ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES, SCHOOL OF ALLIED HEALTH SCIENCES, DEPARTMENT OF MEDICAL LABORATORY SCIENCES

PREVALENCE OF GROUP B *STREPTOCOCCI* COLONIZATION AND SUSCEPTIBILITY PATTERN AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE CLINICS OF HEALTH INSTITUTIONS, ADDIS ABABA, ETHIOPIA

By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AAERC	AHRI/ALERT Ethical Review Committee
AAU	Addis Ababa University
ACOG	American College of Obstetricians and Gynecologists
AHRI	Armaur Hansen Research Institute
ALERT	All African Leprosy, Tuberculosis, Rehabilitation and Training Center
ANC	Antenatal Care
AST	Antimicrobial Susceptibility Testing
ATCC	American Type Culture Collection
CAMP Test	Christie, Atkins, Munch, Peterson Test
CDC	Center for Disease Control and Prevention
CLSI	Clinical and Laboratory Standard Institute
DRERC	Departmental Research and Ethics Review Committee
EOGBSD	Early-Onset Group B *Streptococcal* Disease
GBS	Group B *Streptococci*
HIV	Human Immunodeficiency Virus
IAP	Intrapartum Antibiotics Prophylaxis
LOGBSD	Late- Onset Group B *Streptococcal* Disease
MHA	Muller Hinton Agar
NRERC	National Research Ethics Review Committee
PROM	Premature Rupture of Membranes
SBA	Sheep Blood Agar
SOPs	Standard Operating Procedures
SPSS	Statistical Package for Social Sciences
THB	Todd Hewitt Broth

ABSTRACT

Background: Group B *Streptococci* (GBS) is the leading cause of septicemia, meningitis and pneumonia in neonates. Maternal colonization with GBS is the principal risk factor for early-onset of invasive GBS disease in infants. GBS is now recognized to be an important cause of maternal and neonatal morbidity and mortality in many parts of the world; however, it has been little studied in Ethiopia.

Objectives: To determine the prevalence of GBS colonization, antibiotic susceptibility pattern and identify risk factors related to GBS among pregnant women attending antenatal care clinics of Health Institutions in Addis Ababa, Ethiopia.

Methods: A cross sectional prospective study was conducted from May-August, 2014. Consented participants' information was collected using structured questionnaire. A total of 281 vaginal swabs were collected by consecutive sampling technique and inoculated into Todd Hewitt Broth and later sub cultured on 5% blood agar for isolation of GBS. Antimicrobial susceptibility testing was performed according to the criteria of the clinical and laboratory standard institute (CLSI) guidelines 2013 by disk diffusion method. Data was entered and analysed using SPSS version 20.0 software. Chi-square test and binary logistic regression analysis were used. A 95 % CI and P- value of < 0.05 were considered statistically significant.

Result: The overall prevalence of GBS colonization among pregnant women was 14.6% (41/281). GBS colonization was significantly associated with health institutions and inversely related with history of contraceptive use ($P<0.05$). All GBS isolated were susceptible to chloramphenicol. Resistance to tetracycline, cefotaxime, clindamycin, penicillin, vancomycin, ampicillin and erythromycin was 90.2%, 34.1%, 26.8%, 19.5%, 17%, 14.6% and 7.5% respectively.

Conclusion and Recommendation: There was high isolation frequency of GBS colonization (14.6%) and resistance to the commonly used antibiotics which suggests the importance of the screening of GBS colonization in pregnant women at 35-37 weeks of gestation and testing their antimicrobial susceptibilities in order to provide antibiotic prophylaxis.

Key words: Group B *Streptococci,* prevalence, antimicrobial susceptibility testing, risk factors

1. INTRODUCTION

1.1. Background

Group B *Streptococcus* (GBS) is originally known for causing bovine mastitis in the 1920's and was not demonstrated to be a human pathogen until 1938 (1-3). Since the mid-1960s, GBS has become the major cause of bacterial infections in the perinatal period in pregnant women as well as focal and systemic infections in newborns(4). In 1970s, GBS emerged as the leading cause of neonatal morbidity and mortality in the United States, with a frequency of 2-3 cases per 1,000 live births and case-fatality ratios as high as 50%(1, 5-8).

Group B *Streptococcus* is encapsulated Gram-positive cocci that usually produce a narrow zone of beta-hemolysis on blood agar. It belongs to Lancefield group B (4, 9). There are 10 GBS serotypes (Ia, Ib, and II to IX) based on variations in the capsular polysaccharide (CPS), a major virulence factor that helps the microorganism to evade the host's defense mechanisms (10-14).

Group B *Streptococcus* is the most frequent pathogen isolated from neonates with invasive bacterial disease and responsible for serious infections in newborns such as pneumonia, septicemia and meningitis (4, 15, 16). GBS neonatal infection is divided into two categories: early-onset GBS disease (EOGBSD), which occurs within the first week of life, and late-onset GBS disease GBS (LOGBSD), which occurs between one week to 3 months of age (2, 17, 18). The most likely reservoir of GBS is the gastrointestinal tract, and the most frequent site of secondary spread is the genitourinary tract. The neonates get colonized with GBS by the aspiration of infected amniotic fluid, or by vertical transmission during the passage through the colonized vaginal canal. It is also a cause of cystitis, amnionitis, endometritis, and stillbirth in the pregnant women. The infants who survive are often left with developmental disabilities, including mental retardation, hearing or vision loss and speech problems (19).

In pregnancy, GBS can infect the amniotic fluid, and during labor, vertical transmission may infect the newborn leading to neonatal sepsis and meningitis. Approximately 10-30 % of women are colonized with GBS in vagina during pregnancy and 50-75 % of their infants acquire this organism through birth canal (20, 21). Colonization during pregnancy may be transient, chronic or intermittent, and is asymptomatic in the majority of cases (22, 23).

Maternal colonization with GBS in the genitourinary or gastrointestinal tract and transmission to the infant during the labor and delivery process is the principal risk factor for early-onset invasive GBS disease (6, 7, 24, 25). Moreover, urinary tract infection sustained by GBS, either symptomatic or asymptomatic, is considered a risk factor for neonatal infections (22).

Universal bacteriological screening of mothers for vaginal or rectal GBS colonization at 35 to 37 weeks of gestation, followed by selective intrapartum antibiotic prophylaxis for all screen-positive women, is the strategy currently recommended to reduce incidence of colonization in neonates and prevent early-onset GBS-related diseases (8, 25-27). In the absence of a licensed GBS vaccine, universal screening and intrapartum antibiotic prophylaxis continue to be the cornerstones of early-onset GBS disease prevention (8).

1.2. Statement of the problem

Since 1970s, more than 7,500 cases of GBS-associated neonatal sepsis and meningitis have been reported annually in the United States, with a financial burden of more than $350 million per year in neonatal costs (28). It is the leading cause of early invasive infections in newborns worldwide and can also cause life-threatening infections in pregnant women and immunocompromised adults (4).

Group B *Streptococcus* is a leading cause of neonatal morbidity and mortality in the US, Western Europe and Australia. Mother to child transmission may lead to neonatal infection in 1 to 2 infants per 1,000 live births with mortality rates ranging from 10 to 20 %. Among pregnant women, the prevalence of colonization with GBS ranges from 3.2 to 36 % (29, 30). In 1970s, GBS emerged as the leading infectious cause of early neonatal morbidity and mortality in the United States with case-fatality ratios as high as 50 % (4, 8, 17, 24, 27). Since this time, GBS has remained the leading infectious cause of neonatal morbidity and mortality in industrialized countries, affecting 0.5–3 newborns in every 1000 live births. In less-developed countries, the incidence of GBS neonatal disease also varies widely: 0.17 per 1000 live births in India to 3 per 1000 live births in sub-Saharan Africa (10).

In Ethiopia, few studies indicated the presence of disease burden caused by GBS among pregnant mothers and neonates (4, 31). In 1989, Schmidt J *et al.* indicated a colonization rate of 9 % to be found among the mothers and 5 % in the neonates in Gondar, Northwest Ethiopia (31)

and recently in 2012, Mohammed M. *et al*., showed colonization rate of 21 % among pregnant women in Hawassa, Southern Ethiopia (4). Therefore, the present study was conducted to determine the prevalence of GBS colonization, antibiotic susceptibility pattern and identify risk factors related to GBS among pregnant women attending antenatal care (ANC) clinics at Health Institutions, Addis Ababa, Ethiopia, in order to reduce the risk of early-onset neonatal sepsis.

2. LITERATURE REVIEW

2.1. Epidemiology of Group B *Streptococci*

Streptococcus agalactiae or GBS is widely distributed in nature, and normal flora of the gastrointestinal tract and may colonize the vagina chronically or intermittently in about a third of women (4, 9, 32). The vagina and the perianal regions/rectum are the major reservoirs for GBS, and the colonization of these regions is a risk factor for subsequent infection in pregnant women and newborns (33). Asymptomatic colonization with GBS is common in pregnant women worldwide. Maternal GBS colonization varies by population characteristics such as age, parity, socio-economic status, presence of sexually transmitted diseases, sexual behavior, ethnic group and geographic area (33, 34) .

Group B *Streptococci* emerged as a significant neonatal and maternal pathogen in the United States (US) and Western Europe with reported mortality rates of 15 to 50 % during the 1970s and 1980s. In the US, 10 to 35 % of pregnant women are asymptomatic carriers of GBS in the genital and gastrointestinal tract at the time of delivery (35).

Prevalence of maternal carriage of GBS in developing countries, including populations in tropical Africa, is almost similar to that identified in populations in the United States. Different Studies from Kenya, South Africa, Zimbabwe and Malawi suggested that GBS is emerging as an important cause of neonatal sepsis in Africa (36).

Worldwide, colonization by GBS is highly prevalent among pregnant women; varying between 4% and 30% (4, 19, 37). The GBS carriage rate is 40–70 % in neonates who are born to the colonized mothers (19). The prevalence of maternal colonization by GBS vary widely throughout the world depends on culture methods, including the number and type of sites cultured and type of medium used, time of pregnancy, origin(region) and race (4, 38, 39). The prevalence of GBS vaginal colonization in pregnant women in different studies was; India/Pakistan 12 % each, America 14 %, Asia-Pacific 19 %, Sub-Saharan Africa 19 %, Middle-East/North Africa 22 % each (19).

A study conducted in the northeast region of Brazil (2008) showed that the prevalence of GBS colonization in the mothers was 20.4 %. In that study, no association was indicated between the

socio demographic variables or gynecological-obstetrical antecedents and a larger presence of GBS colonization (40).

Another study from North-Eastern Italy (2007) showed that among 5020 pregnant women, 901 (17.9 %) were positive for GBS. On 728 positive samples, the results of selective direct plating and selective broth enrichment were compared. A total of 561 (77.1 % of positive samples, corresponding to 13.9 % of patients) were positive on direct selective agar; an additional 167 isolates (22.9 % of samples, 4.1 % of patients) were recovered from the Lim broth (Todd-Hewitt broth supplemented with colistin (10 μg/ml) and nalidixic acid (15 μg/ml) subculture. Results confirm that the combination of selective enrichment broth and selective direct plating is a time-saving and sensitive method (22). Another study conducted among pregnant women in Turin, Italy (2008) showed a prevalence of 18 % GBS colonization (41). A study from Małopolska, Poland (2009) in 340 pregnant women showed that women with a complicated pregnancy were more often colonized than those with a normal pregnancy (20.0 % versus 17.2 %). Regarding neonatal colonization by GBS, they found that neonates born from the colonized mothers with a complicated pregnancy were more often colonized with GBS than those from the mothers with a normal pregnancy (35 % versus 26.7 %) (42). The study conducted in Switzerland (2009) showed that among 1316 pregnant women, the prevalence of GBS colonization was 21 % (43).

A study in Hong-Kong (2009) showed the prevalence of GBS colonization in antenatal population was found to be 10.4% (44). Another study in Taiwan (2012) showed that the positive rate for GBS culture was 6.2% (22/354). Among 107 paired samples, 6 maternal samples and 1 neonatal sample were positive for GBS culture, with an estimated vertical transmission rate of 16.7 % (1/6) (45). A study conducted in Bali-Indonesia (2013) showed that the prevalence of GBS colonization in pregnant women detected with culture method using Blood agar (BA) plates and Chromagar (CA) plates without Todd Hewitt broth (THB) was 9.4 %, whereas the prevalence with culture method using BA and CA enriched by THB was 31.3 %. Moreover, GBS showed resistance to penicillin (10 %), ampicillin (20 %), erythromycin (20 %), and cefazolin (20 %). All GBS isolates were sensitive to chloramphenicol and ceftriaxone. It is indicated that THB enrichment medium seems to be promising as a screening method for GBS colonization in pregnant women in Bali (27). Studies performed at two tertiary care hospitals in Karachi, Pakistan (2013) showed that the overall prevalence of colonization was 69 (17 %)

among 405 pregnant women. The colonization was found to be significantly associated inversely with the body mass index of the carrier of GBS (46). Another study conducted at Hedayat hospital, Tehran, Iran (2009) showed that among the 330 women, 68 (20.6 %) were positive for GBS. In that study, statistical analyses showed no significant relationship between demographics, reproductive histories and obstetric characteristics such as history of abortion, Premature Rupture of Membranes (PROM) and gestational age of subjects with the test results. Solely the antibiotic therapy was associated with GBS colonization (21).

A study from Maputo, Mozambique (2008) showed that among 113 sample taken from rectovaginal swab, 2 (1.8 %) was positive for GBS (47). Mother colonization rate was found to be significantly higher in the rural areas (60 %) as compared to the urban areas (46 %) in the study conducted in Zimbabwe in 2006. GBS colonization persistence was shown to be more in rural (48 %) than in urban women (12 %). Similarly, baby colonization was also shown to be more in the rural (23 %) than in urban area (5 %) (48). A study conducted in AlFayom University Hospital, Egypt showed that among 95 pregnant women, 17 (17.89 %) were GBS positive (1). A study from antenatal clinic of a tertiary hospital in Northeastern, Nigeria (2013) showed that of the 133 pregnant women, 13 (9.8 %) was GBS positive. In that study, statistical significance difference was observed between the age-group and GBS isolates ($p<0.05$). However, other socio-demographic factors such as occupation and education level and obstetric factors did not show any statistically significant association with GBS colonization ($P>0.05$) (9).

In Ethiopia, 200 postpartum women and 80 newborn infants were investigated for GBS carriage at Gondar College of Medical Sciences in 1987, using swabs from the vagina and rectum and from the throat and external ear, respectively. Colonization rate of 9 % was found in the mothers and 5 % in the neonates (31). Another study conducted in Ethiopia, Hawassa health center in 2010 showed a prevalence of GBS colonization to be around 21 %. In that study, no statistically significant association was observed for GBS colonization with any of socio-demographic characteristics of the study subjects including age, occupation, type of contraceptives used, types of gravida, and number of antenatal clinic visits. All GBS strains were susceptible to penicillin, ampicillin, vancomycin and gentamicin. Resistance was observed against erythromycin (6.9 %), tetracycline (48.2 %), ceftriaxone (10.3 %), chloramphenicol (51.7 %), ciprofloxacin (13.8 %) and norfloxacin (10.3 %) (4).

2.2. Risk Factors for Colonization by Group B *Streptococci*

Maternal intrapartum GBS colonization is the primary risk factor for early-onset disease in infants at the time of delivery (34). Other factors that increase the risk for Early-Onset Group B Streptococcal disease (EOGBSD) include delivery before gestational age <37 completed weeks (preterm birth), longer duration of membrane rupture, intrapartum temperature ≥38.0℃, intra-amniotic infection, young maternal age, black race, low birth weight, Chorioamnionitis, maternal diabetes mellitus, low maternal levels of GBS-specific anti-capsular antibody and previous delivery of an infant with invasive GBS disease (1, 3, 8, 10, 19, 25, 34) (49). Moreover, urinary tract infection sustained by GBS, either symptomatic or asymptomatic, is considered a risk factor for neonatal infections (22). A number of factors have been suggested to be related to the prevalence of colonization, including ethnicity, maternal age, marital status, education, smoking and multiple sexual partners. However, the relationships between these factors and actual colonization rates are unclear, and research results are inconsistent (49).

A study at Memorial Hermann Hospital or Lyndon Baines Johnson General Hospital, Texas, USA (2006) showed that of 1264 pregnant women, 154 (12.2%) were GBS positive. The proportion of women with GBS is varied by ethnic background. African-American women were more likely to be colonized with GBS than Caucasians and Hispanics. Resistance to routinely administered antibiotics was common, but there were no statistically significant increases in resistance to antibiotics over the study period (50).

In the study conducted on 499 college women, 90 (18 %) were positive for GBS. In that study, the prevalence of colonization with GBS was not related to sexual practices, history of venereal disease, use of oral contraceptives, presence of gynecologic symptoms, use of antibiotics, race, educational level, marital status, or history of pregnancy (51).

A study conducted in Brazil showed that among 598 pregnant women, the overall maternal GBS colonization prevalence rate was 17.9 %. In that study, there was no association of GBS colonization with maternity hospital and previously identified risk factors, such as age, race, marital status, maternal education, parity, smoking, or alcohol use (52). Another study conducted in Brazil (2007) indicated that among 316 Pregnant women, 46 (14.6 %) were positive for GBS. In that study, GBS was not significantly associated with maternal age, skin color (white and not

white), marital status, residence (urban and rural), gravidity, PROM and intrapartum fever (37). A study conducted in Peru indicated that the prevalence of GBS colonization was 6.0 % in parturient women and 10.6 % in non-pregnant women. In that study, no association of GBS colonization was made with previously identified risk factors such as age, parity, or birth control practices (39).

A study conducted in Oxford shire, UK (2006) showed that of 167 pregnant women, 21.3 % were colonized vagino-rectally with GBS. Risk factors for neonatal GBS disease (maternal fever, prolonged rupture of membranes, and preterm delivery) were present in 34 of 167 women (20.4 %). However, the presence of these factors correlated poorly with GBS carriage (53). A study conducted in the Netherlands (2006), found GBS carrier rate of 21 % in late pregnancy. That study indicated, African women were at a higher risk of GBS carriage (29 %) compared to Europeans and Asian women (13 %). No differences in colonization were found between women with respect to age, parity or socio-economic background in that study (54). The study conducted in Greece (2003) showed that the overall maternal and neonatal colonization rates were 6.6% and 2.4%, respectively. In that study, no association was found between colonization and maternal age, previous obstetric history, marital status, nationality, prematurity, caesarean section, or infant birth weight (55).

Another study conducted in Daejeon, Korea (2011) showed that the overall prevalence of GBS colonization among pregnant women at 35-37 weeks of gestation was 8.3% (219/2,644). In that study, GBS colonization was significantly associated with hospital, age group, education, frequency of pregnancy, gravidity, history of spontaneous abortion and PROM (more than 18 hours) (34). A study conducted at Hedayat hospital, Tehran, Iran showed that among the 330 women, 68 (20.6%) were positive for GBS. In that study, statistical analyses showed no significant relationship between demographics, reproductive histories and obstetric characteristics such as history of abortion, PROM and gestational age of subjects with the test results. Solely the antibiotic therapy was associated with GBS colonization (21).

A study conducted in Trinidad (2003) indicated that the prevalence of vaginal and rectal GBS colonization was 32.9%. In that study, colonization rates were significantly greater among multigravida women than primigravida women ($P < 0.001$) (56). Another study in India (2011) showed that among 300 pregnant women, 7(2.3%) were positive for GBS. In that study, we

observed that multigravida women and those with previous abortion were more often associated with GBS colonization, though it was not statistically significant (33). A study conducted at the Rajavithi Hospital, Thailand (2007) showed that among 320 pregnant women, colonization was present in 58 cases (18.12%). There was no statistically significant association between GBS colonization and socio demographic factors such as, occupations, education levels and obstetric factors such as parity, membrane rupture but there was significant association between older maternal age and lower gestational age (57). Another study in Pakistan (2013) showed that a significant difference was found among the study population of Sobhraj Hospital and Aga Khan University Hospital (AKUH) and with the body mass index of the patient. ($p < 0.05$) (46).

A study conducted in Queen Elizabeth Hospital, Blantyre, Malawi (2005) showed that among 97 pregnant women, 16(16.5%) were GBS positive. All of which were sensitive to penicillin G and erythromycin. In that study, GBS colonization appeared to decrease with age while, it increased with the number of previous bad pregnancy outcomes ($p=<0.05$) (58). Another study in Malawi (2011) showed that GBS carriage was 21.2% and did not differ by HIV status ($P>0.05$) (59).

A study in Ghana showed that 19% of the participants were colonized with GBS where no significance difference between GBS colonization and marital status, age, parity and gestational age of women were observed ($p>0.05$) (60). A study conducted in Tanzania showed that among 300 pregnant women and their newborns, GBS colonization was confirmed in 23% of pregnant women and 8.9% of neonates. A higher proportion of GBS were isolated from the vagina (12.3%) as compared to the rectum (5%). Prolonged duration of labor (>12 hrs) was significantly shown to influence GBS colonization in neonates $P < 0.05$. Other risk factors such as PROM, intrapartum fever, low birth weight, age group, marital status, educational level, history of still birth, history of spontaneous abortion, and HIV infection did not correlate with GBS colonization (61).

A study conducted in Bukavu, Democratic Republic of Congo showed that among 509 pregnant women, the overall GBS colonization rate was 20%. In that study, colonization was significantly associated with low education, history of urinary infection during the pregnancy, history of premature childbirth or abortion, and HIV-positive serology, but was not significantly associated with socio-economic level or parity (62).

In 2010, a study conducted in Zimbabwe showed a prevalence of 21% GBS colonization which indicated that women living in rural areas were significantly more often colonized than those who lived in urban areas ($p < 0.001$). Other socio-economic, demographic and obstetric factors were not statistically associated with GBS colonization (63). A study conducted on GBS carriage during late pregnancy in Ile-Ife Osun, Nigeria (2012) showed that among 150 pregnant women, 17(11.3%) were positive for GBS which increased with age. In that study, there was no significant association between GBS colonization status and age ($p >0.05$)), gestational age ($p >0.05$)), gravidity ($p >0.05$) and obstetric risk factors ($p >0.05$)) (64).

2.3. Antibiotic Susceptibility Pattern

The use of intravenous intrapartum antibiotic prophylaxis (IAP) to prevent early-onset GBS disease in infants was first studied in the 1980s (8). Antibiotic prophylaxis of GBS positive women during labor reduces the risk of early-onset neonatal sepsis (65, 66). IAP is effective in reducing neonatal GBS disease (14) , and indicated for all GBS carriers except for those in whom cesarean delivery is planned in the absence of labor or membrane rupture (4). Penicillin and ampicillin have each been demonstrated in controlled clinical trials to be effective in preventing early-onset GBS disease when administered during labor (7, 8). In cases of history of allergy to penicillin and at high risk for anaphylaxis, clindamycin and erythromycin are recommended. In GBS-colonized mothers with allergy and low risk of anaphylaxis to penicillin, the use of cefazolin is recommended. In those with a high risk of anaphylaxis to penicillin and if the isolate is resistant to clindamycin, vancomycin is recommended (8, 67).

Widespread use of these antibiotics in various clinical conditions as well as their accepted efficacy of IAP in decreasing early-onset neonatal GBS infections has raised the emergence of antibiotic resistance among GBS isolates (8, 38). GBS remain fully susceptible to penicillin as well as to most β-lactams, and penicillin remains the first-choice of antibiotic to prevent GBS-EOD and to treat GBS diseases. However, recently very rare isolates with decreased susceptibility to penicillin have been reported in Japan and USA. A point mutation in the GBS pbp2x gene conferring to this decreased susceptibility was identified (10). Antibiotic resistance amongst GBS is considered an increasing problem so that it was recommended to test the susceptibility of other antibiotics than those recommended as part to establish control measures and that could be used as alternative choices for prophylaxis or treatment of GBS infections (38).

A study in US (2008) indicated that there were 14 573 cases of invasive GBS disease during 1999-2005, including 1348 deaths. All GBS isolates tested were susceptible to penicillin, ampicillin, and vancomycin, but 32% and 15% were resistant to erythromycin and clindamycin, respectively. Serotypes Ia, Ib, II, III, and V accounted for 96% of neonatal cases and 88% of adult cases (5).

A study in Geneva, Switzerland in 2013 showed that the rate of resistance to clindamycin was 28% and to erythromycin was 30%. Only 3 of the 38 erythromycin resistant strains (7.9%) were susceptible to clindamycin, and only 3 out of the 35 clindamycin resistant GBS (8.6%) were identified as "inducible resistance". The rate of co-resistance to clindamycin and erythromycin-resistant strains was 92%. Penicillin remained efficacious in all cases (65). Another study at the University Hospital of Bern in Switzerland (2014) indicated that all isolates were susceptible to penicillin. Resistance rates were 14.5% for erythromycin and 8.2% for clindamycin. Of 364 isolates, 5.8% were susceptible to clindamycin but not to erythromycin, although demonstrating inducible clindamycin resistance. Hence, the final reported clindamycin resistance rate was 14% (67).

In one study that tested 200 GBS isolates collected from vaginal/rectal specimens, the resistance rate was 54% for erythromycin and 33% for clindamycin. Methylase genes erm (B) and erm (TR) and efflux genes mef (E) and mef (A) were detected. All but 3 of 200 isolates were susceptible to telithromycin (68).

A study in Juiz de Fora, Brazil (2010) showed that of 221, 21(9.5%) were positive for GBS. Antimicrobial susceptibility patterns were determined for isolated bacteria by agar diffusion method. Bacterial resistance was not detected against penicillin, ampicillin, cefazolin, vancomycin and ciprofloxacin, whereas 22.7% were resistant to erythromycin and 50% were resistant to clindamycin (12). Another study at University Hospital of Santa Maria (HUSM), Brazil (2011) showed that of 36 pregnant women, the prevalence of GBS were 11.11%. All strains were susceptible to penicillin, ampicillin, and vancomycin. Two strains (50%) were intermediate to clindamycin and one was (25%) intermediate to erythromycin (32).

One study conducted in Misiones, Argentina (2008) showed that carriage rate of GBS among pregnant women was 7.6%. A total of 62 GBS strains were randomly selected for *in vitro*

susceptibility testing. In that study, no resistance to penicillin, ampicillin, quinupristin-dalfopristin, linezolid, and vancomycin was found. Of the isolates examined 96.8%, 98.3%, 46.8%, and 29.0% were susceptible to rifampicin, nitrofurantoin, trimethoprim-sulfametoxazol and tetracycline, respectively (38).

A study in Korea (2013) showed that a total of 418 clinical isolates from pregnant women were screened for antibiotic resistance. The resistance rates were 39.5% for clindamycin and 23.0% for erythromycin (69). A study in Kuwait showed that all isolates were susceptible to penicillin, ampicillin and cefotaxime but were resistant to trimethoprim (92.3 %), tetracycline (89.5 %), minocycline (89.5 %), high-level kanamycin (76.9 %), chloramphenicol (30.0 %), erythromycin (12.6 %), clindamycin (7.0 %), high-level streptomycin (3.5 %) and ciprofloxacin (0.7 %). In that study, most of the isolates belonged to serotypes V (38.5 %), III (20.9 %), Ia (7.7 %) and II (11.2 %). Sixteen isolates (11.2 %) were non-typable (70).

A study conducted in Lebanon (2009) showed that all GBS isolates were susceptible to penicillin G, cefepime, ceftriaxone, and levofloxacin. Resistance to chloramphenicol (C), clindamycin (DA), erythromycin (E), and tetracycline (TE) was found to 4%, 11.8%, 15.8%, and 86.8% respectively (71). A study conducted at Thammasart Hospital, Thailand (2006) showed that among 406 pregnant women, 65 (16%) were positive for GBS colonization. All the isolates were sensitive to ampicillin, penicillin, vancomycin and cephazolin. Resistant was seen with clindamycin (3%) and erythromycin (1.5%) (72).

A study in Beijing, China (2013) showed that from a total of 2850 pregnant women at 35-37 weeks of gestation, 202 (7.1%) were GBS positive. Serotypes III, Ia and V predominated. All isolates were penicillin susceptible, whereas the resistance rates of erythromycin and clindamycin were strikingly high (73). Another study conducted in china (2013) indicated that a total of 146 GBS isolates from 8 cities across China belonged to four serotypes. A high prevalence of resistance was observed for levofloxacin (37.7%), erythromycin (71.2%), clindamycin (53.4%), and tetracycline (81.5%). The levofloxacin and clindamycin resistances among the 4 serotypes differed significantly. Eighty percent of fluoroquinolone-resistant isolates belonged to the sequence type 19 (ST19)/serotype III clone, with GyrAParC- ParE triple substitutions. This clone carried the erm (B), mef (E), and tet (M) genes (74).

A study conducted in Tanzania showed that resistance to clindamycin, erythromycin and penicillin G was found to 17.6%, 13% and 9.4%, respectively (61). A study on the isolation and characterization of GBS and other pathogens among pregnant women in Ibadan, Southwestern Nigeria (2010) showed that all the GBS isolates were sensitive to Penicillin G and Erythromycin; with 80% sensitive to Ampicillin, Vancomycin and Augmentin (15). Another study conducted in Ile-Ife Osun, Nigeria (2012) showed that all GBS isolates were 100% resistance to penicillin, ampicillin, cefoxitin and clindamycin. Resistance to cefotaxime (11.8%), erythromycin (64.7%) and vancomycin 70.6% were observed (64). A study conducted in Nsukka, Enugu State, Nigeria (2014) indicated that of 200 pregnant women and non-pregnant women, the carriage rate of GBS among pregnant women was 18.00%, while in non-pregnant women it was 5.5%. Statistical analyses proved the difference to be significant ($P<0.05$). In that study, no resistance to Ampicillin and Cloxacillin, amoxicillin, cefuroxime and ceftriaxone was found. Of the isolates examined, 6.70, 18.95, 32.75, 50 % were resistance to ciprofloxacin, streptomycin, sulphamethoxazole and trimethoprim and gentamycin, respectively. Erythromycin and perfloxacin showed the same resistance (8.62%) (75).

3. SIGNIFICANCE OF THE STUDY

Group B *Streptococcus* (GBS) is now recognized to be an important cause of maternal and neonatal morbidity and mortality in many parts of the world (32, 35, 61, 75); however, it has been little studied in Ethiopia (4, 31). Until now, there is no study conducted on prevalence of colonization and drug susceptibility pattern of GBS among pregnant women in the current study site. Lack of data in this area of study hinders the implementation of prophylaxis program in pregnant women. Therefore, this study was conducted to determine the prevalence of GBS colonization, antibiotic susceptibility pattern and to identify risk factors related to GBS among pregnant women attending ANC at Health Institutions, Addis Ababa, Ethiopia. The results of this study will provide updated information about the prevalence of colonization of GBS, susceptibility pattern and risk factors related to GBS among pregnant women in the current study site. Moreover, it will provide baseline information to formulate a policy and program for treatment, prevention and control efforts regarding perinatal GBS diseases in country at large.

Antibiotic resistance amongst GBS is considered as an increasing problem so that it was recommended to test the susceptibility of other antibiotics than those recommended as part to established control measures and that could be used as alternative choices for prophylaxis or treatment of GBS infection (8, 38). Therefore, a regional knowledge of the resistance profile of GBS is useful for administration of appropriate antibiotics for prophylaxis and treatment.

4. HYPOTHESIS

- The prevalence of GBS and susceptibility pattern among pregnant women in the present study could be similar with previous studies conducted in Ethiopia.

5. OBJECTIVES OF THE STUDY

5.1. General Objective

- To determine the prevalence of GBS colonization, risk factors and antimicrobial susceptibility pattern of GBS isolates among pregnant women at 35-37 weeks of gestation attending antenatal clinic of Health Institutions, Addis Ababa, Ethiopia.

5.2. Specific Objectives

- To determine the prevalence of GBS from vaginal specimens among pregnant women at 35 to 37 weeks of gestation.
- To identify risk factors associated with GBS colonization in pregnant women
- To determine the susceptibility pattern of GBS isolates to antimicrobial agents that are commonly used for intrapartum antibiotic prophylaxis and treating GBS infection in newborns.

6. MATERIALS AND METHODS

6.1. Study Setting

The study was conducted from May to August, 2014 in pregnant women attending antenatal clinic of ALERT Center, Alem Bank and Woreda 03 Health center, Addis Ababa, Ethiopia. Addis Ababa is the capital city of Ethiopia, with a population of 2,738,248 according to the 2007 population census conducted by the Central Statistical Agency of Ethiopia (CSA) with annual growth rate of 2.1 % (76). ALERT Center was established in 1934 by Sudan Interior Mission as a Leprosarium, and named after the daughter of His excellence Emperor Majesty Haile Silasie I as Prince Zenebework Memorial Hospital (PZWMH). It is one of the specialized tertiary referral hospitals in the country, located in Addis Ababa at 7 kms south west on the way to Jimma (77). Alem Bank Health center is found in west of Addis Ababa, Kolfe Keranio sub city. Woreda 03 Health center is found in the center of Addis Ababa, Nifas silk sub city.

6.2. Study Design

A descriptive cross sectional prospective study was conducted to determine the prevalence of GBS colonization and susceptibility pattern among pregnant women attending antenatal clinic of Health Institutions, Addis Ababa, Ethiopia from May to August, 2014.

6.3. Source Population

All pregnant women attending antenatal care clinics of ALERT Center and Selected Health Centers from May to August, 2014, Addis Ababa, Ethiopia.

6.4. Study Population

During the period of May to August, 2014, a total of 281 pregnant women attending the routine antenatal care follow up were screened for GBS colonization at ALERT Center (n=141), Alem Bank (n=88) and Woreda 03 (n=52) Health center. The screening approach was based on universal screening of all pregnant women for GBS colonization at 35 to 37 weeks of gestation. Study participants were informed about the study and the objective of the study were thoroughly explained to them and were asked for their volunteer participation. Then, written informed consent was obtained from volunteer study participants. Questionnaire was also filled by the

attending midwives and experienced nurses to acquire socio-demographic and other relevant obstetric history. (See annex III-VI).

6.5. Study Participants' Eligibility Criteria

6.5.1. Inclusion criteria

All consenting pregnant women with gestational age from 35-37 weeks were included.

6.5.2. Exclusion criteria

Pregnant women with history of antibiotic(s) use within two weeks prior to recruitment and premature rapture of membrane (PROM) were excluded from the study.

6.6. Sample size

Sample size was calculated based on the prevalence indicated in the previous study which was 21% in 2012 Hawassa, South Ethiopia (4). Expected margin of error (d) was 0.05 and confidence interval (z) was 95%. Contingency for the unknown circumstance was 10%.

$n=\frac{(Z\alpha/2)^2 * p(1-p)}{d^2}$, $\frac{(1.96)^2 * 0.21(1-0.21)}{(0.05)^2}$ =255+10%=281

Where n=sample size estimated

d= Expected margin of error =0.05

Zα/2= 95%confidence interval (C.I) =1.96

p=prevalence of previous study found from literature review=21%

Therefore; the sample size was 255 + contingency for the unknown circumstance (10%) =281 to determine the prevalence within ±0.05.

6.7. Sampling Procedure

ALERT Center, Alem Bank and Woreda 03 Health Centers were selected by convenient method as study sites. A total of 281 samples were collected during the study period by consecutive sampling technique. The study units were selected using inclusion and exclusion criteria.

6.8. Variables of the study

6.8.1. Dependent variables

- Prevalence of GBS
- Antimicrobial susceptibility testing (AST)

6.8.2. Independent variables

- Socio-demographic characteristics (age, health institution, occupation, marital status)
- History of contraceptive use
- Number of ANC visits
- Type of Gravida
- Previous history of abortion
- Previous history of still birth
- HIV status

6.9. Data collection procedures

6.9.1. Data Collection

The questionnaire was initially developed in English and then translated to Amharic by legal translator and then translated to English again to check the consistency of translation by other legal translators. The study participants have been informed about the study by the attending midwives and nurses. After obtaining written informed consent, data was collected using a structured questionnaire designed to obtain socio-demographic data and other relevant information such as maternal age, marital status, previous obstetric history and gravidity. Moreover, recent HIV result was taken from study participants' medical records after obtaining written informed consent. The questionnaire was administered by the attending midwives and nurses. Pregnant women with gestational age from 35-37 weeks of gestation were interviewed.

6.9.2. Timing of Screening

Group B *streptococcal* colonization status can change over the course of a pregnancy, so the timing of specimen collection for determination of colonization status is important. GBS colonization can be transient, hence if colonization detected early in pregnancy it is not predictive of early-onset GBS disease. Late third trimester colonization status has been used as a proxy for intrapartum colonization (8).

6.9.3. Specimen Collection

According to the Center for Disease Control and Prevention (CDC 2010) and American College of Obstetricians and Gynecologists (ACOG Committee opinion) 2011 guidelines (8, 78), vaginal swab was taken from the lower one third of vagina using sterile cotton swab (figure 2.1) and inoculated directly into Todd-Hewitt broth (THB) (OXOID, UK) and immediately transported to the Microbiology Laboratory of ALERT Centre for further analysis.

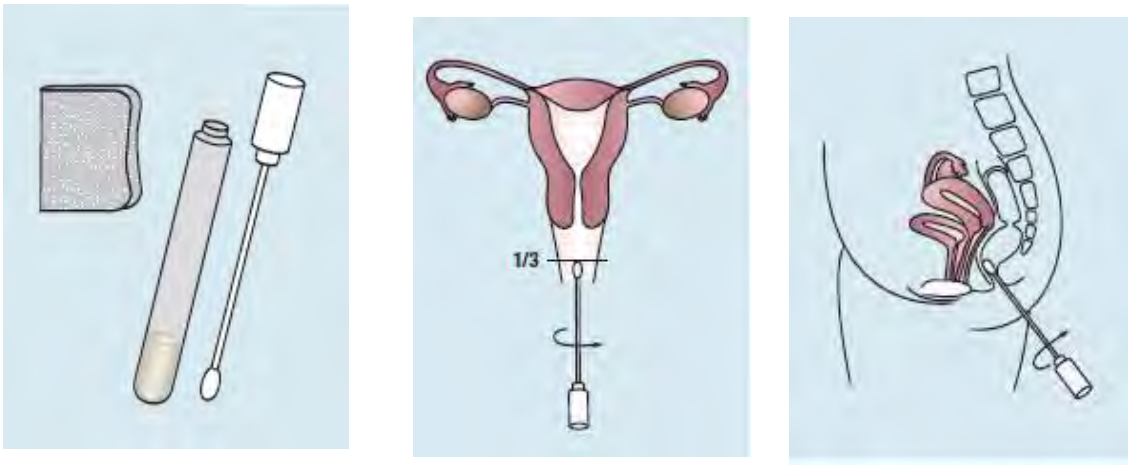

Figure 2.1. Site for collection of rectovaginal specimen for GBS isolation (Adapted from BD diagnostics www.geneohm.com).

6.8.4. Data quality management

Training on data collection procedures was given. Pre-testing of the questionnaire was done to assure the quality of data and for improvement of data collection tool. Supervision during data collection was done to understand how the data collectors handle the questionnaire and each filled questionnaire was checked for its completeness, accuracy, clarity, consistency on daily

basis. Corrective measures was taken accordingly for any gap, then special care was given during data entry, and data cleaning and the whole data was cross checked for reliability before analysis.

6.10. Culture and Identification of Group B *Streptococci*

The vaginal swabs were placed into 1 ml THB (Oxoid, UK) supplemented with gentamicin (8 µg/ml) (Intas pharmaceutical Ltd., India) and nalidixic acid (15 µg/ml) (Sigma Aldrich, Italy) to prevent growth of contaminants (8). The broth was incubated for 18–24 hours at 35-37°C and inoculated on 5% sheep blood agar (Oxoid, UK) and incubated overnight in 5% CO_2 atmosphere for 18-24 hours. All suspected GBS colonies (pin pointed, with narrow beta-hemolysis) were sub-cultured on 5 % sheep blood agar (SBA) and subjected for Gram stain and catalase test. All Gram positive and catalase negative cocci isolates were tested for Christie, Atkins, and Munch-Peterson (CAMP) test. Swab of the sample onto SBA plates produced small and white colonies (pin pointed, beta–hemolysis) surrounded by a clear zone after 18-24 hours of incubation as can be seen in Figure 2.2.

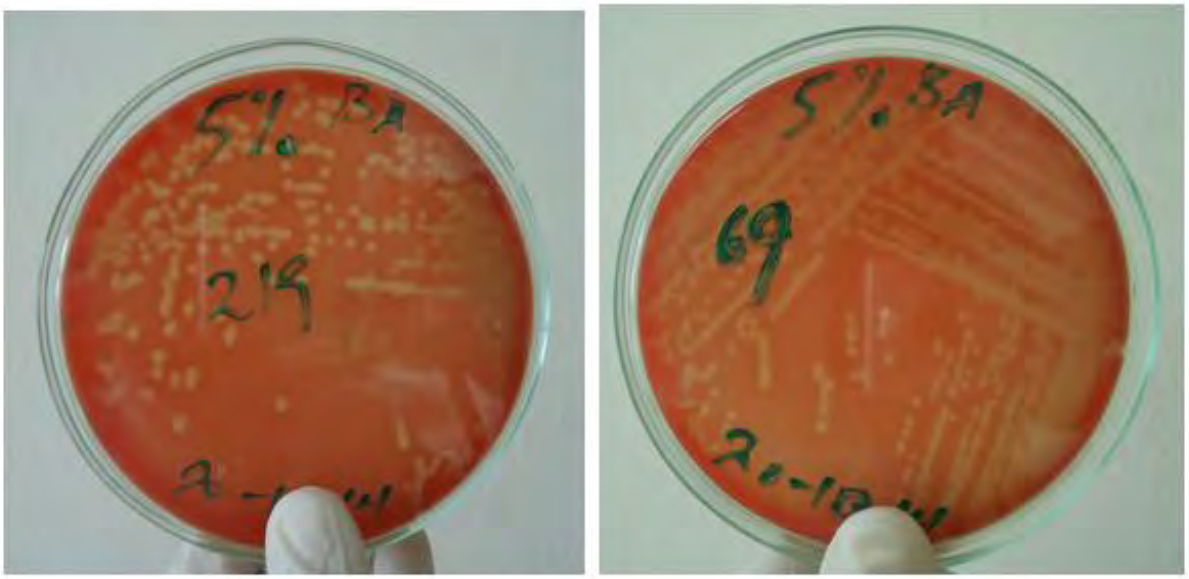

Figure 2.2. Appearance of GBS Colonies on BA after 18-24 hours of incubation

6.11. Christie, Atkins, and Munch-Peterson (CAMP) Test

The CAMP test has been named after Christie, Atkins, and Munch-Peterson, who described it in 1944. CAMP test is used for the presumptive identification of GBS *(Streptococcus agalactiae*) from other CAMP negative *Streptococcus* species (*Streptococcus pyogenes, Enterococcus faecalis)*. It is the only *Streptococcus* which yields a positive CAMP test. It detects a diffusible, heat-stable, extracellular protein produced by GBS that enhances the hemolysis of sheep erythrocytes by *Staphylococcus aureus.* The CAMP factor acts synergistically with the β-hemolysin produced by *S. aureus.* The synergistic reaction results in an enhanced and very visible zone of hemolysis in the region between the two cultures (79-82). A known hemolytic strain of *S. aureus* (ATCC 25923) was streaked in a straight line across the center of the sheep blood agar plate. Test inoculum was streaked in a straight line (2-3 cms in length) perpendicular to *S.aureus* streak but without touching it. A known GBS (ATCC 27956) as a positive control and *Enterococcus faecalis* (ATCC 29212) as a negative control was also streaked similarly. The plate was incubated at 35-37 °C for 18-24 hours. A positive test for CAMP factor appears as "arrowhead" hemolysis between the junction of growth of *S.aureus and* GBS (Figure 2.3 below). No enhanced or "arrowhead" hemolysis was seen when the test isolate was not GBS.

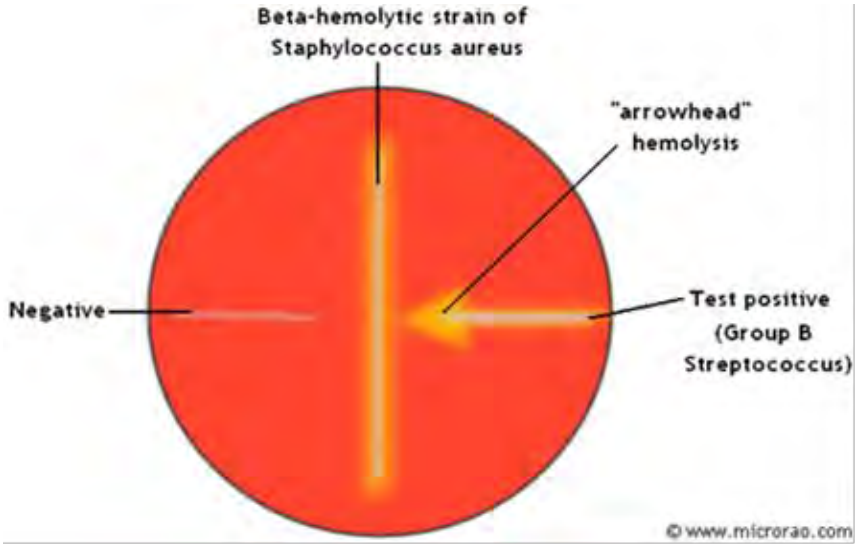

Figure 2.3. CAMP test for the identification of GBS *(Streptococcus agalactiae)* (Adapted from www.microrao.com).

6.12. Antimicrobial susceptibility testing

Antimicrobial Susceptibility Testing was performed according to Clinical and Laboratory Standard Institute guidelines (CLSI) 2013 for disk diffusion (83). Direct colony suspension in sterile saline, equivalent to 0.5 McFarland standard was done and inoculated on Muller-Hinton agar (MHA) (Oxoid, UK) with 5% sheep's blood using a sterile cotton swab. An antibiotic disk (Toxoid, UK) was placed on the agar with clindamycin and erythromycin disks placed 16 mm from each other in order to detect inducible resistance to clindamycin (D-zone test) and incubated at 35-37 °C with 5% CO_2 atmosphere for 18-24 hours. The zones of growth inhibition were measured using oxoid ruler (Oxoid, UK) and Linex ruler (Linex 116, Denmark). The sizes of the inhibition zones were graded according to the CLSI guidelines 2013. The antibiotic used were; penicillin (P) (10µg), ampicillin (AMP) (10µg), erythromycin (E) (15µg), clindamycin (DA) (2µg), vancomycin (30 µg), cefotaxime (CTX) (30µg), chloramphenicol(C) (30µg) and tetracycline (TE) (30µg). The results were interpreted according to CLSI guidelines 2013 as susceptible, intermediate or resistant (83).

6.13. Quality control

As quality control, sterility of SBA and MHA with 5 % sheep blood were checked by incubating overnight at 35-37 ºC without specimen inoculation. The performance of THB was checked by inoculating the broth with known Gram negative bacteria (*Escherichia coli)* (ATCC 25922) and known *Streptococcus agalactiae* (ATCC 27956) to see if it can really inhibit Gram negative bacteria and allow growth of Gram positive bacteria. The performance of catalase reagent (3% hydrogen peroxide) was checked by known *Staphylococcus aureus* (positive control) and *Streptococcus pyogenes* (negative control). For Gram stain reagents *S.aureus* (Gram positive) and *E.coli* (Gram negative) was used as quality control. Before use of any reagents and culture media any physical change like cracks, excess moisture, color, hemolysis, dehydration, and contamination was assessed and expiration date was also checked. Temperature of incubator and refrigerator was monitored daily. *Enterococcus faecalis* (ATCC 29212), *S.aureus* (ATCC 25923), *S.pyogenes* (ATCC 19615) and *S. agalactiae* (ATCC 27956) (obtained from Armaur Hansen Research Institute (AHRI) were used as a quality control throughout the study for culture and antimicrobial susceptibly testing with known susceptibility to the antimicrobial agents. The

purity of the colonies were checked by sub cultured a single colonies into a SBA and by doing Gram stain. All resistance and intermediate isolates were repeated.

6.14. Operational definitions

- **Gravida** is a pregnant woman.
- **Primigravida** or **Gravida 1** is a woman who is pregnant for the first time or has been pregnant one time.
- **Multigravida:** means a woman who is currently pregnant or who has been pregnant two or more times (gravida 2, gravida 3, etc.).
- **Parity** refers to the number of times a woman has given birth.
- **Preterm birth**: is the birth of an infant prior to 37 weeks of pregnancy.
- **Stillbirth**: is a baby dead after 24 completed weeks of pregnancy. If a woman loses a pregnancy after she's past her 20^{th} week, it's called a stillbirth
- **Abortion:** is termination of pregnancy by the removal or forcing out from the womb of a fetus or embryo before it has obtained the ability to survive on its own.
- **Multi drug resistance:** resistance to at least two chemically different drugs (84, 85).

6.15. Data Management

Data were entered into a computer and kept on a secured, password protected computer and back up data was kept at AHRI data management unit. Hard copies of the data collection worksheets were kept securely locked.

6.17. Ethical Consideration

The proposal was approved and ethically cleared by Department Ethics and Research Committee (DERC) of Addis Ababa University (AAU), College of Health Science (COHS), School of Allied Health Sciences (SOAHS), Department of Medical Laboratory Science (DMLS), AHRI/ ALERT Ethical Review Committee (AAERC), Addis Ababa Health Bureau Institutional Review Board, and Ministry of Science and Technology National Research Ethics Review Committee (NRERC). Official permissions from the study sites were obtained. Each study participant was notified about the purpose of the study, their right to refuse to participate in the study, and anonymity and confidentiality of the information gathered. Written informed consent was

obtained from study participants (see Annex III &IV). The laboratory examination result was given to the study participants in case they give birth in another health institution so that they can inform their attending physician. The gynecologists in charge at ALERT center and selected Health Centers were notified for the better management of colonized pregnant women.

6.16. Data Analysis

Data was coded, entered, cleaned and analyzed by using SPSS version 20.0software. Frequency count and percentage was used to clean and check the accuracy of data entry and to analyze the data. Similarly, frequency distribution, percentage, tables and charts were used to present results. Explanatory variables were individually cross tabulated with the outcome variable and statistical significance was assessed using chi-square and binary logistic regression model. Where the numbers in a cell was less than five, a Fisher's exact test. Odd ratio (OR) and 95% confidences interval (CI) were calculated to determine the strength of the association. P-value less than 0.05 was considered statistically significant. Explanatory variables significantly associated with the outcome variable in univariate analysis were included in a multivariate logistic regressions analysis to detect confounding effects.

6.18. Dissemination of Results

Study result was submitted to Addis Ababa University, College of Health Science, School of Allied Health Sciences, Department of Medical Laboratory Sciences (DMLS), AHRI/ALERT, Addis Ababa Health Bureau, and Ministry of Science and Technology. A copy of this research was available at Addis Ababa University, College of Health Science, School of Allied Health Sciences, Department of Medical Laboratory Sciences Library, AHRI/ALERT, Study sites, Addis Ababa Health Bureau, and Ministry of Science and Technology. The findings of this study will be published in peer reviewed journals.

7. RESULTS

7.1. Socio Demographic Characteristics

A total of 281 pregnant women (from 35-37 weeks of gestation) were enrolled from May to August, 2014. The study participants were from three health institutions of Addis Ababa, Ethiopia. One hundred forty one (50.2%) of the study participants were from ALERT Center, while 88 (31.3%) and 52 (18.5%) were from Alem Bank health center and Woreda 03 health center respectively. The study participants were from 5 sub cities of Addis Ababa. Two hundred seventeen (77.2%) of the study participants were from Kolfe Keranio sub city, 61(21.7 %) from Nifas silk sub city, and 1(0.4 %) from each of Arada, Gulele and Addis Ketema sub cities.

The age of the study participants ranged from 18 to 39 years with a mean of 26.46 (± 4.41) years. Most of the participants were between the ages of 25-29 years 120 (42.7%), while 85 (30.2%),49 (17.4%), 19 (6.8%), 8 (2.8%) were between the age of 20-24 years, 30-34 years, ≥35 years, and 15-19 years respectively. Most of the study participants were married (97.5%), but the rest were single (1.4%), divorced (0.7% and widowed (0.4%).

Most of the study participants' ethnic group was Amhara (35.9%) and Gurage (30.2%), while the others were Oromo (13.5%), Seltie (9.3%), Wolene (2.8%), Tigray (2.8%), Wolayta (2.5%), Hadya (1.8%) and Gamo (1.1%). The majority of the study participants were house wives (68.3%), while the rest were business women (19.2%), and civil servants (12.5%). **(See Table 7.1 below).**

Table 7.1. Socio-demographic characteristics of pregnant women (35-37 weeks of gestation) who were investigated for GBS, ALERT Center, Alem Bank and Woreda 03 Health Center, Addis Ababa, Ethiopia from May to August, 2014 (n=281).

Socio-demographic characteristics	Frequency	Percentage
Health Institutions		
ALERT Center	141	50.2%
Woreda 03 Health Center	52	18.5 %
Alem Bank Health Center	88	31.3%
Age groups		
15-19	8	2.8%
20-24	85	30.2%
25-29	120	42.7%
30-34	49	17.4%
≥ 35	19	6.8%
Address		
Kolfe Keranio sub city	217	77.2%
Nefas silk sub city	61	21.7%
Arada sub city	1	0.4%
Addis Ketema sub city	1	0.4%
Gulele sub city	1	0.4%
Marital status		
Married	274	97.5%
Single	4	1.4%
Divorced	2	0.7%
Widowed	1	0.4%
Ethnicity		
Amhara	101	35.9%
Gurage	85	30.2%
Oromo	38	13.5%
Seltie	26	9.3%
Wolene	8	2.8%
Tigray	8	2.8%
Wolayta	7	2.5%
Hadya	5	1.8%
Gamo	3	1.1%
Occupation		
Civil Servant	35	12.5%
House Wives	192	68.3%
Business Women	54	19.2%

7.2. Prevalence of Group B *Streptococci* and Antimicrobial susceptibility Testing

7.2.1. Prevalence of Group B *Streptococci*

A total of two hundred eighty one (281) pregnant women (from 35-37 weeks of gestation) from three health institutions of Addis Ababa, Ethiopia were enrolled in this study from May to August, 2014. One hundred forty one (141) of the study participants were from ALERT Center, of these, 17(12.1%) were GBS positive, 20(22.7%) among 88 pregnant women and 4(7.7%) among 52 pregnant women were positive for GBS in Alem Bank and Woreda 03 health center respectively. The overall prevalence of GBS colonization among pregnant women at 35-37 weeks of gestation was 14.6% (41/281).

7.2.2. Antimicrobial Susceptibility Testing

The antimicrobial susceptibility pattern of GBS isolated from pregnant women is summarized in Table 7.2. All GBS isolated were 100% susceptible to chloramphenicol. Most isolates (80.5% to 92.5%) were susceptible to penicillin G, vancomycin, ampicillin and erythromycin. Most GBS isolated (90.2%) were resistance to tetracycline. Resistance to cefotaxime, clindamycin, penicillin G, vancomycin, ampicillin and erythromycin was found to be 34.1 %, 26.8 %, 19.5 %, 17%, 14.6%, and 7.5 % respectively. Intermediate to tetracycline was found to be 2.4%.

Table 7.2. Antimicrobial susceptibility pattern of GBS isolated from pregnant women, ALERT Center, Alem Bank and Woreda 03 Health Center, Addis Ababa, Ethiopia from May to August, 2014 (n=41).

Antibiotics	Disk Potency (µg)	Susceptible	Intermediate	Resistant
Chloramphenicol (C)	30	41(100%)	0	0
Erythromycin (E)	15	38(92.5 %)	0	3(7.5 %)
Ampicillin (AMP)	10	35(85.4%)	0	6(14.6%)
Vancomycin (VA)	30	34(83%)	0	7(17%)
Penicillin G (P)	10	33(80.5%)	0	8(19.5%)
Clindamycin (DA)	2	30(73.2 %)	0	11(26.8%)
Cefotaxime (CTX)	30	27(65.9%)	0	14(34.1%)
Tetracycline (TE)	30	3(7.3%)	1(2.4%)	37(90.2%)

7.2.3. Multi Drug Resistance pattern

The multi drug resistance pattern of GBS isolated was summarized in the table 7.3.below. Multi drug resistance (MDR) was detected in 43.9 % (18/41) of the isolates. Resistance to 2, 3, 4 and 5 drugs was found to be 43.6 %, 23.9%, 23.9 % and 8.4 % respectively.

Table 7.3. Multi drug resistance pattern of GBS isolated from pregnant women, ALERT Center, Alem Bank and Woreda 03 Health Center, Addis Ababa, Ethiopia from May to August, 2014 (n=41)

Drugs resistance pattern (Antibiogram)	No. of drug resistance	No. of resistant strains (%)
TE: CTX	2	12 (16.9)
TE: VA	2	6 (8.45)
TE: DA	2	10 (14.08)
TE: E	2	3 (4.23)
CTX: TE: DA	3	9 (12.68)
CTX: DA: P	3	8 (11.27)
CTX: TE: AMP: E	4	2 (2.82)
CTX: TE:AMP: DA	4	5 (7.04)
CTX:TE:DA: P	4	7 (9.86)
CTX:TE: DA:VA	4	3 (4.23)
CTX:TE:DA:AMP: E	5	1 (1.41)
CTX:TE:DA:E: P	5	1 (1.41)
CTX:TE:DA:P: VA	5	2 (2.82)
CTX:TE:DA: VA: AMP	5	2 (2.82)

7.3. Risk factors for Group B Streptococci

7.3.1. Socio-demographic factors

The association of socio-demographic variables with GBS colonization is summarized in Table 7.4. In univariate analysis, GBS colonization showed statistically significant association with health institutions (P<0.05). However, there were no significant association between GBS colonization and age group, marital status and occupation **(Table 7.4).** The GBS colonization rates in pregnant women of different age groups were: 15-19 years, 25%; 20-24 years, 15.3%; 25-29 years, 15%; 30-34, 12.2% and ≥35 years, 10.5%. This study revealed a higher GBS colonization rate among pregnant women of age group 15-19 years (25%) but lower among age group ≥ 35years (10.5%). However, the difference was not statistically significant (P > 0.05).

A multivariate analysis was carried out to detect confounding effects. In multivariable logistic regression analysis, health institution was associated with GBS colonization. GBS colonization differ significantly with health institution, being 22.7% from Alem Bank health Center, 12.1% from ALERT Center and 7.7 % from Woreda 03 health center (P<0.05). Those pregnant women who were managed in Alem Bank Health Center were 2.3 times more likely to be colonized with GBS compared to those pregnant women who were managed in ALERT Center (AOR: 2.26, 95% C.I: 1.02-5.02) and those mothers who were managed in Woreda 03 Health center were 66% times more likely to be colonized by GBS than those who were managed in ALERT Center (AOR: 0.66, 95% C.I: 0.21-2.12). **(See table 7.4 below).**

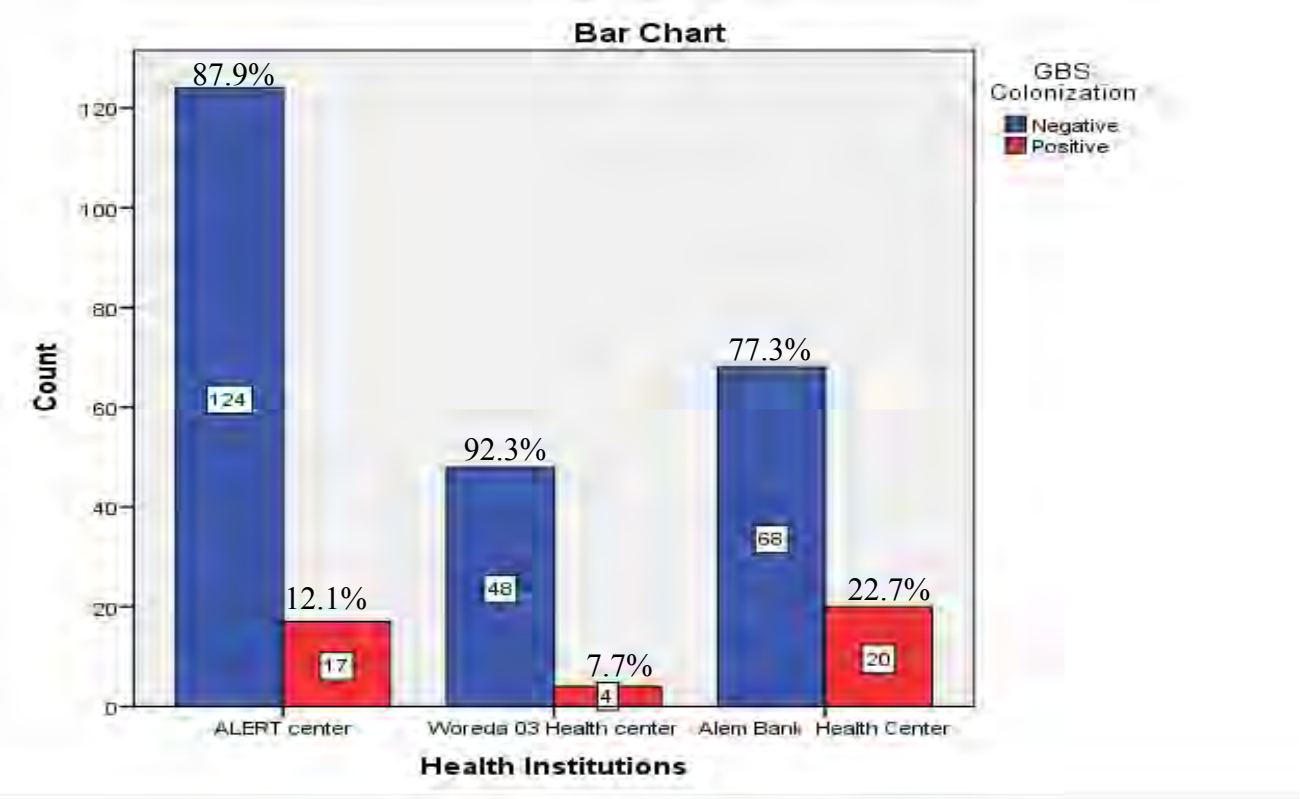

Figure 2.4. Distribution of GBS colonization among pregnant women attending ANC of Health institutions, Addis Ababa, Ethiopia from May to August, 2014 (n=281).

In the present study, GBS colonization did not differ significantly with occupation, being 16.7% among business women, 15.1% among house wives and 8.6 % among civil servants (P > 0.05) **(See Table.7.4 below).**

Table 7.4. Association between socio-demographic factors and GBS colonization among pregnant women in ALERT Center, Alem Bank and Woreda 03 Health Center, Addis Ababa, Ethiopia from May to August, 2014 (n=281).

Socio-demographic factors	Total	GBS Culture		COR (95%C.I)	p-value[a]	AOR (95%C.I)	p-value[b]
		GBS negative (n=240) f (%)	GBS positive (n=41) f (%)				
Age group							
15-19	8	6(75)	2(25)	2.83(0.32-24.8)	0.35		
20-24	85	72(84.7)	13(15.3)	1.54(0.32-7.45)	0.59		
25-29	120	102(85)	18(15)	1.50(0.32-7.06)	0.61		
30-34	49	43(87.8)	6(12.2)	1.19(0.22-6.47)	0.84		
≥35	19	17(89.5)	2(10.5)	1			
Health Institutions							
ALERT Center	141	124(87.9)	17(12.1)	1		1	
Woreda 03 health Center	52	48(92.3)	4(7.7)	0.61(0.20-1.90)	0.392	0.66 (0.21-2.12)	0.485
Alem Bank health Center	88	68(77.3)	20(22.7)	2.15(1.05-4.37)	0.035*	2.26 (1.02-5.02)	0.045*
Marital Status							
Married	274	234(85.4)	40(14.6)	1			
Single	4	3(75)	1(25)	1.95(0.20-19.2)	0.57		
Divorced	2	2(100)	0(0.0)	0.000	0.99		
Widowed	1	1(100)	0(0.0)	0.000	1.00		
Occupation							
Civil servant	35	32(91.4)	3(8.6)	1			
House wife	192	163(84.9)	29(15.1)	1.9(0.55-6.61)	0.3144		
Business women	54	45(83.3)	9(16.7)	2.13(0.54-8.51)	0.283		

*Significant at p-value<0.05 [1] is Logical reference CI: Confidence Interval
COR: Crude odds ratio AOR: Adjusted odds ratio
P-value [a] obtained by binary, p-value [b] obtained by multiple logistic regression

7.3.2. Obstetric factors

The association between maternal obstetric factors and GBS colonization is summarized in Table 7.5 below. In univariate analysis, GBS colonization showed inversely related with history of contraceptive use (P<0.05). However, there were no significant association between GBS colonization and the number of antenatal visit, gravidity, history of spontaneous abortion and stillbirth/neonatal loss. A multivariate analysis was carried out to detect confounding effects. In multivariable logistic regression analysis, history of contraceptive use was associated with GBS colonization. Those pregnant women who had a history of using contraceptive were 54% less likely to be colonized with GBS compared to those pregnant women who had no a history of using contraceptive (AOR: 0.46, 95% C.I: 0.23-0.94). (**Table 7.5).**

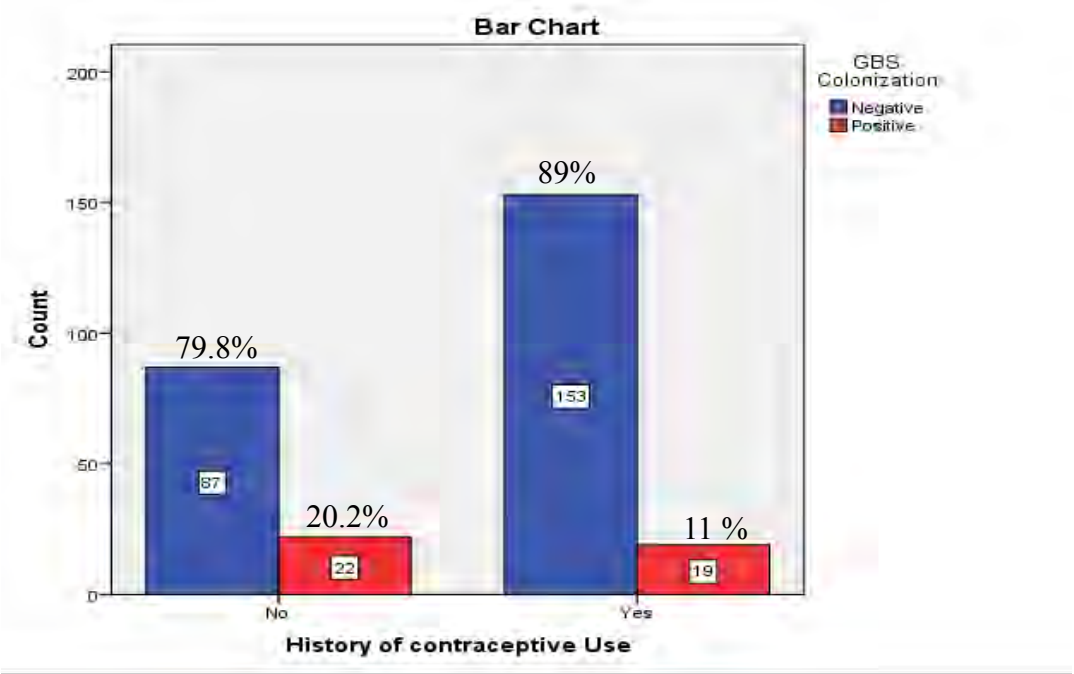

Figure 2.5. Frequency of GBS colonization along with contraceptive use among pregnant women attending ANC of Health Institutions, Addis Ababa, Ethiopia from May to August, 2014 (n=281).

Based on gravidity, the GBS colonization rates were higher in primigravida (15.9 %) than multigravida (14%). However, these difference was not statistically significant (P > 0.05). In this study, pregnant women who had no a previous history of still birth or neonatal loss (14.9 %) had higher rate of GBS colonization than those who had a previous history of still birth or neonatal loss (7.7%). However, this difference was not statistically significant (P > 0.05). Pregnant women with no previous history of spontaneous abortion (14.7 %) had higher GBS colonization rate than those with previous history spontaneous abortion (14.3 %). However, this difference was not statistically significant (P > 0.05). **(See Table 7.5. below).**

Table 7.5. Association between obstetric factors and GBS colonization among pregnant women in ALERT Center, Alem Bank and Woreda 03 Health Center, Addis Ababa, Ethiopia from May to August, 2014 (n=281).

Obstetric Factors	Total	GBS culture		COR (95%C.I)	P-value [a]	AOR (95% C.I)	P-value [b]
		GBS negative (n=240) f (%)	GBS positive (n=41) f (%)				
History of contraceptive use							
No	109	87(79.8)	22(20.2)	1		1	
Yes	172	153(89)	19(11)	0.49 (0.25-0.96)	0.037*	0.46(0.23-0.94)	0.032*
Number of ANC visit							
One times	6	5(83.3)	1(16.7)	1.12(0.13-10.1)			
Two times	37	32(86.5)	5(13.5)	0.88(0.31-2.51)			
Three times	99	85(85.9)	14(14.1)	0.93(0.45-1.92)			
Four times	139	118(85.4)	21(14.6)	1			
Type of Gravida							
Primigravida	88	74(84.1)	14(15.9)	1.16(0.58-2.35)			
Multigravida	193	166(86)	27(14)	1			
History of still birth / Neonatal Loss							
No	268	228(85.1)	40(14.9)	1			
Yes	13	12(92.3)	1(7.7)	0.48(0.06-3.76)			
History of Abortion							
No	211	180(85.3)	31(14.7)	1			
Yes	70	60(85.7)	10(14.3)	0.97(0.45-2.09)			

*significant at P-value <0.05 [1] logical reference CI: Confidence interval
COR: Crude odds ratio AOR: Adjusted odds ratio
P-value [a] obtained by binary p-value [b] obtained by multiple logistic regression

7.3.3. HIV infection

Of the 281 pregnant women screened for GBS colonization, 25(8.9%) were HIV positive and 256(91.1%) were HIV negative. HIV prevalence was higher in ALERT Center (15.6% (22/141)), but lower in Woreda 03 (3.8% (2/52)) and Alem Bank Health centers (1.1% (1/88)). This might probably be due to ALERT Center is one of antiretroviral treatment (ART) initiative Center. Among the pregnant women with HIV infection, 6(24%) were positive for GBS and among pregnant women with HIV negative, 35(13.7%) were GBS positive. However, this difference was not statistically significant (P>0.05).Those mothers who had HIV infection were two times more likely to be colonized by GBS than those mothers who had not HIV infection (COR: 2, 95 % C.I: 0.75-5.34).

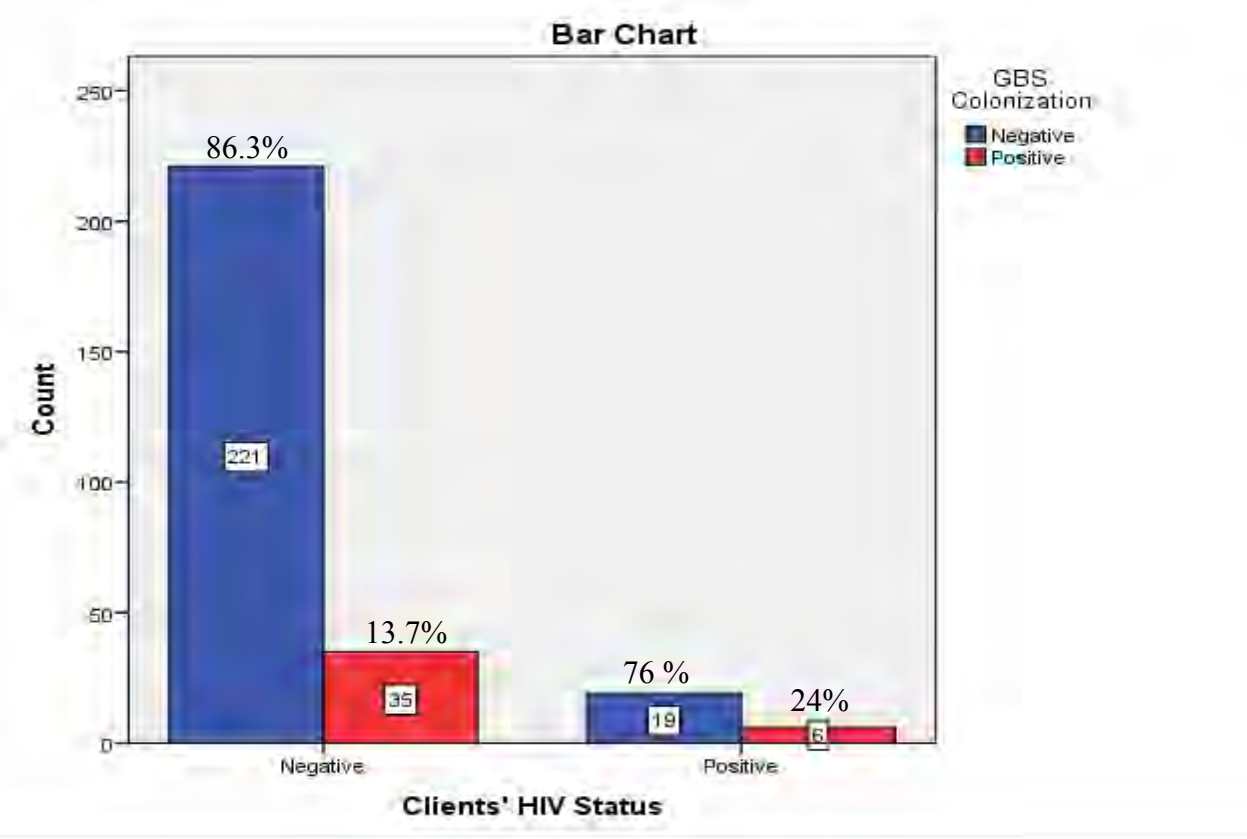

Figure 2.6. HIV status of study participants and GBS colonization among pregnant women attending ANC of Health Institutions, Addis Ababa, Ethiopia from May to August, 2014 (n=281).

8. DISCUSSION

Group B *Streptococcus* (GBS) is now recognized to be an important cause of maternal and neonatal morbidity and mortality in many parts of the world (32, 35, 61, 75); however, there were only two studies in Ethiopia (4, 31). The first one was in 1989 in Gondar and the second one was after 23 years in 2012 in Hawassa. Therefore, this study was conducted to determine the prevalence of GBS colonization, antibiotic susceptibility pattern of the isolates and to identify risk factors related to GBS among pregnant women in Addis Ababa, Ethiopia.

In this study, the overall prevalence of GBS among pregnant women was 14.6%%. This finding is almost similar with reports from other developing countries; in Egypt (2009) (17.89%), Malawi (2005) (16.5%), Nigeria (2012) (11.3%) (1, 58, 64), but higher than those reported in Ethiopia (1989) (9%) and Mozambique (2008) (1.8%) (31, 47) and slightly lower than those reported in Ethiopia (2012) (20.8%), Tanzania (2009) (23%) and Zimbabwe (2010) (21%) (4, 61, 63). The variations between countries could possibly be due to differences in the sample size and type of sites cultured, culture methods, type of culture media used, socio-economic status, sexual behavior and geographic areas (4, 15, 22, 38, 39).

The finding of this study is also comparable to a study done in some European countries; in Italy (2007) (17.9%), Italy (2008) (18%), Poland (2009) (17.2%) (22, 41, 42), but higher than those reported in Northern Greece (2003) (6.6 %) (55) and slightly lower than those reported in Switzerland (2009) (21%), UK (2006) (21.3%) and Netherlands (2006) (21%) (43, 53, 54).

The result of our study is also almost similar with studies conducted in Texas, USA (12.2%) (50) and in Brazil ranging from 11.11 % to 20.4% (32, 37, 40, 52), but higher than those reported in Argentina (2008) (7.6%) and Peru (1998) (6%) (38, 39). These variations in isolation frequency could be due differences in culture methods, and type of culture media used as well as geographical differences.

The finding of this study (14.6%) is similar with reports from some Asian countries; such as Pakistan (2013) (17%) by Najmi N *et al*, Thailand (2006) (16%) by Torudom *et al*, Thailand (2007) (18.12%) by Kovavisarach *et al*, Iran (2008) (20.6%) by Fatemi *et al* (21, 46, 57, 72), but higher than those reported in Indonesia (2013) (9.4 %) performed with culture method using culture media BA and CA without THB, India (2011) (2.3%), Taiwan (2012)(6.2%), China

(2013)(7.1%), Korea (2011)(8.3%) and Hong Kong (2009) (10.4%) (27, 33, 34, 44, 45, 73) and lower than in Indonesia (2013) (31.3%) performed with culture method using culture media BA and CA enriched by THB (27). The difference could be due to geographical variation, sample site, culture methods and type of culture media used.

In this study, GBS colonization was significantly associated with Health institutions (P<0.05). This finding is consistent with reports from other authors (34, 46). Those pregnant women who were managed in Alem Bank Health Center were 2.3 times more likely to be colonized with GBS compared to those pregnant women who were managed in ALERT Center (AOR: 2.26, 95%C.I: 1.02-5.02) and those mothers who were managed in Woreda 03 Health center were 66% times more likely to be colonized by GBS than those who were managed in ALERT Center (AOR: 0.66, 95% C.I: 0.21-2.12). This difference might be due to professional difference /quality of care of the health professionals who managed the pregnant women or could be due to difference in socio economic and hygienic status of pregnant women and specimen collection technique which needs further investigation to confirm the relationship between GBS colonization and health institutions.

Group B *Streptococci* colonization did not differ significantly with occupation, being 16.7% among business women, 15.1% among house wives and 8.6 % among civil servants (P > 0.05). This is consistent with reports from other authors (9, 21, 57). This variation within the occupation could be partly explained on the basis of the difference in hygienic status of different populations, which is more likely to be better among civil servants (educated) than housewives and business women (the less educated women).

Group B *Streptococci* colonization in this study was not associated with HIV status probably due to the small sample of HIV infected pregnant women among the studied population. Similar findings have been reported in studies conducted in Tanzania (2009) and Malawi (2011) (59, 61). However, in another study in Democratic Republic of the Congo, colonization rate was significantly associated with HIV-positive serology (62).

In the present study, we observed that primigravida women were more often associated with GBS colonization, though it was not statistically significant (P>0.05). Similar findings have been reported in studies conducted in Nigeria (2012), Ethiopia (2012), Ghana (2011) and Brazil (2007) (4, 37, 60, 64). However, in another study colonization rates were found to be

significantly greater among multigravida women than primigravida women (P<0.001) (33, 34, 56). This might be due to geographical variation. Therefore, further studies are needed to confirm the correlation between gravidity and colonization by GBS from different geographical locations.

In the present study, history of spontaneous abortion did not influence GBS colonization in pregnant women. Similar findings have been reported in studies conducted in Tanzania (2009), India (2011) and (Iran 2009) (21, 33, 61). However, in another study history of spontaneous abortion showed significant association with GBS colonization (34, 62). Therefore, further studies are needed to confirm the correlation between abortion and colonization by GBS. In this study, previous history of still birth or neonatal loss did not influence GBS colonization. The lack of association with this factor can possibly be explained by the fact that the numbers of participants in this study with such risk factor were small. This finding is consistent with studies from other authors (61).

In this study, history of contraceptive use was significantly shown to influence GBS colonization in pregnant women, being 20.2% among women who had no history of using contraceptive and 11% who had a history of using contraceptive ($p<0.05$). Those pregnant women who had a history of using contraceptive were 54% less likely to be colonized with GBS as compared to those pregnant women who had no a history of using contraceptive (AOR: 0.46, 95% C.I: 0.23-0.94). However, in another study, history of contraceptive use was observed to be unrelated to GBS carriage (39, 51). Therefore, further studies are needed to confirm the correlation between contraceptive and GBS colonization.

The prophylaxis currently recommended for prevention of neonatal disease is the intrapartum use of antibiotics only in women known to be colonized by GBS. Penicillin is the first-choice of drug, while ampicillin is an alternative and, in cases of history of allergy to penicillin and at high risk for anaphylaxis, clindamycin and erythromycin are recommended (8). In GBS-colonized mothers with allergy and low risk of anaphylaxis to penicillin, the use of cefazolin is recommended. In those with a high risk of anaphylaxis to penicillin and if the isolate is resistant to clindamycin, vancomycin is recommended (8, 67). Antibiotic resistance amongst GBS is considered an increasing problem so that it was recommended to test the susceptibility of other

antibiotics than those recommended as part to established control measures and that could be used as alternative choices for prophylaxis or treatment of GBS infection (8, 38).

In this study, all GBS isolated were susceptible to chloramphenicol, however; this antibiotic is not recommended for intrapartum antibiotic prophylaxis for GBS colonization in pregnant women because of its adverse effect during pregnancy such as gray baby syndrome and in women or fetuses with G6PD deficiency, hemolysis (86). This is consistent with reports from other authors (27). In our study, we observed resistance to penicillin (19.5%) and ampicillin (14.6%) which are the first choice of drugs for intrapartum prophylaxis. This did not match with the CDC 2010 guidelines study, which did not find any resistance to penicillin. These findings are comparable to those reported in other studies (27, 64). The expanded use of beta-lactam antimicrobials in the treatment of several infective clinical syndromes and the free accessibility of purchase over the counter might be the cause of emergence of GBS resistance strains in this environment.

The CDC 2010 guidelines recommends testing of GBS isolates for susceptibility to clindamycin and erythromycin, as they are the drugs of choice for penicillin-allergic women at high risk for anaphylaxis (8). An increase in resistance of GBS to erythromycin has been reported (5, 64, 65, 68, 69, 74). In this study, we found that 7.5% of the isolates were resistant to erythromycin. This is consistent with reports from other authors (4, 12, 61, 70, 75). This rate of erythromycin resistance in the GBS isolates strongly supports the current CDC recommendation that antibiotic susceptibility test should be performed if erythromycin therapy is needed to prevent neonatal GBS infection. With respect to resistance to clindamycin, the findings of this study (26.8%) are similar to those reports from other authors (12, 61, 64, 65, 67-69, 74). Since clindamycin is another alternative antibiotic recommended by the CDC for pregnant women who are allergic to penicillin, the resistance level underline the need of carrying out a susceptibility test. This might be due to the widespread use of the antibiotics.

Vancomycin is recommended for GBS-colonized mothers with a high risk of anaphylaxis to penicillin and if the isolate is resistant to clindamycin (8). In this study, we found that 17% of the isolates were resistance to vancomycin. This finding is comparable to those reported in other studies (15, 64). Since vancomycin is another alternative recommended by the CDC for pregnant women who are allergic to penicillin and clindamycin resistant isolates, the resistance level

underline the need of carrying out a susceptibility test. Most GBS isolated (90.2%) were resistance to tetracycline showing that this antibiotic could not be used for prophylaxis. The high proportion of resistant isolates to tetracycline in the present study is consistent with reports from other authors (4, 15, 33, 70, 71, 74). This may probably be due to the widespread use of this antibiotics and ease of procurement of antibiotic and/or could be attributed to indiscriminate use of antimicrobial drugs in this area. Even though we did AST for tetracycline according to the CLSI 2013 guideline, it is generally not recommended during pregnancy because of fetal considerations (87). The cefotaxime resistance (34.1 %) in this study is difficult to explain since cefotaxime is rarely used in Ethiopia. In contrast to this, high susceptibility to cefotaxime was observed in another study (64, 70).

9. LIMITATIONS

- Lack of anti-sera for sero-typing of GBS which will provide an important vehicle for evaluating the public health risks posed by changes in distribution of GBS serotypes and choosing an appropriate vaccine composition.
- Inability to evaluate the neonatal outcome of GBS positive mothers.

10. CONCLUSION

In this study, the overall prevalence of GBS colonization among pregnant women at 35-37 weeks of gestation was 14.6%. This high prevalence of GBS colonization among pregnant women calls for screening of this bacteria in women attending antenatal care so that intrapartum antimicrobial prophylaxis can be offered to all women identified as carriers and requires needs for awareness and concerted effort for intervention preventive measures. GBS colonization in pregnant women showed statistically significant association with health institution and inversely related with history of contraceptive use ($P < 0.05$).Other socio-demographic factors such as maternal age, marital status, occupation, obstetric factors such as history of still birth/neonatal loss, spontaneous abortion, gravidity and HIV did not significantly associated with GBS colonization.

All GBS isolated were susceptible to chloramphenicol, however; this antibiotic is not recommended for intrapartum antibiotic prophylaxis for GBS colonization in pregnant women because of its adverse effect during pregnancy. Most GBS isolated (90.2%) were resistance to tetracycline showing that this antibiotic could not be used for prophylaxis. Resistance to cefotaxime, clindamycin, penicillin G, vancomycin, ampicillin and erythromycin was found to be 34.1 %, 26.8 %, 19.5 %, 17%, 14.6%, and 7.5 % respectively. In this study, we observed resistance to penicillin and ampicillin, the first choice of drugs for intrapartum prophylaxis, which calls for testing of susceptibility of these antibiotics before administration. There is also erythromycin and clindamycin resistance, the drugs of choice for penicillin-allergic women at high risk for anaphylaxis, which strongly supports the current CDC recommendation that antibiotic susceptibility test should be performed if erythromycin and clindamycin therapy is needed to prevent neonatal GBS infection. Multi drug resistance (MDR) was detected in 43.9 % (18/41) of the isolates. Resistance to 2, 3, 4 and 5 drugs was found to be 43.6 %, 23.9%, 23.9 % and 8.4 % respectively.

11. RECOMMENDATIONS

- The high isolation frequency of GBS among pregnant women in this study suggests a risk based approach and culture based screening approach for all pregnant women should be done at 35–37 weeks' gestation to provide antibiotic prophylaxis to GBS carrier and it signifies that GBS infection might be a silent clinical problem that is undiagnosed in the present study area, therefore, requires needs for awareness and concerted effort for preventive measures.
- In this study, the health institution showed statistically significant association with GBS colonization which shows the need of focused antenatal care (FANC) follow up by trained health professionals and needs further investigation to confirm the relationship between GBS colonization and health institutions.
- In this study, there is resistance to the commonly used antibiotics such as penicillin, ampicillin and clindamycin, which calls for performing susceptibility testing before administration of any of these antibiotics.
- Sero-typing of GBS should be done to develop and implement effective vaccine for prevention of neonatal GBS disease. Serotyping is an effective epidemiological tool for studying GBS.
- The studies on neonatal outcome of GBS positive mothers should be done.
- Antibiotic resistant GBS may occur with more widespread use of antibiotics. Therefore; surveillance of antibiotic resistance patterns among several antimicrobial classes will be important in determining optimal prophylaxis and treatment of GBS infections.
- Indeed, the problem of antibiotic resistance is global. Therefore; health care providers worldwide should be encouraged to join public health authorities, to control the inappropriate use of antibiotics and promote responsible prescribing. This will greatly help to improve prevention and control of drug resistant organisms in communities.
- It would have an impact on the management of pregnant women before giving birth and their babies if these kind of studies are repeated in many health institutions. So the outcome of these studies will be used in formulating guidelines for the antenatal care.

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ANNEX I: ENGLISH VERSION OF THE INFORMATION SHEET

Addis Ababa University Post graduate School

Principal Investigator: Solomon Assefa

Name of Organization: AAU, College of Health Sciences, School of Allied Health Sciences, Department of Medical Laboratory Sciences

Information sheet for the prevalence of Group B *Streptococci* colonization and susceptibility pattern among pregnant women attending antenatal clinic of Health Institutions, Addis Ababa, Ethiopia

Title: The "prevalence of Group B *Streptococci* colonization and susceptibility pattern among pregnant women" attending antenatal care clinics of Health Institutions, Addis Ababa, Ethiopia.

Aim: The aim of this study is to determine prevalence of GBS colonization and susceptibility pattern in pregnant women attending antenatal clinic of Health Institutions, Addis Ababa, Ethiopia. Pregnant women with GBS colonization during 35 and 37 weeks of gestations will pose a risk to their children during delivery. Infection with this organism can cause sepsis and meningitis in new born. Therefore; this study identified colonization rate in pregnant women so that those who are colonized with GBS received prophylaxis before delivery to reduce the risk of neonatal sepsis and meningitis.

Duration: The duration of this study depend upon the availability of study subjects. It might take about three months or more.

Procedures to be carried on: For this study to be successful we need your participation. If you are voluntary to participate in this study, you are expected to understand and sign the informed consent. Then, socio demographic and clinical information related to GBS which are important for this study will be taken. Vaginal swab samples will be collected by attending gynecologist, experience nurse and/or midwives. Collected samples will be transported to ALERT microbiology laboratory as soon as possible and will be analyzed for the presence of GBS by using standard operating procedures (SOPs). In addition, recent HIV result was taken from study participants' medical records.

Risk: The risk associated with the specimen collection is minimal because the collection of these specimens will follow the routine procedures for the laboratory investigation. Administering Intrapartum antibiotics to colonized women are effective in reducing the risk of neonatal mortality. However rarely, IAP may cause maternal allergic reactions and increase in drug-resistant organisms if not properly managed.

Expected benefits: We will notify the GBS positive laboratory results to gynecologist in charge at Health Institutions for the better management of colonized pregnant women. In this study, you are not directly benefited however; the new born will have a reduced risk of neonatal sepsis and meningitis by administering appropriate antibiotics to colonized women.

Confidentiality: All your personal information collected for the purpose of this study will be kept confidential.

Payment: No payment will be provided by participating in this study.

Right: Participation in the study is voluntary, and refusal to participate involves no penalty or loss of benefits to which you are otherwise entitled. The study participants have a right to withhold information, decline to cooperate in the study and refuse provision of specimens.

Approval: This research project has got ethical clearance from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Medical Laboratory Science, AHRI/ALERT Ethical Review Committee (AAERC), Addis Ababa Health Bureau Institutional Review Board, and Ministry of Science and Technology National Research Ethics Review Committee (NRERC).

Whom to contact: If you have any question or description about this study, you can communicate on the following address:

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ANNEX II: AMHARIC VERSION OF THE INFORMATION SHEET

ቅፅ 2፡ ለተሳታፊዉ በቂ መረጃ ለመስጠት የተዘጋጀ ቅጽ (ትርጉም በአማርኛ)

አዲስ አበባ ዩኒቨርሲቲ የድህረ ምረቃ ትምህርት

የተመራማሪዉ ስም፡ ሰለሞን አሰፋ

የድርጅቱ ስም፡ አአዩ ፣ የጤና ሳይንስ ኮሌጅ፣የአላይድ ጤና ሳይንስ ት/ት፣ ህክምና ላቦራቶሪ ሳይንስ ክፍል

በጤና ተቋም የቅድመ ወሊድ ክትትል በሚያደርጉ እርጉዝ እናቶች መሃከል ግሩፕ ቢ ስትሮፕቶኮካይ የተባሉ በሽታ አምጪ ባክቴሪያዎች የስርጭት መጠናቸዉን እና መድሀኒት የመላመድ ሁኔታቸዉን ለማጥናት ለተሳታፊዉ በቂ መረጃ ለመስጠት የተዘጋጀ ቅጽ ነው፡፡

<u>ርዕስ፡-</u> በጤና ተቋም የቅድመ ወሊድ ክትትል በሚያደርጉ እርጉዝ እናቶች መሃከል ግሩፕ ቢ ስትርፕቶኮካይ የተባሉ በሽታ አምጪ ባክቴሪያዎችን የስርጪት መጠናቸዉን እና መድሀኒት የመላመድ ሁኔታቸዉን ምን ያህል ነዉ የሚል ነዉ፡፡

<u>የጥናቱ ዋና አላማ፡-</u> በጤና ተቋም የቅድመ ወሊድ ክትትል በሚያደርጉ እርጉዝ እናቶች መሃከል ግሩፕ ቢ ስትርፕቶኮካይ የተባሉ በሽታ አምጪ ባክቴሪያዎች የስርጪት መጠናቸዉ እና መድሀኒት የመላመድ ሁኔታቸዉን ለማጥናት ነው፡፡ ከ35 እሰከ 37 ሳምንት የእርግዝና ወቅት ባክቴሪያዉን በተሸከሙ እናቶች ላይ ህፃኑ የበሽታ ሰለባ ሊሆን ይችላል ፡፡ ህፃኑ በባክቴሪያዉ ከተጠቃ ለተላያዩ በሽታዎች(ኒወናታል ሴብሲስ) እና ማጅራት ገትር(ሜኒንጃትስ) ሊጋለጥ ይችላል፡፡ ስለዚህ ይህ ጥናት የስርጭቱ መጠን ከለየ በኋላ እናቶች የቅድመ መከላከያ መድሀኒት እንዲወስዱ በማድረግ ህፃኑን ከተላያዩ በሽታዎች(ኒወናታል ሴብሲስ) እና ማጅራት ገትር(ሜኒንጃትስ) አደጋ እንዲቀንስ ማድረግ ይቻላል፡፡

<u>የጥናቱ ጊዜ፡-</u> ክትትል በሚያደርጉ እርጉዝ እናቶች ብዛት የሚወሰን ሲሆን 3 ወር እና ከዛም በላይ ሊወስድ ይችላል፡፡

<u>የጥናቱ ሂደት፡-</u> ለዚህ ጥናት እዉን መሆን የእርስዎን ተሳትፎ እንፈልጋለን፡፡ በዚህ ጥናት ለመሳተፍ ፈቃደኛ ከሆኑ የስምምነት ቅጹን መረዳትና መፈረም ይጠበቅብዎታል፡፡ከዛም ለጥናቱ አስፈላጊ የሆኑ ህብረተሰብ ነክ እና የህክምና መረጃዎችን እንወስዳለን ፡፡ በመቀጠል ናሙና ከ ብልትዎ አካባቢ ክትትል በሚያደርግሎት ሀኪም፣ ልምድ ባላቸዉ ነርስ እና አዋላጅ ነርስ የምንወስድ ሲሆን የምርመራው ስራ ደግሞ በአለርት

ማይክሮባዮሎጂ ላቦራቶሪ ይከናወናል፡፡ እንዲሁም የኤች.አይ.ቪ ምርመራ ዉጤት ከ ህክምና ካርድዎ ላይ ይረጋገጣል፡፡

<u>ሊከሰቱ ስለሚችሉ ስጋቶችና የምችት መጓደሎች፡-</u> ለጥናቱ በሚወሰደዉ ናሙና ምክንያት ከትንሽ ስሜት ዉጪ የተለየ ችግር/ስጋት አይኖረዉ ምክንያቱም የጥናቱ ናሙና አወሳሰድ ከተለመደዉ የአገልግሎት አሰጣጥ የተለየ አይደለም፡፡ የቅድመ መከላከያ መድሀኒት ባክቴሪያዉ ላለባቸዉ እናቶች መስጠት የህፃናትን ሞት ለመቀነስ ዉጠታማ ቢሆንም አንዳንዴ ግን በተገቢዉ መንገድ መቆጣጠር ካልተቻለ እናቶች ላይ የሰዉነት መቆጣት እና መድሃኒት የተላመደ ተዋስያን ሊያስከትል ይችላል፡፡

<u>የተሳታፊዎች ጥቅሞች፡-</u>ባክቴሪያዉ ያለባቸዉን እርጉዝ እናቶች የላቦራቶሪ ዉጤታቸዉን በ ጤና ተቋም በማገልገል ላይ ለሚገኘዉ ጋይናኮሎጂስት(የማዕፀን)ሃኪም በማሳወቅ የተሻለ ክትትል ይደረግላቸዋልል፡፡በዚህ ጥናት ላይ እርሰዎ ቀጥተኛ ተጠቃሚ አይሆኑም ነገርግን የቅድመ መከላከያ መድሃኒት እንዲወስዱ በማድረግ ልጅዎን ከተላያዩ በሽታዎች (ኒወናታል ሴብሲስ) እና ማጅራት ገትር (ሜኒጃትስ) አደጋ መቀነስ ይቻላል፡፡

<u>ሚስጥራዊነት፡-</u> ለጥናቱ ተብለው የተሰባሰቡ የግልዎ መረጃ ሚስጢርነቱ የተጠበቀ ነው፡፡

<u>ክፍያ፡-</u> በዚህ ጥናት በመሳተፍዎ የሚያገኙት ምንም አይነት ልዩ ክፍያ የለም፡፡

<u>የተሳታፊዉ መብት፡-</u>ተሳትፎዎ ሙሉ በሙሉ በፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡ ፈቃደኛ ካልሆኑ በዚህ ጥናት ያለመሳተፍ መብትዎ የተጠበቀ ነዉ፡፡ በዚህም ምክንያት ከሆስፒታሉ በሚያገኙት አገልግሎት ላይ ምንም አይነት ተጽዕኖ አይደርስብዎትም፡፡ ከጥናቱ በማንኛዉም ሰዓት ራስዎን የማግለል መብትዎ የተጠበቀ ነዉ፡፡

<u>የጥናቱ ፈቃድ/ህጋዊነት፡-</u> የዚህ ጥናት ህጋዊነት በዲፓርትመንታል ምርምር እና ስነ ምግባር ቅኝት ኮሚቴ፣አዲስ አበባ ዩኒቨርስቲ፣ኮሌጅ አፍ ሄልዝ ሳይንስ ስኩል አፍ አላይድ ጤና ሳይንስ ፣ በአህሪ/አለርት ስነ ምግባር ቅኝት ኮሚቴ፣በአዲስ አበባ ጤና ቢሮ ስነ ምግባር ቅኝት ኮሚቴ፣ በብሄራዊ ምርምር ስነ ምግባር ቅኝት ኮሚቴ ድጋፍ አግኝቷል፡፡

<u>መረጃ ስለማግኘት፡-</u> ይህን ጥናት አስመልክቶ ምንም አይነት ጥያቄ ወይም ማብራሪያ ቢያስፈልግዎት፡

1. አዲስ አበባ ዩኒቨርሲቲ ፣ የጤና ሳይንስ ኮሌጅ፣ የ አላይድ ጤና ሳይንስ ት/ት፣ ህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

   ስ.ቁ.፡- +251-112-75-51-70

ፋክስ፡+251-112-75-46-69

ኢ-ሜል፡: SMLT@ethionet.et

ፒ.ኦ.ቦክስ፡ 1176 ፣አዲስ አበባ፣ኢትዮጲያ.

2. አህሪ/አለርት ምርምር ስነ ምግባር ቅኝት ኮሚቴ ጽ/ቤት

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ኢ-ሜል፡info@ahri-alert.org

ፒ.ኦ.ቦክስ፡ 1005 ፣አዲስ አበባ፣ኢትዮጲያ

ወይብ ሳይት፡ http://www.ethioahrialert.org/Default.aspx

3. የብሄራዊ ምርምር ስነ ምግባር ቅኝት ኮሚቴ

ስ.ቁ፡+251-11-157-11-37

ፋክስ፡+251-11-152-44-00

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ፒ.ኦ.ቦክስ፡2490 ፣አዲስ አበባ፣ኢትዮጲያ

4. የአዲስ አበባ ጤና ቢሮ ስነ ምግባር ቅኝት ኮሚቴ ጽ/ቤት

ስ.ቁ፡+251-11-551-39-11

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ፒ.ኦ.ቦክስ፡30738 ፣አዲስ አበባ፣ኢትዮጲያ

5. የጥናቱ ተመራማሪ፡ ሰሎሞን አሰፋ (በአአ ዩኒቨርሲቲ የማስተር ተማሪ)

ስ.ቁ፡-0913131052/0912729465

ኢሜል:sole.lab2000@yahoo.com or solomonas64@gmail.com

ፒ.ኦ.ቦክስ፡ 165፣ አለርት ማእከል፣ አዲስ አበባ፣ኢትዮጲያ.

ANNEX III: ENGLISH VERSION OF THE CONSENT FORM

Serial no…….

Card no…..……

Name of study participant: _____

I have been requested to participate about this study, which plans to determine prevalence of colonization with Group B *Streptococcus* and Susceptibility Pattern among pregnant women attending antenatal care clinics Health Institutions, Addis Ababa, Ethiopia in which my baby will be protected from GBS infection. I have been informed this study which involves collecting of vaginal specimen. During collection of the specimen I have been told that there is no harm except little discomfort and I have also read the information sheet or it has been read to me. I have been also informed that all information contained within the questionnaire is to be kept confidential. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and drop out of the study if I want and that none of my actions will have any bearing at all on my overall health care and hospital access.

It is therefore with full understanding of the situations that I agreed to give the informed consent voluntarily to the researcher to use the specimen taken from vaginal for the investigation. I also agreed to give my HIV result from my medical record. Moreover, I have had the opportunity to ask questions about the project and I have received clarification to my satisfaction. I was also told that results would be reported timely to the requesting physicians for the appropriate treatment and management of the GBS infection.

I agree that I am contributing to the treatment of my fellows by participating in this project. I have asked some questions and clarification has been given to me. I have given my consent freely to participate in the study, and I_____ hereby to approve my agreement with my signature.

Participants' signature: _____Date_____
Principal Investigator's signature: _____Date _____
Witness (Illiterate) _____Date _____

ANNEX IV: AMHARIC VERSION OF THE CONSENT FORM

ቅፅ 4፡ የስምምነት መግለጫ (ትርጉም በአማርኛ)

ተራ ቁጥር _____

የካርድ ቁጥር _____

የተሳታፊው ስም _____

እኔ ስሜ ከላይ የተጠቀሰዉ በጤና ተቋም በተመላላሽ እርጉዝ እናቶች መሀከል የግሩፕ ቢ ስትሪፕቶኮካይ በተባሉ በሽታ አምጪ ባክቴሪያዎች ስርጪት መጠን እና መድሃኒት የመላመድ ሁኔታቸዉን ለማጥናት የተዘጋጀ ጥናት ላይ እድሳተፍ ተጠይቄ ስለ ጉዳዩም ለመረዳት በቂ መረጃ አግኝቻለሁ፡፡ የጥናቱ ዉጤት ልጄን ከበሽታዉ ሊከላከልልኝ የሚችል ጥናት መሆኑን ተረድቻለሁ፡፡ስለሆነም ናሙና የሚሰበሰበው ከ ብልቴ መሆኑን ስለተርዳሁኝ ናሙና ወስዶ መመርመር አስፈላጊ ስለሆነ ናሙናዉን በመስጠት ልተባበር ሙሉ ፈቃደኛ መሆኔን ገልጫለሁ፡፡ እንዲሁም ከህክምና ካርዴ ላይ የ ኤች.አይ.ቪ ዉጤቴን እንዲወስዱ መፍቀዴን ተስማምቻለሁኝ፡፡ናሙና በሚወስድበት ጊዜ ከትንሽ ስሜት ውጪ ምንም አይነት ጉዳት እንደሌለው ተነግሮኛል እንዲሁም ከመጠይቁ አንብቢያለሁ ወይም ተነቦልኛል፡፡ ከምርመሩ መሳተፍ ወይም አለመሳተፍ መብቴ የተጠበቀ መሆኑን እና ላለመሳተፍ ብወስን በጤና ተቋም በሚደረግልኝ ህክምና ላይ ምንም ተፅዕኖ እንደማይኖረዉ ተረድቻለሁ፡፡

ስለዚህ የጥናቱን ጠቃሚነት አምኜበት የስምምነት ቃሌን የሰጠሁት በፍፁም ፈቃደኝነት ነዉ፡፡በመጨረሻም ልጄ ከጥናቱ ዉጤት ተጠቃሚ ሊሆን እንደሚችል ተገልፆልኝ በመሳተፌና በመተባበሬ ወገኖቼን ልረዳ በመቻሌ ደስተኛ መሆኔን ገልጨ ፤ግለፅ ያልሆኑ ጥያቄዎች ላይ ማብራርያ እንዲሰጠኝ ጠይቄ መልስ ተሰጥቶኛል፡፡ እንዲሁም በጥናቱ ሂደት እንድሳተፍ ፍቃደኝነቴን በፊርማየ አረጋግጫለሁ፡፡

የተሳታፊ ፊርማ_____ቀን_____.

የጥናቱ አስከያጅ ፊርማ_____ቀን_____

ምስክር (ማንበብና መፃፍ ለማይችሉ) _____ቀን_____

ANNEX V: ENGLISH VERSION OF THE QUESTIONNAIRE

The title of this study is **"The prevalence of Group B *Streptococci* and Susceptibility Pattern among pregnant women"** attending antenatal care clinics of Health Institutions, Addis Ababa, Ethiopia.

Interview

We thank gratefully for your agreement to participate in this study. Now we are going to undertake interview with you and the interview is about general socio demographic characteristics and clinical data. All of the answers you provide in this study will be kept confidential. The information you give us is very essential for this study. Therefore, we politely ask you to give us the right response.

A. Background information		
1	Study ID	
2	Participant Card No.	
3	Address	Region:_____ Sub city: _____Kebele: _____ Tel:_____
4	Full name of the Participant:	
5	Age:	
6	Ethnicity	i).Amhara ii).Oromo iii).Tigray iv. others (specify)
7	Marital status	i). Single- Never married ii.) Married iii).Divorced/Separated iv). Widowed
8	Occupation	i.) Civil servant ii).Student iii).Housewife iv).Business women

B. Clinical data		
9	History of Primigravida or multigravida:	i).Primigravida(1^{st}) ii).Multigravida(2^{nd} ,3^{rd} ,4^{th} 5^{th} ,6^{th} ,…) please specify
10	History of previous Abortions	i).Yes, if yes for how many times ii).No
11	Previous History of Still birth or Neonatal loss	i).Yes ii).No
12	Gestational age (weeks)	
13	Number of prenatal visit	
14	History of recent any antibiotic treatment	i).Yes If yes, mention antibiotic/s and time taken…….. ii).No
15	History of any contraceptive use	i).Yes If yes, mention type of contraceptive used…… ii). No
16	Recent HIV result (status)	1.Positive 2.Negative
17	Date and time of vaginal specimen collection	
C. comments		

Thank you very much for taking the time to complete our study!

Name of principal investigator _____

Signature _____ Date _____

ANNEX VI: AMHARIC VERSION OF QUESTIONNAIRE

ቅፅ 6፡ መጠይቅ (ትርጉም በአማርኛ)

<u>የዚህ ጥናት ርዕስ</u>፡ በጤና ተቋም በተመላላሽ እርጉዝ እናቶች መሀከል ግሩፕ ቢ ስትርፕቶኮካይ በተባሉ በሽታ አምጪ ባክቴሪያዎች የስርጪት መጠን እና መድሃኒት የመላመድ ሁኔታቸዉን ለማጥናት የተዘጋጀ ጥናት ነው፡፡

ቃለ መጠይቅ

በዚህ ጥናት ላይ ለመሳተፍ በመስማማትዎ እጅግ አድርገን እናመሰግናለን፡፡አሁን ከ እርሰዎ ጋ ስለ ህብረተሰብ ነክ እና የህክምና መረጃዎች ቃለ መጠይቅ ልናደርግ ነዉ፡፡ለጥናቱ ተብለው የተሰባሰቡ የግልዎ መረጃ ሚስጢርነቱ የተጠበቀ ነው፡፡እርስዎ የሚሰጡን መረጃ ለዚህ ጥናት በጣም አስፈላጊ ነዉ፡፡ስለዚህ ትክክለኛ የሆነ ምላሽ እንዲሰጡን በትህትና እንጠይቀዎታለን፡፡

	ሀ. የተሳታፊዎች መለያ	
ነ	ተራ ቁጥር	
2	የተሳታፊዉ ካረድ ቀጥር	
3	አድራሻ	ክልል:_____ክፍለ ከተማ:___ቀበሌ: ___ ስ.ቁ. _____
4	የተሳታፊው ሙሉ ስም	
5	እድሜ	
6	ብሔር	i). አማራ ii). ኦሮሞ iii).ትግሬ iv. ሌላ (ይገለፅ)
7	የጋብቻ ሁኔታ	i). ብቸኛ አግብቶ የማያቅ ii.) አግብታ የፈታች iii).ባሏ የሞተባት iv). ያገባች
8	ስራ	i.) የመንግስት ሰራተኛ ii). ተማሪ

		iii). የቤት እመቤት iv). የቢዝነስ ሴት(የግል ስራ)
ለ.የህክምና መረጃ		
9	የእርግዝና ሁኔታ	i). ለመጀመርያ ጊዜ ነው (ነኝ) ii). ለሁለተኛ ና ከዛም በላይ ነው(2ተኛ፣3ተኛ፣4ተኛ፣5ተኛ...) እባክዎትን ለስንተኛ ጊዜ እንደሆነ ይግለፁ፡፡
10	ከዚህ በፊት ዉርጃ አጋጥመዎት ያዉቃል	i).አዎ፣ መልስዎ አዎ ከሆነ ለስንተገኛ ጊዜ_____ ii).አያዉቅም
11	ከዚህ በፊት ሞቶ የተወለደ ህፃን አጋጥመዎት ያዉቃል	i).አዎ ii).አያዉቅም
12	የፅንሱ ጊዜ (በሳምንት)	
13	የከትትል ጊዜዎ ለስንተኛ ጊዜ ነው	
14	በቅርቡ መድሃኒት ወስደዋል	i). አዎ፣ መልስዎ አዎ ከሆነ የወሰድቱን መድሃኒት ቢጠቅሱት..... ii). አልወሰድኩም፡፡
15	የወሊድ መቆጣጠሪያ ወስደዉ ያዉቃሉ	i). አዎ ፣ መልስዎ አዎ ከሆነ የወሰዱትን የወሊድ መቆጣጠሪያ ቢጠቅሱት..... ii). አልወሰድኩም፡፡
16	የቅርብ የኤች.አይ.ቪ ዉጤት(ሁኔታ)	i).ፖዘቲቭ ii).ኔጋቲቭ
17	ናሙና የተወሰደበት ጊዜ እና ሰአት	
ሐ.አስተያየት፡		

ጊዜዎትን መሰዋት አርገዉ ስለረዱን እናመሰግናለን፡፡

የጥናቱ ዋና አስከያጅ ስም: _____ፊርማ: _____

ቀን. _____

ANNEX VII: LABORATORY PROCEDURE

A). Sample Collection, Handling and Transport

1. Objective and Scope:

To describe the specimen collection instructions and subsequent handling of specimens by researcher (Laboratory Technologist) for culture of GBS. This document contains standard operating procedures (SOPs) for clinical specimens containing GBS from the vaginal and anorectal swabs for processing at ALERT Center clinical laboratory.

2. Requirements/Materials:

Supplies	Equipment	Reagents	Drugs
• BAB,MHA,THB powder • Cotton swabs • Specimen collection tubes, Petridish • Inoculating loops • Distilled water, normal saline • Graduated cylinder, Flask, Spatula • Candle jar, measuring caliper/ruler • Sterile sheep blood • Ice box, sample rack	• Incubator • Bunsen burner • Safety cabinet • Balance, autoclave, • autoclave tape • Distiller, • PH meter • Hot plate • Refrigerator • Microscope	• 3%H_2O_2 • 0.5 McFerland standard • Crystal violet • Lugol's iodine • Acetone alcohol • Safranin • Methanol	• Gentamicin • Nalidixic • Antimicrobial disks

3. Procedure:

An adequate specimen is essential for the success of GBS culture. Specimens are to be collected with the utmost care and go to the laboratory promptly. Culture-based screening for GBS at 35 to 37 weeks of gestation from swabs collected from the vagina was the specimen of choice in the investigation of GBS.

4. **Specimen Collection**

According to the CDC 2010 and American College of Obstetricians and Gynecologists (ACOG Committee opinion) 2011 guidelines, vaginal swab was taken from the lower one third of vagina using sterile cotton swab in pregnant women attending antenatal clinic of Health Institutions, Addis Ababa, Ethiopia, from May-July, 2014 (Figure 2.1) by attending midwives and experienced nurses.

5. **Transport of specimen to ALERT center microbiology laboratory**

Vaginal swab was placed in THB (Oxoid, UK) and immediately transported to the Microbiology Laboratory of ALERT Center for culture. If delay is unavoidable the specimens should be refrigerated at 4 °C for 24 hrs.

6. **Specimen rejection criteria**

Specimen is liable for rejection for Culture if:

- Specimen is unlabeled or mislabeled.
- Specimen without request form.
- Specimen name and request form does not match.
- Specimen not collected in an appropriate container.

B).Specimen Processing

i). Culture

Procedure:

1) The vaginal swab was placed into 1 ml THB (Oxoid, UK) supplemented with gentamicin (8 µg/ml) (Intas pharmaceutical Ltd., India) and nalidixic acid (15 µg/ml) (Sigma Aldrich, Italy) to prevent growth of contaminants.
2) The broth was incubated for 18–24 hours at 35-37°C
3) Then, inoculated the vaginal swab into 5% % sheep blood agar (Oxoid, UK)
4) Incubated in a humid environment of air containing 3-5 % CO_2.
5) Incubated for a minimum of 48 hrs before discarding the plates.

6) Examined the plates after 18-24 hrs of incubation.
7) Presumptive diagnosis was made by performing Gram stain, catalase, and CAMP test.
8) Antimicrobial susceptibility testing was performed for GBS isolated according to CLSI guideline 2013.

ii). Gram stain

Purpose: This procedure provides instructions to perform gram stain.

Principle: Gram positive bacteria have thick mesh-like cell wall made of peptidoglycan (50-90% of cell wall) which stains purple while Gram-negative bacteria have a thinner layer (10% of cell wall),which stains pink.

Clinical Utility: The gram stain is used to classify bacteria on the basis of their forms, sizes, cellular morphologies, and gram reactions. It is a critical test for rapid presumptive diagnosis.

Materials:

Reagents	**Supplies**
• Crystal violet • Lugol's iodine • Acetone alcohol • Safranin	• Disposable plastic loops • Glass microscope slides • Slide warmer, dry heat block, absolute methanol

Quality control: - Gram positive: *S.aureus* (ATCC 25923

-Gram negative: *E.coli* (ATCC 25922)

Procedure:

1) A glass microscope slide was labeled with the laboratory accession number.
2) The vaginal swab was rolled gently across the slide surface, covering the area of the size of a quarter.

-For those samples taken from colony, one drop of saline on a slide was placed and picked one colony using loop and mixed with saline on the slide.

3) Placed air dried smears in a coplin jar with methanol for one minute, drained slides and allowed to dry before staining.

4) The prepared slide was flooded with crystal violet for one minute
5) The slides was rinsed gently with tap water
6) The slide was flooded with Gram's iodine for one minute
7) The slide was rinsed gently with tap water
8) The slide was decolorized by acetone-alcohol for 5 seconds and rinsed with tap water.
9) The slide was flooded with Safranin for one minute
10) The slide was rinsed gently with tap water
11) The slide was drained in an upright position. The slide was blotted and placed on a slide warmer or heating block to completely dry.
12) Scanned 20-40 fields using oil immersion.

Result interpretation:

- Gram-positive bacteria and yeast stained blue to purple
- Gram-negative bacteria stained pink to red.

Limitations:

- Recovery of organisms not observed on direct gram stains should prompt a review of both the smear and the culture.
- Application of excessive heat during fixation of smear may affect the morphologic appearance of host cells and microorganisms.
- Treatment with antimicrobial agents may cause gram positive bacterial to appear gram negative.
- The gram stain is not an infallible tool for diagnosis, identification, or phylogeny; however, it is extremely limited use in environmental microbiology.

iii).Catalase test (3%H_2O_2)

Purpose: This procedure provides instructions to perform catalase test.

Principle: Catalase is an enzyme which acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water.

Clinical utility: This test is used to differentiate those bacteria that produce the enzyme catalase such as *staphylococci*, from non-catalase producing bacteria such as *streptococci.*

Materials:

Reagent	Supplies
• 3% H_2O_2	• Test tube • Sterile wooden sticks or glass rod • H_2O_2 • Distilled water • Bunsen burner

Reagent preparation of 3% H_2O_2:

i). Measure 97 ml of distilled water and pour into sterile flask.

ii). Measure 3 ml of concentrated H_2O_2 and dispense into distilled water.

-Reagent stability and storage: H_2O_2 is unstable and should be stored in spark proof fridge, avoid direct sunlight exposure.

Quality control: *S.aureus* (ATCC 25923)

Procedure:

1) Poured 2-3 ml of the hydrogen peroxide solution into a test tube
2) Using a sterile wooden stick or glass rod removed several colonies of the test organism & immersed in the hydrogen peroxide solution
3) Looked for immediate active bubbling

Result interpretation:

- Active bubbling…….positive catalase test
- No bubbling……..negative catalase test.

Limitations: care must be taken when testing an organism cultured on a medium containing blood because catalase is present in red cell.

iv). CAMP test

Purpose: This procedure provides instructions to perform CAMP test

Principle: This is a screening test for the presumptive identification of GBS which requires the use of a beta-lysin producing strain of *S.aureus* (ATCC 25923) to detect the CAMP factor, i.e. extracellular diffusible protein produced by *S.agalactiae.* This protein interacts with the *S.aureus* beta-lysin on sheep blood agar producing enhanced hemolysis.

Clinical Utility: The test is used for the presumptive identification of GBS (*Streptococcus agalactiae*) (CAMP positive) from other CAMP negative streptococci (*Streptococcus pyogenes*, *Enterococcus faecalis*).

Materials:

Supplies	**Equipment**
• BAP • Cotton swab • Inoculating loops	• Incubator • Bunsen burner • Safety cabinet

Procedure:

1) Inoculated *Staphylococcus aureus* onto a sheep blood agar plate by making a narrow streak down the center of the plate with a loop.
2) Streaked the test organism (suspect GBS) in a straight-line at right angles to the *S. aureus.*
3) Made the *Streptococcus* streak within 2 mm without touching the *S. aureus* streak.
4) A known GBS (ATCC 27956) as a positive control and *Enterococcus faecalis* (ATCC 29212) as a negative control was also streaked similarly.
5) The plate was incubated at 35-37 °C for 18-24 hours.
6) A positive test for CAMP factor appeared as “arrowhead” hemolysis between the junction of growth of *S.aureus and* GBS with the "arrow point" toward the *S. aureus* streak. (Figure 2.7 below). No enhanced zone of beta-hemolysis was observed in a CAMP negative reaction.

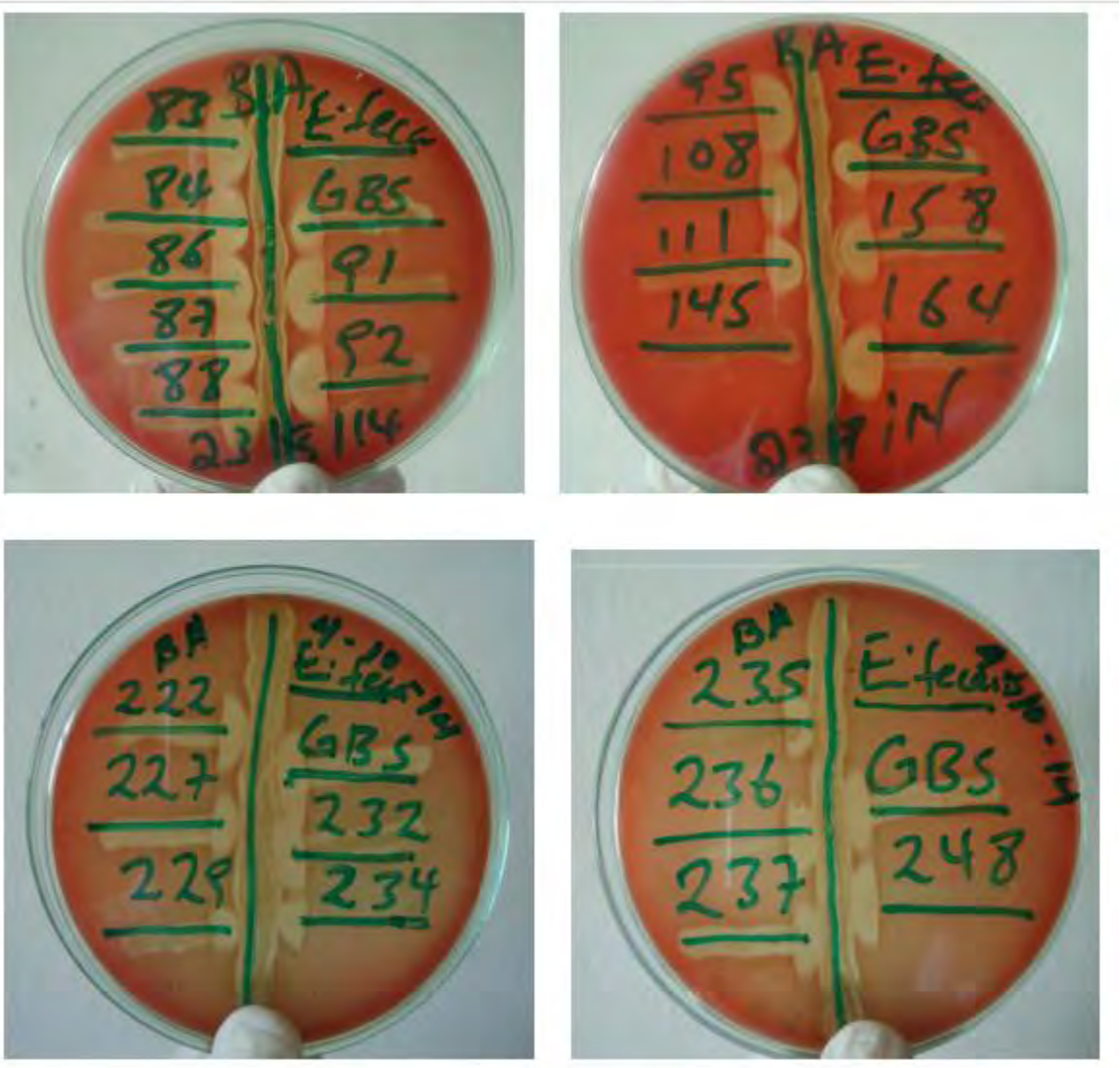

Figure 2.7. CAMP positive results of GBS in BA after 18-24 hours of incubation indicated by an "arrowhead"-shaped enhanced zone of beta-hemolysis in the area between the two cultures with the "arrow point" toward the *S. aureus* streak.

v). Antimicrobial susceptibility test

Principle: The antibiotic will diffuse in radial manner from the disc and will inhibit bacterial growth around it.

Purpose: This procedure provides instructions to determine the drug susceptibility pattern of bacteria using Kirby-Bauer disk diffusion method.

Clinical utility: To detect the in vitro relationship between an organism and an antibiotic to predict the failure or success of therapy in vivo (in patient).

Materials:

Reagent	**Supplies**	
0.5 McFerland standards	• MHA with 5 % sheep blood • Normal saline • Test tube • Wooden applicator sticks with cotton • antimicrobial disks	• Safety cabinet • Bunsen burner • Incubator • Measuring caliper/ruler • Candle jar

Preparation of 0.5 McFerland standards:

Reagent preparation:			
Turbidity standard number	Barium chloride dehydrate (1.175 %)	Sulfuric acid (1%)	Corresponding approximately to density of bacteria
0.5	0.5 ml	99.5 ml	1×10^8
Reagent stability and storage: for six months at 2-8 ºC			

Limitations: comparing the inoculum turbidity with the standard McFerland is subjective.

Procedure

1) Pure colony suspension was prepared into normal saline equivalent to 0.5 McFarland standards.
2) The suspension was inoculated the entire surface on Muller-Hinton agar (Oxoid, UK) with 5% sheep's blood using a sterile cotton swab.
3) According to CLSI 2013 guideline an antibiotic disk (Toxoid, UK) was selected and deposited on the agar and
4) Incubated at 35-37 °C with 5% CO2 atmosphere for 18-24 hours.
5) Measure zone of inhibition and the result based on CLSI 2013 guideline break point.

Result interpretation:

Susceptible (S): The 'susceptible' category implies that isolates are inhibited by the usual achievable concentration of antimicrobial agent when the recommended dosage is used for the site of infection.

Intermediate (I): The intermediate category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated or when a higher than normal dosage of a drug can be used.

Resistance (R): The resistance category implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules, and/or that demonstrate zone diameters that fall in the range where specific microbial resistant mechanisms are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies.

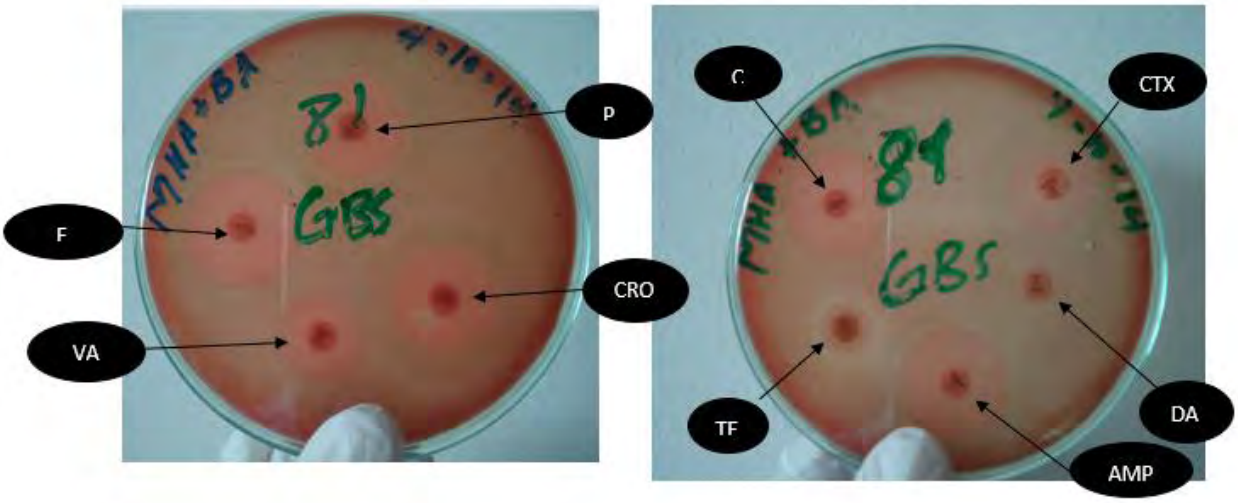

Figure 2.8. Appearance of Antimicrobial susceptibility pattern for GBS isolated from pregnant women attending ANC of Health Institutions, Addis Ababa, Ethiopia from May to August, 2014.

Limitations: The response to antimicrobial therapy in vivo may not always reflect results of in vitro.

C). Media Preparation

i). 5% Sheep blood agar (SBA)

Purpose: This procedure provides instructions how to prepare blood agar media.

Principle: Blood agar base formulation has been used as a base for preparation of blood agar and to support good growth of a wide variety of fastidious microorganisms. Because it is a highly nutritious medium it can also be used as a general purpose growth media without adding blood. The medium contains sodium chloride for the osmotic balance. Blood agar bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of beta-hemolytic streptococci.

Clinical utility: A non-selective medium for the isolation and cultivation of many pathogenic and non-pathogenic microorganisms like *Neisseria, streptococci* etc. the medium is often used to observe the different form of hemolysis from pathogenic microorganisms.

Materials:

Supplies	Equipment	
• Blood agar base powder • Weighting paper • Distilled water • Spatula • Sterile sheep blood • Refrigerator	• Balance • Autoclave • Bunsen burner • Distiller • Graduated cylinder • Flask	• Test tube • PH meter • Autoclave tape

Formula / Liter Supplements:

To make about 35 blood agar plates:

Blood agar base…… 40 g

Distilled water……1000 ml

Defibrinated blood . . . . . ……..50 ml

Procedure:

1) 40 grams of blood agar base powder was weighted and suspended in 1000 ml of distilled water.
2) The medium was mixed by boiling until completely dissolved.
3) The medium was sterilized by autoclaving at 121 °C for 15 minutes.
4) The agar has been cooled to 50 °C water bath before adding the sheep blood.
5) 50 ml of sterile defibrinated sheep blood was added aseptically and mixed gently.
6) Forming air bubbles was avoided.

   Important: The blood must be allowed to warm to room temperature before being added to the molten agar.
7) 12-15 ml of blood agar was dispensed aseptically in sterile Petridish.
8) The medium was allowed to solidify and dated the medium.
9) Stored the plates at 2–8 °C. Preferably in sealed plastic bags to prevent loss of moisture.

Limitation: blood agar is not a selective media so we couldn't differentiate microorganisms from the agar.

ii).Todd Hewitt Broth

Intended use: Todd Hewitt Broth is a general-purpose medium, which primarily is used for the cultivation of beta-hemolytic streptococci, especially for serological studies. THB with Gentamicin and Nalidixic acid is used for the selective enrichment of GBS (*Streptococcus agalactiae*), especially from genital specimens.

Principles: Todd Hewitt Broth is highly nutritious due to its content of peptones, dextrose and salts. Dextrose stimulates hemolysin production. Sodium phosphate and sodium carbonate provide buffering action to counteract the acidity produced during fermentation of dextrose, thereby protecting the hemolysin from inactivation by the acid. Selectivity for GBS is obtained by the inclusion of gentamicin and nalidixic acid in the medium. Selective enrichment broths include the advantages of both enrichment and selection by providing conditions conducive to the growth of GBS while inhibiting the growth of contaminants.

Procedure:

1) 36.4 grams of THB powder was dissolved in 1000 ml of distilled water
2) The powder was boiled until completely dissolved.
3) The medium was transferred into screw-cap bottles and sterilized (with caps loosened) by autoclave at 115°C for 10 minutes.
4) When cooled, the gentamicin and nalidixic acid was added into the medium and the bottle caps was tightened.
5) Dated the medium and give it a batch number. Stored the medium at 2-8 ^{O}C.

iii). Mueller Hinton Agar (MHA) with 5% Sheep Blood

Purpose: This procedure provides instructions to prepare MHA

Intended Use (Clinical utility): Mueller Hinton Agar is used in antimicrobial susceptibility testing by the disk diffusion method.

Principles: Beef Extract and Acid Hydrolysate of Casein provide nitrogen, vitamins, carbon, and amino acids in Mueller Hinton Agar. Starch is added to absorb any toxic metabolites produced. Agar is the solidifying agent. A suitable medium is essential for testing the susceptibility of microorganisms to sulfonamides and trimethoprim. Antagonism to sulfonamide activity is demonstrated by para-aminobenzoic acid (PABA) and its analogs. Reduced activity of trimethoprim, resulting in smaller growth inhibition zones and inner zonal growth, is demonstrated on medium possessing high levels of thymide. The PABA and thymine/thymidine content of Mueller Hinton Agar are reduced to a minimum, reducing the inactivation of sulfonamides and trimethoprim.

Materials:

Supplies	**Equipment**
• MHA powder • Distilled water • Flask • Petridish • Graduated cylinder	• Balance • Distilled water • Autoclave • PH meter • Autoclave tape

Procedure:

1) 38 grams of MHA powder was suspended and transferred in to a flask containing one liter of distilled water.
2) The powder was boiled until the medium completely dissolved.
3) The medium was autoclaved at 121°C for 15 minutes.
4) 5% sheep blood was added and cool to room temperature.
5) OPTIONAL: Supplement as appropriate. Pour cooled MHA with into sterile petri dishes on a level, horizontal surface to give uniform depth. Allow to cool to room temperature.
6) Check prepared MHA with 5% sheep blood to ensure the final pH is 7.3 ±0.1 at 25 °C.

D). Quality control

- As quality control, sterility of SBA and MHA with 5% sheep blood was checked by incubating overnight at 35-37 ºC without specimen inoculation.
- The proficiency of TH broth was checked by inoculating the broth with known Gram negative bacteria (*Escherichia coli)* (ATCC 25922) and known *S.agalactiae* (ATCC 27956) to see if it can really inhibit Gram negative bacteria and allow growth of Gram positive bacteria.
- The proficiency of catalase reagent (3 % hydrogen peroxide) was checked by known *S.aureus* (ATCC 25923) (positive control) and *S.pyogenes* (ATCC 19615) (negative control).
- For Gram staining reagents *S.aureus* (ATCC 25923) (gram positive) and *Escherichia coli* (ATCC 25922) (gram negative) was used as quality control.
- Before use of any reagents and culture media any physical change like cracks, excess moisture, color, hemolysis, dehydration, & contamination was assessed and expiration date was also checked. Temperature of incubator and refrigerator was monitored daily. *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), *Streptococcus agalactiae* (ATCC 27956) (obtained from AHRI) was used as a quality control throughout the study for culture and antimicrobial susceptibly testing.

ASSURANCE OF PRINCIPAL INVESTIGATOR

I, the undersigned, declare that this thesis is my original work in partial fulfillment of the requirement for the degree of Master of in clinical laboratory sciences (clinical laboratory management and quality assurance specialty track).

Name of the student:

Signature: _____

Date: _____

Place of Submission: Department of medical laboratory science, school of allied sciences, college of health science, Addis Ababa University

Date of Submission_____

This thesis work has been submitted for examination with my approval as University advisor.

Name of the primary advisor:

Signature: _____

Date: _____