozi J. Group B *Streptococcus* colonization Among Pregnant Women Attending Antenatal Care at Tertiary Hospital in Rural Southwestern Uganda. *Internl J Microbiol.* 2016;5:7.
15. Alemseged G, Selam N, Haftamu H, Mehamud A, Muthupandian S and Tsehaye A. Isolation and anti-microbial susceptibility pattern of Group B Streptococcus among pregnant women attending Antenatal clinic in Ayder Referral Hospital and Makelle Health Centre, Makelle, Northern Ethiopia. *BMC Res Notes.* 2015; 8:518.
16. Ezeonu IM and Agbo MC. Incidence and anti-microbial resistance profile of Group B Streptococcus (GBS) Infection in Nsukka, Enugu, Nigeria. *Afr J microbiol Res.* 2014;1:1-
17. Shabayek SA, Abdalla SM, Abouzeid AM. Vaginal carriage and antibiotic susceptibility profile of group B Streptococcus during late pregnancy in Ismailia, Egypt. *J Infect Public Health.* 2009;2(2):86-90.
18. Najmi, N, Sikandar, R., Zuberi NF, Jehan I. Maternal genital tract colonization by Group B Streptococcus: A hospital based study. *J Pakistan Med Assoc.* 2013; 63(9),1103-1107
19. Gerald K. Epidemiology of early-onset neonatal group B streptococcal infection; implication for screening. *Can Fam Physician.* 2007; 53(6): 1054-5.
20. Were E, Esamai F, Liechty E. The vaginal and ano-rectal colonization by Group B *Streptococcus* among term pregnant women in Moi Teaching and Referral hospital, Eldoret. Presentation made at the annual Kenya Obstetrical and Gynaecological Society (KOGS) meeting, 2006. 32(4);173-6.
21. American College of Obstetricians and Gynecologists Committee on Obstetric Practice ACOG Committee Opinion No. 485: Prevention of early-onset group B Streptococcal disease in newborns. *Obstet Gynecol.* 2011; 117(4):1019-27.
22. Melissa KVD. Evaluation of Universal Antenatal Screening for Group B Streptococcus. *N Engl J Med,* 2009; 360:2626-2636.