

**ADDIS ABABA UNIVERSITY**  
**COLLEGE OF HEALTH SCIENCES**  
**DEPARTMENT OF MEDICAL LABORATORY SCIENCES**



Prevalence of *Streptococcus agalactiae* (GBS) In Pregnant Women and its effect on outcome of neonates at Zewditu Memorial Hospital, Addis Ababa, Ethiopia

A research thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology Speciality)

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June, 2021

Addis Ababa, Ethiopia

## Research Project Submission Form

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Semira Mustefa, entitled:

Group B Streptococcus agalactiae Prevalence in Pregnant Women and outcome of neonates in Zewditu Memorial Hospital, Addis Ababa, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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## Acknowledgment

First of all, I would like to acknowledge Addis Ababa University, Department of Medical Laboratory Sciences for providing me good inspiration in the scientific teaching and for providing me a chance to conduct research which is a chance to learn and to contribute.

Secondly I would like to thank my advisors Mr. Kassu Desta for his important suggestions and comments to the writing of this thesis.

My heartfelt regards to all pregnant mothers who participated in this study and for their contribution in improving pregnant mothers and neonates health and also for making this study possible.

Finally, I would like to thank all Laboratory personnel and delivery and antenatal care unit staff working in Zewditu memorial hospital and also my families and friends for their kind assistance are acknowledged.

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## Abbreviations

AAU:	Addis Ababa Univerisity
CAMP:	Christie,Atkins,and Munch-Peterson
CLSI:	Clinical and laboratory Standards Institute
DC:	Data Collector
DRERC:	Departmental Research and Ethics Review Committee
EOD:	Early-onset disease
GBS:	Group B streptococcus
IAP:	Intrapartum antibiotic prophylaxis
LOD:	Late onset disease
MHA:	Muller Hinton Agar
PI:	Personal Investigator
SOP:	Standard Operating Procedure
THB:	Toddd-Hewitt Broth

## Abstract

**Background:** Group B streptococcus (GBS) is gram-positive coccus which is a normal flora of the intestines, vagina or rectum and around 25% of healthy women carry this bacteria without showing any symptom. Bacteraemia, endometritis, amnionitis and urinary tract infection are major cause of bacterial infections caused by GBS in pregnant women in perinatal time, and in neonates it causes sepsis and meningitis. New born get infected through intra-amniotic route or at time of delivery.

**Objective:** To determine GBS prevalence in pregnant women and outcome of neonates in Zewditu Memorial Hospital Addis Ababa

**Methods:** A cross-sectional study design conducted at Zewditu hospital in city of Addis Ababa, Ethiopia from January 2019 to June 2019. A total of 192 pregnant women participated on isolation of group B *S.agalactiae*. For GBS isolation from vaginal swabs and antimicrobial susceptibility pattern standard operating procedures were followed. Culture, gram stain, catalase reaction and CAMP test, and testing for their susceptibility to antimicrobial agents were performed. All collected data was organized and tabulated using SPSS version 20, Multiple Logistic regression analysis was used to see the association between variables finally the results was summarized by using tables.

**Results:** The age was between 18 to 38 years with a mean of  $28.34 \pm 3.66$  years. From 192 pregnant mothers 30(15.6%) were GBS culture positive. The highest participation 161(83.9%) recorded between the ages of 25-35 years. From all participants the highest 70(36.4%) were house wives. GBS colonization showed a statistically significant association with primary level of maternal education and newborn birth weight. High susceptible of GBS isolate was seen to penicillin G & chloramphenicol (93.3%) for each, ampicillin, and ceftriaxone (90%) each, following vancomycin (80%) and erythromycin (70%). Relatively, GBS showed high resistance to tetracycline (86.7 %), ciprofloxacin (46.7 %) and clindamycin (33.3%). In this study the babies born to women with GBS colonization had statically significant association with clinical sepsis and low birth weight.

**Conclusion:** In this study, the overall prevalence of GBS colonization was 15.6%. Primary educational level and low birth weight was statistically significant with GBS colonization. This study used to give attention during management of pregnant women by making GBS culture as one of routine diagnosis during ANC follow up and to prevent newborn infection by early detection.

**Key Words:** Group B streptococcus, prevalence, pregnant women, Ethiopia

## 1. Introduction:

### 1.1 Background:

GBS emerged as the leading cause of sepsis and meningitis in the 1970s, in the first week of life.(1) By 1996 the Centers for Disease Control and Prevention (CDC) made an issue on intrapartum chemo-prophylaxis for perinatal disease prevention control.(2)

In newborns and adults invasive bacterial infections and severe inflammatory response remains a significant cause of morbidity and mortality.(1,2 ) The most common cause of life threatening bacterial infection in newborns is Group B streptococci (GBS), or *Streptococcus agalactiae*. These bacteria are gram positive, B hemolytic, chain forming cocci that are normal residents of the vaginal flora in 20% of healthy women (9) Transmission of GBS from colonized mothers to the newborn can occur in utero or during birth when the neonate aspirates contaminated amniotic/vaginal fluids. Low birth weight, preterm and full-term infants may be infected(4)

GBS is the leading cause of neonatal infections, cause of illness in pregnant as well as elderly people suffering from diabetes or immunosuppressive disease. (1–3). 10–40 % of pregnant women may be colonized with GBS.(7) It can cause urosepsis, chorioamnionitis, endometritis, pneumonia, skin and soft tissue infections in adults and pregnant women (6, 7). Also a cause of neonatal sepsis, pneumonia, and meningitis in newborns (8–9).

Without causing serious cervicitis GBS can pass the cervix and cross intact amniotic membrane causing amnionitis and infecting the fetus within the uterus (4). Neonate get infected during delivery or through amniotic fluid (8). Infant GBS infection is classified when occurring from birth to 6 days early-onset disease (EOD) which is accountable for (70–80 %) of cases and late-onset disease (LOD) from 7–90 days after birth which get infection from mother or health care personnel to infants (11–13).

GBS positive mothers have a risk of transmitting the bacteria to their newborns so to save neonates clinically identifying colonized women before or during delivery is important(8). Around 50 % of neonates born from colonized women who didn't take chemoprophylaxis may be positive for GBS and 1–2 % of these neonates shows high burden of disease [11].

Nowadays GBS became repeatedly isolated pathogen in neonates with high disease burden. The intrapartum fetal transmission may lead to invasive disease in 1 to 2 infants per 1,000 live births (8). The early onset disease accounts for approximately 80% of GBS infection in neonates and mortality rate is 10 to 20%.

Infants suffering from GBS infection require longer hospitalization, and those who survive may have neurological disorder (2, 3). Risk factors for neonatal colonization include intrapartum fever and heavy maternal colonization and lack of intrapartum antibiotic prophylaxis (IAP). And also risk factor for neonatal infection includes preterm delivery 18hrs, intrapartum fever temperature of at least 38oc.

GBS colonization rate at pregnancy time is different in many countries (12). The estimated mean prevalence of GBS colonization shows 17.9 % overall and in Africa high rate recorded (22.4 %) followed by the Americas (19.7 %) and Europe (19.0 %). And Southeast Asia shows the lowest mean prevalence around (11.1%) (12). Significant heterogeneity showed in this study across and within regions and differences in specimen collection time in pregnancy, culture technique and sample size did not describe the heterogeneity [12].

Although infant mortality is high in Ethiopia only few studies have been conducted related to GBS. GBS colonization rates varies in different study; study done in Hawassa health center showed (20.6 %) which is higher than reported in Gonder hospital(7.2 %) and the study conducted in Jimma hospital showed(19%) [13, 14,20].

In Ethiopia there are no organized health policies or strategies regarding at the reduction of GBS neonatal infection. Therefore, this study provides updated data on GBS positivity rate and neonatal infection rate in the study area.

## **1.2 Statement of the problem**

Group B streptococcus (GBS) is major cause of neonatal sepsis and meningitis with high burden of mortality and morbidity. Worldwide under five mortality is decreasing from 12.7 million in 1990 to 6.3 million in 2013, but continuous effective measures should be made to decrease mortality of newborns in developing countries.(6) In Africa the mortality rate is 4 times higher compare to America and Europe. So strategies on prevention of neonatal GBS have a crucial role on under five mortality.(6)

Effectively use of intrapartum antibiotic prophylaxis(IAP) has direct impact around 80% reduction in early onset GBS disease. So a strategy on IAP evaluation for prevention of EOD should be done in developed countries and to decrease burden of GBS disease, development of vaccine or other preventive plan should be considered.(6)

In Ethiopia there is no definite rate of maternal GBS carriage and neonatal disease which made it critical. There are many studies conducted in different cities of Ethiopia which shows high prevalence of the disease in mothers as well as their infants.(13,14,18,20), however there is no strategic plan developed to minimize the disease onset.

Therefore, the result of this study may show the current updated burden of the disease and its consequence regarding to newborns. So by considering the importance of the possible findings of the study, we evaluated the prevalence of GBS in pregnant mothers in Zewditu memorial hospital in Addis Ababa, Ethiopia and will provide updated information for responsible bodies to formulate policies, to implement prevention plan by universal screening for GBS in ANC unit; and also effective using of prophylaxis to prevent early onset GBS-neonatal disease. (13)

### **1.3 Significance of the study**

As GBS is a cause of illness in pregnant mothers and the leading cause of neonatal infection. In this study we try to show the outcome of neonate which is a serious issue. So, understanding the outcome would help to plan strategies focusing on identifying and medicating pregnant before delivery and control the transmission to their infants early.

This study would be useful for pregnant mothers to know the risk factors about their newborn and take a preventive measure by routinely following ANC service and taking prophylaxis.

Also to provide clinicians awareness of this bacteria and giving early treatment to prevent transmission to newborns.

This study would also provide more data to give attention to this organism to control and to set standard diagnostic criteria to screen GBS as one of routine ANC program starting from 30 weeks of gestational period and give a valuable input to the policy makers then which leads to hospitals should provide prophylaxis for positive mothers.

In addition, the results of this study can be used as reference for further similar studies that will be conducted in Addis Ababa, Ethiopia in the future.

## 2. Literature Review

Since GBS infection being one of the challenging problem, many research have been done in the world .These research showed the prevalence of etiologic and their antimicrobial pattern has been changed from place to place and from time to time. so, it needs to updated epidemiological data for a given place and time .

Worldwide Studies done on GBS colonization rate in vagina or rectum samples seems similar in some variations ranging from 10 to 30% in United States, 6.5% up to 36% in Europe, 7.1 to 16% in Asia, 9.1 to 25.3% in the Middle East, and 11.9 to 31.6% in Africa(20). And low prevalence of GBS recorded, 4% in Mexico City (9), 6% in Peru (10), 6.6% in Italy (7), 8.7% in Turkey (13)

The study done in Jiangsu, East China at 34 to 37 weeks' gestation revealed the overall GBS positive rate was 8.7% by PCR and 3.5% by culture. Seven thousand one hundred sixty-two pregnant women received GBS screening by bacterial culture and GBS-positive samples were tested for antibiotic resistance(48)

According to the study conducted in Thammasart Hospital in Thailand in 2006 from 406 pregnant women 65 were positive for GBS. This result reflects a high prevalence of 16% of pregnant women receiving antenatal care at the hospital.(22)

In Jakarta, Indonesia. vaginal swabs were collected from 177 pregnant .Bacterial culture identification tests and latex agglutination were performed for GBS. Antibiotic susceptibility testing done.GBS colonization was found in 53 (30%) pregnant women. All isolates were susceptible to vancomycin, penicillin, ampicillin, cefotaxime, daptomycin and linezolid. The majority of GBS were resistant to tetracycline (89%) followed by clindamycin (21%), erythromycin (19%), and levofloxacin (6%).(39)

At Rio de Janeiro State, Brazil, in between March 2008 and December 2015, a total of 3,647 pregnant women between the 35<sup>th</sup> and 37<sup>th</sup> gestational weeks diagnosed and 956 (26.2%) were GBS culture positive. All 689 GBS isolates evaluated were susceptible to ampicillin and vancomycin. Resistance to chloramphenicol and levofloxacin was observed in thirty-five (5%)

isolates. Five hundred and ninety-two (86%) isolates showed resistance to tetracycline. Resistance to erythromycin and clindamycin was observed in ninety-seven (14%), and fourteen (2%) isolates, respectively.(37)

Another review study conducted in Parana state in Brazil in 2014; 496 pregnant women analyzed and 141 (28.4%) were positive for GBS which 23.7% for vaginal samples and 21.9% for anorectal based on the combination of the three culture media with vaginal and anorectal samples (26).

Other similar study was done in Dr George Mukhari Academic Hospital, a University Teaching Hospital in Pretoria, South African 2012; Out of 413 samples collected, 128 (30.9 %) were positive for GBS. The susceptibility testing revealed that 100 % of isolates were sensitive to penicillin, ampicillin, vancomycin and high level gentamicin. Erythromycin and clindamycin resistance was 21.1 and 17.2 %, respectively.(21)

In 2006 at the Maxaquene Health Centre, in Maputo, 113 pregnant women were enrolled and two pregnant women were found to be positive for GBS, yielding a prevalence of 1.8%. Both colonized women had abortion history in the past. The prevalence in this study is the lowest carriage rate reported in Maputo. (24)

A Cross-sectional study conducted in Rural Southwestern Uganda indicates that GBS culture positive found in 89 (28.8 %) of the 309 pregnant women between January and March 2015.

A study done at Ghandi Memorial(GMH) and Black Lion Hospital on pregnant mothers in Addis Ababa in 2010; 300 pregnant mothers analyzed and 22 (7.2%) were GBS culture positive. Among those 12(55%) were penicillin sensitive and 20(91%) were ampicillin sensitive. Only one isolates were resistance to erytromycin. (14)

In 2010 a study in Hawasa health center, Adare hospital, Hawasa, Ethiopia, 139 pregnant women who attending antenatal clinic were screened for GBS and 29(20.9%) were colonized by GBS. Penicillin, gentamicin, vancomycin and ampicillin show 100% susceptibility. Most common

resistance was seen in erythromycin, tetracycline, ceftriaxone, chloramphenicol, ciprofloxacin and norfloxacin ranged from (51.7%-6.9%)(13).

In 2012, in Jimma hospital 126 pregnant women screened and 24 (19 %) were GBS culture positive. The vaginal and rectal carrier rates shows 13(10.3 %) and 18(14.3 %), respectively. Among isolates 6(25 %) were from the vagina and 11(45.8 %) from the rectum only. And from vaginal and rectal at the same time colonization showed 7(29.2 %). Also antimicrobial susceptibility testing done and 100% susceptibility were seen on vancomycin, penicillin G, and ampicillin and shows resistant to some antibiotics like; erythromycin (6.5 %), ciprofloxacin (9.7 %), ceftriaxone (9.7 %), clindamycin (3.2 %), norfloxacin (12.9 %), tetracycline (45.2 %) and cotrimoxazole (29 %) (20).

Also a study conducted in 2014 in different health institutions of Addis Ababa shows among 281 pregnant women screened 41 (14.6%) were colonized with GBS (31).

To detect GBS, the center for disease control and prevention (CDC) recommends, Lim broth (Todd-Hewitt broth supplemented with selective antibiotics) an enrichment medium, for isolating the bacterium from vaginal or anorectal swab followed by subculture on sheep blood agar and it needs 48 h to complete.

Different culture and sampling techniques performed in the study done at Belgium for the detection of GBS. At 35-37 weeks of gestation from hundred pregnant women three hundred swab was taken, and one vaginal, one rectal and one recto-vaginal swab were taken. And 100% GBS positivity detection observed on recto-vaginal sampling technique compared to vaginal sampling which shows 50% detection and rectal sampling 82% detection rate. In conclusion of this study recto-vaginal sampling increased the number GBS detection rate compared to those two methods apparently done. (25)

Recently study conducted in Germany Ulm University, as many as 10 serotypes (Ia, Ib, and II–IX) are recognized. In the United States and Europe Ia, Ib, II, III, and V Serotypes are prevalent colonizers and VI and VIII Serotypes are mainly found in Japan. Serotypes V found in Egypt and IV serotype reported from United Arab Emirates. Currently Denmark approved Serotype IX was diagnosed. (19)

### **3. Objective**

#### **3.1 General Objective**

- ✚ To determine prevalence and drug susceptibility pattern of GBS colonization in pregnant women and outcome of neonates in Zewditu Memorial Hospital Addis Ababa, Ethiopia from Jan 2019 to June 2019

#### **3.2 Specific Objective**

- ✚ To determine GBS prevalence
- ✚ To determine the antibiotic Susceptibility pattern of GBS bacteria
- ✚ To assess the outcome of neonates with GBS and non GBS colonized mothers

#### **4. Hypothesis**

The prevalence of Group B streptococcus in pregnant women and the antimicrobial susceptibility pattern of isolates are similar with the study done in Ethiopia.

## **5 .Materials And Methods**

### **5.1 Study Area**

In 1925 E.C Zewditu Memorial Hospital established around the current Finfine hall then in 1963 E.C transferred in front of Fil-wiha, which is located in the Kirkos Sub-City of City Government of Addis Ababa position. According to the data obtained from the hospital now the hospital has 600 health professionals and 274 administrative staff with providing different medical services. This hospital is referral hospital providing comprehensive antepartum, intra-partum and post-partum care. The hospital has 60 beds for delivery service and nearly 600 mothers deliver in one month.

### **5.2. Study design and period**

A cross-sectional and prospective observational study was conducted at Zewditu Memorial hospital Addis Ababa, Ethiopia from January to June, 2019 G.C

### **5.3. Population**

#### **5.3.1. Source population**

All pregnant mothers who attend ANC follow up at Zewditu Memorial Hospital Addis Ababa.

#### **5.3.2. Study Population**

All pregnant mothers with 35-37 gestational week that fulfill the inclusion criteria were our study population.

### **5.4. Inclusion and exclusion criteria**

#### **5.4.1. Inclusion criteria**

All consented mothers with gestation age 35–37 weeks  
Who are volunteered and give informed consent

#### **5.4.2. Exclusion criteria**

Participants who had received antibiotics therapy 2 weeks prior to the study

## 5.5. Study variables

### 5.5.1. Dependent variables

- Colonization of GBS from pregnant mothers and neonatal outcome

### 5.5.2. Independent variables:

- Socio-demographic characteristics(Age, Sex, Professional status, Educational level)
- Gravidity
- History of abortion
- History of still birth
- History of contraceptive use
- Status of neonate
- Weight of newborn
- Abnormality of neonate

## 5.6. Measurement and data collection

### 5.6.1. Sample size determination

The sample size (n) is calculated using formula the single proportion

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

By using 95% confidence level, Z value will be 1.96

5% margin of error (d)

Proportion from other = 14.6% from prevalence of study conducted by Solomon Assefa(31)

$$n = \frac{(1.96)^2 0.146 (1- 0.146)}{(0.05)^2} = 192$$

Thus, the study was include 192 subjects,

### 5.6.2. Sampling Method

Convenient sampling technique was used

## 5.7. Measurement and data collection procedures

### 5.7.1. Data collection

Data collectors (attending midwives) was selected and informed to collect the data by pre-structured questionnaire to obtain all the necessary information and the mothers will be interviewed. The pregnant women were followed during delivery and the characteristics of their babies were noted. Fetal outcomes after childbirth; presence of clinical symptoms suggestive of sepsis, low birth weight, fetal distress and other events were recorded and analyzed.

### 5.7.2 Specimen collection

Sample collection was performed according to center for disease control and prevention (CDC, 2018) standard for GBS. Using a sterile cotton swab vaginal swab was taken for culture.(30) Then each swab was placed in a sterile test tube and transported to the Microbiology laboratory at recommended time to get viable organism.

### 5.7.3 Culture and Identification

We prepare Todd-Hewitt broth, an enrichment medium for GBS and swabs was inoculated in 1 ml broth supplemented with 8 µg/ml gentamicin and 15 µg/ ml nalidixic acid to prevent contaminant growth and will be incubated at 37 °C aerobically for 18–24 h then sub cultured onto sheep blood agar plates and re-incubated at the recommended temperature. After 24 h we observe and all negative culture plates re-incubated for an additional 18–24 h and then re-observed. Plate that shows growth was identified by their characteristics appearance and biochemical test such as catalase and Christie, Atkins, Munch-Petersen (CAMP) factor. β-hemolytic on sheep blood agar were subjected to Gram's staining, catalase test and CAMP test. Colonies those are Gram positive cocci, catalase negative CAMP positive will be identified as *S. agalactae*. After birth the characteristics of their neonate were noted. Presence of any abnormalities like; low birth weight, sepsis, preterm delivery were recorded and analyzed. And if they take any antibiotic were also recorded.

#### 5.7.4 Antibiotic susceptibility testing

Kirby Bauer disc diffusion technique was used to test Antibiotic susceptibility test (AST). The media used were Muller Hinton agar (MHA) supplemented with 5 % sheep blood. From a fresh non-selective agar plate pure colonies was selected and transferred to 5 mL sterile normal saline and thoroughly mixed to make the suspension homogenous and turbidity was adjusted using McFarland densitometer to match with a 0.5 McFarland standard, then inoculated following the standard over the entire surface of a MHA plate using a sterile swab. Then using sterile forceps, the antibiotic discs was placed on MHA by considering 24 mm distance between each disk and 15 mm from the border, zone of inhibition was measured by metric scale and reported as susceptible (S), intermediate (I), or resistance (R) using the updated CLSI guide line.

**Table 1: Antibiotics disk used for Group B streptococcus S.agalactiae**

<b>Antibiotic</b>	<b>Concentration(<math>\mu</math>g)</b>	<b>Antibiotic</b>	<b>Concentration(<math>\mu</math>g)</b>
Penicillin G	10 IU	Ceftriaxone	30
Ampicillin	10	Ciprofloxacin	5
Erythromycin	15	chloramphenicol	30
Clindamycin	2	Tetracycline	30
Vancomycin	30		

## **5.8 Data Quality Assurance**

### **5.8.1 Data collection tool**

Data collection procedures training were given. To understand how the data collectors handle the questioner, supervision during data collection was done and each filled questioner was checked for its completeness and accuracy on daily basis. Corrective measure was taken for any gap and whole data was cross checked before analysis.

### **5.8.2 Pre-analytical**

The processes of selecting appropriate site and samples, collecting, identifying and labeling (every sample container had a label which will include information's about: subject code, ID number, Date and time accordingly and documented in the study questionnaire), handling, and transporting biological samples. The Process of accepting samples by the laboratory and sorting the biological samples all the process of pre-analytical steps was performed according to standard operating procedure. Aseptic techniques will be implemented in all the steps of specimen collection and inoculation on the culture media to minimize contamination.

### **5.8.3 Analytical**

Materials and procedures have been correctly controlled. All culture media was prepared according to the direction of the manufacturer and tested for sterility and performance. Sterility of media was checked by incubating overnight at 37oc. Moreover; culture growth, biochemical test and antimicrobial susceptibility test results was confirmed by specially trained experienced microbiologists working in the microbiology unit of the study site.

### **5.8.4 Post-analytical**

All of the extracted information (filled questionnaire, laboratory findings) was checked for legibility, completeness, consistency and placed in secure location. During data cleaning and cross-checking missing information was obtained by going back to the

questionnaire and laboratory records. The data was also stored in a CD as a backup. All laboratory isolates were stored and interpreted as per the SOP of the study site.

## **5.9 Data analysis and interpretation**

All statistical calculations were done using SPSS (the Statistical package for Social Sciences for Windows for windows version 20. During analysis frequencies of the different variables were determined; chi-square test and logistic regression were used for statistical analysis of data. When 'P' value were less than 0.05 taken as statistically significant.

## **5.10 Operational Definitions**

- ❖ Neonatal outcome: occurrence of low birth weight(< 2.5kg),preterm delivery, or severe neonatal condition
- ❖ Gravida: pregnant woman
- ❖ Primigravida: a woman has been pregnant once or currently pregnant
- ❖ Multigravida: a woman who has been pregnant more than once
- ❖ Preterm birth: the birth of an infant prior to 37 weeks of pregnancy
- ❖ Stillbirth: a baby dead after 24 completed weeks or a woman loses her pregnancy after past her 20<sup>th</sup> week
- ❖ Abortion: termination of pregnancy

## **5.11 Ethical considerations**

Ethical clearance was obtained from Departmental Research and Ethics Review Committee (DRERC) of Medical Laboratory Sciences, from Addis Ababa Health Bureau Ethical Review Committee prior to data collection and permission letter was obtained from study hospital administration. The purpose and procedure of the study was explained for each study participant at study sites. Those who agreed to participate was asked to sign consent form. Code numbers are used to assure the confidentiality of participant's information. Any participant who were not voluntary was not force to be included as study subject.

## **5.12. Dissemination of the result**

The findings of this study will be presented to the department of Medical Laboratory Sciences for public defense. The result will also be communicated to AHB and to the study site ZMH. Effort will be made to publish the findings in peer reviewed journals.

## 6. Work Flow

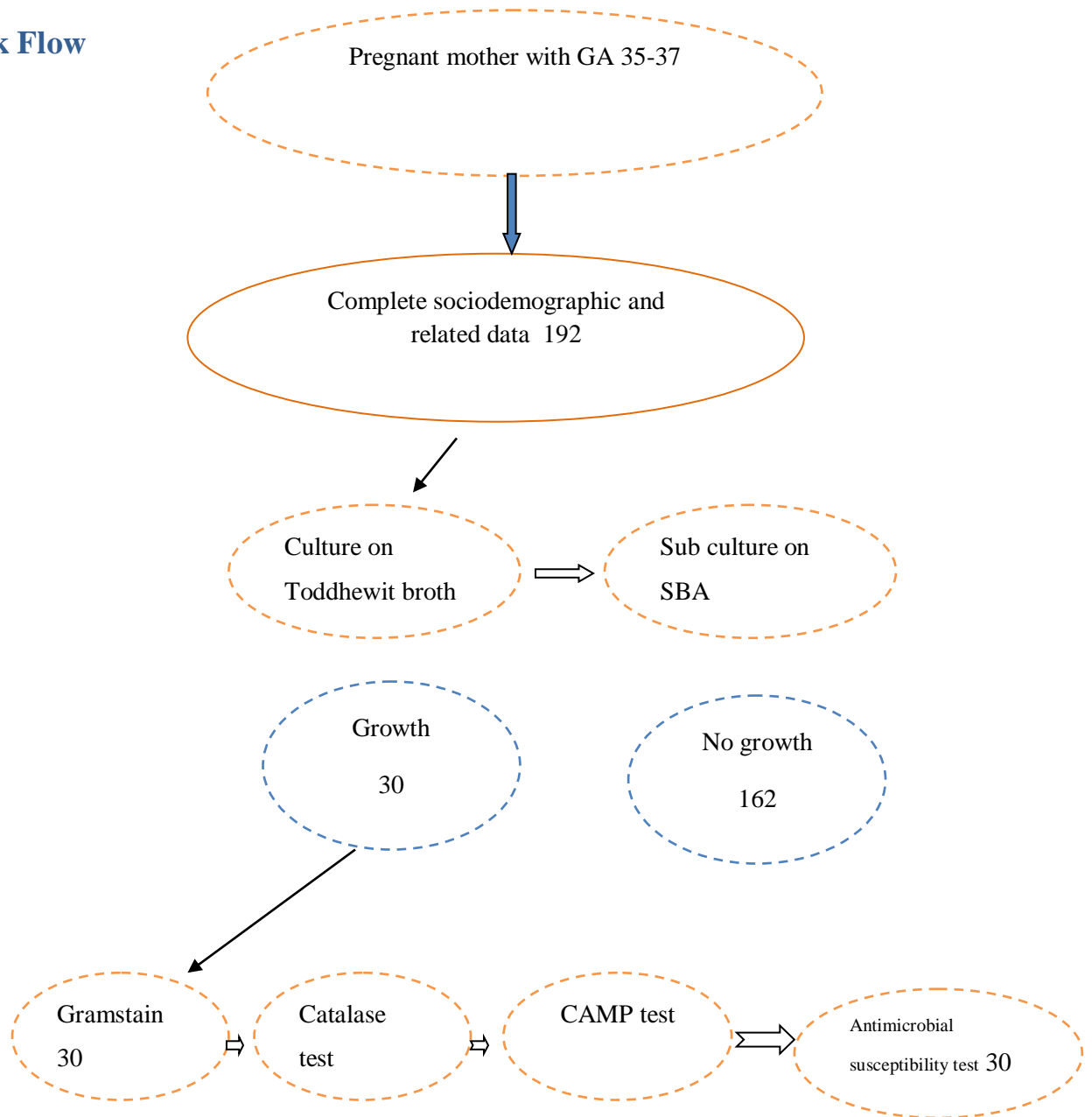


Figure 1: work flow

## 7. Results

### 7.1. Socio-demographic characteristics

During the study period 192 met the inclusion criteria. Of 192 women that undergone GBS culture analysis 30(15.6%) had positive GBS culture results. All participants were from zewditu memorial hospital. The age was between 18 to 38 years with a mean of 28.34 years. The highest participation 161(83.9%) recorded between the ages of 25-35 years. From all participants the highest 70(36.4%) were house wives followed by merchant, civil servants and students. Sociodemographic characteristics of the pregnant women are described in Table 2.

**Table 2:** Socio-demographic characteristics of pregnant women (n=192) at ZMH, Addis Ababa, Ethiopia, 2019

Characteristics		Frequency	Percent
Variables	Categories		
Age	<25	23	11.9
	25-35	161	83.9
	>35	8	4.2
Educational status	Illiterate	0	-
	Primary	44	22.9
	Secondary	75	39.1
	College	72	37.5
Occupational status	Housewife	70	36.4
	Civil servant	44	22.9
	Student	27	14.1
	Merchant	51	26.6

### 7.2 Obstetric characteristics and outcome of neonate

From the study participants 107 (55.7%) were primigravida and multigravida was 85 (44.3%). Of the 192 156(81.3%) had history of abortion and 3(1.6%) of women had history of still birth. Fifty three (27.6%) of the participants were with gestational age 35, 73(38%) were GA of 36 and the rest 66(34.4%) were 37 week of GA. Three fourth of the participants had a history of hormonal contraceptives usage. From 192 delivery 44% were male and 56% were female with birth weight ranging 2.0 to 3.7kg with a mean birth weight of 2.87kg, there were 18(9.3%) babies weighing <2.5 kg and 174(90.7%) babies weighing 2.5 to 3.7kg.(Table 3).

**Table 3:** Obstetric characteristics of pregnant women at ZMH Addis Ababa, Ethiopia, 2019

Variables	Categories	Frequency	Percent
No of gravid	Primigravida	107	55.7
	Multigravida	85	44.3
Gestational age	35	53	27.6
	36	73	38
	37	66	34.4
Still birth history	Yes	3	1.6
	No	189	98.4
Abortion history	Yes	36	18.8
	No	156	81.3
Contraceptive usage	Yes	142	74
	No	50	26
Neonatal status	Survival	186	96.9
	Death	6	3.1
Newborn's weight(kg)	<2.5	18	9.3
	2.5-3.7	174	90.7
Duration of labor	4-12	118	61.4
	13-24	74	38.6

### 7.3 Association of GBS colonization with socio-demographic factor & obstetrics factor

The association of the socio-demographic factors with GBS colonization and transmission is demonstrated on (Table 4). During the study period, a total of 192 mothers were analyzed and GBS was confirmed in 30 (15.6 %) cases. From there categories highest prevalence observed; between age group 25-35 which is 28/161 (17.3%), house wife 11/70(15.7%) and college graduate 18/72(25%) were mentioned. High GBS colonization rate was observed in newborn whose weight is within 2500-3700g range. Out of 6 newborn who were born dead, 1(16.7%) of them was colonized with GBS. Also high rate of GBS observed in hormonal contraceptive users 23(16.3) compared with non-users. Out of 30 GBS positive pregnant 15(14%) was primigravida and 12(21.4%) were multigravida-2. And mothers who were on 37 week of gestation 13(19.6%) and whose duration of labor stays longer 19(38.3%) showed higher colonization rate as compared with short duration of labor 11(82.7%). It was also seen that women with no history of abortion had 11.5% rate of colonization as compared to those women with recent history of abortion which was 33.3%. a multivariable logistic regression analysis showed that mothers

whose educational status was at primary level had a significant association with an increased risk of GBS colonization( $p=0.01$ ). In this study history of abortion and maternal age showed association with GBS colonization in binary logistic regression but not on multi logistic regression.

**Table 4:** Association of GBS and socio-demographic & obstetric factors of pregnant women at ZMH, Addis Ababa, Ethiopia, 2019

Characterstics		GBS+	COR a; 95% CI c	AOR b; 95% CI c	P-
Variables	Catagories	n(%)			value
Age	<25	2(6.7)			0.194
	25-35	28(93.3)			
	>35	0			
Educational status	Illiterate	0			
	Primary	13.33	0.376(0.202-0.699)	0.05(0.005-0.485)	0.01
	Secondary	11 36.7			0.153
	College	18 60			
Occupational status	Housewife	11 36.7			0.77
	Civilservan t	7 23.3			
	Student	6 20			
	Merchant	6 20			
Gravida	Primigravi da	15 50			0.94
	Multigravi da-2	12 40			
	Multigravi da-3	3 10			
	Mutigravid a-4	0			
Gestational age	35	6 20			0.21
	36	11 36.7			
	37	13 43.3			
Contraceptive use	No	7 23.3			0.56
	Yes	23 76.7			
Duration of labour	4-12	11(36.6)			0.34
	13-24	19(63.3)			

Crude odds ratio(a) , Adjusted odds ratio(b) , Confidence Interval (c)

#### 7.4 AST of the GBS isolate

High susceptible of GBS isolate was seen to penicillin G & chloramphenicol (93.3%) for each, ampicillin, and ceftriazone(90%) each, following vancomycin(80%) and erythromycin(70%). Relatively, GBS showed high resistance to tetracycline (86.7 %), ciprofloxacin (46.7 %) and clindamycin (33.3%). Susceptibility to all antibiotics observed in 1 (3.3 %) isolate and one or more antibiotics resistant observed in 29 (96.6%) tested isolates. According to the study the most active drugs for GBS isolates were penicillin, chloramphenicol, ampicillin and ceftriazone with susceptibility results of 93.3, 93.3,90 and 90%, respectively. Moreover, 2(6.7%) isolates of GBS showed intermediate sensitive to erythromycins and chloramphenicol for each and 1(3.3%) showed for tetracycline (Table 5).

**Table 5:** Antibiotic susceptibility patterns of GBS isolated from pregnant mothers

Antibiotics	Disc content	Colonizing GBS isolates		
		Susceptible (%)	Intermediate (%)	Resistant (%)
Penicillin	10 IU	28/30 (93.3%)	-	2 (6.7%)
Ampicillin	10µg	27 (90%)	-	3 (10%)
Erythromycin	15µg	21 (70%)	2 (6.7%)	7 (23%)
Clindamycin	2µg	20 (66.7%)	-	10 (33.3%)
Vancomycin	30µg	24 (80%)	-	6 (20%)
Ceftriazone	30µg	27 (90%)	-	3 (10%)
Ciprofloxacin	5µg	14 (46.7%)	-	16 (53.3%)
Chloramphenicol	30µg	28 (93.3%)	2 (6.7%)	-
Tetracycline	30µg	3 (10%)	1 (3.3%)	26 (86.7%)

#### 7.5 Outcome of neonate

Neonatal outcomes including; low birth weight, sepsis, respiratory distress & neonatal asphyxia were recorded. And analyzed the clinical correlation of GBS with neonatal outcomes. From 192 delivery 44% were male and 56% were female with birth weight ranging 2.0 to 3.7kg with a mean birth weight of 2.87kg, there were 18(9.3%) babies weighing <2.5 kg and 174(90.7%) babies weighing 2.5 to 3.7kg. From 18 low birth weight neonates 4(22.2%) of them had GBS infection; which 2 of them develop sepsis and 2 were develop respiratory distress. From 6 death reported 3 of them had low birth weight, four of them develop sepsis, one with respiratory distress and one with neonatal asphyxia. Out of 30 GBS infection 1 death was reported with

early neonatal sepsis and 13 neonates had abnormalities like; sepsis 6(46.1%),respiratory distress 5(38.5%) and neonatal asphyxia 2(15.4%). From 192 total births; 18 develop neonatal asphyxia, 34 sepsis and 27 were with respiratory distress. The results showed that GBS status was statistical significant difference with neonatal sepsis (p=0.002). As shown below in binary logistic regression there was a statistical significant difference between low birth weights with GBS infection as compared to those normal birth weight(p=0.013) (Table 6).

**Tale 6:** The association between GBS colonization and neonatal outcome

<b>Outcome characteristics</b>	<b>GBS(+)n (%)</b>	<b>GBS(-)n (%)</b>	<b>AOR(a) ; 95% CI(b)</b>	<b>P value</b>
Neonatal status				
Survival	29(96.6)	157(96.9)	0.92(0.104-8.197)	0.94
Death	1(3.33)	5(3.1)		
Newborn's weight(kg)				
Low birth weight	4(22.2)	14(77.8)	0.263(0.088-0.792)	0.013
Normal birth weight	26(15)	148(85)		
Sepsis	6(17.6)	28(82.4)	3.833(1.639-8.963)	0.002
Respiratory distress	5(18.5)	22(81.5)	0.653(0.337-1.264)	0.84
Neonatal asphyxia	2(11.1)	16(88.9)	0.94(0.662-1.361)	0.77

Adjusted odds ratio(a), Confidence Interval(b)

## 8. Discussion

The overall prevalence of Group B streptococcus (GBS) in the present study among pregnant women was 15.6%. Such a result in this study is comparable with studies worldwide ranging from 10-30% in USA, 6.5-36% in Europe, 7.1-16% in Asia and 11.9-31.6% in Africa (20). The study is relatively similar with studies in different part of Ethiopia; 20.9% in Hawasa health centers (13), 19% in Jimma hospital (20) and 14.6% in different health centers in Addis Ababa (31). The rate of GBS colonization in this study is lower than study conducted in Brazil 28.4 % (26) and South Africa 30.9 % (21).

The rate of GBS found in this study and in some countries of Europe is comparable for example in Italy two studies done and reported GBS rate as 17.9% (26), and 18% (27). In Switzerland and Poland positivity rate were 21% (29) and 17.2% (28) respectively and the study done in Netherlands shows 21% (4). Lower GBS colonization rate recorded from Istanbul and Elazin in Turkey giving 8% (30) and 8.7% (31), respectively and study in Northern Greece reported a lowest rate of 6.6% (32).

Providing adequate knowledge for pregnant women on GBS risk factors plays a crucial role in decreasing the morbidity and mortality related to maternal and neonatal GBS infections. The geographical differences, variability in the sample size, methods employed for GBS detection might be possibly explained the disparities.

In this study socio-demography (age and occupation); obstetrics (gravidity; gestational age; history of still birth, neonatal death, contraceptive use; history of abortion; duration of labor and neonate status) has no relation to the GBS colonization like many studies done (3, 25–28). But primary educational level was significantly associated with maternal colonization ( $p=0.01$ ) as the study done in Poland (25) and Bangladesh (28). And also in this study newborn birth weight were significantly associated factors to maternal colonization unlike other study. Maternal age, occupation, education and gravidity were identified as risk factors for GBS in studies done before but no association seen in current study at all (25–28).

Thailand researchers reported that older maternal age and lower gestational age were risks for colonization by GBS (30). The relationship between these factors and GBS colonization however showed marked inconsistencies. In some studies colonization increased with age reported (29), while others reports confirmed younger age group shows the highest (30, 31). Possible reason for this difference seems to be seasonal differences globally, availability of laboratory facilities for detecting GBS and also shorter study period mentioned. In this study history of abortion and maternal age showed association with GBS colonization in binary logistic regression but not on multi logistic regression.

The susceptibility pattern of GBS isolates to Penicillin(93.3%), Vancomycin(80%), ampicillin(90%), Ceftriazone(90%), chloramphenicol(93.%) ,erythromycin(70%), clindamycin(66.7%) and is comparable with previous studies conducted in different countries. However high resistance was observed against tetracycline(86.7%), ciprofloxacin(53.3%), clindamycin(33.3%), erythromycin(23%), vancomycin(20%), ceftriaxone and ampicillin (10%) and in which similar records were found from Tanzania (18), USA (35, 36), Canada (37) and Lebanon (41)

In this study, GBS resistant with reduced Penicillin susceptibility have been detected. Penicillin is the first agent for prevention and treatment of GBS infections; however, nowadays GBS strains with reduced susceptibility to Penicillin have been reported periodically as seen in this study.

To prevent GBS Erythromycin and Clindamycin are the alternative antibiotics for Penicillin-allergic pregnant women with high risk of anaphylaxis. Rising of GBS strains resistant to erythromycin and clindamycin from time to time is complicating management of pregnant women who are allergic to Penicillin (32). In contrast to reports from many other countries, highest susceptibility in the present study was seen to Erythromycin (70%) and Clindamycin (66.7%) and only few isolates were resistance to erythromycin and clindamycin. In this study 23% erythromycin and 33.3% for clindamycin resistance reported which is similar to studies done in Ethiopia shows that 22.7% erythromycin resistant and 17.6% to 18.2% resistant to clindamycin (17). Also in South African 17.2% clindamycin and 21.1% erythromycin resistant reported (15). Tanzania 17.6% (34) and USA 21.0% (39) clindamycin resistance reported which is comparable to our study.

Generally, in contrast to this study, worldwide studies reported high resistance rate to Erythromycin which ranges from 18 to 54%.(28) Absence or low antibiotic resistance of GBS strains in the present study may indicate the suitability of Penicillin, ampicillin, and Chloramphenicol for Ethiopia to prevent EOD-GBS until vaccine is available on the market. Similar to the present study low level of resistance to Erythromycin was reported from Australia (6.4%) (23), Brazil (4.1%) (24), Thai-Myanmar border (8.5%) (25) and France (4%) (28).

No resistant to chloramphenicol is observed in this study and 2 (6.7%) and 3(10%) of the isolates showed resistance to penicillin and ampicillin respectively.

High resistant to Tetracycline (86.7%) in this study was reported and Similar reports from other countries, Brazil (83%) (32), Australia (85.9%) (34), Kuwait (89.5%) (36), Canada (89%) (37), and Island (85%) (38) also reported.

CDC approved patients can take penicillin or ampicillin if they are not allergic to penicillin. Clindamycin or vancomycin is drug of choices with those who had major penicillin allergy and cefazolin for minor allergy to penicillin (40). However, it is difficult for developing vaccine for GBS because multiple serotypes are found and which varies in geographical location (44). As more data regarding GBS and its serotypes became available, it is possible to consider implementation of prevention guideline used by other countries. In the long run it is also possible to develop vaccine to prevent early onset neonatal disease caused by GBS.

Nowadays clindamycin and erythromycin resistance which are first line drugs for those with an allergy to penicillin, increased rapidly (44). Resistance to erythromycin ranged from 7 to 40 % and clindamycin from 3 to 26.4 % and related to some serotypes (39, 42, 43). Inappropriate use of antimicrobial drugs leads to high resistance of drugs. In Ethiopia, peoples easily go to pharmacy shops without prescription to buy antibiotics and this type of uses of antibiotics might responsible for high drug resistance rates observed currently.

In this study, we tried to analyze the relationship of GBS infection with neonatal outcomes. Neonatal outcomes including; low birth weight, sepsis, respiratory distress & neonatal asphyxia were recorded. In our study six neonate develop clinical sepsis which is similar to the study done in India (45). In this study 18(9.3%) babies had low birth weight but in similar study done in India; no risk of low birth weight noted in the GBS colonized group in comparison to the mother

without GBS colonization (46). This difference might be low habit of ANC follow up in Ethiopia and care and treatment of preterm.

The results showed that GBS status was statistical significant difference with neonatal sepsis ( $p=0.002$ ) AOR 3.833(1.639-8.963) in this study; which is similar to the study done in China (47). Also there was a statistical significant difference between low birth weights with GBS infection as compared to those normal birth weight ( $p=0.013$ ) AOR 0.263(0.088-0.792) were similar to the study in China(47) Availability of early diagnosis method and early treatment of neonates is very helpful on decreasing newborn infection. For suspected clinical sepsis cases in GBS colonization group which required treatment ampicillin, cefotaxime, gentamicin and phenytoin were mostly used drugs until confirmed by lab diagnosis.

## 9. Limitation

- Not serotyping done
- Using only disc diffusion for antibiotic susceptibility test

## **10. Conclusion**

The overall prevalence of GBS in the study area was 15.6%. The carriage rate of GBS was high among those aged 25-35 years. Primary level of maternal education and newborn birth weight were significantly associated factors to maternal colonization in my study.

Highest susceptibility was shown for penicillin and chloramphenicol each (93.3%). High resistance was observed against tetracycline (86.7%), ciprofloxacin (53.3%).

Neonatal outcome like; sepsis, low birth weight neonatal distress and neonatal asphyxia are common abnormalities seen in this study.

Including GBS as one of routine ANC follow up test and early diagnosis and proper management and treatment of neonates is very important to reduce the neonatal infection caused by GBS.

## **11. Recommendations**

Since there is high rate of maternal GBS colonization among pregnant mothers developing early universal screening program is recommended

Type of GBS serotype in the society must be studied

The low sensitivity and high resistance to the commonly used antibiotics calls for routine test for antibiotics susceptibility before administration of antibiotics.

Since there are regional variations in serotype distribution it is better if effective vaccine will be developed and implemented for prevention of GBS disease.

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## **Annex I. General information for the study participants (English version)**

### **Introduction**

My name is Semira Mustefa and I am MSc student of Addis Ababa University, School of Medical Laboratory Sciences. I am doing research entitled Group b streptococcus agalactiae prevalence in pregnant women and outcome of neonates in Zewditu memorial hospital Addis Ababa, Ethiopia. As many studies shows prevalence of GBS is increasing time to time it is neglected by responsible bodies. So this study will indicate prevalence of streptococcus agalactiae in pregnant mothersand neonatal outcome in zewditu memorial hospital in Addis Ababa, Ethiopia,antimicrobial drug resistance patterns and associated risk factors.

**What is the reason of this study?** The objective of this research is to study the prevalence of GBS and antimicrobial drug resistance patterns inZewditu memorial hospital in Addis Ababa, Ethiopia. All consented pregnant mothers who agree to participate in the study will give a vaginal swabfor culture as per the standard. Additionally some clinical information will be interviewed from the HCWs.

**Is the information be confidential?** All the data obtained will be kept confidentially only the study personnel will have accesses to the files. Anonymous testing will be undertaken, that means samples will be coded and positive results will not be identified by names.

**What are the costs?** All the investigations performed for the participants of this study will be free of charge.

**What about compensation?** You will not be compensated for your participation in this study but the patient may benefit from the study because more intensive investigations will be done which may allow detecting and treating an infection earlier than otherwise.

### **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 or you may refuse to answer some of the questions if you feel uncomfortable. You are free to participate in this study or you can withdraw your consent anytime, which will not involve any penalty or loss of benefits to which you are entitled.

**What about the harm which may happen in the study?**

This research involves taking vaginal swab from pregnant women selected in the study with professional midwives working in this hospital. There are no major risks to mothers and will not cause any harm to this hospital.

### **Assurance of the principal investigator**

I put on my signature here to verify you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of reports for all stakeholders of the research project.

SemiraMustefa (PI): signature\_\_\_\_\_Date\_\_\_\_\_

**Note:** if you have any questions about this study, feel free to ask now or anytime throughout the study by contacting:

**PI Address:**SemiraMustefa: Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia.

E-mail: [semiramustefa35@gmail.com](mailto:semiramustefa35@gmail.com): Tele: +251911934597

**Annex II. Information sheet (Amharic version)**

**መግቢያ**

ስሜሶሚራሙስጠፋበአዲስአበባዩኒቨርሲቲ በጤና ሳይንስ ኮሌጅ በሜዲካል ለቦራቶሪ ትምህርት ክፍል ማስትሬት ዲግሪ ተማሪ ስምን በዘውዲቱ ሆስፒታል የሚመጡ ነፍሰጡሮች ከ 35-37

ሳምንት ሲሆናቸው ከማህጸናቸው ፈሳሽ በመውሰድ በብዛት ነፍሰጡሮችንና ዲስፎራሚያትን የተወለዱ ህጻናትን የሚያጠቃውን ባክቴሪያ ላይ ምርመራ እሰራለሁ።

ስለዚህ ይህ ጥናት በዘውዲቱ ሆስፒታል ነፍሰጡሮች ላይ ያለውን ባክቴሪያ መጠን ለማየት ይረዳል።

**የጥናቱ ምክንያት**

ይህ በሳይንቲፊክ መጠሪያው *S.agalactiae* የሚባል ሲሆን በተገኘው ነፍሰጡሮች ተኮችን የሚያጠቃው ሳይንቲፊክ ባለወልዲና ልጆቻቸውን ምሊሳዳ ብሎ ምክንያት ሊያደርስ የሚችል ሲሆን ምንም እንኳን ግንብ ምትኩረት ያልተሰጠው በሽታ ነው።

ስለዚህ ይህ ጥናት በዘውዲቱ ሆስፒታል ነፍሰጡሮች ላይ ያለውን ባክቴሪያ መጠን ለማየት ይረዳል። ከተስማሙ በዚህ ጥናት ላይ ለመሳተፍ ፈቃደኛ ከሆኑ ስለሁኔታው በደንብ እንዲያውቁ ይደረጋል።

በተጨማሪም ሌሎችም ለጥናቱ ምክንያት ሊሆኑ ይችላሉ።

**የጥናቱ ሚስጥራዊነት**

የተገኘው መረጃ ሁሉ ሚስጥራዊነት በተቆለፈ ክፍል ወይም ሳጥን ውስጥ በማስቀመጥ የጥናቱ ባለበት ብቻ እንዲገኝ ይደረጋል። በምርመራ ወቅት የተለየ ክፍል ወይም ሌላ ሰው ለመጠቀም የተመረጠው ሆኖ ለሌላው ማህተም ይረጋገጣል።

**የክፍያ ጉዳይ**

በጥናቱ ላይ በሚሳተፉ ላይ ለሚደረጉ ሁሉም አይነት የላቦራቶሪ ምርመራዎች ወጭ ከክፍያ ነፃ ሆኖ በተመራ ማሪ ወይም ይሸፈናል።

**የማካካሻ ጉዳይ**

በጥናቱ ላይ ስለተሳተፉ ምንም ዓይነት ማካካሻ አያገኙም ነገር ግን ህመም ማኑክሚ ደረገው ጥልቅ ምርመራ በሽታ ወይም በጊዜ ተገኝቶ ለትብብር ጥንት ይታከማል።

ከጥናቱ ላይ ያለ መሳተፍና የመገለጫ መብት ጉዳይ

እርሶ በጥናቱ ላይ የተሳተፉት ሙሉ በሙሉ በፈቃደኝነት ላይ የተመሰረተ ነው።

ስለዚህ እርሶ ማንኛውም ሰዓት በጥናቱ ላይ መሳተፍ ማቆም ይችላሉ። ያለ መሳተፍ ምልክት ያደረጉ

አንዳንድ ጥያቄዎች ካልመሰኙ ያለ መመለስ ምልክት አለዎት።

ይህንን ማድረግ ምንም ዓይነት ቅጣት አያስከትል በዎትም፤ ማግኘት የሚገባዎትን ሁሉንም ነገር አያሰጥም።

ከጥናቱ ጋር የተያያዙት ጉዳዮች ላይ ጉዳይ

ና ሙና በሚወሰድበት ጊዜ ከሚሰማዉ ያለ መመሰኛ በስተቀር ምንም ዓይነት የከፋ ጉዳት በጥናቱ ምክንያት አይከሰትም።

የተመራ ማሪያ ማረጋገጫ

እኔ ከስርፈር ማየን ያስቀመጥኩት ሰይንሳዊ ጥናቱን በግብረ ገብ በተሟላ መልኩ እንደሚከናወን ያደናገርኩት ለሚመለከታቸው ባለድርሻ አካላት እንደሚቀርብ በፈርማዎ አረጋግጣለሁ።

ሰሚራ ሙስጠፋ (ዋና ተመራ ማሪያ)፡ ፈርማ----- ቀን

ማስታወሻ፡ ስለ ጥናቱ ማንኛውም ጥያቄ ካለዎት በሚቀጥለዉ አድራሻ ነፃ ሆነ ወይ ጠይቁን፡

የዋና ተመራ ማሪያ አድራሻ፡ ሰሚራ ሙስጠፋ በአዲስ አበባ ዩኒቨርሲቲ፣ የጤና ሰይንስ ኮሌጅ፡

የህክምና ላቦራቶሪ ትምህርት ክፍል፤ አዲስ አበባ ኢትዮጵያ። ኢሜል፡ [semiramustefa35@gmail.com](mailto:semiramustefa35@gmail.com)

ስልክ፡ +25191934597

Annex III. Consent form for pregnant mothers (English version)

I have read the information about the study which plans to determine prevalence of group B streptococcus agalactiae and antimicrobial drug resistance pattern in Zewditu memorial hospital, Addis Ababa Ethiopia or it has been read to me. I was given the opportunity to ask questions and my questions were answered to my satisfaction. I voluntarily consent that I would participate in this study.

To give sample necessary for study and be a participant in this study and understand that I have the right to withdraw from the study at any time .

Print name of participant, date and signature or thumb impression of participant

\_\_\_\_\_ / \_\_\_\_ / \_\_\_\_ (dd/mm/yy) \_\_\_\_\_

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person to be selected by the participant and should haven't any connection to the studies team)

\_\_\_\_\_ / \_\_\_\_ / \_\_\_\_ (dd/mm/yy) \_\_\_\_\_

Phone number:

\_\_\_\_\_

Print name of researcher, date and signature of researcher

\_\_\_\_\_ / \_\_\_\_ / \_\_\_\_ (dd/mm/yy) \_\_\_\_\_



## Annex V. Questionnaire

### V. Questionnaire

Addis Ababa University Collage of Health Sciences, Department of Medical Laboratory Science.  
Questionnaire for group B *Streptococcus agalactiae* Prevalence in Pregnant Women and outcome of neonates in Zewuditu Memorial Hospital. This information is very valuable to us. The information you given is absolutely voluntary and anonymous. We value any information you will be able to provide for us.

#### Patient Identification

Facility name \_\_\_\_\_ Year \_\_\_\_\_ Participant code \_\_\_\_\_

Participants address (Sub city) \_\_\_\_\_ Telephone \_\_\_\_\_ signature \_\_\_\_\_

Data collector name \_\_\_\_\_ date \_\_\_\_\_ signature \_\_\_\_\_

1. Mothers code: \_ \_ \_ \_ \_

2..Adress ; Region----- Sub city\_\_\_\_\_ Kebele\_\_\_\_\_ Tel:\_\_\_\_\_

3. Age of the pregnant mother \_\_\_\_\_

4. Level of Education-----

Allilltrate B.Primary C. Secondary/High school D.College/university

5.Occupation-----

A.House wife B. civil servant C.student D.Merchant

6. Number of Gravida

A. Primigravida B. Multigravida (2<sup>nd</sup> 3<sup>rd</sup> 4<sup>th</sup> 5<sup>th</sup> ..... ) please specify

7.Gestational week of the current pregnancy-----

8. History of still birth or neonatal death

A. Yes                      B. No

9. History of abortion

A. Yes      B. No

10. History of recent any antibiotic use

A. Yes                      B. No

If yes mention antibiotics and time taken

11. History of any contraceptive use

A. Yes if Yes mention                      B. No

### Outcome of neonates

1. Neonate status

A. survival                      B. death

2. Body weight

A. Underweight                      B. Normal      C. Average

3. Any complication -----

Annex VI. Questionare(Amharic version)

ይህ መረጃ ለሻብጣም አስፈላጊ ነው። ይህ መረጃ በፍቃድ ንጉሳይ የተመሰረተ ነው።

የምትሰጡንን ጥያቄዎንም መረጃ እንቀበላለን።

- 1. የእናት የውሎ ስያ -----
- 2. አድራሻ ክልል ----- ክፍለ ከተማ ----- ቀበሌ ----- ስ.ቁ. -----
- 3. የእናት የውሎ ደረጃ -----

4. የትምህርት ደረጃ

U. ያልተማረችላ. 1ኛ ደረጃ ሐ. 2ኛ ደረጃ መ. ኮሌጅ/ዩኒቨርሲቲ

5. የስራ ሁኔታ

U. የቤት እመቤት ለ. የመንግስት ስራ ተኛ ሐ. ነጋዴ

6. የእርግዝና ሁኔታ

U. የመጀመሪያ ጊዜ ለ. ሁለተኛና ከዛ በላይ (2ተኛ፣ 3ተኛ፣ 4ተኛ፣ 5ተኛ----) እባክዎትን ይጠቀስ

7. የጽንሱ ጊዜ (በሳምንት) -----

8. ከዚህ በፊት ሞቶር የተወለደህ ዳን አጋጥሞ ምት ያውቃል

U. አዎ ለ. አያውቅም

9. ከዚህ በፊት ውርጃ አጋጥሞ ምት ያውቃል

U. አዎ ለ. አያውቅም

10. በቅርቡ መድሃኒት ወስደዋል

U. አዎ የወሰዱትን መድሃኒት ቢጠቅሱት ----- ለ. አልወሰድኩም

11. የወሊድ መቆጣጠሪያ ወስደው ያውቃሉ

U. አዎ የወሰዱትን የወሊድ መቆጣጠሪያ ቢጠቅሱት ----- ለ. አልወሰድኩም

## Annex VII: Standard operating procedures (SOPs)

SOPs that are required for media preparation, reagent preparation, specimen collection and laboratory investigation are described below. But all procedures that should be followed for media preparation are not included. In general manufacturers' instructions will be followed to prepare, to store, to perform quality control for each reagents and medium used in this study.

### Amies transport media with charcoal Preparation procedure

**Purpose:**This procedure provides instructions to prepare amies transport media.

**Principle:**Used to preserve the viability of anaerobes .it has inorganic phosphate buffer and also contains charcoal that absorbs inhibitory substances released in the medium during transport of sample

#### Materials, supplies and equipment required

- Charcoal
- Sodium chloride
- Sodium hydrogen phosphate
- Potassium dihydrogen phosphate
- Potassium chloride
- Sodium thioglycollate
- Calcium chloride
- Magnesium chloride
- Agar
- PH meter

#### Procedure:

- 1.Dissolve the required amount of amies transport medium in distilled water and mix well
- 2.Dispense the medium in screw cap bottles
- 3.Autoclave the bottles at 121oc for 15 minutes with cap loosen

4. when medium has cooled down, tighten the cap of bottle and store with labeled date and batch

5. During storage, invert the bottle to ensure even distribution of charcoal

Stability: stored amies transport medium can be used up to 9 months.

Clinical utility: It is a semisolid media used to transport anaerobes and useful in isolation of fastidious organisms

Todd Hewitt broth/THB

Purpose: This procedure provides instructions how to prepare Todd Hewitt broth

Principle: primarily used for the cultivation of B hemolytic streptococci, especially for serologic studies. The medium 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 group B streptococci is obtained by the inclusion of gentamycin and nalidixic acid in the medium. Selective enrichment broths include the advantage of both enrichment and selection by providing conditions conducive to the growth of Group B streptococci while inhibiting the growth of contaminants.

Materials and reagents

- Purified water
- Peptone
- Dextrose
- Sodium chloride
- Sodium phosphate
- Sodium carbonate
- Gentamycin
- Nalidixic acid

Procedure:

1. Dissolve 36.4g in 1 litre of distilled water

2. Mix well
3. Adjust the Ph to 7.8
4. Autoclave at 115oc for 10 minutes
5. Store the prepared medium at 2-8oc

Limitation: For identification, organisms must be in pure culture. Further morphological and biochemical test should be performed for final identification

Clinical utility: For the selective enrichment of group B streptococci (*Streptococcus agalactiae*)

### Blood agar media preparation

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

Principle: Meat extract and Peptone provide nitrogenous compounds, vitamins, carbon, sulphur and amino acids in Blood Agar Base. 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. Sheep blood gives best results for Group A Streptococci. When horse blood is used, *Haemophilus haemolyticus* colonies produce haemolysis and mimic Streptococcus. Haemolytic patterns may vary with the source of animal blood or type of base medium used. Norton found that slight acidic pH ( $6.8 \pm 0.2$ ) favours distinct haemolytic reaction and is advantageous for cultivation of Streptococci and Pneumococci. The low pH helps in stabilization of red blood corpuscles and favours the formation of clear haemolysis zone.

Abbreviation:

BA= blood agar

Materials, Supplies and equipment

- Blood agar base powder
- weighing paper
- distilled water
- spatula

- Sterile sheep blood
- Refrigerator
- Balance
- Autoclave
- Hot plate
- Bunsen burner
- distiller
- dispenser
- graduated cylinder
- flask
- test tube
- PH meter

Procedure:

1. Weigh and Suspend 40grams of powder in 1littr distilled water
2. Mix thoroughly and heat to boiling to dissolve the medium completely with frequent agitation
3. When cool adjust the ph to 7.3
4. Autoclave at 15 lbs pressure at (121oc)for 15 minute
5. Cool the medium at 50 oc
6. Add 50ml of difibrnated and mix with gentle rotation sheep blood
7. Dispense 20ml of the solution in to sterile Petri dish
8. Allow the medium to solidify label with date and store at 4 oc

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

Clinical utility: A non-selective medium for the isolation and cultivation of many pathogenic and non-pathogenic microorganisms like Neisseria, Streptococci etc. The medium is often used to observe the forms of haemolysis from pathogenic microorganisms.

### SOP for gram staining

**PURPOSE:** The gram staining reaction is used to help identify pathogens in specimens and cultures by their gram reaction and morphology. Abbreviations: ATCC=American type culture collection.

**PRINCIPLE:** Difference in gram reaction between bacteria is thought to be due to differences in the permeability of the cell wall of gram positive and gram negative organisms during the staining process. Following staining with a triphenyl methane basic dye such as crystal violet and treatment with iodine, the dye-iodine complex is easily removed from the more permeable cell wall of gram negative bacteria but not from the less permeable cell wall of gram positive bacteria. Retention of crystal violet by gram positive organisms may also be due in part to the more acidic protoplasm of these organism binding to the basic dye(helped by the iodine).

### Materials,Reagents, supplies and equipment

1. Crystal violet (or gentian violet)
2. Lugol's iodine
3. Aceton-alcohol decolorizer (or ethanol-iodine)

**Reagent Stability and storage:** Store at room temperature. When kept with tightly stopper, the stain is stable for several weeks.

- 3" X 1" single frosted, pre-cleaned glass slides.
- Wooden applicator sticks
- Immersion oil
- Disposable glove
- Light Microscope
- Staining rack/jar

- Timer with a second hand
- Staining jars
- Racks for drying slides

Sample: Sample type: smear can be prepared from broth cultures and colonies

Amount of sample required: One drop Storage: Fresh colonies at room temperature

Sample retention: Samples are discarded after the test has been done.

**QUALITY CONTROL:**

Before doing any gram staining test always check new batches of stain and reagents for correct staining reactions using a smear containing known gram positive and gram negative organisms.

**PROCEDURE**

1. Fix the dry smear
2. Cover the fixed smear with crystal violates stain for 30 -60 seconds.
3. Rapidly wash off the stain with clean water
4. Tip off all the water, and cover the smear with lugol’s iodine for 30 -60 seconds.
5. Wash off the iodine with clean water.
6. Decolorize rapidly (few seconds) with acetone-alcohol. Wash immediately with clean water
7. Cover the smear with neutral red stain for 2 minutes.
8. Wash off the stain with clean water.
9. Wipe the back of the slide clean; place it in a draining rack for the smear to air-dry.
10. Examine the smear microscopically, first with 10X objective to check the staining and to see the distribution of material, and then with oil immersion objective to report the bacteria and cells.

**RESULT INTERPRETATION:**

Gram positive bacteria ..... Dark purple

Gram negative bacteria ..... Pale to dark red

Yeast Cells..... Dark purple

The reporting should include:

1. Number of bacteria present, whether many, moderate, few or scanty.
2. Gram reaction of the bacteria, whether gram positive or negative.
3. Morphology of the bacteria, whether cocci, diplo cocci, streptococci, rods, or Cocco bacilli.
4. Presence and number of pus cells.
5. Presence of yeast cells and epithelial cells.

Limitation:

1. Gram positive organisms may lose their ability to retain crystal violet and stain gram negatively because of:

- 1.1 Cell wall damage due to antibiotic therapy or excessive heat-fixation of the smear.
- 1.2 Over-decolonization of the smear.
- 1.3 Use of an iodine solution which is too old, i.e. yellow instead of brown in color (always store in a brown bottle or other light opaque container)
- 1.4. Smear has prepared from an old culture.

2. Gram negative organisms may not be fully decolorized and appear as gram positive when a smear is too thick.

Clinical utility: The Gram's stain is used to classify bacteria on the basis of their forms, sizes, cellular-morphologies, and gram reaction. It is a critical test for rapid presumptive diagnosis.

SOP for Catalase

Purpose: This procedure provides instructions to detect the production of catalase by bacteria.

Principle: Catalase is an enzyme that breaks hydrogen peroxide ( $H_2O_2$ ), a toxic metabolic byproducts of aerobic and facultative anaerobic bacteria into non toxic products water ( $H_2O$ ) and oxygen ( $O_2$ ).  $H_2O_2$  is toxic to cells. It is highly reactive molecule that damage cell components. So the bacteria living in presence of oxygen produces enzyme catalase that breakdown  $H_2O_2$  into  $H_2O$  and  $O_2$ .

Requirements:

- Pure culture of staphylococcus and streptococcus spp

- 3% Hydrogen peroxide
- A glass slide/test tube
- Glass rod

#### Procedure

##### Slide test:

1. Prepare a fresh hydrogen peroxide 3% solution
2. Place a drop of 3% H<sub>2</sub>O<sub>2</sub> on opposite ends of a clean grease free glass slide with the help of dropper
3. Transfer as small portion of both cultures onto drop of 3% H<sub>2</sub>O<sub>2</sub> with the help of a sterile glass rod
4. Examine for immediate bubbling of O<sub>2</sub>

Note: slide test is not recommended because of risk of contamination from active bubbling

##### Tube test:

1. Pour 2-3ml of 3% hydrogen peroxide into a test tube
2. Transfer the culture with the help of glass rod and immerse into H<sub>2</sub>O<sub>2</sub> solution
3. Observe for immediate bubbling

##### Result interpretation:

- Staphylococcus spp: give bubbling of gas, catalase positive
- Streptococcus spp: give no bubbling of gas, catalase negative

Limitation: Do not use wire loop to transfer the culture as H<sub>2</sub>O<sub>2</sub> may reacts with iron to give false positive; H<sub>2</sub>O<sub>2</sub> is toxic to human so handle carefully; observe the result within 30 seconds; culture should not be more than 24 hours old

#### CAMP Test

Purpose: This test is useful in the identification of *S.agalactiae*

Principle: CAMP test detects extracellular protein known as CAMP factor, produced by group B streptococcus. The CAMP factor acts synergistically with the beta lysine produced by

staphylococcus aureus to produce a zone of enhanced lysis of sheep or bovine erythrocytes. The standard CAMP test depends on the elaboration of two toxins during growth to form a typical arrowhead or flame-shaped clearing at the junction of the two organisms when they are placed perpendicular to each other.

Procedure:

1. Using an inoculating loop streak a beta-lysin producing staphylococcus aureus in a straight line across the center of sheep blood agar plate
2. Streak test organism in a straight line perpendicular to the S.aureus leaving 1 cm space between the two streaks
3. Incubate the plate at 37 degree Celsius in ambient air for 18-24 hours

Result interpretation:

Positive: Enhanced hemolysis is indicated by an arrow head-shaped zone of beta-hemolysis at the junction of the two organisms

Negative: No enhancement of hemolysis

Limitation: Some group A streptococcal will be CAMP positive if incubated under anaerobic conditions; extended incubation time or elevated incubation temperature may give false positive results; Sheep blood agar are only used, human or other blood plates will not give a proper reaction.

Antibiotic sensitivity testing procedure

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

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

Abbreviation:

ATCC= American Type Culture Collection

CLSI= Clinical and laboratory standards institute

Materials Reagents: 0.5 McFarland standards

Reagent preparation:

Turbidity standard number

Barium chloride dihydrate (1.175%)	Sulfuric acid (1%)	Corresponding	approximate
density of bacteria			

0.5ml

0.5ml 99.5ml 1x10<sup>8</sup>

Reagent stability: for six months at +2-+8 oc

Material, Supplies, reagent and equipment

- Muller hinton agar
- Muller Hinton agar with 5% sheep blood
- Normal saline
- Test tube
- Wooden applicator sticks with cotton
- Antimicrobial disks
- Safety cabinet
- Bunsen burner
- Incubator
- Measuring caliper
- Vortex
- Candle jar

Sample            Sample type

Amount required      Transport and storage      Stability

Pure colony equivalent to 0.5 McFarland      2ml      The test should be done immediately after the suspension has been made      Stable up to 24 hours

Limitations: Comparing the inoculum turbidity with the standard McFarland is subjective.

Sample retention: Samples are discarded after the test has been done.

Quality Control

Control	Stability	Frequency	Preparation Y/N
ATCC 6 weeks at room temperature			Weekly sub-cultured      Y

Control Preparation

1. Reconstitute the lyophilized standard strain in to 1ml TSY broth or Normal Saline.
2. Inoculate in to BAP & MAP
3. Incubate for 16 – 24 hrs at 35 – 37 oC
4. Perform sensitivity test
5. Compare the sensitivity result with CLSI guideline.

Note: If the results are out of expected value, repeat the test and take corrective action.

Procedure:

1. Prepare pure colony suspension in to normal saline equivalent to 0.5 Mcfarland standards.
2. Streak on appropriate media the entire surface.
3. Select antimicrobial agents according to the CLSI guideline & Put the disc on the plate aseptically
4. Incubate for 16 – 24 hrs at 35 +/- 2oC
5. Measure zone of inhibition and interpret the result based on CLSI break point.

Result interpretation:

1. 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.

2. Intermediate (I)

The ‘intermediate’ category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated or when a higher than normal dosage of a drug can be used. This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretation, especially for drugs with narrow pharmacotoxicity margin.

3. Resistant (R): The ‘resistant’ category implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules, and/or that demonstrate MICs or 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.

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

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

#### Muller Hinton media preparation

Purpose: This procedure provides instructions to prepare Mueller Hinton agar.

Principle: 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.

#### Abbreviation:

MHA= Mueller hinton agar

RT= Room temperature

PABA =para-aminobenzoic acid

#### Materials, Supplies and equipment

- MHA powder
- Distilled water
- Flask
- Petri dish
- Graduated cylinder
- Balance
- Distiller
- Bunsen burner

- Autoclave
- Hot plate
- PH meter

Procedure:

1. Suspend 38 gm of MHA powder & transfer in to a flask containing 1000 ml of distilled water.
2. Boil until the powder completely dissolved
3. Autoclave at 1210c for 15 minute.
4. Final PH at 25oc is 7.3 +/- 0.2.
5. Mix well and dispense aseptically in to sterile Petri dish.

Clinical Utility: MHA is used in antimicrobial susceptibility testing by the disk diffusion method.

Annex IX. Declaration

The undersigned declares that this proposal complies with the regulations of the University and meets the accepted standards with respect to originality and quality. PI also agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports.

M.Sc. candidate: SemiraMustefa(BSc.)

Signature: \_\_\_\_\_

Date of submission: \_\_\_\_\_

This proposal has been submitted with our approval as advisors.

Advisor: Kassu Desta (MSc, PhD candidate)

Signature: \_\_\_\_\_

Date: \_\_\_\_\_

Place: Addis Ababa, Ethiopia.

