DEBRE BERHAN UNIVERSITY

ASRAT WOLDEYES HEALTH SCIENCE CAMPUS

SCHOOL OF PUBLIC HEALTH

DEPARTMENT OF PUBLIC HEALTH

Prevalence of Streptococcus agalactiae, Antimicrobial Susceptibility Pattern and Associated Factors Among Pregnant Women At Debre birhan Comprehensive Specialized Hospital, North East Ethiopia

By:

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A Research Thesis Submitted to School of Public Health, Asrat Woldeyes Health Science Campus, Debre Berhan University for Partial Fulfillment of the Requirements for The Master's degree of Public Health in Epidemiology

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Signed approval sheet by the board of examiner

Debre Berhan University

Asrat Woldeyes Health Science Campus

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Abstract

Background: *Streptococcus agalactiae*, also known as Group B Streptococci (GBS), is a typical component of the microbiota in the female genital tract and anal areas of healthy people. Preterm labor (PTL), premature rupture of the membranes (PROM), chorioamnionitis, postpartum endometritis, and perinatal transmission of the organism are all pregnancy-related disorders that may be brought on by maternal GBS colonization. Neonates born to mothers who have GBS colonization in their genital tracts are susceptible to acquiring Streptococcus agalactiae during delivery. In infants, GBS can result in meningitis and sepsis.

Objective: To assess the prevalence of *Streptococcus agalactiae,* antimicrobial susceptibility pattern and associated factors, among pregnant women at Debre birhan Comprehensive Specialized Hospital.

Methods: an institution-based cross-sectional study was conducted from May 24, 2023 to June 15, 2023. Consecutive sampling technique was used taking all until sample reach. Questionnaires were used to obtain data on socio-demographic, obstetric, and clinical factors. Vaginal swabs was collected and transported to the Microbiology laboratory by using amies transport media, then it was inoculated on a Blood agar plate and incubated at 37 °C for 24 h. Growth (Beta haemolysis) and gram stain and catalase test, Christie, Atkins, Munch, Peterson factor were performed. Antimicrobial susceptibility testing was done by the disk diffusion technique following the Clinical and Laboratory Standards Institute (2022) guideline. The data was entered into Epidata version 3.1 then exported to SPSS version 25 and descriptive statistics and logistic regression was performed. P-value <0.05 is considered for statistical significance.

Result: Overall prevalence of *Streptococcus agalactiae* among pregnant women was 9.9% (95% CI 5.5-15.7). Maternal Urinary tract infection history (AOR 7.017, 95% CI 1.599-30.791), and Premature rapture of membrane (AOR=8.638, 95% CI 1.639, 27.387) has been found statistically significant for maternal colonization. All GBS isolates were susceptible to penicillin G, ampicillin, Cefotaxime, Ceftriaxone, and vancomycin whereas showed resistance to clindamycin (11.1%), chloramphenicol (5.6%), and erythromycin (16.7%), but there were no multiple drug resistance seen.

Conclusion: The *Streptococcus agalactiae* (GBS) carriage rate lower than studies in Ethiopia as well as global estimates. Maternal urinary tract infection history on current pregnancy and history of premature rapture of membrane (PROM) have been found statistically significant. Antimicrobial resistance pattern showed alarming for not prescribe antimicrobial before drug sensitivity test. **S**creening of Antenatal care attendants for GBS and antimicrobial susceptibility testing should be performed.

Keywords: Group B streptococcus, prevalence, Antimicrobial susceptibility pattern

Acknowledgment

I would like to express my gratitude to Debre Berhan University, Asrat Woldeyes health science Campus, department of public health for giving me the opportunity to undertake this research. My sincere and special thanks also go to my advisors Mr. Muluken Tessema (BSc, MPH), and Ms Woineshet Bedru (BSc, MPH) for unresevesed support during this thesis developement. Also I would like express my thanks to Debre birhan Comprehensive Specialized hospital for providing materials and allowing laboratory set ups, Ms. Abeb Mulugeta (BSc) Midwifery staff at Debre birhan Comprehensive Specialized hospital, for collecting the swab samples, the study participants who consented to cooperate, Mr. Getinet Mamo (BSc, MSc) and Mr Behailu Adnew (BSc, MSc) who helped me during sample processing as well as on the analysis process.

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Abbreviation &acronyms

ANC	Antenatal Care
AST	Antimicrobial Susceptibility Testing
BMI	Body Mass Index
BAP	Blood Agar Plate
CAMP	Christie, Atkins, Munch, Peterson
CDC	Center For Disease Control
CLSI	Clinical Laboratory Standard Institute
CS	Caesarean section
DBCSH	Debre birhan Comprehensive Specialized Hospital
DBU	Debre Berhan University
EOD	Early Onset Disease
EOGBS	Early-Onset Group B Streptococcus
EONS	Early-Onset Neonatal Sepsis
IAP	Intrapartum Antibiotic Prophylaxis
GBS	Group B streptococcus
LOD	Late Onset Diseases
MDR	Multi Drug-Resistant
MHA	Muller-Hinton Agar
MLS	Medical Laboratory Sciences
MLS_B	Macrolide, Lincosamide–Streptogramin $_B$
PROM	Premature Rupture Of Membranes
SVD	Spontaneous vaginal delivery
WHO	World Health Organization
UTI	Urinary Tract Infection

1. Introduction

1.1.Background of the study

Streptococcus agalactiae, the sole member of the Lancefield group B, a gram-positive coccus, is referred to as Group B. The gastrointestinal tract acts as a natural reservoir and source of vaginal colonization for streptococci (GBS), which may be present in the female genital tract and anal regions of healthy people as a typical component of the microbiota (1, 2). Pregnancy-related disorders such urinary tract infection (UTI), bacteremia, chorioamnionitis, postpartum endometritis, preterm labor (PTL), premature rupture of membranes (PROM), and perinatal transmission of the organism may be brought on by maternal GBS colonization (1). Potential of GBS to go up from the lower genital tract and colonize the upper genital tract has been linked to intrauterine infection (3).

Despite a recent reduction in prevalence, Group B Streptococci infection in newborns continues to be the leading cause of neonatal morbidity and mortality worldwide, with the highest incidence of invasive illness occurring in low-middle income nations (4).

The majority of invasive diseases caused by Group B Streptococcus affect young newborns, pregnant or postpartum women, older adults, and people with impaired immune systems.(5). Many people have asymptomatic GBS colonization in their gastrointestinal and vaginal tracts, however pregnant women who have colonization are more likely to experience unfavorable obstetric outcomes, premature birth, and perinatal transmission to their newborns. Additionally, GBS is one of the leading causes of cystitis, chorioamnionitis, endometritis, genitourinary tract, and surgical site infection in pregnant women. Nearly one-third of premature births are caused by genital infections, which cause protease activity and cervical softening (6).

Additionally, Group B Streptococcus is known to be able to infect newborns, increasing neonatal morbidity and mortality. Amniotic fluid during pregnancy can become infected with GBS, and the newborns can contract the infection by aspirating it or by vertical transmission when passing via a colonized vaginal canal. Later, the neonates may also contract GBS through breast-feeding or from the hospital environment, which can cause neonatal sepsis and meningitis (7).

This mode of transmission can be prevented by intrapartum antibiotic prophylaxis (IAP), and there are two main approaches for choosing which pregnant women will receive IAP: risk-based and culture-based techniques (8). The Centers for Disease Control and Prevention recommends that if GBS colonization is apparent, IAP should be administered as soon as possible to all pregnant women in the United States between the 35th and 37th gestational weeks. However, in other nations, such as the United Kingdom and the Netherlands, IAP is given while taking other clinical risk factors into

account, such as preterm labor, premature or prolonged (rupture of membranes, GBS bacteriuria, a previous infant with GBS disease (9) . Since 2011, the Brazilian Society for Pediatrics has recommended a policy based on culture, but only about 20% of Brazilians appear to be following these recommendations (10).

Although sporadic reports of isolates with decreased susceptibility to penicillin have been reported since 2008, *S. agalactiae* continues to be considered to be consistently responsive to this medication (11). The use of clindamycin or erythromycin was recommended as alternatives in IAP for penicillin-allergic women with a high risk of anaphylaxis or when therapeutic failure is suspected (8). However, increasing rates of clindamycin and erythromycin resistance have been detected in several regions of the world, including Europe (12, 13) Asia (11, 14), North America (15) and South America (16, 17); for this reason, clindamycin does no longer constitute an empiric reliable alternative (9).

In this study, I have plan to do evaluation of GBS Prevalence and antimicrobial susceptibility pattern of GBS isolates recovered from pregnant women at Debre birhan Comprehensive specialized Hospital, Debre birhan Town, during months, and analyzed the association of clinical, and demographic aspects with GBS colonization.

1.2.Statement of the problem

Globally, *Streptococcus agalactiae* (Group B Streptococcus; GBS) is the most common pathogen to infect newborns. The peripartum period is when mother-to-child transmission happens most frequently. In African contexts, there is still a shortage of information on the incidence of maternal colonization (18), specifically in Ethiopia where few studies revealed maternal colonization ranges from 7.2% (19) to 19% (20).

An important risk factor for early neonatal sepsis worldwide is colonization of Group B Streptococcus in the anogenital region. (21). Worldwide, GBS colonization varies between 11 and 35% (22); however this prevalence varies from place to place(14), so we cannot rely on the prevalence of a neighboring country or region to estimate the prevalence in our setting. Specific sites in sub-Saharan Africa revealed contradicting prevalence (23).

Streptococcus agalactiae, group B streptococcus (GBS), is a species of the normal flora of the lower gastrointestinal and genitourinary tracts (24). GBS is present in the lower genital tract of 10–30 % of pregnant women. When untreated, approximately 50–75 % of infants born to GBS positive mothers will become colonized (18) .GBS continues a significant cause of infection, morbidity, and mortality in newborns, pregnant, and non-pregnant women (25). Vaginal colonization by GBS during pregnancy is associated with premature rupture of membrane, stillbirth, younger women age, and low birth weight babies (26).

The invasive GBS illness has two clinical syndromes: early-onset disease (EOD) and late-onset disorders (LOD). In the first week of birth, sepsis and pneumonia are usual presentations for EOD, while meningitis is the most common diagnosis for LOD from day >7 to 3 months of age (27). According to estimates, 4-6% of infants with early-onset GBS disease die. Infants who survive may develop long-term impairments include hearing loss, visual loss, or mental retardation (28, 29). Early and effective treatment of neonatal meningitis and septicemia in infants, especially in those who had low birth weight, premature delivery, early membrane rupture, and extended labor, depends on the early detection of GBS in pregnant women (30).

Additionally, there is a concern regarding the growth in antibiotic resistance among GBS isolates, making it essential to do antibiotic susceptibility testing on such isolates prior to prescribing antibiotics to determine their susceptibility pattern (31). In South Africa, AST of GBS isolates revealed that they were completely susceptible to penicillin, vancomycin, and high-level gentamicin (120 g). To erythromycin, however, 21.1% were resistant, and to clindamycin, 17.2%. These had lincosamide-streptogramin B (MLSB) constitutively in 69% and inducible in 17.4% of them.

Additionally, 6.8% of each M- and L-phenotype were found (32). Of the 10 isolates in Bosinia, 14.3% revealed erythromycin resistance and 29.6 % to clindamycin (33). Studies conducted in Ethiopia revealed 36.4% to 17/22 (77.3%) penicillin resistance, 9.1% to 18.2% clindamycin resistance, and 4.5% to 22.6% erythromycin resistance (34).

Important information that can be used to develop prevention strategies in the area is provided by maternal colonization prevalence, related risk factors, and its antimicrobial resistance patterns. The purpose of the current study is to identify the prevalence of maternal colonization, related risk factors, and GBS resistance patterns to eight routinely administered antibiotics.

In order to reduce the morbidity and mortality of GBS-associated newborn illness, the Centers for disease Control and Prevention (CDC) advised that all pregnant women at 35–37 weeks gestation should be screened for GBS colonization using vaginal–rectal specimens. Through the research of more regions across more continents, it may be possible to comprehend the worldwide variances in GBS colonization and serotype prevalence, providing the knowledge required to guide toward the development of viable vaccines (35).

The frequency and the consequences of infections transmitted from mother-to-child differ according to the socio-economic conditions of each geographic area and are more common and devastating in low-income countries with high exposure to infectious organisms, nutrient deficiencies (36), low immune response, and limited access to health care services (5).

In Ethiopia, There is limited information regarding prevalence of GBS among pregnant women and there is a paucity of published data concerning maternal GBS colonization and antimicrobial susceptibility pattern in Northeast Ethiopia particularly in the study area. According to the hospital annual report neonatal sepsis is major cause of admission and death of neonates. The present study, therefore, aimed to determine prevalence and antibiotic susceptibility pattern of GBS among pregnant women attending antenatal care (ANC) of Debre birhan comprehensive specialized in Debre birhan Town, Northeast Ethiopia.

1.3.Significance of the study

The Centers for Disease Control and Prevention recommended that all pregnant women at 35–37 weeks gestation should be screened for GBS colonization using vaginal specimens to decrease the morbidity and mortality of GBS-associated neonatal disease. In my study area even if mothers attend their anti natal care (ANC), there is no Screening for GBS colonization. In other side neonatal sepsis is major cause of admission in DBCSH which may be descending infection from mothers. And also WHO recommended prophylaxis of antibiotics. Before administration of antibiotics knowing anti-microbial resistance pattern is important. Therefore, my current study was to address this issue.

Knowing the prevalence, associated factors and sensitivity pattern of GBS will help the clinicians who work at the health facilities whether there was a need for GBS colonization screening of pregnant women attending the antenatal clinic. In addition to that they can also identify factors associated with its colonization and selecting of appropriate antimicrobial. This in turn has led to targeted screening of high-risk pregnant women using minimum resources available, all of which will hopefully contribute to a reduction of cases of neonatal sepsis, infection, newborns diseases such as pneumonia, septicemia, and meningitis caused by both GBS infection at DBCSH.

Policy maker, provide insights in planning intervention programmer for maternal and neonatal survival in our study area and other similar settings of the country, by policies are formulated and strengthened to guide clinicians in the application of safe interventional procedures geared towards reducing the risk of maternal GBS colonization.

Health institutions will help to apply the recommended approach derived from the result of this study in tackling the progression of the GBS colonization. It hopefully create awareness concerning the GBS among pregnant women. The infected individual get early treatment so it is possible to minimize the complications of the disease. Additionally, it is also important to design prevention and control strategies. This information will be useful in establishing the clinical relevance and need for incorporating GBS screening into the routine assessment of Antenatal Care to improve infants and maternal health.

2. Literature review

2.1. Prevalence of Streptococcus agalactiae with Socio-demographic Characteristics

In the study conducted in Libya, Maternal GBS colonization was associated with tertiary education, weight, gravidity. Neonatal GBS colonization was associated with full term delivery (FTD) and low birth weight (LBW). (37). A study conducted in Beijing, China *S. agalactiae* isolates were identified in 863 pregnant women (6.5%) (38).

Sample of vaginal-rectal swabs from women who presented for labor and delivery at Al-Bashir Hospital, Jordan, Amman was collected and In April and May 2015, 200 women were enrolled Overall, 39 (19.5%) of women were GBS-positive on blood agar media and CHROM agar, while 67 (33.5%) were positive by rapid test (36% sensitivity, 67% specificity) (39).

A meta-analysis that has been performed in Africa as a whole shows that 83 articles were assessed and the overall estimate of recto-vaginal colonization was 19.3%. The highest estimate in Southern Africa, 23.8% , then by Northern Africa, 22.7% and the lowest was driven from the Eastern Africa, 15.4% (40).

Four separate prenatal clinics in South Africa's Western Cape were used to find participants, the rate of GBS colonization was 16.6% (41). A cross-sectional study over 6 months analyzing vaginal and anorectal samples obtained from 100 pregnant women at a tertiary hospital in Cameroon and the detected colonization rate was 14% (42).

Prevalence of *Streptococcus agalactiae* infection in women residents in Calabar, Cross River State of Nigeria was investigated Out of the 122 samples screened, 26 were positive for *S. agalactiae* giving an overall prevalence rate of 21.3%. Furthermore, prevalence rates of 23.6% and 18.0% were obtained for the pregnant and non-pregnant women, respectively. Based on the age groups of the participants, prevalence rates of 13.7%, 17%, 26.5%, and 40% were obtained from age groups of ≥20, 21-29, 30-39, and ≥ 40 years, respectively. The higher prevalence rate is seen in married, increased age (40 year and above), primary education, poor hygiene practice. (43).

A prospective cross-sectional study was conducted from May to August 2014 at selected public antenatal care (ANC) centers in Addis Ababa, Ethiopia, and the overall prevalence of GBS colonization among pregnant women was 14.6% (41/281) (20).

Another prospective cross-sectional study was conducted between March and May 2016 in Nekemte Referral Hospital (NRH) shows that the total prevalence of maternal GBS colonization from vaginal swab culture was 12.2% (22/180) (44).

A Hospital-based cross-sectional study was conducted at Hawassa University Comprehensive Specialized Hospital from November 05, 2014, to March 25, 2015, and a result of Prevalence of GBS among pregnant women, were 44(15.7%). Among 26 GBS colonized newborns one developed signs and symptoms of early-onset disease (37).

A prospective cross-sectional study was conducted from 1st December 2016 to 30th November 2017 at the University of Gondar Referral hospital delivery ward and the result that has been found was the overall prevalence of maternal GBS colonization was 25.5% (23).

A hospital-based cross-sectional study was conducted from August to December 2014 at selected health facilities at antenatal clinics in Ayder Referral Hospital and Mekelle Health Center, and a result of 139, 19 (13.7 %) were positive for GBS (27).

In another study which was performed at Ethiopia a cross-sectional study involved 126 pregnant women at 35–37 weeks of gestation at Jimma University Hospital showed that overall carriage rate of GBS was 19.0 % (24/126), and the rectal and vaginal carrier rates were 14.3 % (18/126) and 10.4 % (13/126), respectively (45) .

2.2.Factors associated to S. agalactiae colonization

In the study conducted in Libya, Maternal GBS colonization was associated with tertiary education, weight, gravidity. Neonatal GBS colonization was associated with full term delivery (FTD) and low birth weight (LBW) (37)

Study done from 200 sample of vaginal-rectal swabs from women who presented for labor and delivery at Al-Bashir Hospital, Jordan, Amman April and May 2015, Overall prevalence was 19.5%, but no demographic or clinical differences were noted between GBS+ and GBS-negative women (39).

A study from four different antenatal clinics in the Western Cape, South Africa, risk factors for early-onset invasive GBS in newborns include rupture of membranes for more than 18 h before delivery, fever in the mother during labor, preterm delivery and a history of GBS disease in previous infants (41).

Prevalence of Streptococcus agalactiae infection in women residents in Calabar, Cross River State of Nigeria was investigated Out of the 122 samples screened, 26 were positive for S. agalactiae with the higher prevalence rate is seen in married, increased age (40 year and above), primary education, poor hygiene practice (43).

A cross-sectional study was conducted from May to August 2014 at selected public antenatal care (ANC) centers in Addis Ababa, Ethiopia, GBS colonization did not show statistically significant association with the number of antenatal visits, gravidity, history of spontaneous abortion and stillbirth. Pregnant women without history of stillbirth (14.9%) was higher GBS colonization rates than those mothers with a history of stillbirth (7.7%) (46).

A cross-sectional study was conducted between March and May 2016 in Nekemte Referral Hospital (NRH) shows that the prevalence of GBS colonization was significantly high and differed by gestational age, higher in those pregnant women above 37 weeks of gestation and marital status , married ones (44).

Another study done in Bahirdar No the independent variables had statistically significant association with GBS anogenital colonization but history of preterm PROM, mode of delivery and practice of douching during perineal wash were showed significance in bivariate analysis (47).

2.3.Anti-Microbial Resistance Pattern of S. agalactiae isolates

A study conducted in Beijing, China S. agalactiae 6.5% found that all isolates were susceptible to penicillin and ceftriaxone (38). A meta-analysis that has been performed in Africa as a whole shows that Eighty-three articles were assessed, GBS showed the highest resistance to tetracycline (23).

A cross-sectional study over 6 months analyzing vaginal and anorectal samples obtained from 100 pregnant women at a tertiary hospital in Cameroon and the detected colonization rate was 14%. No resistance to ampicillin, oxacillin, amoxicillin-clavulanate, cefotaxime, pristinamycin, vancomycin, and clindamycin was found. And 12, 94 and 82% of strains showed sensitivity to gentamycin, erythromycin, and cefoxitin respectively (43).

A cross-sectional study was conducted from May to August 2014 at selected public antenatal care (ANC) centers in Addis Ababa, Ethiopia, and All GBS isolates were susceptible to chloramphenicol. Resistance to tetracycline, cefotaxime, clindamycin, penicillin, vancomycin, ampicillin and erythromycin was 90.2%, 34.1, 26.8%, 19.5, 17%, 14.6 and 7.5% respectively (20).

Another cross-sectional study was conducted between March and May 2016 in Nekemte Referral Hospital (NRH) shows that the twenty (91%) of GBS isolates were sensitive to vancomycin and the highest resistance was observed against penicillin G (77.3%) (34).

A cross-sectional study was conducted from December 1, 2016 to November 30, 2017 in the obstetrics department of Gondar University Referral Hospital, GBS was 0 (8.2%), 1 (25.5%), 3 (39, 8%) or higher. of antibiotics have been identified. D-test revealed 15.2% inducible clindamycin-resistant GBS (23).

A hospital-based cross-sectional study was conducted from August to December 2014 at selected health facilities at antenatal clinics in Ayder Referral Hospital and Mekelle Health Center, Mekelle, Northern Ethiopia and All the GBS isolates were susceptible (100 %) to penicillin G, vancomycin, ampicillin, erythromycin, and gentamicin. Two of the GBS isolates showed multidrug resistance against norfloxacin and ciprofloxacin (27).

In another study which was performed at Ethiopia a cross-sectional study involved 126 pregnant women at 35–37 weeks of gestation attending the antenatal clinic at Jimma University Hospital showed that all GBS isolates were susceptible to penicillin G, ampicillin, and vancomycin, but a considerable proportion was resistant to clindamycin (3.2 %), erythromycin (6.5 %), ciprofloxacin (9.7 %), ceftriaxone (9.7 %), norfloxacin (12.9 %), cotrimoxazole (29 %), and tetracycline (45.2 %) (45) .

Summary of Literature Review

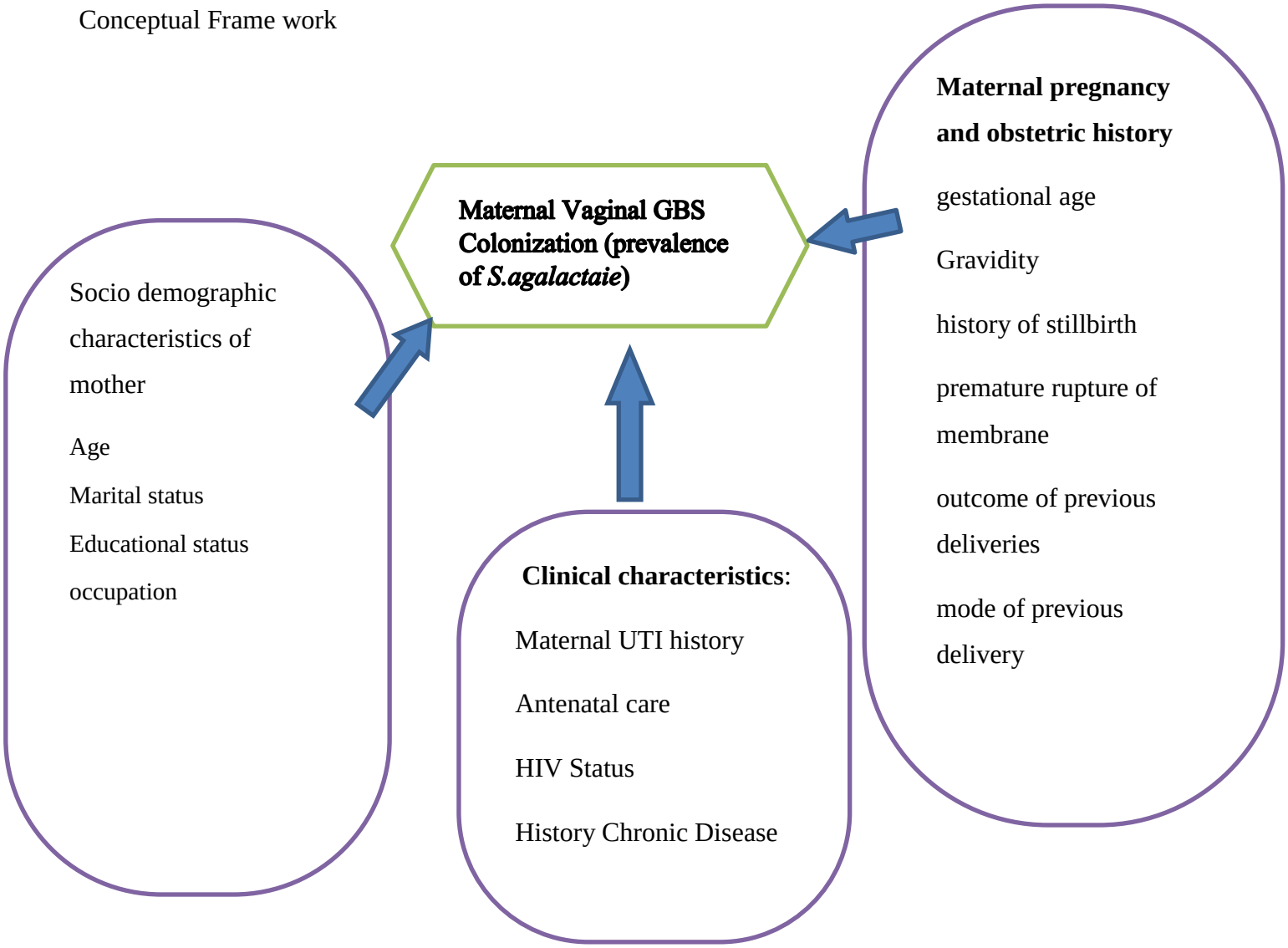
The overall prevalence of GBS varies from 6.5 % in Beigjing China to 34% in Turkey while in Ethiopia from 7.2% Addis Ababa to 25.5% in Gonder. The prevalence differs by socio demographic characteristic, maternal and clinical conditions.

Knowledge about risk factors contributing for GBS colonization in pregnant women is relevant to minimize the morbidity, mortality associated with maternal and neonatal GBS infections. The universal prenatal screening and the risk-based approach are commonly used strategies proposed by the CDC in order to decrease the incidence of early-onset GBS neonatal infection (45).

2.4.Conceptual Frame work

According to different literature the prevalence of *S.agalactiae* differs from place to place as well as determined by clinical conditions of mother and maternal history.

Conceptual Frame work



➢ Figure : Conceptual frame work about Prevalence of Streptococcus agalactiae, Antimicrobial Susceptibility Pattern And Associated Factors Among Pregnant Women at Debre birhan Comprehensive Specialized hospital, northeast Ethiopia, June 2023, (37, 41, 43, 44, 47)

3. Objectives

3.1.General objective

- To assess the prevalence of *Streptococcus agalactiae*, associated factors and Antimicrobial susceptibility pattern among pregnant women at Debre birhan Comprehensive Specialized hospital, northeast Ethiopia

3.2.Specific objectives

- To determine the prevalence of Group B streptococci (*Streptococcus agalactiae*
- To determine Antimicrobial susceptibility pattern of Group B streptococcus (*Streptococcus agalactiae*)
- To identify factors associated with Group B streptococci (*Streptococcus agalactiae)* colonization

4. Methods & Materials

4.1.Study area

The study was conducted at Debre birhan Comprehensive specialized Hospital which is located in Debre birhan town Northeast of Addis Ababa in the Debre birhan region-politan Town Admin zone of the Amhara National Regional State. The hospital serves more than 3,000,000 people and it has about 7,000 delivery services per year with 24 gynecology and obstetrics beds available.

4.2. Study design and period

A health institution based cross-sectional study was conducted from 24th May 2023 to 15th June 2023.

4.3.Source population

All pregnant women who attend at DBCSH were the source population.

4.4.Study population

Pregnant women who were come for antenatal care (ANC) at the maternity ward of the hospital during the study period with a gestational age of ≥ 35 weeks were the study population.

4.5. Eligibility criteria

4.5.1. Inclusion criteria;

All pregnant women, who gave consent for participation in the study with a gestational age of ≥ 35 weeks at Debre birhan comprehensive specialized hospital were eligible in the study.

4.5.2. Exclusion criteria

Pregnant women, who use vaginal cream, lubricants, or traditional sterilizer (vinegar) and antibiotics in the last 2 weeks before sample collection; and those who were in the emergency room and severely ill were not include in the study.

4.6.Sample size determination

The sample size was determined using sample size determination for estimation of single population proportion formula and by considering the following assumptions: 95 % confidence interval (Zα/2 = 1.96), 12.2% proportion a previous study conducted in Nekemte, Ethiopia (44) and 5 % margin of error.

n = $n=\frac{\left(\frac{Z\alpha}{2}\right)^2 * P(1-P)}{d^2}$

Where n = the sample size to be determined.

$Z^{\alpha 2}$ = the z-value at 95 % confidence interval = 1.96.

p = proportion of GBS colonization (pregnant women infected with GBS) 12.2 % = 0.122.

d = absolute sampling error (margin of error) that can be tolerated = 5 %.

$n = ((1.96)^2 \times 0.122 \times (1-0.12))/ (0.05)^2 = 165$

Considering 10 % non-response rate 0.1× 165 =17

So the final sample size is 182.

When I take by factor it 3.1 % by marital status, the sample size become 46.

While I take by Antimicrobial resistance pattern of study conducted Addis Ababa (23), the sample size become 154.

So final selected largest sample sized was 182 based on prevalence.

4.7.Sampling technique

Consecutive technique was applied by taking all pregnant mothers during study period until sample size reach 182 pregnant mothers.

4.8.Data collection

A structured questionnaire was used to obtain data on socio-demographic, obstetric, and clinical factors. The questionnaire was developed in English and translated into Amharic. Participants were interviewed using the local language; Amharic. Midwifes were trained in data collection for this particular study. The data collectors were regularly supervised by the investigator. All culture testing procedures were supervised by senior microbiologist while vaginal swab sample collection procedure was reviewed by Gynecologist.

4.8.1. Collection, transportation and culture processing

Vaginal swabs was collected by brushing the lower vagina with a sterile cotton swab by trained nurses following universal precautions (45). The swabs was immediately transported to the Medical Microbiology laboratory of Debre birhan Comprehensive specialized hospital by Amies transport media in a sterile test tube by within room temperature within 2-4 hr and was analyzed by following the methods described in the CDC and Clinical and Laboratory Standards Institute (CLSI) guidelines. For *Streptococcus agalactiae* (group B) Swabs were inoculated on a Blood agar plate and was incubated at 37 °C for 24 h. Growth (Beta hemolysis) and gram stain and catalase test was performed. All gram-positive cocci and catalase-negative isolates were tested for CAMP factor for presumptive identification of beta-hemolytic streptococci; then a CAMP test was used to identify In brief, *Staphylococcus aureus* was inoculated onto a sheep blood agar plate by making a narrow streak down the center of the plate with a loop. The test organism (group B Streptococcus) is then streak in a straight-line inoculum at right angles to the *S. aureus* within 2 mm far. Then the plates were incubated at 35 °C for 24 h. A positive CAMP is indicated by an "arrowhead"-shaped enhanced zone of beta-hemolysis in the area between the two cultures with the "arrow point" toward the *S. aureus* streak. No enhanced zone of beta-hemolysis is observed in a CAMP negative reaction.

All samples with no growth were discarded after result reading whereas isolates of *Streptococcus agalactiae* were stored by Tryptone Soya Broth (TM 018) with 15 % glycerol for further molecular analysis. But due to limitation of resource and technical skill, I have no plan to do further molecular method of testing.

4.8.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility test was done using a Kirby–Bauer disk diffusion method based on the Clinical and Laboratory Standards Institute (CLSI) guideline 2022 (48). The inoculum was prepared by suspending 4–5 isolated colonies of the same morphology in 5 ml of sterile normal saline equal to a 0.5 McFarland's standard to use as a reference to adjust the turbidity of bacterial suspensions. Sterile cotton swabs were dipped and rotated several times and were pressed against the wall of the test tube. Colonies were inoculated on Mueller–Hinton agar (MHA) plates supplemented with 5% defibrinated sheep blood. Then antibiotic disks were placed and incubated at 35–37 °C. The antibiotics used in this study were based of CLSI 2022 guideline recommendation which include (Oxoid, Basingstoke, UK): penicillin G (P, 10IU), ampicillin (AMP, 10 µg), clindamycin (CLY, 2 µg), erythromycin (E, 15 µg), chloramphenicol (C, 30 µg), ceftriaxone (CRO, 30 µg), vancomycin (VA, 30 µg), and cefotaxime(30 µg) on 5% sheep blood containing Mueller–Hinton agar by Kirby–Bauer method (disk diffusion) following the CLSI 2022 guideline. The zone of inhibition around antibiotic disks were measured by a calibrated ruler and interpreted as sensitive, intermediate, or resistant by using a standard chart (48)

4.9. Study Variables

4.9.1. Dependent variables

Prevalence of group B streptococcus (streptococcus agalactiae)

Antimicrobial Susceptibility Pattern

4.9.2. Independent variables

Socio-demographic characteristics :(Age, Residence, Marital status, Occupation, Educational status)

Clinical characteristics: (antenatal care, maternal UTI history, HIV status, Chronic illness,

Maternal pregnancy and Obstetric Chracterstics: gestational age, history prolonged rupture of membrane >18 h, preterm labor (<37 weeks), Gravidity, mode of previous delivery, Practice of douching,

4.10. Operational definition

Colonization: the presence and multiplication of microorganisms without tissue invasion or damage.

Douching: when women uses soap or water to wash the internal parts of vagina or introitus

Preterm delivery: delivery before 37 completed weeks of pregnancy.

Premature rupture of membrane (PROM), or pre-labor rupture of membrane: a rupture of membrane (breakage of the amniotic sac), commonly called breaking of the mother's water(s), more than 1 h before the onset of labor.

ANC status: women having at least 2 prior antenatal visits

Resistant: Isolates that are resistant or intermediate resistant to antimicrobial categories.

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4.11. Data processing and analysis

To generate quality and reliable data, all quality control was done. All the questions in a structured questionnaire was prepared in a clear and precise way and translated into the local language (Amharic). Questionnaire was pre tested 10 mothers in Debre berhan health center ANC clinic and adjustment was made based on comments. Midwifes were trained; questionnaires were checked for completeness, during, and after data collection by the data collectors. Moreover, all laboratory assays were done by maintaining quality control procedures. The raw data (the laboratory, clinical and demographic data) was checked for completeness and representativeness before entry to the database.

Standard Operating Procedures (SOPs) were strictly followed. Visual inspections of cracks in media or plates, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination were performed. Quality control and sterility test was performed to check the quality of the medium.

Reference strains of *S. pneumoniae* (ATCC 49619), *S. agalactiae* (ATCC 12386), were used as quality control for culture and susceptibility testing throughout the study.

After the collected data checked repeatedly for its completeness, each response was cleaned and entered in to EPI-Data 3.1 and then it was exported to IBM SPSS version 25 for data analysis. Bivariate logistic regression analysis was used to see the association between dependent and independent variables with the cutoff point p-value of <0.2 and multiple logistic regression

analysis was employed to see which variables are the most explanatory cases for dependent variables as well as to extract the cofounding one. The p- value of < 0.05 is considered significant to identify statistical significant factors associated with the outcome variable. The Hosmer and Lemeshow goodness of fit test was used to see the fitness of the model. The result is presented by using different summaries of descriptive statistics like frequency, table and graphs.

4.12. Ethical consideration

Ethical clearance was obtained from the ethical review committee of Debre berhan University, Asrat Woldeyes health science campus. All study records that identify subjects was kept confidential.

All information collected in this study was code numbers and no names were recorded. The key to this code numbers and paper files were kept in a locked file and computerized files were password-protected and all only accessible to the principal investigator.

All the investigations done for participants of this study were free of charge but hospital care and treatments were paid according to the rule of the hospital.

All GBS culture positive finding are reported to ANC clinic for appropriate treatment.

4.13. Dissemination of results

After the study had been accomplished, it will be presented to Debre Berhan University Professor Asrat Woldeyes health sciences, department of public health. Moreover, attempts will also be made to present it on scientific conferences and publish it on scientific journals here or overseas. Reports will be submitted to Amhara Health Bureau, Debre berhan Town Admin zone health department, and the DBCSH in which the research was performed.

5. Result

5.1.Socio-demographic characteristics of Respondent

A total of 181 pregnant women attending ANC service were recruited, with a response rate of 99.5 %. The mean age ± SD of the study participants was 26.7 ± 5.2 years. Almost half (50.8%) of the study participants were in the age of 25 to 34 years and Majority 170(93.9%) of the study participants were married and. Most participants 136 (75.1%) were from urban dwellers. Above a third of study participants (39.2%) attended college education. (Table 1)

Table : Socio-demographic characteristics of the study participants (n=181) in Debre birhan Comprehensive Specialized Hospital (DBCSH), North East Ethiopia, June, 2023

Variables	Frequency	Percent (%)
Age in years		
≤24	75	41.4
25-34	92	50.8
≥ 35	14	**7.7**
Residency		
Rural	45	24.9
Urban	136	75.1
Occupation		
Government Employed	38	21
Self Employed	42	23.2
House wife	84	46.4
Merchant	17	9.4
Educational level		
No Formal Education	10	5.5
Primary	37	20.4
Secondary	63	34.8
College and Above	71	39.2
Marital Status		
Married	170	93.9
Unmarried	11	6.1

5.2.Prevalence of GBS among pregnant women

Among 181 study participants, 18(9.9%) (95% CI 5.5-15.7) were found to be GBS positive. Regarding to gravidity 108(59.7) were multigravida women. Almost half 10(55.6%) of study participants were housewife followed by self-employed 4(22.2). Seventeen (94.4%) of GBS colonization were isolated from married pregnant women. (Table 2)

Table : Socio-demographic characteristics in GBS colonization of study participants (n=181) in Debre birhan Comprehensive Specialized Hospital (DBCSH), North East Ethiopia, June, 2023

Variables	**GBS**		**Total (%)**
	Positive (%)	**Negative (%)**	
Age in years			
≤24	6(33.3)	70(42.9)	76(41.4%)
25-34	11(61.1)	80(49.1)	92(50.8)
>35	1(5.6)	13(8)	14(7.7)
Residency			
Rural	4(22.2)	41(22.6)	45(24.9)
Urban	14(77.8)	122(77.4)	136(75.1)
Occupation			
Government Employed	3(16.7)	35(21.5)	38(21)
Self Employed	4(22.2)	38(23.3)	42(23.2)
House wife	10(55.6)	74(45.4)	84(46.4)
Merchant	1(5.5)	16(9.8)	17(9.4)
Educational level			
No Formal Education	1(5.5)	9(7.7)	10(5.6)
Primary	2(11.1)	35(90.1)	37(20.4)
Secondary	10(55.6)	53	63 (34.8)
College and Above	5(27.8)	66	71 (39.2)
Marital Status			
Married	17(94.4)	153(93.9)	170(93.9)
Unmarried	1(5.6)	10(6.1)	11(6.1)

5.3.Obstetric and medical characteristics in GBS colonization of study participants

Most 14(87.8%) of GBS isolate were from multigravida pregnant mothers. In our study, 24(13.3%) of the study participants had history of UTI during the current pregnancy. Only 3(1.7%) of GBS were isolated from participants with HIV positive. Almost all (98.8%) study participants have at least two and above antenatal care visit during current pregnancy. Above half 10(62.5%) GBS were from participants with history of premature rapture of membrane (PROM). (Table 3)

Table : Obstetric and medical characteristics in GBS colonization of study participants (n=181) in Debre birhan Comprehensive Specialized Hospital (DBCSH), North East Ethiopia, June, 2023

Variables	**Total (%)**	**GBS**	
		Positive (%	**Negative (%)**
Gestational age			
35-37 weeks	104(57.5)	7(38.9)	97(59.5)
>37 weeks	77(42.5)	11(61.1)	66(40.5)
Gravidity			
Primigravida	73(40.3)	4(22.2)	69 (42.3)
Multigravida	108(59.7)	14(87.8)	94 (57.7)
Hospital admission history			
Yes	10(5.5)	2(11.1)	8()
No	171(94.5)	16(88.9)	155()
Surgical procedure			
Yes	8(4.5)	2(11.1)	6(3.7)
No	173(95.6)	16(88.9)	157(96.3)
Preterm labor			
Yes	8(8.7)	3(16.7)	5(6.8)
No	84(91.3)	15(83.3)	69(93.2)
PROM			
Yes	14(15.2)	6(37.5)	8(19.9)
No	79(84.8)	10(62.5)	68(90.1)

Variables	Total (%)	GBS	
		Positive (%	Negative (%)
Still birth			
Yes	13(14.1)	4(30.8)	9(11.8)
No	79(85.9)	12(69.2)	67(88.2)
Neonatal death (N=92)			
Yes	12(13)	1(6.7)	11(14.3)
No	80(87)	14(93.3)	66(85.7)
HIV status			
Yes	3(1.7)	1(5.6)	2(0.6))
No	178(98.3)	17(94.4)	179(99.6)
Maternal UTI History			
Yes	24(13.3)	7(46.7)	17(10.4)
No	157(86.7)	11(73.3)	146(89.6)
Mode of previous delivery			
SVD	78(84.8)	15(100)	63()
CS and instrumental	14(15.2)	0(0)	14()
Abortion History			
Yes	8(4.4)	2(11.1)	6(33.3)
No	173(95.6)	16(88.9)	157(66.7)
Chronic illness			
Yes	10(5.5)	1(5.5)	9(14.3)
No	171(94.5)	17(94.5)	154(85.7)
ANC follow up			
Yes	179(98.9)	0(0)	2(1.2)
No	2(1.1)	18(100)	161(98.8)
Douching Practice			
Yes	93(51.4)	5(33.3)	88(53.9)
No	88(48.6)	13(66.7)	75(46)

N.B. statically significant at $p<0.05$

SVD (spontaneous vaginal delivery), CS (cesarean section), PROM (premature rupture of membranes, ANC (Antenatal Care), GBS (Group B *streptococcus agalactiae*)

5.4. Associated risk factors of maternal GBS colonization

Of the 181 study participants 18 (9.9 %) were found positive for GBS. From six variables included in bivariate analysis, surgical procedure, PROM, stillbirth, history of last pregnancy as preterm, history of abortion, history of UTI, practice of douching during perineal wash, only two variables , PROM and history of UTI, show statistically significant association in multivariate analysis.

Pregnant mothers with current UTI has 7 time more exposed to be colonized by *streptococcus agalactiae* than their counter parts (AOR 7.017, 95 CI 1.599-30.791). The pregnant women who had history of longer duration of PROM were 8 time (AOR=8.638, 95% CI 1.639, 27.387) more likely to have an increased risk of being colonized than those who had no history of PROM.

(Table 4)

Table : Determinants of GBS colonization of study participants (n=181) in Debre birhan Comprehensive Specialized Hospital (DBCSH), North East Ethiopia, June, 2023

Variables	GBS		COR:95%,CI	AOR:95%,CI	P-Value
	Positive (N=18)	Negative (N=163)			
Surgical procedure					
Yes	2(11.1)	6(3.7)	3.271(.609-17.566)	0.379(0.027-5.306)	0.471
No	16(88.9)	157(96.3)			
Preterm labor					
Yes	3(16.7)	5(6.8)	4.312(1.176-15.813)	3.236(0.438-23.906)	0.250
No	15(83.3)	69(93.2)			
PROM					
Yes	6(37.5)	8(19.9)	6.667 (1.830-24.281)	8.638(1.639-27.387)	**0.011**
No	10(62.5)	68(90.1)			
Maternal UTI History					
Yes	7(46.7)	17(10.4)	6.871 (2.388-19.766)	7.017(1.599-30.791)	**0.010**
No	11(73.3)	146(89.6)			
Still birth					
Yes	4(30.8)	9(11.8)	4.467(1.308-15.254)	2.294(0.448-11.748)	0.319
No	12(69.2)	67(88.2)			
Abortion					
Yes	2(11.1)	6(33.3)	3.271(.609-17.566)	5.043(0.270-94.341)	0.279
No	16(88.9)	157(66.7)			
Douching Practice					
Yes	5(33.3)	88(53.9)		0.323(0.083-1.261)	0.104
No	13(66.7)	75(46)	3.051 (1.040-8.951)		

5.5. Antimicrobial susceptibility pattern of GBS isolates

In this study, 8 antimicrobial agents were used to investigate the antimicrobial susceptibility pattern of GBS isolates. All GBS isolates were susceptible to ampicillin, penicillin G, Cefotaxime, Ceftriaxone, and vancomycin. Relatively, GBS showed resistance to erythromycin (16.7%) clindamycin (11.1%) and chloramphenicol (5.6%) and there was no multiple drug resistance seen. (Table 5)

Table : Antimicrobial susceptibility pattern of GBS (n =18) isolated from pregnant women in Debre birhan Comprehensive Specialized hospital, June 2023

Antibiotics	**Susceptible (%)**	**Resistant (%)**
Ampicillin	18(100%)	0
Penicillin	18(100%)	0
Erythromycin	15(83.3%)	3(16.7%)
Clindamycin	16(88.9%)	2(11.1%)
Cefotaxime	18(100%)	0
Ceftriaxone	18(100%)	0
Vancomycin	18(100%)	0
Chloramphenicol	17(94.4%)	1(5.6%)

6. Discussion

Group B *streptococcus agalactiae* (GBS) is frequent cause of postpartum maternal and neonatal sepsis. Planning and putting into practice preventative efforts require an understanding of the GBS carriage rate of pregnant women. There hasn't been a study done yet to determine the prevalence of GBS and associated variables in this geographic area.

The present study revealed the overall prevalence of genital GBS carriage among pregnant mothers at DBCSH was 9.9%. This finding was consistent with study conducted in Jimma (10.4%) (45) , Arba Minch, Ethiopia (8.5%)(7), Nekemte (12.2) (44).

The finding of this study was lower than the study conducted in Mbarara university hospital, Uganda(28.8%) (49), University of Gonder hospital (25.5%) (23) Hawassa (15.7%) (50). This study showed a lower prevalence of maternal colonization with GBS than the adjusted global estimates of colonization (18%) (22). This could be due to differences in sampling techniques, socioeconomic conditions, or geographic differences in GBS colonization. In addition the variation might be explained by differences in type of culture media used, and genetic factors. The lower detection rate might be due to vaginal swab cultured on Todd Hewitt broth but sample were inoculated on 5 % sheep blood agar only in the current research, differences in geography differences in sample size higher than the current sample size, differences in gestational age (some studies recruited pregnant women gestational ages of 35–37 weeks but pregnant women with gestational ages of ≥35 weeks were recruited the current study), and also the time of screening for GBS. Accessibility of pregnant women screening for GBS, Intrapartum antibiotic prophylaxis provision, and detection techniques employed for GBS may also attribute to these variations.

Specimen storage conditions, duration of sample transportation, use of antibiotics, and antiseptic products may also cause disparities of GBS detection among studies. Swabbing cotton tips used, epidemiological characteristics, and different study designs might be the additional contributing reason for this variability across the studies.

However, the results of this study were higher than those of a study conducted in Nigeria Urban Hospital (1.33%) (51), India (kerala) (4.8%) (52). This could be due to differences in sampling techniques, socioeconomic conditions, or geographic differences in GBS colonization.

Knowledge about risk factors associated to maternal colonization of GBS is useful to reduce morbidity and mortality related to GBS diseases. In this study, possible risk factors such as age, gravidity, occupation, residence, previous preterm labor , PROM, history of surgical procedure, mode of delivery, history of abortion or still birth, Presence of UTI , presence of chronic disease, HIV status, frequency of perineal wash, and practice of douching during perineal wash were considered. Maternal UTI history P-value 0.010 AOR 7.017(95% CI 1.599-30.791), Premature rapture of membrane (PROM) P-value = 0.11 AOR 8.638(95% CI 1.639-27.387) has been found significant in multivariate logistic regression.

Premature rapture of membrane (PROM) were significantly associated factors to maternal colonization in our study which is supported by studies done in Adigrat (53), Southern Ethiopia (54) Nigeria (51) and India (55). Increased duration of rupture of membranes (ROM) promotes the process of ascending colonization and infection of the uterine compartment and fetus.

Maternal UTI history in current pregnancy have 7 time high risk AOR 7.017(95% CI 1.599-30.791), compared to women with no such history which supported by study conducted in South west Ethiopia(54, 56).

Streptococcus agalactiae resistance Chloramphenicol, erythromycin and clindamycin was observed but the study shows no resistance observed in ampicillin, ceftriaxone, Cefotaxime, penicillin, and vancomycin which consistence with other study no resistance to penicillin, ampicillin and/or vancomycin (45). Also supported by studies conducted in Ethiopia 22.7% GBS resistant to erythromycin, 17.6% to 18.2% to Clindamycin (44). In contrast to this finding research done in Ethiopia that resistance to penicillin is ranged from 36.4 to 77.3% (44). In the current study, few GBS isolates showed resistance to erythromycin, chloramphenicol and clindamycin. This may limit prophylaxis options for pregnant women who are allergic to penicillin.

7. Conclusion

Overall prevalence of Streptococcus agalactiae (GBS) was 9.9% (95 CI 5.5-15.7). This study showed lower prevalence from similar studies in Ethiopia as well as global estimates. Maternal UTI history in current pregnancy and PROM has been found risk factor for maternal GBS colonization at their late third trimester. All GBS isolates were susceptible to penicillin G, ampicillin, Cefotaxime, Ceftriaxone, and vancomycin. Relatively, GBS showed resistance to clindamycin (11.1%), chloramphenicol (5.6%), and erythromycin (16.7%), and there was no multiple drug resistance seen.

8. Recommendation

Based on the findings and the conclusions made, the following recommendations were forwarded

For Debre birhan Comprehensive Specialized hospital, it is advised to use preventive strategies like IAP at least for mothers with UTI history on current pregnancy and PROM history that were identified as factor which is not yet practiced earlier in the hospital as well as in Ethiopia. And also during maternal antenatal care follow up, better to investigate vaginal colonization of GBS and antimicrobial susceptibility test should be done for isolates before prescription of antimicrobials.

For Amhara regional state health bureau, during pregnant mothers attend ANC, Vaginal swab test for GBS colonization should be incorporated as service package.

For researchers, additional studies are needed to follow up the fate of pregnancies and the status of neonates born to mothers colonized by GBS was recommended.

9. Strength and Limitations

9.1.Strengths of study

It is more informative as the study used primary data with culture and structured questionnaire to collect data.

9.2. Limitations of study

Failure to assess the outcome on neonates, whose mother detected to be colonized by GBS on the study.

Serotyping and molecular characterization of GBS isolates were not performed because of budget constraints.

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11. Annexes

A. DATA COLLECTION FORM

DEBRE BERHAN UNIVERSITY

ASRAT WOLDEYES HEALTH SCIENCE CAMPUS

DEPARTMENT OF PUBLIC HEALTH (EPIDEMIOLOGY)

Annex I: General information for the study participants English version

Date.....

Introduction

My name is …… and I am MPH student of Debre Berhan University, Asrat Woldeyes Health Sciences Campus department of Public health. I am doing research entitled "Prevalence of Streptococcus agalactiae, Antimicrobial Susceptibility Pattern and Associated Factors Among Pregnant Women At Debre birhan Comprehensive Specialized Hospital, North East Ethiopia ". Currently Group B streptococci, have a great burden on pregnant women as different studies indicate. So this study will indicate burden and their Antimicrobial pattern of Group B streptococci at Debre birhan Comprehensive specialized Hospital it will help the physicians to treat based on culture results.

What is the reason for this study?

The objective of this research is to study the Prevalence of Group B streptococci, associated factor, and Antibiotic among pregnant women at Debre birhan Comprehensive specialized Hospital. If you agree to participate in the study, you will give us the necessary information and a vaginal swab sample will be collected from you for laboratory analysis**.**

Will my information be kept confidential?

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, which 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.

What about my rights 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

አጠቃላይ መረጃ

ደብረ ብርሃን ዩንቨርሲቲ የማህበረሰብ ጤና ትምህርት ክፍል

በጥናቱ የሚሳተፉ ግለሰቦች የፈቃድ መጠየቂያ እና መቀበያ ፎርም

መግቢያ

ስሜ----------እባላለሁ::የደብረ ብርሃን ዩኒቨርሲቲ የማህበረሰብ ጤና የትምህርት ክፍል የማስተርስ ድግሪ ተማሪነኝ በአሁኑ ሰአት የGroup B streptococci በነፍሰጡር እናቶች ላይ የሚያመጣውን ህመም እና ያለዉን የስርጭት መጠን ለማወቅ ጥናት እያካሄድኩ ነው፡የ Group B streptococci በነፍሰጡር እናቶች ህሙማን ላይ የተለያዩ ችግሩች ሲያመጡ ይታያል፡፡ይህ ጥናት ደብረ ብርሃን ኮምፕሪሄንሲቭ ስፔሻላይዝድ ሆስፒታል ነፍሰጡር እናቶች፡የ Group B streptococci ምልክት የሚሳዩትን መለየት 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>

የዚህጥናት አላማየ Group B streptococci በነፍሰጡር እናቶች ላይ ያለዉን ስርጭት መጠን፣ተዛማጅ ምክንያቶች እንዲሁም የመድሃኒት መላመድ ለመዳሰስ እና ለማወቅ ነው፡፡እርስዎ በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ ለጥናት የሚያስፈልገዉን ከብልትዎ ላይ ናሙናና እንዲሁም ለጥናቱ የሚያስፈለጉ መረጃዎችን ይሰጣሉ።

<u>ስለ እኔ የሚያዙ መረጃዎች በሚስጥር ይጠበቃለ?</u> የሚሰጡት መረጃ ሚስጥራዊነቱ የተጠበቀነው በስም አይጻፋም የዚህ ኮድ መፍቻ በፋይል ተቆልፎ የሚቀመጥ ሲሆን የተፈቀደለትሰው ብቻ ፋይሉን ማየት ይችላል፡፡ከዚህ ጥናት በሚወጡ ዘገባዎች ወይንም የህትመት ውጤቶች ላይ ስም ወይም ሌላ የእርስዎን ማንነት የሚገልጽ መረጃ አይኖርም፡፡ከምርመራ የሚገኝውም ውጤት ወይም ሌላ መረጃ ለሚመለከታቸው አካላት ለምሳሌ፤እርስዎን የሚንከባከቡ የህክምና ባለሙዎች እና ጥናቱን ለሚያካሄዱት ባለሙያዎች እንዲሁም ጥናቱ ስነምግባርን ጠብቆ መከናወኑን ለሚከተተሉት የኮሚቴ አባላት ብቻ ይገለፃል፡፡ኮምፒውተር ላይ ያለ መርጃዎች ምስጢራዊነታቸው የተጠብቀ ሲሆን በወረቀት ያለ መረጃዎችም ደህንነቱ በሚጠበቅ ቦታ የሚቆለፉና የተፈቀደለት ሰው ብቻ ሊያያቸው እንዲችል ተደርጎ ይጠበቃል፡፡ ዉጤቱ ተጨማሪ ምርመራ የሚያስፈልገዉ ከሆነ እና ህክምና ካሰፈለገዉ ለሀኪሙ ዉጤቱ ይሰጠዋል፡፡

<u>በጥናቱ ለመሳተፍ ፈቃደኛ አለ መሆን ወይም መሳተፍ ከጀመሩ በኋላ ራስን የማግለል መብት</u>

በጥናቱ የሚሳተፉት ፈቃደኛ ከሆኑ ብቻ ነው፡፡ስለዚህ መሳተፍ አለመሳተፍ ከጀመሩ በኃላ ማቋረጥ ወይም መመለስ የማይፈልጉት ጥያቄከ ሆነ 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 English version

Dear participants,

I have been informed about the objective of the study entitled **"*Prevalence of Streptococcus agalactiae, Antimicrobial Susceptibility Pattern and Associated Factors Among Pregnant Women At Debre birhan Comprehensive Specialized Hospital, North East Ethiopia".*** 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, with a full understanding of the situations, I agree to give the entire necessary information and vaginal swab sample for laboratory analysis. 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 results for the Group B streptococci will be given to the health facility and that I may ask the information if I want. This questionnaire and sample collection are designed for the research study. The main objective of this questionnaire and sample collection is to study the Prevalence of Group B streptococci (*Streptococcus agalactiae*), associated factor, and Antimicrobial susceptibility pattern among pregnant women used for partial fulfillment of MPH in Epidemiology. 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 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!!

Annex III. Consent form Amharic version

የዚህ ጥናት አላማየ Group B streptococci በነፍሰጡር እናቶች ላይ ያለዉን ስርጭት መጠን ተዛማጅ ምክንያቶች እንዲሁም የመድሃኒት መላመድ ለመዳሰስ መሆኑን ተረድቻለሁ በዚህ ጥናት የሁሉም መረጃዎች ደህንነት የተጠበቀ መሆኑን:፣ከጥናቱ በፈለኩ ጊዜ መዉጣት እንደምችል፣ በማቋረጤ ምክንያት በህክምና ክትትል ላይ ምንም አይነት ችግር እንደም ይደርስብኝ፣የዚህ ጥናት ዉጤት ለሆስፒታሉ እንደሚሰጥና ደህንነቱ በተጠበቀ ሁኔታ እንደሚቀመጥ እንዲሁም መረጃዉን በፈለኩ ጊዜና ሰአት የማግኘት ሙሉ መብት እንዳለኝ ግልጽ በሆነና በሚገባኝ ቋንቋስ ለተነገረኝ በሚገባ ተረድቻለሁ በመሆኑም ለዚህ ጥናት የሚያስፈልገዉን ከብልት ላይ የሚወሰደዉን ናሙናና እንዲሁም ለጥናቱ የሚያስፈለጉ መረጃዎችን ለመስጠት ሙሉ ፈቃደኛ መሆኔን እግልጻለሁ

ቀን--------

ፊርማ--------

Annex IV. Questionnaire English version

Section 1: Demographic Information:

Patient ID

Serial No.	Questions	Coding categories
101	Age	_____
	Residence area	1.Rural 2.Urban
102	Occupation	1.farmer 2.civil servant 3.self-employee 4.daily labor 5.house wife 6.merchant 7.others_____
103	Educational level	1.No formal education 2.primary 3.secondary 4.college/university
104	Marital status	1.Married 2.Single 3.Divorced 4.Widowed

B. Maternal and Obstetric Information

Sr	Questions	Coding categories
201	Gestational age (week)	1.<37 2.≥37
202	Gravidity	1.Primigravida 2.Multi gravida
203	Hospital admission history	Yes No
204	Surgical procedure	1.Yes 2.No
205	Have Have give birth earlier? Multi-Parity (If Q204 No, What 206-210)	1.Yes 2.No
206	Mode of delivery?	1.C/S 2.SVD 3.Instrument

207	last pregnancy was preterm labor (<37 weeks)	1.Yes 2.No
208	Prolonged rupture of membrane >18 h in the previous pregnancy?	1.Yes 2.No
209	Was there a history of stillbirth?	1.Yes 2.No
210	Have you had a history of neonatal death?	1.Yes 2.No
211	Antenatal care visits?	1.yes 2.No
212	Have you faced maternal UTI history in the current pregnancy?	1.Yes 2.No
213	If Yes for 212; Have Treated	1.Yes 2.No
214	Have you had a history of abortion?	1.Yes 2.No

C. Medical Informations

301	HIV status?	1.Yes 2.No
302	If 301 Yes	1.Yes 2.No
303	How Long you Take ART?	1.Yes 2.No
304	Have you got Chronic illness at the current pregnancy?	1.Yes 2.No
305	If Q304 Yes, What	Diabetic mellitus Hypertension Asthma Others….
306	How frequent do you wash your perineum?	1 Daily 2 Twice daily 3 More than twice daily
307	Do you practice douching or used soap during perineal wash?	1.Yes 2.No

Annex V. Questionnaire Amharic version

ሀ. ማህበራዊ እና ኢኮኖሚያዊ

የካርድቁጥር.....

ተራቁጥር	ጥያቄዎች	መለያ ኮድ
101	እድሜ	_____
102	መኖሪያ ቦታ	1.ገጠር 2.ከተማ
103	የስራ መስክ	1. ገበሬ 2. የመንግስት ሰራተኛ 3. የግል ሰራተኛ 4. የጉልበት ሰራተኛ 5.የቤት እመቤት 6. ነጋዴ 7. ሌላ
004	የትምህርት ደረጃ	1. ያልተማረ 2. የመጀመሪያ ደረጃ 3. ሁለተኛ ደረጃ 4. ኮሌጅ/ዩኒቨርስቲ
005	የጋብቻ ሁኔታ	1.ያገባ 2.ያላገባ 3.የፈታች 4. የተለያየች

ለ. የእናቶች የወሊድና ተያያዥ መረጃዎች

ተ.ቁ	ጥያቄዎች	መለያኮድ
201	የእርግዝና ጊዜ (ሳምነት)	1. 35 WK 2. 36 Wk 3. ≥37 Wk
202	ስንተኛ እርግዝናዎ ነው;	1.የመጀመሪያ 2.ከአንድ በላይ
203	ካሁን በፊት ሆሰፒታል ተኝተዉ ታክመዉ ያዉቃሉ	1.አዎ 2.የለም
204	**የቀዶ ህክምና አድርገዉ ያዉቃሉ·**	1.አዎ 2.አላደረኩም
205	**ከአሁን በፊት ወልደው ያውቃሉ·** **(መልሱ የለም ከሆነ ከ**206-209 **ይዘለል**)	1.አዎ 2.የለም
206	የአወላለድ ሁኔታ (Q205 አዎ ከሆነ)	1.ቀዶጥገና 2.በማሀጸን 3.በመሳሪያ
207	ከዚህ በፊት በነበረዉ እርግዝና ቀድሞ የተፈጸመ ዉልደት ነዉ? (≤37 ሳምንት)	1.አዎ 2.የለም
208	ከዚህ በፊት በነበረዉ እርግዝና የሽንት ዉሀ ቀድሞ ፈሶ ነበረ (premature Rapture of membrane)	1.አዎ 2.የለም

209	ከዚህ በፊት በነበረዉ እርግዝና ቀድሞ ሞቶ ወልደዋል?	1.አዎ 2.የለም
210	ከዚህ በፊት በነበረዉ እርግዝና ከተወለደ በኋላ ሞቶበዎት ያዉቃል?	1.አዎ 2.የለም
211	የእርግዝና ክትትል መኖር (በአሁኑ እርግዝና)	1.አዎካሉ: 1.1. 0–3 1.2. 4–5 2.የለም
212	በአሁኑ እርግዝና የሽንት ቧንቧ ህመም አለበዎት?	1.አዎ 2.የለም
213	ለ 212 መልሶ አዎ ከሆነ፤ ታክመዋልን	1.አዎ 2.የለም
214	ውርጃ አጋጥሞዎት ያውቃል	1.አዎ 2.የለም

ሐ. የህክምናመረጃ		
301	ኤችአይቪ አለበዎት	1.አዎ 2.የለም
302	ለ301 ***መልስዎ አዎ ከሆነ፣መድሀኒት ጀምረዎል***	1.አዎ 2.የለም
303	***መድሀኒት ከጀመሩ ለምን ያህል ግዜ***	
304	ሌሎች ተያያዥ በሽታዎች አለበዎት	1.አዎ 2.የለም
305	ለ ጥያቄ 304 መልስዎ አዎን ከሆነ	1. ስኳር 2. ግፊት 3. አስም 4. ሌላ.....
306	ማህጸንዎን በምን ያህል ግዜ ይታጠባሉ	1 በቀን 2 በቀን 2 ግዜ 3 ከዛ በላይ
307	ማህጸንዎን ሲታጠቡ ሳሙና የመጠቀም ልምድ አለዎት	1.አዎ 2.የለም

Annex VI. Laboratory Test procedures

This procedure provides instructions for inoculating, reading, and interpreting urogenital discharge culture. This is a procedure for the Identification of Group b streptococci (*Streptococcus agalactiae*) from urogenital specimen culture

Principle

Specimens from genital sites are sent to the clinical microbiology laboratory for detection of microorganisms from females presenting with clinical syndromes such as cervicitis, vulvovaginitis, urethritis, bacterial vaginosis (BV), salpingitis (pelvic inflammatory disease [PID], endometritis, or genital ulcers and from males exhibiting urethritis, epididymitis, prostatitis, or genital ulcers Specimens are also submitted from pregnant females to diagnose the presence of organisms that may cause disease in the neonate. Less commonly, specimens are sent from children and postmenopausal women.

Specimen Handling and Preparation

Along with the specimen, design a system to provide the following.

1. Demographics of the patient: name, address, age, sex, location in the hospital , unique patient identifying number, name of the physician of record, name of the physician who is ordering the test, and ICD9/10 code or diagnosis.

NOTE: Most jurisdictions require a clinical specimen to be labeled with two unique patient identifiers for it to be accessioned and tested. Besides, the patient identifiers on the requisition must match that provided on the specimen label.

2. Details of the specimen: type of specimen, anatomic site of collection (if variable), whether the specimen was collected from an invasive procedure (e.g., during surgery), and gross description, if variable.

3. Specific culture, stain, and antigen test requests. Provide the above information on each request form, specimen container, and transport carrier. The patient demographic information, specimen information, and specific test requests provided on the laboratory requisition should match those on the specimen container label and the transport carrier.

4. Vaginal swabs will be collected by brushing the lower vagina with a sterile cotton swab by trained nurses following universal precautions and transported to the Medical Microbiology laboratory section of the hospital.

Reagents, Materials, & Equipment

The media that will be used for identification of *Streptococcus agalactiae* are 5% Blood Agar and, Muller-Hinton Agar, Petri dish 90mm diameter, Petri dish 150mm diameter.

B. Stain reagents and supplies

Cristal violate of 500ml

Gram iodine of 500 ml

Acetone alcohol of 500 ml

Safranin of 500 ml

Microscope slide (27x75mm) of 100 pcs

C. Other supplies

1. Sterile Petri dishes

2. Pasteur pipettes

3. Sterile scissors, forceps, and scalpels

4. Sterile test tubes

5. Sterile swabs and sticks

6. Inoculating loops

D. Equipment

1. Biological safety cabinet

2. Incubators (35 to 37————————————C; both 5% CO2 and ambient air)

Storage and Handling

2 cotton swabs of vaginal discharge (one for culture and the other for microscopy)

Vaginal swabs for culture will be inserted in Amies transport medium.

Specimen will not freeze or incubated.

The patient did not passed urine for 2 hours before the specimen is collected.

Specimen collection, transportation and culture processing

1. Vaginal swabs will be collected by brushing the lower vagina with a sterile cotton swab by trained midwives following universal precautions (45).

2. The swabs will be immediately transported to the Medical Microbiology laboratory of Debre birhan comprehensive specialized hospital laboratory by Amies transport media in a sterile test tube by controlling the appropriate temperature within 2-4 hour. and Swabs inoculated on a Blood agar plate and were incubated at 37 °C for 24 h.

3. Growth (Beta hemolysis) and gram stain and catalase test will be performed.

4. All gram-positive cocci and catalase-negative isolates will be tested for CAMP factor for presumptive identification of beta-hemolytic streptococci; CAMP test uses to diferentiate GBS (CAMP positive) from Streptococcus pyogene (beta-hemolytic CAMP negative). In brief, *Staphylococcus aureus* is inoculated onto a sheep blood agar plate by making a narrow streak down the center of the plate with a loop.

5. The test organism (group B *Streptococcus*) is then will be streaked in a straight-line inoculum at right angles to the *S. aureus* within 2 mm far.

6, the plates will be incubated at 35 °C for 24 h. A positive CAMP is indicated by an "arrowhead"-shaped enhanced zone of beta-hemolysis in the area between the two cultures with the "arrow point" toward the *S. aureus* streak.

7. No enhanced zone of beta-hemolysis was observed in a CAMP negative reaction. The CAMP test positive colonies are presumptively considered as GBS. (48).

Gram stain procedure

1. **Heat fixation**

a. Pass air-dried smears through a flame two or three times. **Do not overheat**.

b. Allow slide to cool before staining.

2. Flood the prepared slide with crystal violet for one minute.
3. Rinse the slide gently with tap water.
4. Flood the slide with Gram's iodine for one minute.
5. Rinse the slide gently with tap water.
6. Working with one slide at a time, flood the slide with decolorizer for 5 seconds and rinse with tap water.
7. Flood the slide with safranin for one minute.
8. Rinse the slide gently with tap water.

Antimicrobial susceptibility pattern

1. The inoculum is prepared by suspending 4–5 isolated colonies of the same morphology in 5 ml of sterile physiological saline equal to a 0.5 McFarland's standard use as a reference to adjust the turbidity of bacterial suspensions.
2. Sterile cotton swabs are dipped and rotated several times and will be pressed against the wall of the test tube.
3. Colonies are inoculated on Mueller–Hinton agar (MHA) plates supplemented with 5% defibrinated sheep blood.

4. Then antibiotic disks will be placed and incubated at 35–37 °C. The antibiotics used in this study include (Oxoid, Basingstoke, UK): penicillin G (P, 10IU), ampicillin (AMP, 10 µg), clindamycin (CLY, 2 µg), erythromycin (E, 15 µg), chloramphenicol (C, 30 µg), ceftriaxone (CRO, 30 µg), vancomycin (VA, 30 µg), and cefotaxime(30 µg)on 5% sheep blood containing Mueller–Hinton agar by Kirby–Bauer method (disk diffusion) following the CLSI 2022 guideline.

5. The zone of inhibition around antibiotic disks is measured by a calibrated ruler and interpreted as sensitive, intermediate, or resistant by using a standard chart (48).

Annex VII: Laboratory results

Participant code_____

Bacteria identified from culture is GBS? Yes ☐ No ☐

Break point of drug susceptibility of GBS from CLSI 2022

	Antimicrobial Disk	Disk content	Zone of diameter (mm)			Result	Interpretation	Remark
			S	I	R			
1.	Ampicillin	10 units	≥24	-	≤23			
2.	Chloramphenicol	30 μg	≥21	18-20	≤17			
3.	Cefraxone	30 μg	≥24	-	≤23			
4.	Cefotaxime	*30* μg	≥24	-	≤23			
5.	Clindamycin	*2* μg	≥19	16-18	≤15			
6.	Erythromycin	15 μg	≥21	16-20	≤15			
7.	Penicillin	10 μg	≥24	-	≤23			
8.	Vancomycin	*30* μg	≥17	-	≤16			

Based on CLSI 2022: Table 2H.1 Streptococcus B-haemolysis M002 and M007

Annex VIII: Declaration

I hereby declare that this MPH thesis is my original work and has not been presented for a degree in any other university. Where, the work of other people has been used, reference has been provided. In this regard, I declare this work to be our unique work.

Name: Getaw Alemayehu

Signature: _____ Date _____

Annex IX: Advisors Approval sheet

This is to certify that the thesis entitled "Prevalence Of *Streptococcus agalactiae*, Anti-microbial resistance And Associated Factors Among Pregnant Women At Debre birhan comprehensive specialized hospital, North East Ethiopia." is submitted in partial fulfillment of the requirements for the degree of MPH in Epidemiology to the Graduate Program of the Asrat Woldeyes Health science campus, Debre Berhan University and was carried out by Getaw Alemayehu ID No: PGE/92/13 under my supervision. Therefore, I recommend that the student has fulfilled the requirements and hence hereby can submit the thesis to the Department.

Name of Advisors:

1. Muluken Tessema (BSc, MPH)

   Signature _____ Date _____

2. Woineshet Bedru (BSc, MPH)

   Signature _____ Date _____