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Prevalence, Vertical Transmission, Antimicrobial Susceptibility Profiles of Group B Streptococci and Associated Factors Among Pregnant Women at Selected Public Health Facilities In Bahir Dar, Northwest Ethiopia

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ACRONYMES

ACOG……..American College of Obstetrics and Gynecology

AST…….…….....Antimicrobial Susceptibility Test

BAB…….…….....Blood Agar Base

BAP…….…….....Blood Agar Plate

BDAZHO…….…….....Bahir Dar Administrative Zone Health Office

BDHC……Bahir Dar Health Center

CAMP…….…….....Christie, Atkins, Munch-Peterson

CLSI…….…….....Clinical Laboratory and Standard Institute

EDHS…….…….....Ethiopian Demographic and Health survey

EOND…….…….....Early Onset Neonatal Disease

FHCSH……..…….....Felege Hiwot Comprehensive Specialized Hospital

GBS…….....Group B Streptococci

IAP…….....Intra-partum Antibiotic Prophylaxis

LOND…….....Late Onset Neonatal Disease

MHA…….....Muller Hilton Agar

PROM…….....Premature Rapture of Membrane

SBA…….....Sheep Blood Agar

SSA…….....Sub-Saharan Africa

TASH…….....Tikur Anbesa Specialized Hospital

THB…….....Todd Hewitt Broth

ABSTRACT

Background: *Streptococcus agalactiae* also called Group B Streptococci (GBS) is the leading cause of early onset neonatal sepsis, pneumonia and meningitis. However, there is no screening practice of pregnant women for the recto-vaginal colonization of GBS and data on the vertical transmission of GBS and its antibiotic susceptibility profiles are scarce in Ethiopia.

Objectives: To determine the prevalence of Group B streptococci colonization, and its proportion of vertical transmission and antimicrobial susceptibility profile of isolates among pregnant women at selected public health facilities in Bahir Dar, Northwest Ethiopia.

Methods: Health facility based cross-sectional study was conducted from March 1 to May 30, 2021. Recto-vaginal swabs from 292 pregnant women and the same number of pooled ear, nasal and umbilical swabs from newborns were collected. The swabs were processed and GBS isolates were identified following standard microbiological protocols. Antimicrobial Susceptibility Testing was performed using modified Kirby-Bauer disc diffusion method and results were interpreted based on the 2020 Clinical Laboratory and Standard Institute Guideline. Descriptive statistics and logistic regression analysis were computed. P-value < 0.05 was considered as cut-off for statistical significance.

Results: The overall prevalence of GBS colonization among pregnant women was 18.5% (n=54) with a vertical transmission proportion of 40.7% (n=22). Group B Streptococci colonization was significantly associated with preterm delivery (AOR=2.77, 95% C-I: 1.14-6.68) and history of still birth (AOR=3.13, 95% C-I: 1.13-8.70) in pregnant women. GBS isolates showed high level of resistance to tetracycline (77.8%) and considerable level of resistance to clindamycin (35.2%), erythromycin (25.9%) and Ceftriaxone (22.2%). Highest susceptibility was observed to vancomycin (92.6%) and chloramphenicol (87%). Overall, 19 (35.2%) GBS isolates were MDR.

Conclusion: Maternal GBS colonization, its transmission to their newborns and resistance of isolates to the commonly used and recommended drugs connected with MDR are critical problems. Therefore, regular antenatal care follow up, timely screening of pregnant women for GBS colonization and antimicrobial susceptibility testing of isolates are necessary for proper antibiotic prophylaxis, and prevention of disease consequences to newborns.

Key words: GBS, maternal colonization, vertical transmission, antimicrobial susceptibility.

1. INTRODUCTION

1.1 Background

Streptococcus agalactiae (S. agalactiae), also known as Group B Streptococcus (GBS), was first recognized in 1887 by Edmond Nocard as a source of bovine mastitis that resulted in agalactia (lack of milk production). Decades later, this organism identified as a human pathogen responsible for infections, most commonly in pregnant women and newborns. In 1970s, this pathogen emerged as a predominant causative agent of bacteremia and meningitis in newborns and young infants less than three months old with a frequency of 2-3 cases per 1,000 live births and case-fatality rates as high as 50% (Phares *et al.*, 2008, Verani *et al.*, 2010, Cheng *et al.*, 2019).

Group B streptococci can be found in up to one-third of healthy asymptomatic pregnant women colonizing normal gastro-intestinal and genitourinary tracts (Russell *et al.*, 2017a). This colonization may be transient, chronic or intermittent (Kwatra *et al.*, 2014). Besides to colonization, GBS can cause urinary tract infection (UTI), bacteremia, cystitis, amnionitis, endometritis, puerperal sepsis and pelvic abscesses during the gestational and postpartum period. It can also result stillbirth following infection of the endometrium, placenta or amniotic sac (Darvin Scott Smith, 2018).

Group B streptococcus is also a leading cause of neonatal sepsis, pneumonia and meningitis, often associated with high morbidity, mortality and developmental disabilities. Neonatal GBS disease can be classified as early-onset neonatal disease (EOND), that occurs in less than 7 days after birth in which 90% occur in the first 12 hours and usually resulting from vertical transmission of GBS from colonized pregnant women during or just before delivery, and late-onset neonatal disease (LOND) (onset of disease in 7 to 89 days of age), that can be acquired from the mother or from the environmental sources horizontally (Hanna and Noor, 2020).

Recto-vaginal colonization of pregnant women with GBS is the primary risk factor for EOND. Prolonged rupture of membrane, prematurity, chorioamnionitis, young maternal age, history of UTI and previous newborn with EOND are among the factors that can increase risk of disease among newborns (Russell *et al.*, 2017b). Hence, it is important to identify clinically these women before delivery and provide chemoprophylaxis. The American College of Obstetricians and Gynecologists (ACOG) recommends performing universal GBS screening between 36 0/7-37 6/7 weeks of gestation and all women whose recto-vaginal

cultures are positive should receive appropriate intra-partum antibiotic prophylaxis (IAP), unless a pre-labor cesarean birth is performed in the setting of intact membranes. In the absence of IAP, up to 50% of neonates born from colonized women acquire GBS and 1–2 % of these neonates develop invasive diseases (ACOG, 2020).

The widespread screening of pregnant women for GBS colonization in the third trimester and subsequent provision of IAP dramatically reduces the risk of EOND. For instance, an 80% IAP coverage is expected to lower the risk of EOND from 1.1% to 0.3% (Russell *et al.*, 2017b). However, most of the sub-Saharan African (SSA) countries including Ethiopia do not have clear strategies for prevention of GBS (Gizachew *et al.*, 2020). Though 7.5 - 25.5% prevalence of vaginal GBS colonization in pregnant mothers has been reported in previous studies from other parts of Ethiopia, to date, there is no practice of IAP provision (Woldu *et al.*, 2014, Gizachew *et al.*, 2019a). Thus, studies on maternal GBS colonization, its vertical transmission and factors linked with colonization are necessary to device preventive strategies.

In recent years, the widespread use of IAP coupled with over-prescription and unregulated use of antibiotics exacerbated the development of antimicrobial resistance among GBS isolates. The emergence of GBS isolates resistance to first-line antibiotics such as penicillin, increasing trend of resistance to second-line antibiotics (erythromycin, clindamycin), and the increased necessity of third-line antibiotics (vancomycin), in turn results emergence of multidrug resistant (MDR) GBS isolates. Moreover, evidences showed the increasing trend of anti-microbial resistance (AMR) in GBS isolates (Gizachew *et al.*, 2019a, Hayes *et al.*, 2020). Therefore, updated regional knowledge on the susceptibility profile of GBS isolates is also indispensable for administration of appropriate antibiotics for prophylaxis and treatment.

1.2 Statement of the problem

Group B streptococcus colonizes pregnant women in all regions of the world with an estimated worldwide maternal colonization prevalence of 18% with regional variation ranging 11–35% (Russell *et al.*, 2017a). The colonization prevalence of GBS is estimated to be as high as 22.4% in Africa (Kwatra *et al.*, 2016), 21.8% in SSA (Sinha *et al.*, 2016) and up to 25.5% in Ethiopia (Gizachew *et al.*, 2019a).

Group B streptococcus is a leading cause to adverse maternal and newborn outcomes. It is a leading cause of infant disease, particularly in the first week after birth with an estimated annual EOND of 205,000 cases worldwide. Furthermore, there are a minimum of 33,000 maternal GBS cases, 114,000 LOND cases and up to 3.5 million preterm births that could be attributed to GBS infection/colonization worldwide (Seale *et al.*, 2017).

Group B streptococcus is also a significant causes of death, with 57,000 stillbirths and 90,000 infant deaths estimated in 2015, which accounts for more than the total number of deaths from mother-to child transmission of human immunodeficiency virus (HIV), and more than the combined neonatal deaths from tetanus, pertussis, and respiratory syncytial virus. In addition, more than 10,000 new cases of neuro-developmental impairments per year are attributed to infant GBS meningitis (Seale *et al.*, 2017).

Africa had the highest burden of GBS cases, accounting 54% of all estimated worldwide GBS cases and 65% of stillbirths and infant deaths (Seale *et al.*, 2017). Particularly to SSA, incidence of EOND is high with 1.3 cases per 1000 live births, a burden of disease that is comparable to burden in high income countries prior to the practice of IAP (Sinha *et al.*, 2016).

Ethiopia is one of the SSA countries with high neonatal deaths. The 2016 Ethiopian Demographic and Health Survey (EDHS) reported neonatal mortality rate of 29 deaths per 1,000 births indicating that the progress of the country is far to achieve the Sustainable Development Goal (SDG) that plans all countries reduce neonatal mortality rate to 12 per 1,000 live births (EDHS and ICF, 2016). About 34.3% of neonatal deaths in the country is attributed to infection (Debelew *et al.*, 2014) and burden of neonatal disease due to GBS is estimated to be as high as 1.7% among infants less than 3 months (Ali *et al.*, 2020b). Though the specific contribution of GBS is not known, sepsis is the leading cause of neonatal admission (42%) and the second most common causes of neonatal death at Felege Hiwot Comprehensive Specialized Hospital (FHCSH, 2020).

The widespread use of antibiotics in various clinical conditions as well as their accepted efficacy of IAP in decreasing GBS EOND raised the emergence of antibiotic resistance among GBS isolates. Emergence of antibiotic resistance among GBS isolates is also a rising concern in Africa (Gizachew *et al.*, 2019b). Moreover, previous studies in Ethiopia isolated GBS that exhibited considerable level of resistance to penicillin (77%), ampicillin (54.3%) and vancomycin (17%) (Assefa, 2014, Mengist *et al.*, 2017, Fantahun *et al.*, 2020) which contradicts with reports from Western countries. Therefore, it is indispensable to conduct research on the susceptibility of GBS isolates to other antibiotics besides to those recommended as an alternative choice for prophylaxis or treatment of GBS infection.

Despite GBS is recognized to be an important cause of maternal and neonatal morbidity and mortality in many parts of the world, there is no routine screening program of GBS in Africa. The prevalence of GBS also varies across regions and timing of pregnancy. Data on the magnitude of GBS colonization among pregnant women, its proportion of vertical transmission, associated factors and drug susceptibility profiles of GBS isolates are scarce in Ethiopia, particularly to Bahir Dar city. Therefore, the present study was undertaken to provide updated information on the prevalence of GBS colonization, rate of vertical transmission and antibiotic susceptibility profiles of GBS isolates among pregnant women at selected public health facilities of Bahir Dar, Ethiopia.

1.3 Significance of the study

The result of this study will provide a local knowledge on the prevalence of GBS, rate of vertical transmission and antibiotic susceptibility profiles among pregnant women in the study area. The finding of the study is important to reduce and prevent the burden of GBS in pregnant women and neonates. It will be an input for the implementation of regular screening of pregnant women for GBS and subsequent prevention strategies such as IAP to protect EOND and its complications in the country. Results of antibiotic susceptibility profiles are vital to select the better drug for better treatment & management of patients. In addition, the finding of this study is important in slowing the spread of AMR. Pregnant mothers and their newborns benefited from this study in that their laboratory findings were communicated for their attending physicians for further follow up and management. Moreover, the findings of this study will be used as a baseline data for future related studies particularly on the outcomes of vertical transmission of GBS.

2. LITRATURE REVIEW

2.1 Bacteriology of Group B Streptococcus

Group B Streptococcus is the species representation for *Streptococcus* belonging to the Lancefield group B. It is an encapsulated facultative anaerobic chain forming Gram positive cocci that can grow well on blood agar plate (BAP). GBS on BAP forms colonies of 1-3 mm diameter, ß-haemolytic and grayish-white mucoid color. It is nutritionally fastidious, catalase and mannitol salt negative, pyrrolidonyl arylamidase (PYR) test negative, sodium hippurate hydrolysis test positive, bacitracin resistant, and Christie Atkins Munch Peterson (CAMP) test positive (Rosa-Fraile and Spellerberg, 2017, Apurba Sankar Sastry, 2018).

Group B streptococci possesses different virulence factors that contribute for host cell attachment, invasion, colonization and progression of invasive disease (Armistead *et al.*, 2019). The sialylated GBS capsular polysaccharide (CPS) represents one of the most critical virulence factors and, has two different antigen types; the group B specific antigen which is common for all strains and the type specific CPS which further divide GBS into 10 serotypes (Ia, Ib and II–IX). Serotypes Ia, Ib, II, III, and V accounted for the majority of diseases caused by GBS worldwide. Beyond this, GBS has also been classified by sequence type (ST) based on an allelic profile of seven different housekeeping genes and serotype III strains belonging to the ST-17 represent a hyper-virulent lineage, which causes a disproportionately high incidence of neonatal invasive disease and meningitis (Furfaro *et al.*, 2018). In addition, β-hemolysin/cytolysin (β-H/C), serine-rich repeat surface glycoproteins (Srr1/2), pilus proteins, and surface adhesins known to interact with extracellular matrix components are additional well-studied GBS virulence factors (Burcham *et al.*, 2019).

2.2 Acquisition of Group B Streptococcus in pregnant women and newborns

Having multiple sexual partners, frequent sexual intercourse, concurrent vaginal yeast cell infection and absence or decrease in hydrogen peroxide producing *Lactobacillus* are reported to be risk factors for acquisition of GBS colonization in pregnant mothers (Meyn *et al.*, 2002), though there are inconsistencies between reports. On the other hand, the pathogenesis of GBS in neonates starts with the asymptomatic colonization of recto-vaginal tract of pregnant women. Neonates acquire GBS infection vertically either by ascending infection through the placental membranes that initiate in utero infection of the fetus or by aspiration of infected vaginal fluids during the process of vaginal delivery (Vornhagen *et al.*, 2017).

2.3 Prevention of vertical transmission of Group B Streptococcus

The use of intravenous IAP to prevent EOD of GBS in infants was first studied in the 1980s (Verani *et al.*, 2010). IAP is effective in reducing neonatal GBS disease and indicated for all GBS carriers before 4 hours of delivery except for those in whom cesarean delivery is planned in the absence of labor or membrane rupture (ACOG, 2020). Penicillin and ampicillin have each been demonstrated in controlled clinical trials to be effective in preventing EOND caused by GBS when administered during labor. First generation cephalosporin's (cefazolin) is recommended for women who reported penicillin allergy and indicates a low risk of anaphylaxis or is of uncertain severity. For women with a high risk of anaphylaxis, clindamycin is the recommended alternative to penicillin, only if the GBS isolate is known to be susceptible to clindamycin. If isolates are resistant to clindamycin and clients are at high risk of anaphylaxis to penicillin, vancomycin is recommended (ACOG, 2020).

2.4 Epidemiology of maternal Group B Streptococcus colonization

The prevalence of GBS colonization in pregnant women varies widely throughout the world. This could be due to methodological difference to identify GBS, time of GBS screening in pregnancy or true differences in GBS maternal colonization prevalence with variation by region, socio-economic status, ethnicity and other socio demographic and clinical factors (Lekala *et al.*, 2015, Russell *et al.*, 2017a, Akkaneesermsaeng *et al.*, 2019).

A review on 390 articles retrieved from 85 countries with a total of 299,924 pregnant women documented an adjusted estimate of 18% maternal GBS colonization worldwide with regional variation ranging 11-35%. The highest prevalence of GBS colonization was from the Caribbean (35%) and lower prevalence was reported in Southern Asia (12.5%) and Eastern Asia (11%) (Russell *et al.*, 2017a).

A meta-analysis study on the prevalence of vaginal GBS colonization and preterm births in India, on 9778 pregnant women, reported 7.8% prevalence of GBS colonization. Specifically, 4.8%, 5.9% and 7% Prevalence of GBS colonization was documented from vaginal, rectal, and ano-rectal samples swabbed, respectively (Ashary *et al.*, 2020).

In another systematic review and meta-analysis of 30 studies in Iran, the pooled prevalence of GBS colonization in 10,090 Iranian pregnant women was estimated to be 13.65%. In this study, GBS was isolated in 11.96%, 13.65% and 25.63% of vaginal, vaginal and rectal, and anal and vaginal samples, respectively (YektaKooshali *et al.*, 2018). On the other hand,

relatively higher colonization prevalence was reported in Jordan (19.5%), that was conducted on vaginal-rectal swabs (Clouse *et al.*, 2019).

On a historical cohort study between 2003 and 2015 at Duke health affiliated hospitals, North Carolina, USA, 21.6% of the 60,029 deliveries were colonized with GBS (Edwards *et al.*, 2019). On the other hand, a high prevalence (35%) of GBS colonization were reported in retrospective cross-sectional study carried out in Virginia, USA (Kum-Nji *et al.*, 2020). Similarly, a relatively higher prevalence of maternal colonization was reported in a study from UK (Rao *et al.*, 2017).

A retrospective study that retrieved data from7,726 pregnant women in China between 2009 and 2014 reported a prevalence of 8.2% GBS vaginal colonization (Ji *et al.*, 2017). Similarly, another study in western china in 2017 also reported a colonization rate of 6.1% (Chen *et al.*, 2018a).

A meta-analysis of GBS maternal colonization of Africa from 1989 to 2019 on 57 studies with 22,206 pregnant women documented 19.3% pooled estimate of the recto-vaginal maternal GBS colonization. In this review, the highest estimate was observed in Southern sub-region of Africa with a prevalence of 23.8%, followed by 22.7% in Northern Africa, while the lowest was driven from the Eastern Africa with 15.4% prevalence (Gizachew *et al.*, 2019b).

In another recent studies from African countries, a high prevalence of GBS colonization of pregnant mothers were reported in South Africa (48.2%) (Lekala *et al.*, 2015), Gambia (33.7%0 (Le Doare *et al.*, 2016), Uganda (28.8%) (Namugongo *et al.*, 2016), Egypt (26.5%) (Sadaka *et al.*, 2018), Gabon (19%) (Belard *et al.*, 2015) and a relatively lower maternal GBS colonization prevalence was reported in Kenya (12%) (Seale *et al.*, 2016). The high prevalence report in South African study was attributed probably due to adequate culture methods used which included use of enrichment and selective media, and multiple site sampling.

In Ethiopia, published studies starting from 1987 to now reported variable GBS colonization rate that ranges from 7.5% to 25.5% (Woldu *et al.*, 2014, Gizachew *et al.*, 2019a). On studies conducted at different times in Addis Ababa, Ethiopia; variable GBS colonization results were reported. The first one was conducted at Gandhi Memorial Hospital and Tikur Anbessa Specialized Hospital (TASH) in 2010 on 300 pregnant women from lower genital tract and

rectum samples, and 7.5% colonization rate was reported (Woldu *et al.*, 2014). On another study in 2014 on a total of 281 pregnant women from three antenatal clinics (ALERT Center, Alem Bank and Woreda 03 Health center) in which vaginal swab samples were taken, an overall prevalence of GBS colonization among pregnant women was 14.6% (Assefa, 2014). In another study from March to August 2015 at TASH on 280 pregnant women screened for GBS, colonization rate were reported to be 23.2% (Ali *et al.*, 2020b). In addition, Fantahun et al recently reported an overall GBS colonization prevalence of 23.6% from maternal vaginal swabs of 250 pregnant women coming for delivery at TASH (Fantahun *et al.*, 2020).

On another prospective cross-sectional study conducted from December 2016 to November 2017 at University of Gondar Referral hospital on 385 pregnant women from combined recto-vaginal swabs, an overall maternal GBS colonization prevalence of 25.5% was reported (Gizachew *et al.*, 2019a). Other studies in Ethiopia has also reported different GBS colonization prevalence including; 13.7% in Mekelle (Alemseged *et al.*, 2015), 11.3% in Adigrat (Gebremeskel *et al.*, 2015), 13.2% in Adama (Ali *et al.*, 2020a), 15.7% in Hawassa (Ali *et al.*, 2019), 12.2% in Nekemte (Mengist *et al.*, 2017), 13.68% in Harar (Yadeta *et al.*, 2018a), and 8.5% in Arbaminch (Shiferawu *et al.*, 2019).

Studies show that high rate of GBS colonization is often obtained if women are followed up for a certain period of time, both the rectal and vaginal sites are investigated and adequate culture methods are used which included use of enrichment and selective media. Falsely lower rates are attributed to false negative culture due to inadequate swabbing technique or poor handling, specimen storage conditions, and prolonged transport (Lekala *et al.*, 2015). Different positive culture rates are also attributed to use of different culture media (Shirazi *et al.*, 2014).

2.5 Magnitude of Group B Streptococcus vertical transmission

Vertical transmission of GBS can occur during pregnancy or at time of birth process from genitourinary or gastrointestinal tract of colonized pregnant women(Scasso *et al.*, 2015). Worldwide vertical transmission of GBS shows considerable variation within and between geographic regions, ranging from 7.6% in china (Chen *et al.*, 2018a) and 11.2% in Germany (Kunze *et al.*, 2011) to 65% in India (Sridhar Santhanam *et al.*, 2017).

Lower rate of vertical transmission was reported from studies conducted in China with 7.6% vertical transmission rate that takes one sample swab from newborns (Chen *et al.*, 2018a) and

14.1% (13/92) on another study using sample from the ear canal, throat, and umbilical swab immediately after birth (Chen *et al.*, 2018b).

A relatively high (65%) vertical transmission was reported in a similar study done in India from umbilical and external ear swabs taken within 30 minutes of delivery (Sridhar Santhanam *et al.*, 2017). Similarly, in a study conducted in Gambia in which nasopharyngeal and rectal swabs were taken from neonates within four hours after birth, a vertical transmission of 57.7% (146/253) were reported (Le Doare *et al.*, 2016). On the other hand, a relatively moderate vertical transmission (38%) was reported in a study conducted in Bangladesh (Saha *et al.*, 2017).

On a mother and newborn pair study by Ali et al. in three hospitals of Ethiopia in which swabs from external ear, nasal area, throat and umbilical area were collected, a vertical transmission rate of 49.2% (32/65), 56.8% (21/37) and 59.1% (26/44) was reported in Addis Ababa, Adama and Hawassa respectively (Ali *et al.*, 2019, Ali *et al.*, 2020b, Ali *et al.*, 2020a).

In another study at Addis Ababa, Ethiopia on a total of 250 newborns from oropharyngeal swab samples collected immediately after delivery; a vertical transmission rate of 47.4% was reported (Fantahun *et al.*, 2020). Similar to this, another study conducted in Harar, Ethiopia; a vertical transmission rate of 45 % was reported from swab samples taken at least from two sites (the ear canal, umbilicus, axilla, groin, or nose) within 6 hours after birth (Yadeta *et al.*, 2018b). Furthermore, high proportion of vertical transmission (63.3%) was reported in a study conducted in Gondar, Ethiopia; in which swabs were collected from ear, nasal and umbilicus of newborns (Gizachew *et al.*, 2020).

The variation in the percentage of vertical transmission of GBS among regions and different parts of Ethiopia might be due to differences in sampling sites. Significant difference is observed when multiple sampling sites are used and when only two or less sampling sites are used (Chen *et al.*, 2018b). In addition; variability in the sample size, methods employed for GBS detection, level of laboratory facilities, newborn body surface sites swabbed and time of sample collection after birth might be also the possible explanations for the disparities. Furthermore; the presence or absence of the IAP administration, variations of maternal colonization and density of GBS colony and mode of delivery are the reason for the existing differences (Chen *et al.*, 2018b, Gizachew *et al.*, 2020).

2.6 Antibiotic susceptibility profile of Group B Streptococcus isolates

The accepted efficacy of IAP in decreasing GBS EOND coupled with the widespread use of these antibiotics in various clinical conditions has raised the emergence of antibiotic resistance among GBS isolates. Emergence of antibiotic resistant GBS isolates is considered as an increasing problem so that it necessitates testing 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 (Hayes *et al.*, 2020).

On a study conducted in Brazil on 136 identified GBS isolates, all were found susceptible for penicillin, vancomycin and cefotaxime, whereas, 5.9%, 8.1% and 82.3% were found resistant to erythromycin, clindamycin and tetracycline, respectively (Melo *et al.*, 2016).

A 100% sensitivity to the antibiotics penicillin, ceftriaxone, linezolid and vancomycin were reported at a study conducted in china on 636 identified GBS isolates, whereas 52.4% were resistant to clindamycin, 25.9% were resistant to levofloxacin and 64.9% were resistant to erythromycin (Ji *et al.*, 2017). On the other hand, in another study conducted in China on 119 isolate, 79.9% were found MDR (Gao *et al.*, 2018). Similarly, a study from Saudi Arabia, all (178) GBS isolates, were sensitive to penicillin G, ampicillin and vancomycin. However, GBS isolates revealed 15.7% and 5.1% of resistance to erythromycin and clindamycin, respectively in this study (Khan *et al.*, 2015).

A relatively similar finding was reported from a study done in Alexandria, Egypt, on a 53 GBS isolates, in which all were susceptible to penicillin, ampicillin, ceftriaxone, cefotaxime, cefepime, vancomycin, and linezolid. However, 43.4%, 28.3%, 22.6%, and 15% of isolates were found to be resistant to levofloxacin, azithromycin, erythromycin, and clindamycin, respectively (Sadaka *et al.*, 2018).

A meta-analysis done in Ethiopia on the pooled proportion of antibiotic resistance to GBS isolated from pregnant women in Africa from 1996 to 31th January 2019 against 10 antibiotics showed a resistance of 19.6% to clindamycin, 19.7% to vancomycin, 20.8% to erythromycin, 24.5% to ciprofloxacin, 25.9% to ceftriaxone, 26.7% to ampicillin, 27.3% to chloramphenicol, 31.4% to amoxicillin, 33.5% to penicillin, and 82.6% to tetracycline (Gizachew *et al.*, 2019b).

Group B Streptococcus isolates show a relatively increased level of resistance to first line antibiotic (penicillin) in a study conducted in Addis Ababa, Ethiopia in 2010, in which 10 out

of 22 GBS isolates (45%) were found to be resistant against penicillin. In this study, 20 (91%) were sensitive to ampicillin and all isolates except one were sensitive to erythromycin (Woldu *et al.*, 2014). In another study in Addis Ababa, Ethiopia in 2014; out of 41 GBS isolates, all were susceptible to chloramphenicol. In this study, resistance to tetracycline, cefotaxime, clindamycin, penicillin, vancomycin, ampicillin and erythromycin was found in 90.2%, 34.1%, 26.8%, 19.5%, 17%, 14.6% and 7.5% of GBS isolates, respectively. In this study, 43.6% 0f the isolates were found MDR (Assefa, 2014). In another recent study in 2018 in Addis Ababa, Ethiopia, out of 59 identified GBS isolates, the highest resistance level was recorded to cefepime (59.4%) followed by penicillin (57.7%) and ampicillin (54.3%). Vancomycin, clindamycin and chloramphenicol were the most active drugs for GBS isolates with susceptibility results of 93.3, 86.4, and 79.6%, respectively. Moreover, 5% isolates of GBS isolates showed intermediate sensitivity to erythromycin and chloramphenicol for each (Fantahun *et al.*, 2020).

A study conducted in Jimma, Ethiopia in 2012, all (24) GBS isolates were susceptible to penicillin G, ampicillin, and vancomycin but a considerable proportion was found to be resistant to clindamycin (3.2 %), erythromycin (6.5 %), ciprofloxacin (9.7 %), ceftriaxone (9.7 %), norfloxacin (12.9 %), cotrimoxazole (29 %), and tetracycline (45.2 %) (Mengist *et al.*, 2016). In another study in this town in 2015; out of 22 GBS isolates, majority of them were sensitive to ampicillin and penicillin G with 95.5% and 90.1%, sensitivity respectively. Erythromycin and clindamycin were resisted by 50% and 40.9% of the GBS isolates, respectively, whereas gentamicin was resisted by all isolates (Girma *et al.*, 2020).

Similar to most above studies, a 100% (n=29) susceptibility to penicillin, ampicillin and vancomycin were reported on a study conducted at Hawassa, Ethiopia in 2012. In this study, resistance was observed against erythromycin (6.9%), tetracycline (48.2%), ceftriaxone (10.3%), chloramphenicol (51.7%), ciprofloxacin (13.8%) and norfloxacin (10.3%) (Mohammed *et al.*, 2012). Similarly, in a study conducted at Adigrat, Ethiopia in 2012; out of 17 isolates, all were susceptible to ampicillin, penicillin G, vancomycin, and amoxicillin. However, 2 isolates were resistant to erythromycin (11.8%) and three isolates to clindamycin (17.6%). Common resistant to erythromycin and clindamycin was found in two isolates (Gebremeskel *et al.*, 2015). In another similar study conducted in Mekelle, Ethiopia in 2014, all (n=19) of the GBS isolates were found susceptible to penicillin, ampicillin, vancomycin and erythromycin. However, in this study, 10.5% of the isolates were found MDR (Alemseged *et al.*, 2015).

High level of resistance to penicillin G (77.3%) was observed in a study conducted at Nekemte, Ethiopia in 2016. In this study, out of 22 GBS isolates 20 (91%) were sensitive to vancomycin, 41% of the isolates were intermediate against ceftriaxone, half of the isolates were sensitive to clindamycin and 36.4% of them were intermediate against erythromycin. In addition, all of the GBS isolates were found MDR in this study (Mengist *et al.*, 2017).

In a study conducted at Arbaminch, Ethiopia in 2016, out of 24 isolates, all were susceptible to penicillin, ampicillin and vancomycin. Resistance to ciprofloxacin, ceftriaxone, clindamycin, erythromycin, chloramphenicol and gentamicin was found to be 37.5%, 29.2%, 29.2%, 20.8%, 8.3%, and 4.2%, respectively and two showed MDR (Shiferawu *et al.*, 2019).

A considerable level of resistance to penicillin (10.2%) and ampicillin (9.2%) were observed in a study conducted at Gondar from 2016 to 2017 on 98 GBS isolates. In this study, the highest resistance was observed to tetracycline 72 (73.4%) followed by 31 (31.6%) to ceftriaxone, 26 (26.5%) to erythromycin, and 21 (21.4%) to clindamycin (Gizachew *et al.*, 2019a).

The observed differences in antimicrobial susceptibility patterns of GBS isolates against different drugs might be due to the occurrence of differences regarding bacterial strain, sample size, laboratory procedures, bacterial load, laboratory facility, drug control policies and awareness of the community towards drug resistance.

2.7 Factors associated with maternal Group B Streptococcus colonization

Identifying risk factors and reducing risk is one of the most important mechanism in prevention of maternal and neonatal GBS colonization (WHO, 2017). A number of factors have been suggested to be related to the prevalence of colonization, including ethnicity, culture, maternal age, marital status, education, smoking and multiple sexual partners (Darling and Saurette, 2010). However, it is unclear that the relationships between these factors and actual colonization rates, and research results are inconsistent.

In a study conducted at Saudi Arabia; GBS colonization was found to be significantly associated with age, occupation, number of antenatal clinic visits, and gravidity (Arain *et al.*, 2015). In another study in Saudi Arabia, a higher GBS colonization rate was seen in pregnant women >40 years of age. In this study, women with gestational age >42 weeks were more frequently colonized than women with a gestational age from 41-42 weeks and an increased

rate of colonization was found in women who delivered >5 times and no colonization in women who delivered once (Khan *et al.*, 2015).

Contrary to the above Saudi Arabian study, in a study conducted at western china in 2017; age younger than 40 years was significantly associated (p=0.04) with higher GBS colonization rate in pregnant women (Chen *et al.*, 2018a). Similarly, in a meta-analysis study at India that analyzed 36 studies; maternal age showed a negative association with GBS colonization. In addition, this study showed pregnant women who had preterm delivery and premature rapture of membrane (PROM) were 7.9 and 5.5 times more likely to have GBS colonization, respectively than their counterparts (Ashary *et al.*, 2020).

In a study conducted at Thailand; teenage pregnancy, multi-parity and non-Buddhist religions were significantly associated with GBS colonization with a respective AOR of 3.83, 3.59 and 1.87. However, intra partum risk factors (gestational age <37 weeks, rupture of membranes >18 hours, body temperature $>38^0$c) were not associated with GBS colonization (Akkaneesermsaeng *et al.*, 2019).

In a retrospective study at a tertiary care university teaching hospital in Virginia, USA, from 2011-2019; tobacco smoking during pregnancy was found the most significant predictor for GBS colonization. Women who smoked during pregnancy were twice more likely to be colonized than their non-smoking counterparts. In this study, maternal age was the only other significant predictor with younger mothers more than one and half times more likely to be colonized than their older counterparts (Kum-Nji *et al.*, 2020). In another cohort study in USA (North Carolina), in which a total of 60,029 deliveries were included for analysis; GBS colonization was significantly associated with maternal age at delivery, Black or African American race, and preexisting diabetes (Edwards *et al.*, 2019).

In a study conducted at southwestern Uganda, obesity was the only significant factor associated with ano-genital GBS colonization with odds ratio of 3.78 (Namugongo *et al.*, 2016). Whereas, history of more than one previous still birth was significantly associated with maternal GBS colonization in a study of Gambia (Le Doare *et al.*, 2016). On the other hand, in a study conducted at South Africa shows the frequency of GBS colonization was significantly associated with women who had no matric education, unemployed, having history of miscarriages and stillbirths, and HIV co-morbidity (all with p<0.0001) (Lekala *et al.*, 2015). Whereas, in a study at Alexandria, Egypt, prevalence of GBS colonization was

significantly associated with age group 20–30 (p=0.01), at 37 weeks of gestation (p=0.002) and among women who had frequent antenatal visits (p<0.001) (Sadaka *et al.*, 2018).

In a study conducted at Jimma, Ethiopia maternal GBS colonization was significantly associated with a history of preterm delivery (PTD) (AOR=6.3) and history of UTI during current pregnancy (AOR=6.4) (Girma *et al.*, 2020). Similarly, in another study in Harar, Ethiopia, maternal GBS colonization was significantly associated with still birth (AOR=8.93), frequency of ANC follow up (AOR=0.53), hypertensive disorders (AOR=4.66), prolonged labor (AOR=3.65) and low birth weight (AOR=1.81) (Yadeta *et al.*, 2018a).

In previous studies conducted in other parts of Ethiopia, GBS colonization was significantly associated with experiencing meconium stained amniotic fluid (AOR=3) and longer duration of PROM (AOR=1.8) in a study conducted in Gondar (Gizachew *et al.*, 2019a). On the other hand, history of contraceptive use (inversely related (AOR=0.43)) in a study conducted in Addis Ababa (Assefa, 2014), with gestational age >37 weeks (AOR=2.1) and married ones (AOR=1.8) in a study conducted in Nekemte (Mengist *et al.*, 2017), and with occupation and parity (being house wife and para 2-4 has decreased risk of colonization) in a study conducted in Addis Ababa (Fantahun *et al.*, 2020).

2.8 Factors associated with vertical transmission of Group B Streptococcus to newborns

Worldwide, vertical transmission of GBS shows considerable variation within and between geographic regions. Factors such as maternal fever during labor, PROM, preterm delivery, chorioamnionitis, maternal GBS sepsis, and lack of IAP administration are attributed for the occurrence of vertical transmission (Berardi *et al.*, 2014, Shah *et al.*, 2014).

In a study conducted at China, longer duration of rupture of membrane (≥12 h) (p<0.001) and longer duration of labor (≥4 h) (p<0.001) were significant risk factors for GBS colonization in infants (Chen *et al.*, 2018a). In another study in China, GBS vertical transmission was associated with the mode of delivery and sexually transmitted diseases (Chen *et al.*, 2018b). On the other hand, in a case control study conducted in France, GBS vertical transmission was significantly associated with obesity, fetal tachycardia and late preterm birth (35 to 36 weeks) (Dahan-Saal *et al.*, 2011). Similarly, in another study from Gambia, infants born before 34 weeks of gestation had increased odds of GBS colonization at birth (Le Doare *et al.*, 2016).

Term PROM, prolonged rapture of membrane ≥18 hours before delivery, intra-partum maternal fever, and mothers receiving of IAP ≥4 hours (negatively associated) were factors that were significantly associated with vertical transmission, in a study conducted at Harar, Ethiopia. In this study, all newborns exposed to IAP ≥4 hours were found to be not colonized (Yadeta *et al.*, 2018b). In another study in Ethiopia conducted at Gondar, Ethiopia; lower maternal educational level, employment and lower ANC visit were significantly associated risk factors to GBS vertical transmission (Gizachew *et al.*, 2020).

3. OBJECTIVES

General objective:-

- To determine the prevalence of GBS colonization and its antimicrobial susceptibility profiles among pregnant mothers and their newborns, and identify the associated risk factors at selected public health facilities in Bahir Dar city, Northwest Ethiopia.

Specific objectives:-

- To determine the prevalence of GBS colonization among pregnant women in selected public health facilities of Bahir Dar city.
- To determine the proportion of GBS vertical transmission to newborns.
- To determine susceptibility profile of GBS isolates to selected antibiotics.
- To identify factors associated with GBS colonization in pregnant women.
- To identify factors associated with vertical transmission of GBS.

4. MATERIALS AND METHODS

4.1 Study area

This study was conducted in Bahir Dar town, Northwest Ethiopia. Bahir Dar is the capital city of Amhara National Regional State which is located at 565 km from Addis Ababa. Based on the 2020 population projection, the current population size of the city is estimated to be 389,177, among which 46% are females (BDAHO., 2020). The city has two public specialized hospitals (Felege Hiwot Comprehensive Specialized Hospital (FHCSH)) and Tibebe Ghion Specialized Hospital (TGSH), one public primary hospital (Addisalem Hospital), and ten public health centers. In 2020, a total of 14,612 deliveries were documented in health facilities of Bahir Dar town (BDAHO., 2020). In this study, FHCSH and Bahir Dar Health Center (BDHC) were included based on their high client flow for ANC and delivery services.

4.2 Study design and period

A health facility based cross-sectional study was conducted from March 1 to May 30, 2021

4.3 Population

4.3.1 Source population

All pregnant women attended in FHCSH and BDHC for delivery service and their newborns

4.3.2 Study population

All pregnant women who were admitted at FHCSH and BDHC for vaginal delivery service and their newborns

4.3.3 Study participants

Pregnant women who were admitted at FHCSH and BDHC for vaginal delivery service and their newborns that were actually participated in this study

4.3.4 Inclusion criteria

All pregnant women admitted to labor and delivery room for vaginal delivery and their newborns were included in the study

4.3.5 Exclusion criteria

Pregnant women with history of antibiotic use within two weeks prior to recruitment, those who used vaginal cream, lubricants or traditional sterilizer, those who plan cesarean delivery, and those who are mentally unstable were excluded.

4.4 Sample size determination

The sample size was determined using single population proportion formula ($n= (Z\alpha/2)^2 * p (1-p)/d^2$) where, n=sample size, z=level of confidence according to the standard normal distribution, p=sample proportion and d= tolerated margin of error. Therefore, by taking z= 1.96 for a level of confidence of 95%, p= 0.255: proportion of GBS colonization among pregnant women taken from Gondar, Ethiopia (Gizachew *et al.*, 2019a) and 5% margin of error. The sample size was calculated as $n = (1.96)^2 \times (0.255) (1-0.255)/ (0.05)^2 = 292$. Thus, a total of 292 pregnant women and equal number of their newborns were included in the study. This sample size was proportionally allocated into FHCSH and BDHC based on their last year average number of 3 month delivery report from March to May, 2012 E.C. Thus, 192 and 100 pregnant women and their newborns were taken from FHCSH and BDHC, respectively.

4.5 Sampling technique

Convenient sampling technique was used. Samples were collected consecutively from those pregnant mothers who fulfilled the inclusion criteria until the determined sample size was reached.

4.6 Variables of the study

4.6.1 Dependent variables

Maternal GBS colonization, its vertical transmission and AST

4.6.2 Independent variables

Socio-demographic factors: (Age of the mother, marital status, residence, occupation and level of education)

Clinical and obstetric factors: Gravidity, gestational age, number of ANC visits, history of hormonal contraceptive use, history of abortion, history of still birth, history of neonatal death, history of UTI during current pregnancy, history of STI during current pregnancy, chronic illness during current pregnancy, history of antibiotic use during current pregnancy, PROM, duration of labor, meconium stained amniotic fluid, HIV status, status of newborn, sex of newborn, birth weight of newborn, 5^{th} minute Apgar score.

4.7 Data collection

4.7.1 Demographic and clinical data collection

Data on demographic, obstetric and other clinical related variables were collected by attending midwives using a pre-tested structured questionnaire with face-to face interview and was complemented with medical record review.

4.7.2 Specimen collection and transportation

A combined recto-vaginal swab was collected from the mother at the point of labor by trained midwives following universal precautions using a sterile cotton applicator swab. Maternal samples were collected as per the ACOG Committee opinion and American Society for Microbiology (ASM) recommendations. Accordingly, a single swab specimen was collected first from the lower vagina and then from the rectum (through the anal sphincter) without use of a speculum (ACOG, 2020, Filkins *et al.*, 2020). Immediately after delivery, swabs were collected from the newborns. In order to assure vertical transmission, sampling from the newborns was made within 30 minutes of birth and before any further handling and/or wiping of the newborns. Ear, nasal and umbilical samples were swabbed from newborns using different sterile cotton applicator swab and pooled in to a test tube with Amies transport medium. The collected samples were transported to Microbiology laboratory of Bahir Dar University immediately and processed following standard bacteriological techniques as described in the ACOG opinion, ASM and Monica Cheesbrough (ACOG, 2020, Cheesbrough, 2006, Filkins *et al.*, 2020).

4.7.3 Specimen processing

The collected swabs were placed in Todd Hewitt selective enrichment broth (Oxoid, UK) supplemented with gentamicin (8µg/ml) and nalidixic acid (15µg/ml). The inoculated selective medium was incubated for 24 h. at 37 °C. Growth (turbidity) was sub-cultured onto 5% Defibrinated Sheep-Blood Agar (SBA) (Oxoid, UK) and incubated for 24 h at 37 °C in 5% CO2 atmosphere. Pin pointed, with narrow beta-hemolysis colonies were considered as presumptive GBS and subjected to Gram stain and catalase test. All Gram positive cocci and catalase negative isolates were further tested for CAMP factor and bacitracin resistance test as a final identification of GBS (ANNEX VI).

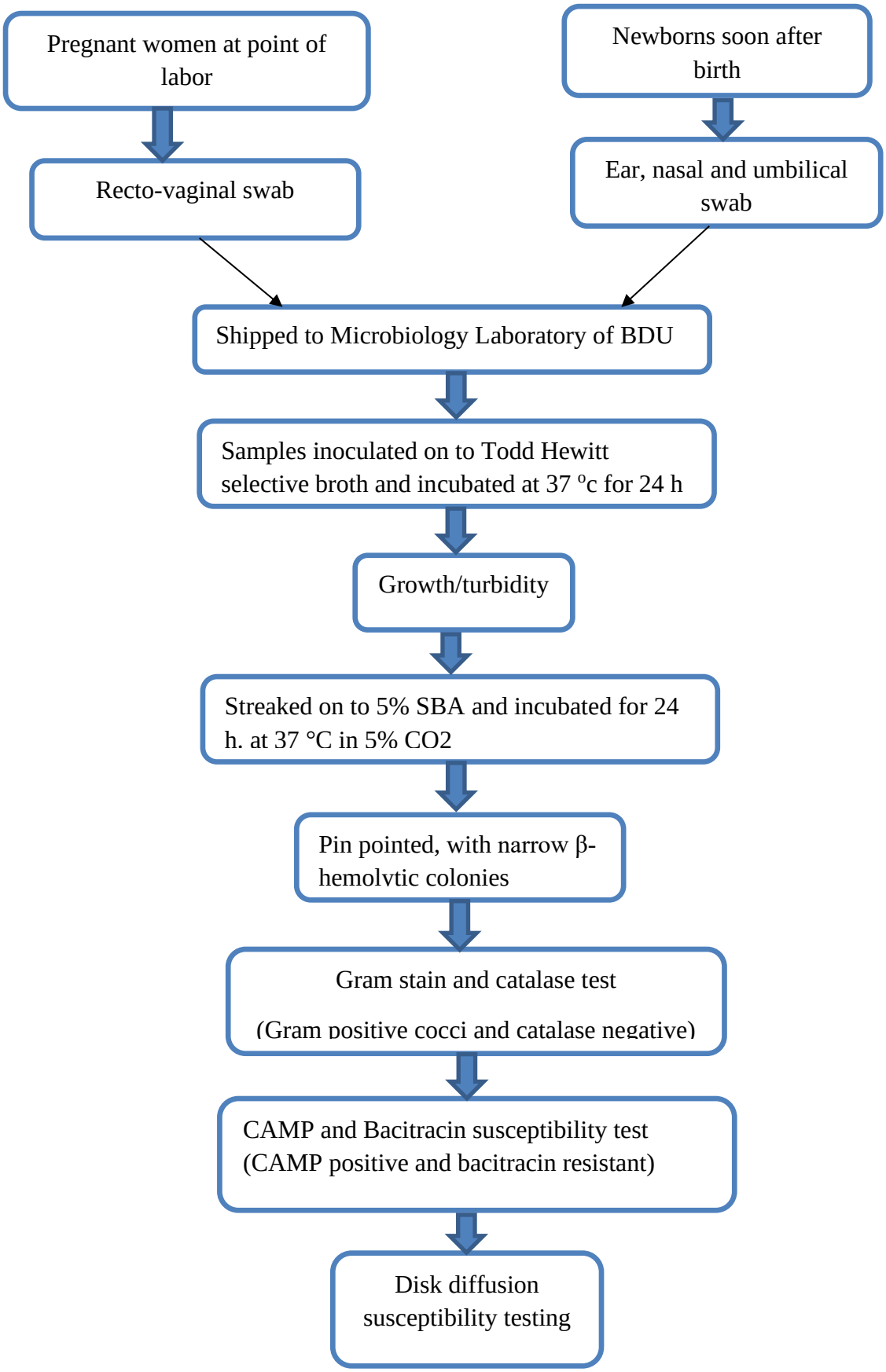

Figure 1: Flow chart for specimen processing, identification of GBS, and AST

4.7.4 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) for all GBS isolates of the mother was tested using the Kirby-Bauer disk diffusion method. Bacterial inoculum was prepared from 4-5 freshly grown GBS colonies in 3-5 ml sterile physiological saline and the turbidity was adjusted using 0.5 McFarland standards. Sterile cotton swab was dipped and rotated several times, and was pressed against the wall of the test tube. It was then streaked over the entire surface of the Mueller Hinton Agar (MHA) (Oxoid, UK) containing 5% Defibrinated sheep blood. Then, antibiotic impregnated paper disks were placed on the plate and incubated in 5% CO2 atmosphere at 37 °C for 24 h. The antibiotics tested were selected based on CLSI 2020 guideline and their availability, and includes penicillin G (P, 10IU), ampicillin (AM, 10µg), clindamycin (CLY, 2µg), erythromycin (E, 15µg), chloramphenicol (C, 30µg), ceftriaxone (CRO, 30µg), vancomycin (VA, 30µg), and tetracycline (TE, 30µg) (Oxoid, UK). Zone of inhibition around antibiotic disks were measured by calibrated ruler and interpreted as sensitive, intermediate or resistant by using standard chart (CLSI, 2020). MDR profile of GBS isolates were calculated by counting isolates that are resistant to three or more antibiotics from different classes and dividing it to total number of isolates.

4.8 Quality control

The questionnaire was adapted from other similar studies and initially prepared in English and was translated to Amharic and then translated back to English by other translator to check for consistency. Before data collection, a pretest was conducted in Tibebe Ghion Specialized Hospital on 5% of the sample size. Based on the result of the pretest, adjustments were made accordingly to the data collection tool. Spot checks on the quality of data collection were made at the study site and completeness of questionnaires was also checked regularly. Data collectors were given one day training on the study objectives, method of data and sample collection, and the tools for data collection.

Sterility of SBA and MHA with 5 % sheep blood, and Todd Hewitt Broth (THB) were checked by incubating 5% of the media overnight at 35-37 ºC without specimen inoculation. American Type Culture Collection (ATCC) standard reference strains ((*E. coli)* (ATCC 25922), *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 25923), *S. pyogenes* (ATCC 19615) and *S. agalactiae* (ATCC 27956)) were used as a quality control throughout the study for THB, bacitracin test, CAMP test and AST with known susceptibility to the antimicrobial agents. The performance of THB was checked by inoculating the broth with known Gram negative bacteria (*E. coli)* (ATCC 25922) and known *S. agalactiae* (ATCC 27956) to check

its selectivity for the growth of only Gram positive bacteria. The purity of the colonies was checked by sub culturing single colonies onto a SBA and by doing Gram stain.

4.9 Operational definitions

Vertical transmission of GBS: transmission of GBS from colonized mother to her neonate before or during delivery.

Gravidity: The number of times that a woman has been pregnant.

Premature rapture of membrane (PROM): Rapture of membrane one hour before the start of labor.

Meconium stained amniotic fluid: Release of amniotic fluid that is mixed with first stool (meconium) of the baby.

4.10 Data management and analysis

Data was cleaned, coded and entered first into Epi Data version 4.6 to minimize error and was exported to IBM SPSS Statistics Version 26 for further analysis. Descriptive statistics were computed to describe relevant variables. Chi-square and fisher's exact tests were calculated to identify variables significantly associated with vertical transmission of GBS.

Logistic regression analysis was carried out to assess the association between independent variables and maternal GBS colonization. Majority of the variables were fitted to the bivariable logistic regression. Then all variables with a p-value of <0.2 in the bivariable analysis were further included in the multivariable logistic regression analysis. Multivariable logistic regression model was fitted to control confounders and to get the independent predictors of maternal GBS colonization. Hosmer & Lemeshow goodness of fit test was checked for insignificance ($p>0.05$) to indicate that the model is adequate and it was found insignificant ($p=0.904$). Presence of multicollinearity was also checked by using variance inflation factor (VIF), and no multicollinearity was detected. Backward stepwise regression was used. Variables with p-values <0.05 were considered as significant predictors.

Ethical considerations

Ethical approval was obtained from Institutional Review Board of College of Medicine and Health Sciences, Bahir Dar University. Informed written consent from the mothers and assents from them for the newborns was obtained after explaining the purpose and benefits of the study, confidentiality of the information and the voluntary nature of participation in the

study. Mothers were assured that refusal to participate in the study did not affect their service utilization in the health institution. The laboratory findings were linked to the attending physician/s for further follow up and management of mothers and neonates with culture confirmed GBS colonization. Confidentiality of results were kept by anonymizing and numbering/coding names of the study participants, storing in a locked cabinet, and by documenting data files on password protected personal computer.

5. RESULTS

5.1 Socio-demographic characteristics of pregnant women

Overall 292 pregnant women who came for vaginal delivery services were took part in the study. Of whom, 192 were from FHCSH and 100 were from BDHC. The median age of mothers was 26 years and more than half (51%) were below or equal to the age of 26. Near to all (95.9%) of the mothers were married and majorities (77.4%) were urban dwellers. The majority of mothers (33.9%) had a primary level of education and 43.2% were housewives (Table 1).

Table 1: Socio-demographic characteristics of pregnant women from FHCSH and BDHC Bahir Dar, 2021

Variables	**Frequency N (%)**
Health facility	
Felege Hiwot Comprehensive Specialized Hospital	192 (65.8)
Bahir Dar Health Center	100 (34.2)
Age of the mother (years) (median age=26)	
≤ 26	149 (51.0)
> 26	143 (49.0)
Marital status	
Not married	8 (2.7)
Married	280 (95.9)
Divorced	4 (1.4)
Residence	
Urban	226 (77.4)
Rural	66 (22.6)
Level of education	
No formal education	33 (11.3)
Grade 1-8	99 (33.9)
Grade 9-12	67 (22.9)
College or University	93 (31.8)
Occupation	
Civil servant	27 (9.2)
Student	5 (1.7)
Farmer	64 (21.9)
Housewife	126 (43.2)
Business woman	60 (20.5)
Daily laborer	10 (3.4)
Total	**292 (100)**

5.2 Demographic characteristics of newborns

Of the 292 newborns participated in this study, 153 (52.4%) were females and majorities (93.5%) had ≥ 2500 gram of birth weight. From the total newborns, 5 (1.7%) were stillbirths and 14 (4.8%) had < seven 5th minute Apgar score (Table 2).

Table 2: Demographic characteristics of newborns from FHCSH and BDHC, Bahir Dar, 2021

Variables	**Frequency N (%)**
Sex of newborns	
Male	139 (47.6)
Female	153 (52.7)
Weight of newborns (Grams)	
< 2500	19 (6.5)
≥ 2500	273 (93.5)
Status of newborn at delivery	
Alive	287 (98.3)
Still birth	5 (1.7)
5th minute Apgar score	
< 7	14 (4.8)
7-10	278 (95.2)
Total	**292 (100)**

Apgar: appearance, pulse, grimace, activity, respiration

5.3 Prevalence of GBS colonization and its proportion of vertical transmission to newborns

Overall, 54 (18.5%) (95% CI: 14 -22.6) of the pregnant women had GBS colonization. Out of 292 screened newborns, 22 (7.5%)(95% CI: 4.9 -10.6) harbored GBS on their body surface (Figure 2).

The overall proportion of vertical transmission of GBS from the colonized pregnant women to their newborn was 22/54 (40.7%) (95% CI: 22.8 -57.4) (Figure 3).

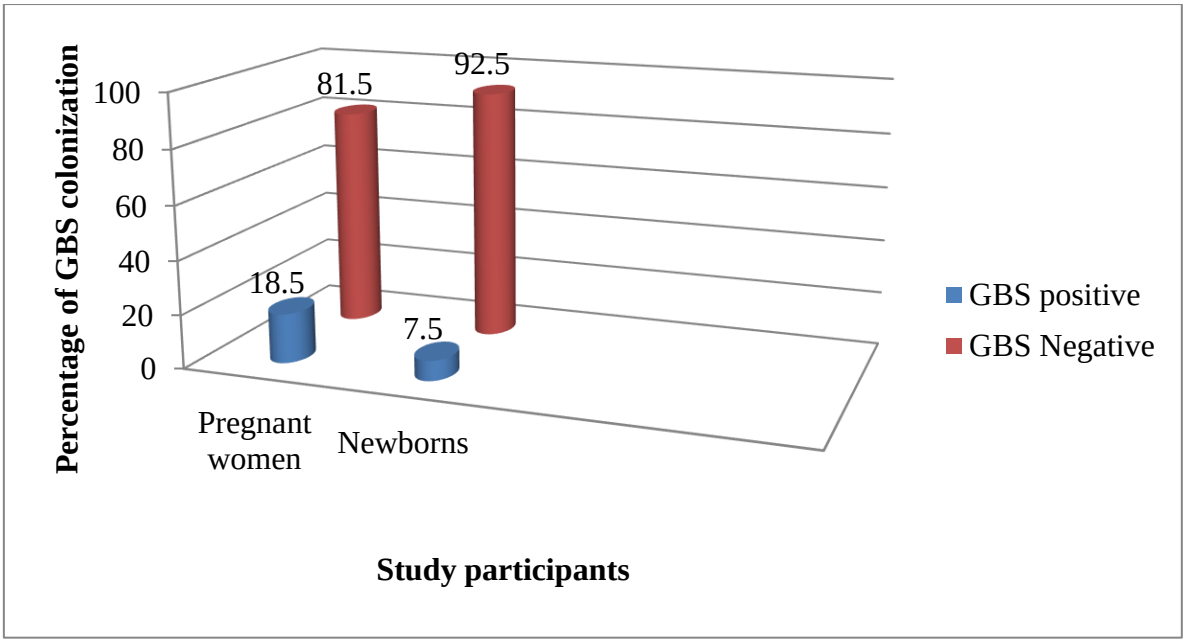

Figure 2: Prevalence of GBS colonization among preganant women and their newborns in FHCSH and BDHC, Bahir Dar, 2021

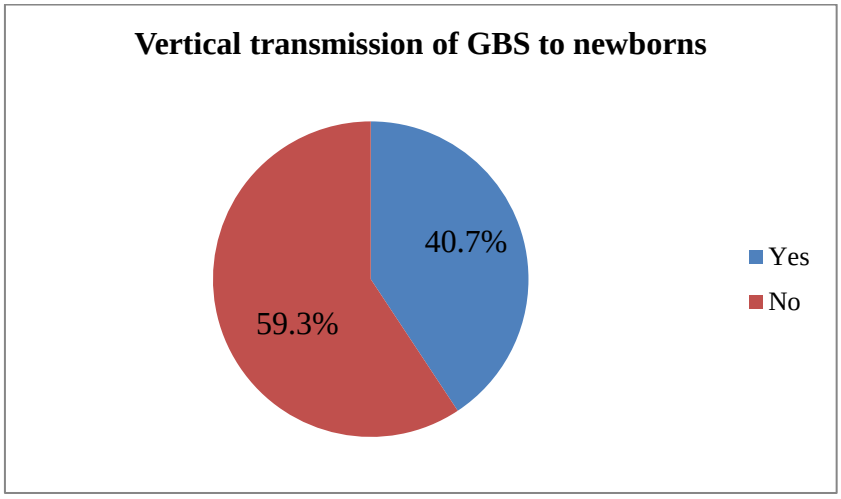

Figure 3: Proportion of vertical transmission of GBS from colonized mothers to their newborns in FHCSH and BDHC, Bahir Dar, 2021

5.4 Obstetric characteristics and GBS colonization among pregnant women

Among 292 pregnant women participated in this study, 28 (9.6%) delivered before 37 weeks of gestation. Majorities (84.9%) of pregnant women had ≥ 4 times history of ANC visit and those with primigravida accounted 28.8% of the participants. Most of the pregnant women (91.1%) had history of hormonal contraceptive use and 12.3%, 6.2% and 1% of them had history of abortion, still birth and neonatal death, respectively (Table 3).

In terms of HIV status, 6 (2.1%) of pregnant women were HIV positive. Majority of the pregnant women (66.4%) delivered within 12 hours of the start of labor. Of the total participants, 8.2% and 11.3% of them had experienced PROM and meconium stained amniotic fluid during delivery (Table 3).

The percentage of GBS colonization was higher among pregnant women who had: < 37 weeks of gestational age (39.3%), multigravida (19.7%), history of abortion (25%), history of still birth (38.9%), HIV infection (33.3%), membrane rupture before the start of labor (29%) and meconium stained amniotic fluid (30.3%) than their counter parts (Table 3).

Table 3: Obstetric characteristics and GBS colonization among pregnant women in FHCSH and BDHC, Bahir Dar, 2021

Variables	**Number of pregnant women N (%)**	**GBS culture positives N (%)**
Gestational age (weeks)		
< 37	28 (9.6)	11 (39.3)
≥ 37	264 (90.4)	43 (16.3)
Number of ANC visits at current pregnancy		
< 4	44 (15.1)	9 (20.4)
≥ 4	248 (84.9)	45 (18.1)
Gravidity		
Primigravida	84 (28.8)	13 (15.5)
Multigravida	208 (71.2)	41 (19.7)
History of hormonal contraceptive use		
Yes	266 (91.1)	48 (18)
No	26 (8.9)	6 (23)
History of abortion		
Yes	36 (12.3)	9 (25)
No	256 (87.7)	45 (17.6)
History of still birth		
Yes	18 (6.2)	7 (38.9)
No	274 (93.8)	47 (17.1)
History of neonatal death		
Yes	3 (1)	1 (33.3)
No	289 (99)	53 (18.3)
History of UTI during current pregnancy		
Yes	20 (6.8)	5 (25)
No	272 (93.2)	49 (18)
History of STI during current pregnancy		
Yes	5 (1.7)	1 (20)
No	287 (98.3)	53 (18.5)
History antibiotic use at current pregnancy		
Yes	32 (11)	5 (15.6)
No	260 (89)	49 (18.8)

Chronic illness at current pregnancy		
Yes	19 (6.5)	4 (21)
No	273 (93.5)	50 (18.3)
HIV status		
Positive	6 (2.1)	2 (33.3)
Negative	286 (97.9)	52 (18.2)
Premature rapture of membrane		
Yes	24 (8.2)	7 (29)
No	265 (91.8)	47 (17.7)
Duration of labor (hours)		
< 12	194 (66.4)	38 (19.6)
≥ 12	98 (33.6)	16 (16.3)
Meconium stained amniotic fluid		
Yes	33 (11.3)	10 (30.3)
No	259 (88.7)	44 (17)
Total	**292 (100)**	**54 (18.5)**

Key: STI: Sexually transmitted infection, UTI: Urinary tract infection, ANC: Ante Natal Care

5.5 Bivariable analysis on factors associated with maternal GBS colonization

On bivariable analysis, age, marital status, educational status, residence and occupation were not statistically significant with GBS colonization at p=0.05, 95% C.I. However, from the obstetric and clinical related variables, history of still birth (COR: 3.32, 95% CI: 1.45-7.59) and pre-term delivery (COR: 3.07, 95% CI: 1.13-8.34) were statistically significant with maternal GBS colonization (Table 4).

Table 4: Bivariable logistic regression analysis on factors associated with maternal GBS colonization in FHCSH and BDHC, Bahir Dar, 2021

Variables	**Maternal GBS colonization**		**COR (95% C.I)**	**P-value**
	Positive (n=54)	Negative (n=238)		
Health facility				0.427
Felege Hiwot Comprehensive specialized hospital	33	159	0.78 (0.42-1.43)	
Bahir Dar Health Center	21	79	1	
Age of pregnant mothers (years)				0.285
≤ 26	24	125	0.72 (0.39-1.31)	
> 26	30	113	1	
Residence				0.775
Urban	41	185	0.90 (0.45-1.81)	
Rural	13	53	1	

Educational status				0.650
No formal education	8	25	1.80 (0.67-4.80)	
Grade 1-8	20	79	1.42 (0.67-3.02)	
Grade 9-12	12	55	1.23 (0.52-2.86)	
College/university	14	79	1	
Gestational age at delivery (weeks)				0.004*
< 37	11	17	3.32 (1.45-7.59)	
≥ 37	43	221	1	
Number of ANC visits at current pregnancy				0.716
< 4	9	35	1.16 (0.52-2.58)	
≥ 4	45	203	1	
Gravidity				0.400
Primigravida	13	71	0.74 (0.37-1.47)	
Multigravida	41	167	1	
History of hormonal contraceptive use				0.530
Yes	48	218	0.73 (0.28-1.92)	
No	6	20	1	
History of abortion				0.286
Yes	9	27	1.56 (0.68-3.54)	
No	45	211	1	
History of still birth				0.027*
Yes	7	11	3.07 (1.13-8.34)	
No	47	227	1	
History of UTI during current pregnancy				0.440
Yes	5	15	1.51 (0.52-4.37)	
No	49	223	1	
History of antibiotic use				0.658
Yes	5	27	0.79 (0.29-2.17)	
No	49	211	1	
Premature rapture of membrane				0.166*
Yes	7	17	1.93 (0.76-4.93)	
No	47	221	1	
Duration of labor (hours)				0.498
< 12	38	156	1.24 (0.65-2.37)	
≥ 12	16	82	1	
Meconium stained amniotic fluid				0.106*
Yes	10	25	1.93 (0.86-4.31)	
No	44	213	1	
Sex of newborn				0.696
Male	27	112	0.88 (0.49-1.60)	
Female	27	126	1	
Birth weight (grams)				0.368
< 2500	5	14	1.63 (0.56-4.74)	
≥ 2500	49	224	1	
5th minute Apgar score				0.100*
< 7	5	9	2.59 (0.83-8.08)	
7-10	49	229	1	

*=candidates for multivariable logistic regression, 1= reference category, COR=Crude Odds Ratio, ANC=Ante Natal Care, Apgar: appearance, pulse, grimace, activity, respiration

Multivariable analysis on maternal GBS colonization

From multivariable analysis, pre-term delivery (<37 weeks of gestation) (AOR: 2.7, 95% CI: 1.14-6.68) and having history of still birth (AOR: 3.1, 95 % CI: 1.13-8.7) were significantly associated with maternal GBS colonization. The odd of maternal GBS colonization was 3.1 times higher for those mothers having history of still birth when compared to their counterparts. On the other hand, the likelihood of maternal GBS colonization was 2.7 times higher for those mothers who delivered at <37 weeks of gestational age than those who delivered at ≥37 weeks of gestational age in the current pregnancy (Table 5).

Table 5: Multivariable logistic regression analysis of factors associated with maternal GBS colonization in FHCSH and BDHC, Bahir Dar, 2021

Variables	Maternal GBS colonization		AOR (95% C.I)	P-value
	Positive (N)	Negative (N)		
Gestational age at delivery (Weeks)				0.023*
< 37	11	17	2.77(1.14-6.68)	
≥ 37	43	221	1	
History of still birth				0.028*
Yes	7	11	3.13(1.13-8.70)	
No	47	227	1	
Premature rapture of membrane				0.330
Yes	7	17	1.64(0.60-4.48)	
No	47	221	1	
Meconium stained amniotic fluid				0.280
Yes	10	25	1.62(0.67-3.92)	
No	44	213	1	
5th minute Apgar score				0.369
< 7	5	9	1.79(0.50-6.39)	
7-10	49	229	1	

*=significant at p=0.05, 95% C.I, 1= reference category, AOR=Adjusted Odds Ratio, Apgar: appearance, pulse, grimace, activity, respiration

5.6 Univariable analysis of factors associated with vertical transmission of GBS

From 54 pregnant women with GBS, 22 of the newborns had GBS yielding a vertical transmission rate of 40.7%. Relatively higher proportion of vertical transmission was observed among those who delivered at gestational age ≥37 weeks (46.5%), having history of abortion (55.6%), multigravidas (46.3%), meconium stained amniotic fluid (50%), and in those giving female newborns (59.7%) than their counterparts. However, there was no

statistical significant association between any of the variables tested with vertical transmission of GBS from colonized mothers to their newborn (Table 6).

Table 6: Univariable analysis of factors associated with vertical transmission of GBS in FHCSH and BDHC, Bahir Dar, 2021

Variables	**Transmission of GBS from mothers to newborns**		**P-value**
	Yes	**No**	
	N (%)	**N (%)**	
Health facility			
Felege Hiwot Comprehensive Specialized Hospital	15 (45.5)	18 (54.5)	0.41
Bahir Dar Health Center	7 (33.3)	14 (66.7)	
Age of the mother (years)			
≤ 26	10 (41.7)	14 (58.3)	1.00
> 26	12 (40)	18 (60)	
Residence			
Urban	18 (43.9)	23 (56.1)	0.306
Rural	4 (30.8)	9 (69.2)	
Educational status of the mother			0.353*
No formal education	2 (25)	6 (75)	
Grade 1-8	10 (50)	10 (50)	
Grade 9-12	3 (25)	9 (75)	
College/university	7 (50)	7 (50)	
Gestational age at delivery (weeks)			
< 37	2 (18.2)	9 (81.8)	0.084*
≥ 37	20 (46.5)	23 (53.5)	
Number of ANC visit during current pregnancy			
< 4	2 (22.2)	7 (77.8)	0.195*
≥ 4	20 (44.4)	25 (55.6)	
Gravidity			
Primigravida	3 (23.1)	10 (76.9)	0.121*
Multigravida	19 (46.3)	22 (53.7)	
History of contraceptive use			
Yes	18 (37.5)	30 (62.5)	0.211*
No	4 (66.7)	2 (33.3)	
History of abortion			
Yes	5 (55.6)	4 (44.4)	0.266*
No	17 (37.8)	28 (62.2)	
History of still birth			
Yes	3 (42.9)	4 (57.1)	0.606*
No	19 (40.4)	28 (59.6)	

History of UTI at current pregnancy			
Yes	2 (40)	3 (60)	0.676*
No	20 (40.8)	29 (59.2)	
History of antibiotic use at current pregnancy			
Yes	2 (40)	3 (60)	0.676*
No	20 (40.8)	29 (59.2)	
Premature rapture of membrane			
Yes	2 (28.6)	5 (71.4)	0.394*
No	20 (42.6)	27 (57.4)	
Duration of labor (hours)			
< 12	15 (39.5)	23 (60.5)	0.772
≥ 12	7 (43.7)	9 (56.3)	
Meconium stained amniotic fluid			
Yes	5 (50)	5 (50)	0.723
No	17 (38.6)	27 (61.6)	
Sex of newborns			
Male	10 (37)	17 (63)	0.782
Female	22 (59.5)	15 (40.5)	
Birth weight (grams)			
< 2500	1 (16.7)	5 (83.3)	0.207*
≥ 2500	21 (43.7)	27 (56.3)	
Status of newborns			
Alive	22 (42.3)	30 (57.7)	0.347*
Dead/stillbirth	0 (0)	2 (100)	
5th minute Apgar score			
< 7	2 (40)	3 (60)	0.676*
7-10	20 (40.8)	29 (59.2)	
Total	**22 (40.7)**	**32 (59.3)**	

*: Fisher's exact test

5.7 Antibiotic susceptibility profile of GBS isolates

Among the 54 identified GBS isolates, 9 (16.7%), 7 (13%) and 4 (7.4%) showed resistance to penicillin, ampicillin and vancomycin, respectively. Highest resistance was observed against tetracycline 42 (77.8%) followed by clindamycin 19 (35.2%), erythromycin 14 (25.9%) and ceftriaxone 12 (22.2%). However, 47 (87%) of the GBS isolates were found sensitive to chloramphenicol (Table 7).

Table 7: Antimicrobial susceptibility profile of GBS isolates (n: 54) identified from pregnant women in FHCSH and BDHC, Bahir Dar, 2021

Antibiotics tested	**Susceptible N (%)**	**Intermediate N (%)**	**Resistance N (%)**
Penicillin	45 (83.3)	0	9 (16.7)
Ampicillin	47 (87)	0	7 (13)
Clindamycin	30 (55.6)	5 (9.3)	19 (35.2)
Erythromycin	33 (61.1)	7 (13)	14 (25.9)
Chloramphenicol	47 (87)	2 (3.7)	5 (9.3)
Ceftriaxone	42 (77.8)	0	12 (22.2)
Vancomycin	50 (92.6)	0	4 (7.4)
Tetracycline	7 (13)	5 (9.3)	42 (77.8)

Multi-drug resistance profile of GBS isolates

Overall, 19 (35.2%) of GBS isolates were found to be MDR (showing resistance to 3 or more antibiotics from different classes) (Table 8).

Of the 54 GBS isolates, 4 (7.4%) showed susceptibility to all the tested eight antibiotics. However, 17 (31.5%) and 14 (25.9%) of the isolates showed resistance to 1 and 2 of the tested antimicrobials, respectively. From MDR isolates, isolates resistance to clindamycin, erythromycin and tetracycline at a time was the most frequent (52.6%) followed by penicillin, ampicillin, ceftriaxone and clindamycin (10.5%) and penicillin, ceftriaxone and clindamycin (10.5%) combinations (Table 9).

Table 8: Multi-drug resistance profile of GBS isolates isolated from pregnant women in FHCSH and BDHC, Bahir Dar, 2021

Number of GBS isolates	**R0 N (%)**	**R1 N (%)**	**R2 N (%)**	**R3 N (%)**	**R4 N (%)**	**MDR N (%)**
N=54	4 (7.4)	17 (31.5)	14 (25.9)	16 (29.6)	3 (5.6)	19 (35.2)

Note: R0=Susceptible to all antibiotics tested, R1, R2, R3 and R4= Isolates resistance to 1, 2, 3 and 4 antibiotics taken from different classes, respectively, MDR= Multi-drug Resistance

Table 9: Profile of antibiotics combination resisted by MDR GBS isolated from pregnant women in FHCSH and BDHC, Bahir Dar, 2021

Antibiotic combination	**Number of antibiotic classes**	**Frequency N (%)**
Penicillin, ampicillin, ceftriaxone, clindamycin	3	2 (10.5)
Penicillin, ceftriaxone, clindamycin	3	2 (10.5)
Ampicillin, clindamycin, ceftriaxone	3	1 (5.3)
Clindamycin, erythromycin, tetracycline	3	10 (52.6)
Chloramphenicol, ceftriaxone, tetracycline	3	1 (5.3)
Penicillin, clindamycin, ceftriaxone, tetracycline	4	1 (5.3)
Ampicillin, ceftriaxone, clindamycin, tetracycline	4	1(5.3)
Ampicillin, clindamycin, erythromycin, tetracycline	4	1(5.3)
Total MDR		**19 (100%)**

6. DISCUSSION

The overall prevalence of GBS colonization in pregnant women was 18.5% in the present study. This prevalence of maternal GBS colonization is concurrent with adjusted estimate of global GBS maternal colonization prevalence (18%) (Russell *et al.*, 2017a) and pooled prevalence report in Africa (19.3%) (Gizachew *et al.*, 2019b). Moreover, specific studies done in Hawassa, Ethiopia (15.7%) (Ali *et al.*, 2019), North Carolina, USA (21.6%) (Edwards *et al.*, 2019), Jordan (19.5%) (Clouse *et al.*, 2019), and Gabon (19%) (Belard *et al.*, 2015) reported comparable prevalence of GBS colonization with the current study.

The prevailing prevalence of GBS colonization is lower than previous studies conducted in Gondar (25.5%) and Addis Ababa (23.2% and 23.6%), Ethiopia (Ali *et al.*, 2020b, Fantahun *et al.*, 2020, Gizachew *et al.*, 2019a), South Africa (48.2%) (Lekala *et al.*, 2015), Gambia (33.7%) (Le Doare *et al.*, 2016), Uganda (28.8%) (Namugongo *et al.*, 2016), Egypt (26.5%) (Sadaka *et al.*, 2018), United Kingdom (29.4%) (Rao *et al.*, 2017) and Virginia, USA (35%) (Kum-Nji *et al.*, 2020). On the other hand, the present finding is higher than studies conducted in other parts of Ethiopia ((Addis Ababa (7.5%) (Woldu *et al.*, 2014), Mekelle (13.7%) (Alemseged *et al.*, 2015), Adigrat (11.3%) (Gebremeskel *et al.*, 2015), Adama (13.2%) (Ali *et al.*, 2020a), Nekemte (12.2%) (Mengist *et al.*, 2017), Arbaminch (8.5%) (Shiferawu *et al.*, 2019)), Kenya (12%) (Seale *et al.*, 2016) and China (6.1%) (Chen *et al.*, 2018a). The difference in the percentage of GBS colonization between the current study and previous reports might be strongly linked with variation in microbiological protocols used to isolate and identify GBS, site and composition of specimens, its transportation and storage conditions. Moreover, geographical variation and time of screening in pregnancy might contribute for such variations.

In this study, 22 out of 292 newborns (7.5%) were found colonized with GBS. This finding was in line with studies conducted in Bangladesh (7.4) (Saha *et al.*, 2017) and Nigeria (6.8%) (Elikwu *et al.*, 2016). Different from this study, higher colonization prevalence was reported in studies from Gondar, Ethiopia (16.1) (Gizachew *et al.*, 2018), Gambia (12%) (Roca *et al.*, 2017), South Africa (15.7%) (Madzivhandila *et al.*, 2011) and France (13.9%) (Jost *et al.*, 2014). However, this result was higher when compared with results from Saudi Arabia (1.0%) (Al-Sunaidi *et al.*, 2011) and India (3.2%) (Shah *et al.*, 2014). The discrepancies between the studies might be due to difference in sites of newborns swabbed, presence or absence of provision of IAP and geographical variability in prevalence of maternal GBS colonization.

In this study, the percentage of vertical transmission of GBS from colonized mothers to their newborns was 40.7%. This is in line with studies conducted in Addis Ababa, Ethiopia (47.4% and 49.2%) (Ali *et al.*, 2020b, Fantahun *et al.*, 2020), Harar, Ethiopia (45.02%) (Yadeta *et al.*, 2018a) and Bangladesh (38%) (Saha *et al.*, 2017). However, the present result is lower than studies conducted in Gondar, Ethiopia (63.3%) (Gizachew *et al.*, 2020), Hawassa, Ethiopia (59.1%) (Ali *et al.*, 2019), Gambia (57.7%) (Le Doare *et al.*, 2016) and India (65%) (Sridhar Santhanam *et al.*, 2017). On the other hand, lower percentage of vertical transmission of GBS have been reported in China (7.6% and 14.1%) (Chen *et al.*, 2018a, Chen *et al.*, 2018b) and Germany (11.2%) (Kunze *et al.*, 2011). The observed differences between studies in the percentage of GBS transmission from mothers to newborns might be due to differences in the clinical profile of mothers, sample size, sample source and its composition, methods employed for GBS detection, time of sample collection (soon after birth or later), presence or absence of the IAP administration, and density of vaginal GBS colonization.

Knowledge of risk factors and reducing risk is one of the most important mechanism in prevention of maternal GBS colonization and neonatal GBS disease is a priority in resource limited countries like Ethiopia where routine ante partum screening of GBS is lacked. In the present study, preterm delivery is one of the factors significantly associated with maternal GBS colonization. This is in agreement with a study from Jima, Ethiopia (Girma *et al.*, 2020), South Africa (Lekala *et al.*, 2015) and India (Ashary *et al.*, 2020). This is because vaginal colonization of pregnant mothers results ascending infection and inflammation, chorioamnionitis and preterm premature rapture of membrane which will in turn causes preterm delivery (Surve *et al.*, 2016).

The present study also find out that maternal GBS colonization was significantly associated with having history of still birth. This finding is in line with a study from Harar, Ethiopia (Yadeta *et al.*, 2018a) and Gambia (Le Doare *et al.*, 2016). This is because still birth can be resulted from the GBS cytolysis breach of feto-maternal barrier as a result of ascending infection from the recto vaginal colonization (Randis *et al.*, 2014).

In this study GBS isolates revealed considerable level of resistance to the commonly prescribed antibiotics such as penicillin (16.7%), ampicillin (13%) and vancomycin (7.4%). Similarly, previous studies in Gondar and Addis Ababa, Ethiopia showed a respective 10.2%, 9.2%, 16.3% (Gizachew *et al.*, 2019a) and 19.5%, 14.6%, 17% (Assefa, 2014),

resistance to penicillin, ampicillin and vancomycin. However, the 2020 CLSI guideline reported negligible GBS resistance to the above tested antibiotics (CLSI, 2020). Moreover, studies from other parts of Ethiopia ((Jimma (Girma *et al.*, 2020), Adigrat (Gebremeskel *et al.*, 2015), Arbaminch (Shiferawu *et al.*, 2019)), Egypt (Sadaka *et al.*, 2018), Saudi Arabia (Khan *et al.*, 2015), Brazil (Melo *et al.*, 2016) and China (Ji *et al.*, 2017), reported the absence of GBS resistance to the above antibiotics. However, 77.3% of GBS isolates were resistant to penicillin in a study from Nekemte, Ethiopia (Mengist *et al.*, 2017), and 57.7% and 54.3% of GBS isolates were resistant to penicillin and ampicillin, respectively in a study from Addis Ababa, Ethiopia (Fantahun *et al.*, 2020). The widespread empirical use of these antibiotics for the treatment of different infectious diseases might contribute for the emergence of GBS resistance strains to these antibiotics in the area.

In the present study, 22.2% of GBS isolates showed resistance to ceftriaxone, a result which is parallel with studies conducted in Nekemte, Ethiopia (31.8%) (Mengist *et al.*, 2017) and Arbaminch, Ethiopia (29.2%) (Shiferawu *et al.*, 2019) but contradicts with a study from Egypt (Sadaka *et al.*, 2018), China (Ji *et al.*, 2017) and Brazil (Melo *et al.*, 2016) that revealed no resistance to this antibiotic. Differences in bacterial strain, practice of prophylaxis, number of isolates tested, drug control policies and awareness of the community towards drug resistance might be the possible reasons for the observed difference between these studies. On the other hand, the wide spread use of ceftriaxone for the treatment of various infections coupled with free accessibility to purchase without prescription might be linked for the observed GBS resistance in the present study.

In this study, GBS isolates revealed 35.2% and 25.9% of resistance to clindamycin and erythromycin, respectively. This result is partly consistent with studies from Gondar (Gizachew *et al.*, 2019a), Nekemte (Mengist *et al.*, 2017), Arbaminch (Shiferawu *et al.*, 2019) and Jimma (Girma *et al.*, 2020), Ethiopia which showed a respective resistance profile of 21.4%, 18.2%, 29.2% and 40.9% for clindamycin and 26.5%, 22.6%, 20.8% and 50% for erythromycin. Similarly, 22.6% resistance against erythromycin was reported in Egypt (Sadaka *et al.*, 2018) and 52.4% resistance to clindamycin in China (Ji *et al.*, 2017). The significant level of resistance to these antibiotics might be due to unregulated extended use of these antibiotics for various infections and it strongly supports the ACOG recommendation that states AST should be performed for these antibiotics before using as a prophylaxis in the intra partum period (ACOG, 2020).

Group B streptococci isolates in this study showed a better susceptibility to chloramphenicol (87%), though; it is not recommended for prophylaxis in pregnant women due to its adverse effect during pregnancy. However, most of the GBS isolates (77.8%) were found resistance to tetracycline in the present study. This high proportion of resistance to tetracycline in the present study is consistent with a pooled estimate of tetracycline resistance in Africa (82.6%) (Gizachew *et al.*, 2019b), and studies from Gondar, Ethiopia (73.4%) (Gizachew *et al.*, 2019a) and Addis Ababa, Ethiopia (90.2%) (Assefa, 2014). Similar to these reports, a systematic review by Hayes et.al also showed that >80% of GBS isolates were resistant to tetracycline (Hayes *et al.*, 2020). The high percentage of GBS isolates resistance to tetracycline in the present study combined with others indicate that this antibiotic could no longer be used for either treatment or as a prophylaxis for this isolate.

In the present study, 35.2% of the GBS isolates were found MDR. This finding is in line with a report from Addis Ababa, Ethiopia (43.9%) (Assefa, 2014) whereas higher than studies from Arbaminch (8.3%), Ethiopia (Shiferawu *et al.*, 2019) and Mekelle (10.5%), Ethiopia (Alemseged *et al.*, 2015). On the other hand, the present study MDR report is lower than findings from Nekemte, Ethiopia where 100% of the isolates were MDR (Mengist *et al.*, 2017) and in China where 79.7% of the GBS isolates were MDR (Gao *et al.*, 2018). Majority of the MDR profile observed in our study was covered by resistance to the antibiotics tetracycline, clindamycin and erythromycin. This can be due to an increasing trend of antimicrobial resistance to these antibiotics through time and this can be supported by a study finding in France that showed MDR GBS isolates exhibiting resistance to tetracycline, macrolides and lincosamides showed an increase from 0% in 2007 to 14% in 2019 (Plainvert *et al.*, 2020).

7. LIMITATION OF THE STUDY

This study uses a cross-sectional study design which has a “chicken or egg dilemma” and this makes establishing a cause and effect relationship between the dependent and the independent variables difficult. In addition, this study uses a non-probability sampling technique (convenient sampling technique) which is a weaker sampling technique compared to the probability sampling techniques since it may have sampling error. Lastly, this study does not include a method for detection of non-hemolytic GBS strains due to inaccessibility of serologic tests.

8. CONCLUSION AND RECOMMENDATIONS

This study documented high prevalence of GBS colonization among pregnant women and high proportion of its transmission to newborns. Pre-term delivery and history of still birth are identified factors associated with maternal GBS colonization. Resistance of GBS isolates to the commonly used antibiotics coupled with MDR is becoming a major concern in the study area. Therefore, screening of all pregnant mothers for GBS colonization at 36 0/7 to 37 6/7 weeks of gestation followed by providing antibiotic prophylaxis to GBS carriers are recommended. Administration of antibiotics for prophylaxis or treatment of GBS should be guided by susceptibility testing. Further studies focusing on outcome of newborns colonized with GBS are required in the study area and other parts of Ethiopia.

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10. ANNEXES

Annex I: Information sheet for pregnant women

English version

Date.....

Introduction

My name is Yasab Leykun and I am MSc student of Bahir Dar University, College of Medicine and Health Sciences Department of Laboratory Sciences. I am doing a research entitled "prevalence, vertical transmission, and antimicrobial susceptibility profile of Group B Streptococci isolates among pregnant women at selected public health facilities of Bahir Dar city". Currently Group B streptococci have a great burden on pregnant woman as different studies indicate. So, this study will indicate burden and their antimicrobial susceptibility profile of Group B streptococci at Bahir Dar city public health facilities and it will help the physicians to treat based on culture results.

Purpose of the study

The objective of this research is to study the colonization prevalence of Group B streptococci and its rate of vertical transmission and antimicrobial susceptibility pattern among pregnant women at Bahir Dar city public health facilities. If you agree to participate in the study, you will give us the necessary information and recto-vaginal swab sample will be collected from you for laboratory analysis**.**

Confidentiality

All the data obtained will be kept strictly confidential and locking the data, only study personnel will have access to the files. Anonymous testing will be undertaken, that means samples will be coded and positive results will not be identified by names. There will not be any payment or direct benefit for participating and you are not asked to pay for the laboratory examination. Your result will be reported back to the physicians if it is found significant for further diagnosis and treatment.

Right to decline participation or withdraw from the study

Your participation in this study is purely voluntary, and you may stop the participation at any time or you may refuse to answer some of the questions if you feel uncomfortable. You are

free to refuse to participate in the study or you can withdraw your consent at any time, without giving reasons and this will not involve any penalty or loss of benefits to which you are entitled such as proper care and treatment. Your access to treatment will not be dependent on your participation in the study. If you are not comfortable please feel free to stop it at any level of the study.

I appreciate your cooperation greatly. If you have questions regarding this study or would like to be informed of the results after its completion, please contact me through the following address.

Amharic version

አጠቃላይ መረጃ

መግቢያ

ስሜ ያሳብ ለይኩን እባላለሁ:: የባህር ዳር ዩኒቨርሲቲ የላቦራቶሪ ሳይንስ የትምህርት ክፍል የማስተርስ ድግሪ ተማሪ ነኝ። በአኑ ሰአት የGroup B streptococci በነፍሰጡር እናቶች ላይ ያለዉን የስርጭት መጠን ለማወቅ ጥናት እያካሄድኩ ነው፡ Group B streptococci በነፍሰጡር እናቶች እና በሚወለዱ ህፃናት ላይ የተለያዩ ችግሮችን ሲያመጣ ይታያል። ይህ ጥናት በባህር ዳር ነፍሰ ጡር እናቶች ፡ የGroup B streptococci የስርጭት መጠን መለየት እንዲሁም ወደ ሚወለዱ ህፃናት የመተላለፍ መጠን እና በየትኛዉ መድሀኒት ሊጠፋ እንደሚችል ለማመልከት ሲሆን ይህም ለሃኪሙ ህሙማንን ለማከም የሚያግዝ ሲሆን በተጨማሪም ተያያዥነት ያላቸዉን ችግሮች ለማዉቅ እና የመፍትሔ ዕርምጃ እንዲወሰድ ለማመልከት ነዉ።

<u>የጥናቱ አላማ፡-</u> የዚህ ጥናት አላማ የGroup B streptococci በነፍሰጡር እናቶች ላይ ያለዉን ስርጭት መጠን፣ ወደ ሚወለዱ ህፃናት የመተላለፍ መጠን እንዲሁም የመድሃኒት መላመድ ለመዳሰስ እና ለማወቅ ነው። እርስዎ በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ ለጥናት የሚያስፈልገዉን ከብልትዎ ላይ ናሙናና እንዲሁም ለጥናቱ የሚያስፈለጉ መረጃዎችን ይሰጣሉ።

<u>የመረጃዉ ሚስጥራዊ አያያዝ</u>፡- የሚሰጡት መረጃ ሚስጥራዊነቱ የተጠበቀ ነው። በስም አይጻፋም የዚህ ኮድ መፍቻ በፋይል ተቆልፎ የሚቀመጥ ሲሆን የተፈቀደለት ሰው ብቻ ፋይሉን ማየት ይችላል። ከዚህ ጥናት በሚወጡ ዘገባዎች ወይንም የህትመት ውጤቶች ላይ ስም ወይ ሌላ የእርስዎን ማንነት የሚገልጽ መረጃ አይኖርም። ከምርመራ የሚገኘውም ውጤት ወይም ሌላ መረጃ ለሚመለከታቸው

አካላት ለምሳሌ፤እርስዎን የሚንከባከቡ የህክምና ባለሙዎች እና ጥናቱን ለሚያካሄዱት ባለሙያዎች እንዲሁም ጥናቱ ስነ-ምግባርን ጠብቆ መከናወኑን ለሚከተተሉት የኮሚቴ አባላት ብቻ ይገለፃል።ኮምፒውተር ላይ ያለ መረጃዎች ምስጢራዊነታቸው የተጠብቀ ሲሆን በወረቀት ያሉ መረጃዎችም ደህንነቱ በሚጠበቅ ቦታ የሚቆለፉና የተፈቀደለት ሰው ብቻ ሊያያቸው እንዲችል ተደርጎ ይጠበቃል።ዉጤቱ ተጨማሪ ምርመራ የሚያስፈልገዉ ከሆነ እና ህክምና ካሰፈለገዉ ለህኪሙ ዉጤቱ ይሰጠዋል።

<u>በጥናቱ ለመሳተፍ ፈቃደኛ አለመሆን ወይም መሳተፍ ከጀመሩ በኋላ ራስን የማግለል መብት፡-</u> በጥናቱ የሚሳተፉት ፈቃደኛ ከሆኑ ብቻ ነው። ስለዚህ መሳተፍ አለመሳተፍ ከጀመሩ በኃላ ማቋረጥ ወይም መመለስ የማይፈልጉት ጥያቄ ከሆነ ይለፈኝ ማለት ሙለ መብትዎ ነው። በጥናቱ መሳተፍ አለመሳተፍ አገልግሎት ላይ ምንም አይነት ጥቅምም ሆነ ጉዳት አይኖረውም።ጊዜወትን መሰዋት አድርገው ሰለተባበሩኝ ከልብ አመሰግናለሁ።

Annex II: Consent Form for pregnant women

English version

I have been informed about the study's objective entitled "***prevalence, rate of vertical transmission and antimicrobial susceptibility profile of Group B streptococci among pregnant women at Bahir Dar City Health facilities***." I am also informed that all information contained within the questionnaire is to be kept confidential. Moreover, I have been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want, and none of my actions will have any bearing at all on my overall health care. Therefore, I agree to give the complete necessary information and vaginal swab sample for laboratory analysis with a full understanding of the situation. I have had the opportunity to ask questions about the project and received clarification to my satisfaction in a language I understand. I am also told that Group B streptococci results will be given to the health facility and that I may ask for the information if I want. This questionnaire and sample collection is designed for the purpose of the research study. This questionnaire and sample collection's main objective is to study prevalence, rate of vertical transmission and antimicrobial susceptibility profile of Group B streptococci among pregnant women used for partial fulfillment of MSC in Medical Microbiology. Whatever information you provide will be kept strictly confidential. The success of the study depends on your real responses to the questions.

Please listen carefully and respond to the questions honestly. The questionnaires spend out above 20 minutes.

I have read this form, or the form has been read to me in the language I understand, and I have understood the conditions stated above. I am willing to participate in this study.

I _____ hereby give my consent for giving of the requested information and specimen for this study. Participant code: _____
Signature: _____ Date: _____

Thank you very much for your cooperation!!

Amharic version

መረጃዉን በፈለኩ ጊዜና ሰአት የማግኘት ሙሉ መብት እንዳለኝ ግልጽ በሆነና በሚገባኝ ቋንቋ ስለተነገረኝ በሚገባ ተረድቻለሁ:: በመሆኑም ለዚህ ጥናት የሚያስፈልገዉን ከብልት ላይ የሚወሰደዉን ናሙናና እንዲሁም ለጥናቱ የሚያስፈለጉ መረጃዎችን ለመስየዚህ ጥናት አላማ የGroup B *streptococci* በነፍሰጡር እናቶች ላይ ያለዉን ስርጭት መጠን፣ እንዲሁም ወደሚወለዱ ህፃናት የመተላለፍ ፍጥነት እና የመድሃኒት መላመድ ለመዳሰስ መሆኑን ተረድቻለሁ:: በዚህ ጥናት የሁሉም መረጃዎች ደህንነት የተጠበቀ መሆኑን፣ከጥናቱ በፈለኩ ጊዜ መዉጣት እንደምችል፣ በማቋረጤ ምክንያት በህክምና ክትትሌ ላይ ምንም አይነት ችግር እንደማይደርስብኝ፣ የዚህ ጥናት ዉጤት ለሆስፒታሉ እንደሚሰጥና ደህንነቱ በተጠበቀ ሁኔታ እንደሚቀመጥ እንዲሁም ጠት ሙሉ ፈቃደኛ መሆኔን እግልጻለሁ::

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Annex III: Information sheet for parents of newborn

English version

You are invited to let your child participate in this study. The aim of this study is to determine the rate of vertical transmission of Group B *Streptococcus* at public health facilities of Bahir Dar city and to give recommendation to concerned bodies so as to take appropriate measures to prevent this disease. Group B *Streptococcus* is recognized as a major cause of disease among newborn in different parts of the world.

Purpose: the purpose of this study is to determine rate of vertical transmission of Group B streptococci to newborns

Procedure to be carried out: the procedure is easy and simple; ear, nasal and umbilical swab sample will be collected by attending midwifes. All samples will be transported to Laboratory for analysis.

Risk and discomfort: There will be no discomfort during collection of samples since samples are taken from external surface of the newborn.

Expected benefits: The information gained from yours and others child will help to consider prevention strategy for vertical transmission and neonatal disease caused by GBS in Ethiopia, if your baby is positive for GBS appropriate medical care will be provided to him.

Confidentiality: We respect your child‘s privacy and confidentiality. Any information that identifies your child will not be shared with anyone else outside the study team. If a research article or publication comes from this study, your child will not be identified by name. The information we collect from your child as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study.

Voluntary Participation and Withdrawal from the Study: The participation is completely voluntary and you have the right not to late your child to participate in this study. You can stop your child participating in the study at any time after giving your consent. This decision will not affect in any way yours or your child‘s current or future medical care in the health facility.

Contact information: If you have any questions about this study you can contact the investigator with the following address. Yasab Leykun: Tel; 0912951583

Amharic version

የመረጀ ቅጽ ለተወለዱ ህጻናት ወላጆች

በዚህ ጥናት ልጆትን እንዱያሳትፉ ተጋብዘዋል። የዚህ ጥናት ዋና አላማ ግሩፕ ቢ እስትሪፕቶኮከስ የሚባል ባክቴርያ ያለዉን ከ እናት ወደ ልጅ የመተላለፍ መጠን ለማወቅ ነው። እንዱሁም አስፈሊጊው

የመከላከያ እርምጃ እንዲወሰድ ለሚመለከተው አካል ለማሳወቅ ነው። ግሩፕ ቢ እስትሪፕቶኮከስ በተለያዪ የአለማችን ክፍሎች ላይ ዋና የህጻናት ህመም አምጪ እንደሆነ ታውቆዋል።

አላማ: የዚህ ጥናት አላማ ግሩፕ ቢ እስትሪፕቶኮከስ የሚባለዉ ተዋህሲያን ያለዉን ከ እናት ወደ ልጅ የመተላለፍ መጠን ለማወቅ ነው።

የአሰራር ሁኔታ: ለዚህ ጥናት የሚወሰደዉ ናሙና በቀላል መንገድ የሚከናወን ነው። ናሙና የሚወሰደው ከዉጨኛዉ ጀሮ፤ ከአፍንጫ እና ከእንብርት ጋር ነዉ።

የሚያስከትለው አደጋ: ናሙናዉ የሚወሰደዉ ከዉጨኛዉ የአካል ክፍል ስለሆነ የሚያስከትለዉ ችግር የለም።

የሚያስገኘው ጥቅም: ይህ ከእርሶ ልጅ እና ከ ለሌች ሌጆች የሚገኝ መረጃ ወደፊት ግሩፕ ቢ እስትሪፕቶኮከስ ያለዉን ከእናት ወደ ልጅ የመተላለፍ መጠን ለመቀነስ እና የሚመጣዉን የህጻናት ህመም የመከላከያ ዘዴን ለማሰብ ይጠቅማል።

ሚስጥራዊነት: የልጆትን ሚስጥር እናከብራለን። ልጆትን የሚያመለክት ማንኛዉንም መረጃ ከተመራማሪዎች በስተቀር ለማንም ተላልፎ አይሰጥም። ከዚህ ጥናት በመፅሄት ላይ የሚታተም ነገር ካለ ሌጆት በስም አይጠቀስም። ከልጆት የተገኘ ማንኛዉም መረጃ ሳጥን ዉስጥ ይቆለፍበታል ወይንም በሚስጥር ቁጥር ኮምፒተር ዉስጥ ይቀመጣሌ። ይህንን መረጀ ማግኘት የሚችሉት ከምርምሩ ጋር ግኙኝነት ያላቸዉ ሰዎች ብቻ ናቸዉ።

በፈቃደኝነት የመሳተፍ እና ከጥናቱ ስለመቋረጥ: በዚህ ጥናት መሳተፍ ሙለ በሙሉ በፈቃደኝነት ላይ የተመሰረተ ነው። ልጅዎትን ያለማሳተፍ መብት አልዎት። በማንኛውም ግዜ የልጅዎትን የጥናት ተሳታፊነት የስምምነት ቅፁንም ከፈረሙ ቦኋላም ቢሆን ማቋረጥ ይችላሉ። ይህን በመወሰልዎ ከሌጅዎት ጋር አሁን የሚያገኙትንም ሆነ ወደፊት የሚያያገኙት የጤና አገልግሎት በምንም አይነት መልኩ አይስተጓጎልም።

አድራሻ: ይህንን ጥናት በተመለከተ ጥያቄ ከልዎት በሚከተለው አድራሻ ተመራማሪዉን በመጠየቅ ዝርዝር ማብራርያ ማግኘት ይችላሉ። ያሳብ ለይኩን ስልክ ቁጥር-0912951583

Annex IV: Consent form for Parents of new born

English version

Serial no_____

Card no_____

Name of study participant: _____

I have been requested to let my child participate in this study which involves collection of specimen from my child. The purpose of the study and sample collection procedure has been explained to me. I have also read the information sheet (or it has been read to me); I have understood that this study is about rate of vertical transmission Group B streptococci which is one of leading cause of morbidity and mortality among newborn in the world. I have asked some questions and clarification has been given to me. I have given my consent on behalf of my child to let him participate in the study and I hereby confirm my agreement with my signature.

Date ---------- Signature----------

Amharic version

የስምምነት ቅጽ ለወላጆች

ተራ ቁጥር_____

የካርድ ቁጥር_____

በዚህ ጥናት ልጄን እንዲሳተፍ እና ከልጄ ናሙና እንዲወሰድበት ተጋብዣለው። የዚህ ጥናት አስፈሊጊነት እና የናሙናአወሳሰዱ ተብራርቶልኛል። የመረጃ ቅጹንም አንብብያለው ወይንም ተነቦልኛል። ይህ ጥናት ግሩፕ ቢ እስትሪፕቶኮከስ ያለዉን ከእናት ወደ ልጅ የመተላለፍ መጠን ለማወቅ እንደሆነም ተረድቻለው። ይህ ባክቴርያ በአለም ላይ ዋና የህጻናት ህመም አምጪ እና ገዲይ እንደሆነም ተገንዝብያለው። አንዳንድ ያልተረዳሁትን ነገር ጠይቄ ማብራርያ ተሰቶኛል። በዚህ ጥናት ልጄ እንዲሳተፍ ፍቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

ፊርማ----------ቀን----------

Annex V: Questionnaire

English version

Socio-demographic data of the mother		
Participant study ID_____		
Name of health facility_____ Participant card number_____		
Address: Region _____sub city_____kebele_____Phone_____		
1.	Age (in years)	_____
2.	What is your marital status	Single Married Divorced Widowed
3.	Where is your residence	Urban Rural
4.	What is your educational status	Unable to read and write Elementary (1–8) Secondary (9–12) High grade (College and University)
5.	What is your occupation	Civil servant Student Farmer House wife Merchant(business women) Daily laborer

Clinical and obstetric factors		
6.	Gestational age	_____
7.	Number of ANC visits	_____
8.	Gravidity	Primigravida Multigravida
9.	History of hormonal contraceptive use	Yes No

10.	History of still birth or neonatal loss	Yes No
11.	History of abortion in previous pregnancies	Yes No
12.	History of neonatal death	Yes No
13.	History of UTI during current pregnancy	Yes No
14.	History of STI during current pregnancy	Yes No
15.	History of antibiotic use during current pregnancy	Yes No
16.	History of chronic illness during current pregnancy	Yes No
17.	HIV status of the mother	Positive Negative
18.	Rupture of membrane 1 h. before the start of labor(PROM)	Yes No
19.	Duration of labor	_____
20.	Fever during labor	Yes No
21.	Meconium stained amniotic fluid	Yes No
22.	Sex of newborn	Male Female
23.	Birth weight	_____
24.	Status of newborn	Alive Dead(still birth)
25.	APGAR score at 1st minute	<7 7-10

Amharic version of questionnaire

የተሳታፊዎች መለያ ኮድ_____

ተ.ቁ	የጤና ተቐሙ ስም_____የተሳታፊ ካርድ ቀጥር_____ አድራሻ-ክልል:_____ክፍለ ከተማ:_____ቀበሌ: ____ስ..ቁ._____	
1.	እድሜ	_____
2.	የጋብቻ ሁኔታ	ያላገባች ያገባች አግብታ የፈታች ባል የሞተባት
3.	መኖሪያ አድራሻ	ከተማ ገጠር
4.	የትምህርት ደረጃ	ማንበብ እና መፃፍ የማትችል 1–8 9–12 ኮሌጅ/ዩኒቨርስቲ
5.	ስራ	የመንግስት ሰራተኛ ተማሪ አርሶ አደር የቤት እመቤት የቢዝነስ ሴት(የግል ስራ) የቀን ሰራተኛ
ከእርግዝና ከህክምና ጋር የተያያዙ መረጃዎች		
6.	የእርግዝናዉ እድሜ(በሳምንት)	_____

7.	በዚህ እርግዝና ስንት ጊዜ የነፍሰ ጡር ክትትል አድርገሻል	_____
8.	ስንተኛ እርግዝናሽ ነዉ	የመጀመሪያ ≥2
9.	ከዚህ በፊት የወሊድ መቆጣጠሪያ ትጠቀሚ ነበር	አዎ የለም
10.	ከዚህ በፊት ሞቶ የተወለደ ህፃን አጋጥሞሽ ያዉቃል	አዎ የለም
11.	ከዚህ በፊት ዉርጃ አጋጥሞሽ ያዉቃል	አዎ የለም
12.	ከዚህ በፊት የጨቅላ ህፃን ሞት አጋጥሞሽ ያዉቃል	አዎ የለም
13.	በዚህ እርግዝና ወቅት የሽንት ቱቦ ኢንፌክሽን ታመሽ ታዉቂያለሽ	አዎ የለም
14.	በዚህ እርግዝና ወቅት በግብረ ስጋ ግንኙነት የሚተላለፍ በሽታ አለብሽ ተብለሽ ታዉቂያለሽ	አዎ የለም
15.	በዚህ እረግዝና ወቅት የፀረ-ተዋህሲያን መድሃኒት ወስደሽ ታዉቂያለሽ	አዎ የለም
16.	በዚህ እርግዝና ወቅት የስኩዋር ወይም የደም ግፊት ህመም አለብሽ ተብለሽ ታዉቂያለሽ	አዎ የለም
17.	የእናትዮዋ የHIV ዉጤት	ፖዘቲቭ ነጌቲቭ
18.	ምጥ ከመጀመሩ 1 ሰዓት በፊት የእንሽርት ዉሃ መፍሰስ	አዎ የለም
19.	የምጥ እርዝማኔ	_____

20.	ሙኮኒየም የተቀላቀለበት የእንሽርት ዉሃ	አዎ የለም
21.	በምትወልድበት ጊዜ ትኩሳት	አዎ የለም
22.	የህፃኑ ፆታ	ወንድ ሴት
23.	የህፃኑ ክብደት	_____
24.	የተወለደዉ ህፃን ሁኔታ	በህይዎት የተወለደ ሞቶ የተወለደ
25.	የ 5ኛዉ ደቂቃ APGAR ዉጤት	<7 7-10

ጊዜዎትን መስዋት አድርገዉ ስለተባበሩን እናመሰግናለን።

የጥናቱ መረጃ ሰብሳቢ ስም: _____ፊርማ: _____ቀን_____

Annex VI: Laboratory procedures

A. Sample Collection, Handling and Transport

1. **Objective and Scope:** To describe the specimen collection instructions and subsequent handling of specimens by researcher for culture of GBS. This document contains standard operating procedures (SOPs) for clinical specimens containing GBS from the recto vaginal swab from the mother and from ear, nasal and umbilical swabs from the neonate for processing at Bahir Dar University Microbiology Laboratory.
2. **Specimen Collection**

An adequate specimen is essential for the success of GBS culture. Specimens have to be collected with the utmost care and go to the laboratory promptly. Culture-based screening for the pregnant mother from recto-vaginal swab and for the newborn from ear, nasal and umbilical swab at the point of delivery was taken and investigated for GBS.

According to ACOG Committee opinion 2019 guideline and ASM 2020 recommendations, a single swab sample was taken first from vagina followed by rectal area of the mother. Without using a speculum, specimen was collected first from the vagina (near the introitus) by inserting the swab about two centimeters and then from the rectum by inserting the same swab one centimeter through the anal sphincter. Swab sample was also taken from the ear, nasal and umbilical area of the newborn using sterile cotton swab at point of delivery in Health facilities, Bahir Dar, Ethiopia, from March 1 to May 30, 2021 by attending midwives.

3. Transport of specimen to Bahir Dar University Microbiology Laboratory

Recto-vaginal swabs of the mother and nasal, ear and umbilical swabs of the newborn was placed in Amies transport media and immediately transported to the Microbiology Laboratory of Bahir Dar University for culture. If delay was unavoidable the specimens were refrigerated at 4 °C for 24 hrs.

B. Specimen Processing

i. Culture

Procedure:

1. The recto-vaginal swab and ear, nasal and umbilical swab was placed into 2 ml THB supplemented with gentamicin (8µg/ml) and nalidixic acid (15µg/ml) to prevent growth of contaminants.

2. The broth was incubated for 18–24 hours at 35-37°C
3. Then, if there is growth sample was inoculated into 5% sheep blood agar.
4. Incubated in a humid environment of air containing 5 % CO_2 and examined the plates after 18-24 hrs of incubation. But, if no growth in first 24 hrs, incubated for a minimum of 48 hrs before discarding the plates.
5. Presumptive diagnosis was made by performing Gram stain, catalase, bacitracin test and CAMP test.
6. Antimicrobial susceptibility testing was performed for GBS isolated according to CLSI guideline 2020.

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, size, cellular morphology, and gram reactions. It is a critical test for rapid presumptive diagnosis.

Materials/reagents

Reagents	**Supplies**
• Crystal violet • Lugol's iodine • Acetone alcohol • Safranin	• Disposable plastic loops • Glass microscope slides • Absolute methanol

Quality control:

- Gram positive: *S. aureus*
- Gram negative: *E. coli*

Procedure:

1. A glass microscope slide was labeled with the laboratory accession number.

2. Swab sample 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 was mixed with saline on the slide.

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

Result interpretation:

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

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 • Bunsen burner

Quality control:

- Positive control- *S.aureus*
- Negative control- *S. pyogenes*

Procedure:

1. Pour 2-3 ml of the hydrogen peroxide solution into a test tube
2. Using a sterile wooden stick or glass rod remove several colonies of the test organism & immerse in the hydrogen peroxide solution
3. Look for immediate active bubbling

Result interpretation:

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

iv. Bacitracin test

1. Using an inoculating loop, streak two or three suspect colonies of a pure culture onto a blood agar plate.
2. Using heated forceps place a bacitracin disk in the first quadrant and gently tap the disk to ensure adequate contact with the agar surface.
3. Incubate the plate for 18 to 24 hours at 35-37^oC in CO_2 rich atmosphere.
4. Look for a zone of inhibition around the disk

Results interpretation:

- Bacitracin sensitive: Any zone of inhibition around the disk.
- Bacitracin resistant: No zone of inhibition around the disk.

Quality control:

- Positive control- *S. pyogens*
- Negative control- *S. agalactiae*

v. 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 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. A known hemolytic strain of *S. aureus* will be streaked in a straight line across the center of the sheep blood agar plate. Test inoculums will be streaked in a straight line (2-3cms in length) perpendicular to *S. aureus* streak but without touching it. A known GBS as a positive control and *Enterococcus faecalis* as a negative control was also be streaked similarly. The plate was incubated at 35-37 ℃ for 18-24 hours. A positive test for CAMP factor appears as "arrowhead" hemolysis between the junction of growth of *S. aureus and* GBS. No enhanced or "arrowhead" hemolysis was seen when the test isolate is not GBS.

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* 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

Procedure:

1. Inoculate *S. aureus* onto a sheep blood agar plate by making a narrow streak down the center of the plate with a loop.
2. Streak the test organism (suspected GBS) in a straight-line at right angles to the *S. aureus.*
3. Make the *Streptococcus* streak within 2 mm without touching the *S. aureus* streak.
4. A known GBS as a positive control and *E. faecalis* as a negative control was also streaked similarly.
5. Incubate 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. No enhanced zone of beta-hemolysis observed in a CAMP negative reaction.

vi. Antimicrobial susceptibility test

Principle: The antibiotics diffuse in radial manner from the disc and 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 standard	• MHA with 5% sheep blood • Normal saline • Test tube • Wooden applicator stick with cotton • Antimicrobial disks	• Safety cabinet • Bunsen burner • Incubator • Measuring ruler • Candle jar

Procedure:

1. Prepare pure colony suspension into normal saline equivalent to 0.5 McFarland standards.
2. Inoculate the suspension to entire surface on Muller-Hinton agar with 5% sheep's blood using a sterile cotton swab.
3. Antibiotic disks were selected and deposited on the agar according to CLSI 2020 guideline
4. Incubated at 35-37 °C with 5% CO2 atmosphere for 18-24 hours.
5. Measure zone of inhibition and report the result based on CLSI 2020 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 MIC 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.

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 *streptococci*. 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	• Balance • Autoclave • Refrigerator • Bunsen burner • 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. Weight 40 grams of blood agar base powder and suspended in 1000 ml of distilled water.
2. Mix the medium by boiling until completely dissolved.
3. Sterilize the medium by autoclaving at 121 °C for 15 minutes.
4. Cool the agar to 50 °C water bath before adding the sheep blood.
5. Add 50 ml of sterile defibrinated sheep blood aseptically and mix gently.
6. Avoid forming air bubbles.

Important: The blood must be allowed to warm to room temperature before being added to the molten agar.

7. Dispense 12-15 ml of blood agar aseptically in sterile petridish.
8. Allow the medium to solidify and date the medium.
9. Store the plates at 2–8 °C. Preferably in sealed plastic bags to prevent loss of moisture.

ii. Todd Hewitt Broth (THB)

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. dissolve 36.4 grams of THB powder in 1000 ml of distilled water
2. Boil until completely dissolved.
3. Transfer the medium into screw-cap bottles and sterilize (with caps loosened) by autoclave at 115°C for 10 minutes.
4. Add the gentamicin and nalidixic acid into the medium when cooled and tighten the bottle caps.
5. Date the medium and give it a batch number. Store the medium at 2-8 0C.

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 • Petridishs • Graduated cylinder	• Balance • Distilled water • Autoclave • Autoclave tape

Procedure:

1. Suspend 38 grams of MHA powder and transfer in to a flask containing one liter of distilled water.
2. Boil until the medium completely dissolves.
3. Autoclaved the medium at 121°C for 15 minutes.
4. Add 5% sheep blood and cool to room temperature.
5. Date the medium and give it a batch number. Store the medium at 2-8 0C.

D. Quality control

- As quality control, sterility of SBA and MHA with 5% sheep blood was checked by incubating overnight at 37 ^{0}C without specimen inoculation.
- The proficiency of THB was checked by inoculating the broth with known Gram negative bacteria (*Escherichia coli)* and known *S. agalactiae* 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* (positive control) and *S. pyogenes* (negative control).

- For Gram staining reagents *S.aureus* (ATCC 25923) (gram positive) and *E. 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. *E. coli* (ATCC 25922), *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 25923), *S. pyogenes* (ATCC 19615) and *S. agalactiae* (ATCC 27956) was used as a quality control throughout the study.

Approval sheet

The undersigned examining committee certify that the thesis presented by Yasab Leykun entitled: prevalence, vertical transmission, antimicrobial susceptibility profile of Group B Streptococcus and associated factors among pregnant women at selected public health facilities in Bahir Dar, Northwest Ethiopia, submitted to Bahir Dar University, College of Medicine and Health Sciences, Department of Medical Laboratory Sciences , in partial fulfillment of the requirements for master degree in Medical Microbiology complies with the regulation of the University and meets the accepted standards with respects to originality and quality.

Place of submission: Department of Medical Laboratory Sciences, College of Medicine and Health sciences, Bahir Dar University.

Date of submission _____

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2. Chalachew Genet (Assistant professor) Signature, _____Date, _____

INTERNAL EXAMINER: _____, Signature, _____, Date, _____

EXTERNAL EXAMINER: _____, Signature_____, Date_____

CHAIR OF DEPARTMENT: _____Signature, _____Date, _____

AUGUST, 2021

BAHIRDAR, ETHIOPIA