PUERPERAL SEPSIS: BACTERIAL PROFILE, ANTIMICROBIAL SUSCEPTIBILITY PATTERN AND ASSOCIATED FACTORS AT ASELLA REFERRAL AND TEACHING HOSPITAL, CENTRAL ETHIOPIA.

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CERTIFICATE

This is to certify that the thesis entitled **‘‘Puerperal sepsis: Bacterial Profile, Antimicrobial Susceptibility Patterns and Associated Factors at Asella Referral and Teaching Hospital, Central Ethiopia’’** submitted by Abduselam Abbiso for the Degree of Master of Science in Medical Microbiology was carried out under our supervision and with our signature below we approved that this thesis is an original work of us.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANC	Antenatal Care
AST	Antimicrobial Susceptibility Test
ARTH	Asella Referral and Teaching Hospital
CAP	Chocolate Agar Plate
CI	Confidence Interval
C/S	Cesarean Section
EDHS	Ethiopian Demographic and Health Survey
EUCAST	European Committee on Antimicrobial Susceptibility Testing
MAC	MacConkey agar
MDR	Multi-Drug Resistance
MMR	Maternal Mortality Rate
MRSA	Methicillin Resistance *Staphylococcus aureus*
PPS	Puerperal sepsis
PROM	Prolonged Rupture of Membrane
BAP	Sheep Blood Agar
SPSS	Statistical Package for the Social Sciences
UNICEF	United Nations International Children Emergency Fund
WHO	World Health Organization

ABSTRACT

Background: Puerperal sepsis is any infection of the female reproductive tract or bloodstream occurring at any time between the onset of the rupture of membranes or labor and forty-two days of delivery or abortion/miscarriage. It is among the leading causes of maternal morbidity and mortality both in developing and developed countries.

Objectives: To determine bacterial profiles, their antimicrobial susceptibility patterns, and factors associated with puerperal sepsis.

Methods: A cross-sectional study was conducted among puerperal sepsis suspected postpartum/aborted women attending Asella Referral and Teaching Hospital, central Ethiopia from September 2020 to August 2021 G.C. A total of 174 study participants were enrolled. Sociodemographic and obstetric data of the participants were collected using a pretested structured questionnaire and checklist respectively. About 20 ml blood sample was collected from all study participant into BacT/ALERT® 3D blood culture bottles (about 10 ml each into aerobic and anaerobic) and incubated into BacT/ALERT® 3D automated blood culture system. Endocervical swab was also collected into Aime's transport media. All endocervical swabs and positive blood cultures were inoculated on MacConkey agar, blood agar, and chocolate agar plates for bacterial isolation and identification. Data were entered into EpiData version 4.6 and transferred to SPSS version 25.0 for analysis. Descriptive statistics were calculated to show frequency, bivariate and multivariate regression analysis were calculated to see the association of dependent and independent variables.

Results: The overall positivity rate of bacterial isolates among puerperal sepsis women suspected was 48.9%. Out of these 87.1% of the isolates were Gram negative bacteria. The most common isolates were *E. coli* (54.1%) followed by *Klebsiella spp.* (23.5%) and *S. aureus* (10.6%). *E. coli* were showed a higher resistance rate to Piperacillin (87%) and Trimethoprim-sulfamethoxazole (65.2%). *Klebsiella species* were showed a higher resistance rate to Aztreonam (65%) and Ceftriaxone (65%). *S. aureus* showed a higher resistance rate to Trimethoprim-sulfamethoxazole (66.6%). In this study, 81.2% of the isolates were multi-drug resistant bacterial pathogens. Multivariate regression analysis showed no statistically significant association between sociodemographic, obstetrics factors, and having bacteria.

Conclusion and Recommendation: The overall positivity rate in this study was around half. We reported *E. coli*, *Klebsiella* species, and *S. aureus* were the most common isolated bacteria. High numbers of multidrug-resistant bacterial isolates were identified. Our finding recommends the need for strengthening microbiology services to culture samples before starting antibiotics.

Keywords: Puerperal sepsis, bacterial profile, antimicrobial resistance, multidrug resistance, associated factors.

CHAPTER 1: INTRODUCTION

1.1. Background

The World Health Organization (WHO) defines puerperal sepsis as "any infection of the female reproductive tract or bloodstream occurring at any time between the onset of the rupture of membranes or labor and forty-two days of delivery or abortion/miscarriage" which is characterized by two or more of the following symptoms: pelvic pain, fever (i.e., the temperature of 38°C or higher), abnormal vaginal discharge (e.g. presence of pus), abnormal smell or foul odor of discharge, delay in the reduction of the uterus size (below 2 cm per day within the first eight days), visible evidence of infection in cesarean wounds, any system/organ failure and shock (1).

The most important predisposing factor for puerperal sepsis is delivery by cesarean section, while other factors also have a great contribution including home delivery related to unhygienic conditions using dirty materials, low socioeconomic condition, anemia, parity, prolonged rupture amniotic membrane (PROM), frequent per-vaginal examinations, prolonged labour, and postpartum hemorrhage. Several mechanisms appear to prevent overt infection in the genital tract including the acidity of the normal vagina, tenacious cervical mucus, and maternal antibodies to most vaginal flora. During labour and particularly after rupturing of the membranes some of the protective mechanisms are no longer present. Contractions during labor may spread bacteria present in the reproductive organ into the bloodstream (2–4).

A wide variety of bacteria (Gram negative and Gram positive) are responsible for causing puerperal sepsis. The major pathogens causing sepsis in the puerperium are group A *streptococcus* (*Streptococcus pyogenes*), group B *streptococcus (Streptococcus agalactiae), Staphylococcus aureus*, Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Klebsiella* species., *Pseudomonas aeruginosa, Proteus* species, *Citrobacter* species, *Clostridium septicum,* and *Morganella morganii* (5–7).

In developed country, puerperal sepsis as any other infectious disease is treated by diagnosing the causative agents and choosing the appropriate antimicrobials. But in many developing countries including Ethiopia puerperal sepsis is treated empirically with broad-spectrum antibiotics due to lack

of microbiology facility. The recommended empiric treatment of puerperal sepsis by the Ethiopian Ministry of Health is ampicillin, metronidazole, and gentamycin. ceftriaxone or benzylpenicillin is alternatives to ampicillin, while chloramphenicol replaces gentamycin if the women have renal function abnormality. Recommended antibiotic prophylaxis before the cesarean section is ceftriaxone or ampicillin plus metronidazole (8).

Even though puerperal sepsis is the common cause of maternal morbidity and mortality, it appears to be largely preventable with good antenatal check-up, aseptic delivery practices, and postpartum care and treated with a proper antibiotic regimen (9). Antibiotics are among the most successful drugs ever developed to treat bacterial diseases including puerperal sepsis, but it became evident that bacteria could become resistant to them (10). Diseases like puerperal sepsis and its causative agents that were once thought to be controlled by antibiotics are now returning to new leagues due to resistance (11).

The resistance among various microbial infectious agents to different antimicrobial drugs has emerged as a cause of public health threat worldwide. Due to the pacing advent of new resistance mechanisms and decrease in efficiency of treating common infectious diseases, it fails in microbial response to standard treatment. Almost all the capable infecting agents have employed high levels of multidrug resistance (MDR) with enhanced morbidity and mortality; thus, they are referred to as “superbugs” (12).

It is known that bacterial pattern with antimicrobial susceptibility is dynamic and leading to prolonged illness, higher expenditures for health care, and has an immense risk of death. Though research on this area of interest is needed in healthcare settings; no study, particularly on bacterial etiology and antimicrobial susceptibility pattern has been reported in this study area.

Therefore, this research was girded to determine bacterial pathogens, their antimicrobial susceptibility patterns, and factors associated with puerperal sepsis among suspected women at Asella Referral and Teaching Hospital, Central Ethiopia.

1.2. Statement of the problem

Puerperal sepsis is among the leading cause of preventable maternal morbidity and mortality particularly in developing countries (13). Globally, puerperal sepsis is the third leading cause of mortality accounting for 10.7% of maternal deaths from 2003 to 2012 and developed nations share approximately 5% of maternal deaths from this, while more than double (11%) of maternal deaths is in developing nations (14).

Mortality by region from sepsis was indicated that, 11.7% in Asia, 9.7% in Africa, 7.7%, in Latin America and the Caribbean, which is very high compared to 2.1% in developed countries (15). The greatest attention has been on postpartum hemorrhage and hypertensive disorders, the two leading direct causes of maternal mortality. However, the third most common direct cause of maternal mortality, sepsis received less attention, research, and programming as fifteen years (1990-2015) WHO report indicated (1). Puerperal sepsis causes at least 75,000 maternal deaths per year, particularly in low-income countries (13).

Sepsis is also ranked as the sixth leading cause of disease burden for women of reproductive age, next to depression, HIV/AIDs, tuberculosis, abortion, and schizophrenia (16). Sepsis is estimated to account for about 15% of maternal deaths worldwide (17). But, as the definition of sepsis and sepsis-related conditions are not being uniformly used throughout the studies particularly in obstetrics, as a result, the incidence and prevalence of sepsis reported around the world are varied (18).

In a developed country like the United States of America (USA), the absolute risk of developing sepsis was 10 per 10,000 live births (19). In Ireland from 2005 to 2012 the sepsis rate was 1.81 per 1,000 pregnant women, of which 17% of the episodes occurred antenatally, 36% occurred intrapartum, and 47% occurred postpartum (20). But, in developing countries including Ethiopia national burden of maternal sepsis and puerperal sepsis is less known except few studies showing institutional data (7,21,22).

In developed countries, puerperal sepsis is treated with the support of evidence from patients' microbiology results. However, in many developing countries including Ethiopia, the treatment of puerperal sepsis is empirical/syndromic, mainly due to a deficiency of microbiological diagnosis services. This empirical use of antibiotic classes leads to poorer patient outcomes by developing

resistance to those classes over time and may eventually lead to the development of multidrug-resistant organisms (MDROs). MDROs such as methicillin resistance *S. aureus* (MRSA) and extended-spectrum β-lactamase (ESBL) producing Gram negative bacteria can also cause puerperal sepsis that can make the treatment difficult (23).

Sepsis also consumes considerable human and financial costs of healthcare both in low-income and high-income countries (24). If left untreated, women with puerperal sepsis may develop wound dehiscence, peritonitis, pelvic abscess, necrotizing fasciitis, toxic shock syndrome, chronic pelvic inflammatory disease, chronic pelvic pain, infertility, and even death (25).

1.3. Significance of study

This study provides information on bacterial profiles, antimicrobial susceptibility patterns, and factors associated with puerperal sepsis at Asella Teaching and Referral Hospital, central Ethiopia. This will help the health institution and health professionals to prescribe the appropriate antibiotics for patients according to the result, which will also prevent the chance of occurrence of multidrug-resistant organisms and save the lives of mothers as well as minimize the wastage of resources.

Moreover, the study finding will help as baseline information for researchers, policy makers, non-governmental organizations, and others working on maternal health care by showing a common bacterial pathogen causing puerperal sepsis with their antibiotics resistance patterns and factors significantly associating with the disease at the study setting.

CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

The first epidemic of puerperal fever occurred at the Hotel Dieu in Paris in 1646. Subsequently, maternity hospitals all over Europe and North America reported outbreaks and even between epidemics the death rate from sepsis reached one woman in four or five of those giving birth.

Thomas Watson, Professor of medicine in 1842, wrote puerperal sepsis is when a practitioner has attended anyone example of it, he should use most diligent ablution. He began to write on the subject, and in 1843 published his classic essay the contagiousness of puerperal fever-that contains eight principles to avoid the puerperal sepsis if obstetric cases were being treated (1).

Currently, puerperal sepsis is defined as "infection of the genital tract occurring at any time between the onset of the rupture of membranes or labor and the 42nd day postpartum" (26).

2.2. Epidemiology of puerperal sepsis

Puerperal sepsis is among the leading cause of preventable maternal morbidity and mortality not only in developing countries but in developed countries as well. A literature review was done on low-income and high-income countries in 2010, estimated that puerperal sepsis causes at least 75,000 maternal deaths every year, mostly in low-income countries, while from high-income countries incidence of maternal morbidity due to sepsis of 0.1 to 0.6 per 1000 deliveries (13).

A prospective review study was done for about eight years (2005–2012) at two tertiary referral hospitals in Dublin, Ireland showed the sepsis rate of 1.81 per 1,000 attending women (includes all miscarriages, stillbirths, and live births) (20).

A retrospective study done in India shows the incidence of puerperal sepsis to be 8.68% and puerperal sepsis was responsible for 60.29% of cases of puerperal pyrexia (27). Another retrospective study was done in New Delhi, India, from January 2016 to June 2016, from 14,550 admissions in Obstetrics and Gynecology Department, 366 were due to puerperal sepsis, giving an incidence of 2.5% (28).

In Africa mortality due to puerperal sepsis is about 9.7% as a review of the literature was shown in 2016 (15). A descriptive cross-sectional study done in a tertiary health facility in Nigeria shows the incidence of puerperal sepsis was 9.34% (29). Another study from Nigeria found that the incidence of puerperal sepsis was 0.78%(30). According to a study of laboratory-confirmed puerperal sepsis in the national referral hospital of Tanzania, the incidence of puerperal sepsis was found to be 11.2% (31). A retrospective unmatched case-control study done in a tertiary university teaching hospital in Uganda shows puerperal sepsis was the leading cause of maternal death (30.9 %) followed by postpartum hemorrhage (21.6 %) (32).

A cross-sectional study conducted at Dire Dawa, Ethiopia shows the prevalence of maternal septicemia being 12.9% (7). Another study done in Dessie referral hospital, Ethiopia shows the prevalence of puerperal sepsis accounting for 5.7% (21).

2.3. The common bacterial pathogen causing puerperal sepsis.

According to the Green-top Guideline, the major pathogens causing sepsis in the puerperium are group A *streptococcus* (GAS), *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumonia*, meticillin-resistant *S. aureus* (MRSA), *Clostridium septicum,* and *Morganella morganii* (5).

A literature review done on low-income and high-income countries in 2010, showed the causative microorganisms for puerperal sepsis to be a poly-microbial with beta-hemolytic GAS often being the major cause of severe cases of puerperal fever followed by *Escherichia coli* (13).

According to a retrospective cohort study done in the USA, the most common organisms responsible for causing puerperal sepsis were *Escherichia coli* (17.5%), *Bacteroides* species (10.8%), *Enterococcus* species (10.8%), group B *streptococci* (10.8%), and GAS (5.0%) (6).

According to a prospective review for over eight years at two tertiary referral hospitals in Dublin, Ireland the common bacteria isolated from postpartum women were *E. coli* and group B *Streptococcus* (GBS) (20).

A descriptive cross-sectional study done in a tertiary health facility in Nigeria shows the commonest microorganism causing puerperal sepsis were *Klebsiella* species accounts for 57.7% followed by *Escherichia coli* (22.3%) and *Proteus* species (0.8%) (29). Another study from

Nigeria also shows the most common bacteria isolated were *Staphylococcus aureus* (35.4%) and *Escherichia coli* (20.9%) followed by *Streptococcus* species (6.9%) (30).

According to a hospital-based cross-sectional study done in Tanzania, the most common bacterial isolates causing puerperal sepsis were *Klebsiella* species, followed by *E. coli* and *S. aureus*, with *Enterococci* and *Pseudomonas* were also found in a small amount (31). Another study from Zimbabwe showed the commonest bacterial isolates causing puerperal sepsis were Escherichia coli (30.6%) and Klebsiella pneumoniae (15.3%) (25).

In a prospective study done from January 2011 to January 2012 at Hussein Mustafa Hospital in Sudan among clinically confirmed puerperal sepsis, about 72.9% of patients had positive bacterial blood culture. Out of the positive cases, aerobes were the majority isolates accounting for 62.1% which included *Staphylococcus aureus*, *Staphylococcus epidemics*, and *Listeria monocytogenes*. The anaerobes isolates were *Clostridium perfringens* and *Enterobacter cloacae* (33).

According to a cross-sectional study from eastern Ethiopia, the predominant bacterial isolates to cause puerperal sepsis was Coagulase negative *staphylococci* followed by *E. coli*, and *Salmonella* species (7). Another study from Bahir Dar Ethiopia showed the common organism from puerperal sepsis suspected women were S.aureus (33.9%), E.coli (32.1%) followed by Klebsiella pneumonia (12.5%) (22).

2.4. Antimicrobial susceptibility pattern of bacterial pathogens causing puerperal sepsis.

According to a retrospective cohort study done in the USA, about 81% of *E. coli* were resistant to ampicillin, about 9.5% were resistant to gentamicin, and 9.5% were resistant to both ampicillin and gentamicin. In addition, resistance to extended-spectrum beta-lactamases was noted in 47.6% of cases. *Bacteroides* and *Enterococcus* species were sensitive to ampicillin and gentamicin, and *Bacteroides* species were also sensitive to clindamycin and extended-spectrum beta-lactamases. All GBS and group A *streptococci* isolates were sensitive to ampicillin. Notably, 69.2% of GBS isolates and 66.7% of group A *streptococci* isolates demonstrated resistance to clindamycin (6).

According to a retrospective study conducted in Maharashtra, India in 2016, antimicrobial susceptibility of different bacterial isolates was seen that *K. Aerogenes was sensitive* to ciprofloxacin (92.30%) and Gentamicin (84.61%), *P. aeruginosa* shows sensitivity to

ciprofloxacin (88.88%). Most of the isolates of *Proteus* were sensitive to ciprofloxacin (100%) and Amikacin (80%). *E. coli* shows sensitivity to ciprofloxacin (75%). *S. aureus* shows sensitivity to cephalexin (100%), gentamicin (80%), and ciprofloxacin (70%). *S. pyogenes* was 100% sensitive to cephalexin and ciprofloxacin (34).

According to a study of laboratory confirmed puerperal sepsis in a national referral hospital of Tanzania, *E. coli* were highly susceptible to meropenem (97.0%), while resistance to ceftriaxone, ampicillin, and ceftazidime was 64.7, 67.6, and 63.2%, respectively. *Klebsiella* species were susceptible to meropenem (86.4%) and resistant to ceftriaxone (77.3%), gentamicin (86.4%), ampicillin (81.8%) and ceftazidime (86.4%). *Staphylococcus aureus* isolates were 100% susceptible to clindamycin. The proportion of extended spectrum beta lactamase producers among gram-negative bacilli was 64(69.6%) and 53.8% of *S. aureus* isolates were resistant to methicillin (31).

A cross-sectional study conducted at Dilchora hospital of Dire Dawa, Eastern Ethiopia shows that gram negative bacteria isolates have high frequency of sensitivity to ciprofloxacin (88%) and to ceftriaxone (81.8%) followed by gentamicin (67%). Lower frequency of resistance (< 30%) was observed to ciprofloxacin (6%), ceftriaxone (12.1%), gentamicin (24%), chloramphenicol (18%) and nalidixic acid (30%). Among, the predominantly isolated Gram negative bacteria, *E. coli* was found to be highly sensitive to ciprofloxacin (92.3%), ceftriaxone (92.3%), and gentamicin (84%) whereas the high frequency of resistance was observed to ampicillin (100%), amoxicillin (92.3%), and tetracycline (84.6 %). A susceptibility of Gram positive bacteria isolates showed a high frequency of susceptibility to gentamicin (91.7%), erythromycin (87.5%), ceftriaxone (75%), chloramphenicol (75%), and ciprofloxacin (71%). The proportion of resistance of Gram-positive isolates was (83%) for ampicillin, 79 % for amoxicillin, 54.1% for tetracycline, and 33.3% for chloramphenicol while the lower frequency of resistance (< 30%) was observed against erythromycin, ceftriaxone and ciprofloxacin (7).

2.5. Factors associated with puerperal sepsis.

A case-control study done in 2017 at Uppsala University hospital, Sweden shows post-term delivery (≥42 weeks), induction of labour, prolonged duration of the second stage, oxytocin treatment, cesarean section, and manual placental removal were associated with postpartum infection (35).

A case-control study from two tertiary level hospitals in Bangladesh on socio-demographic factors and puerperal sepsis shows the significant predisposing factors for puerperal sepsis to be an age less than 25 years, respondent's educational level less than secondary school and husband's educational level less than secondary school (36). Another study from Pakistan showed common risk factors found were absent membranes, delivered or undelivered and mismanaged, referred cases (2).

A retrospective study was done in New Delhi, India, from January 2016 to June 2016, shows Anemia, prolonged labour, delivery by an untrained person and unsafe abortion were the main identifiable risk factors (28).

According to a study done in Maiduguri University Teaching Hospital in Nigeria, major risk factors for developing puerperal sepsis were cesarean section (C/S), home delivery, perineal trauma, un-booked status, and maternal age <24 years (30).

According to a case-control study done in the west Shoa zone of Oromia regional state, Ethiopia the major determinants of puerperal sepsis were living in the rural areas, being in labour for greater than 25 hours, amniotic membrane ruptured 24 hours before delivery, referred from other health institutions, delivered by C/S (16)**.** Another retrospective cross-section study from Ethiopia also showed the factors most significantly associated with puerperal sepsis were being in age ranged from 15 to 46 years, primi-gravidity, multiple vaginal examinations, delivery by cesarean section and premature rupture of membrane (21).

2.6. Clinical diagnosis of puerperal sepsis

The current standard for clinical sepsis diagnosis is referred to as sequential organ failure assessment (SOFA) score. This scoring system informs of cumulative organ dysfunction that results from a dysregulated host response to infection. The SOFA score factors in respiration (arterial oxygen partial pressure to fractional inspired oxygen), coagulation (platelet count), liver function (bilirubin levels), cardiovascular function (mean arterial pressure or intervention), central nervous system function (Glasgow Coma Scale), and renal function (creatinine and urine output).

For each poorly functioning organ system, one point is assigned, with a score of 2 points or more representing a positive sepsis diagnosis. Quick SOFA (qSOFA) score offers a rapid alternative to

SOFA by using only a subset of the SOFA scoring including altered mentation, systolic blood pressure below 100 mm Hg, and a respiratory rate of 22 per minute or greater. qSOFA does not require laboratory testing and can be repeated as needed (37).

As the maternal physiology gradually returns to normal postpartum, its recommend that the definition of postpartum sepsis be the same as for non-pregnant patients after the first week postpartum(38). But, before the first week of postpartum and at pregnancy SOMANZ (Society of Obstetric Medicine Australia and New Zealand) recommended using the obstetrically modified SOFA (omqSOFA) score and obstetrically modified qSOFA (omqSOFA) score because woman's gravid state will impact some of the variables used for scoring like systolic blood pressure. Using the omqSOFA, sepsis (as distinct from infection) in pregnant women should be considered where two or more of the following are present: systolic blood pressure of 90 mmHg or less which is 100 mmHg or less for normal adult; respiratory rate of 25/min or greater which is 22/min or greater for normal adults, altered mentation (any state other than 'Alert' on maternal observation charts) which is same to normal adult (37,38).

2.7. Laboratory diagnosis of puerperal sepsis

Blood cultures are essential first-line laboratory investigations for sepsis. Bloods should be sent to the lab before antibiotic therapy, but empirical antibiotic treatment should be started immediately after taking the blood sample. Serum lactate indicates the level of tissue perfusion; levels ≥4 mmol/L indicates tissue hypoperfusion. Sample should be taken within six hours of hospital or ICU admission. Routine blood tests like complete blood count (CBC), urea, electrolytes, and C-reactive protein (CRP). Thrombocytosis, a rising CRP, and swinging pyrexia usually indicate a collection of pus or an infected haematoma in the woman. Swabs for culture and sensitivity like high cervico-vaginal swabs, urine, throat swabs, sputum, cerebrospinal fluid, epidural site swab, cesarean section, or episiotomy site wound swabs and expressed breast milk can be requested based on the clinical situations and suspicion of focus of infection. If the MRSA is unknown, a pre-moistened nose swab may be sent for rapid MRSA screening if a facility for this testing is available. Swabs should be taken before the start of any antibiotics. If there is a history of diarrhea, a stool sample should be sent for *C. difficile* toxin testing and routine culture (e.g. *Salmonella, Campylobacter*) (39,40).

2.8. Conceptual framework

Several factors were identified to influence the occurrence of puerperal sepsis which includes socio-demographic factors of the patients, obstetric factors, and individual factors.

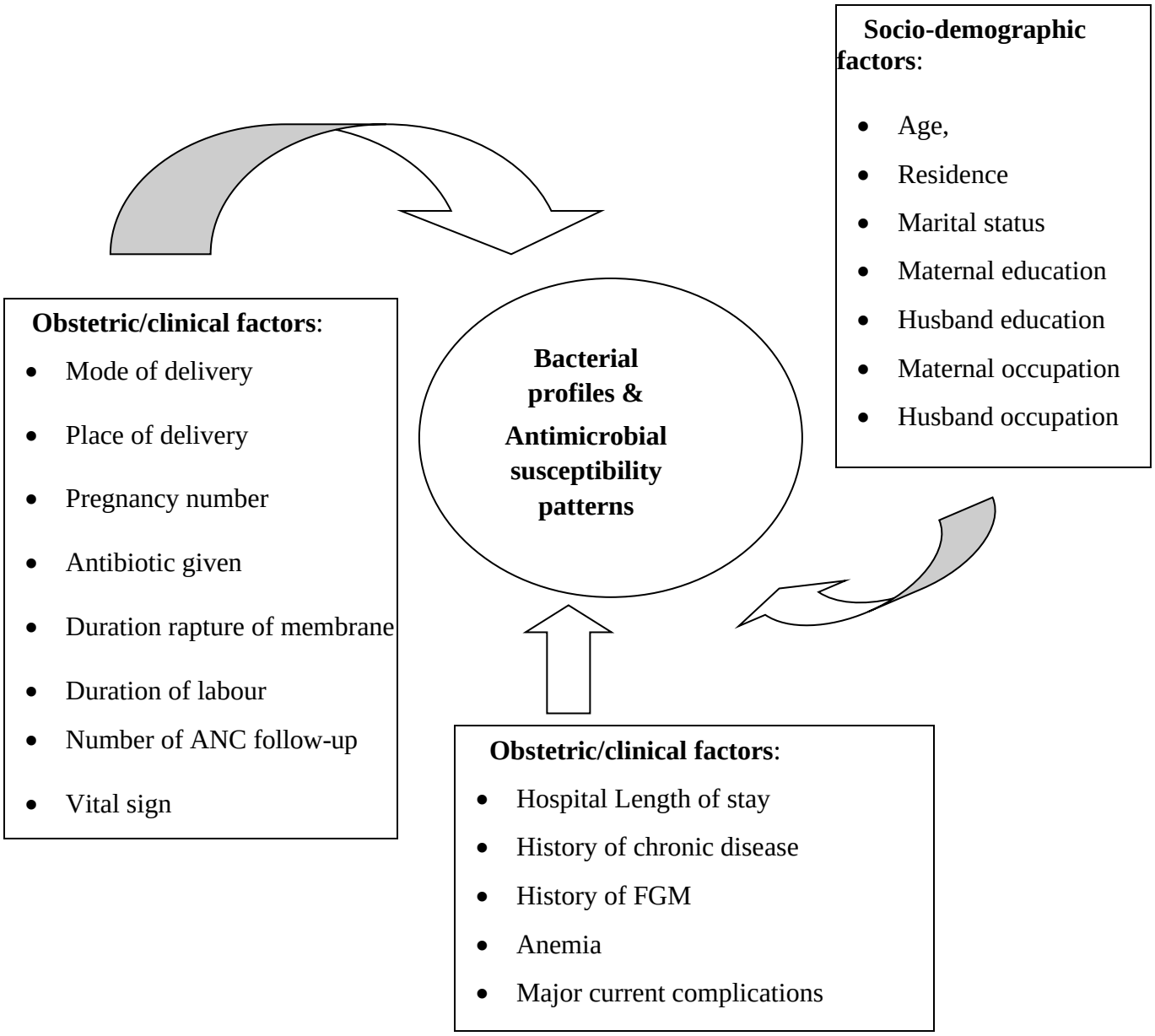

Figure 1: Conceptual framework.

CHAPTER 3: OBJECTIVES

3.1. General objective

To determine bacterial profiles, antimicrobial susceptibly patterns, and risk factors associated with puerperal sepsis among suspected postpartum/aborted women from September 2020 to August 2021 G.C at Asella Referral and Teaching Hospital, central Ethiopia.

3.2. Specific objectives

- To determine bacterial profiles causing puerperal sepsis among suspected postpartum/aborted women.
- To determine the antimicrobial susceptibility patterns of the isolates among suspected postpartum/aborted women.
- To identify risk factors associated with puerperal sepsis among suspected postpartum/aborted women.

CHAPTER 4: METHODS AND MATERIALS

4.1. Study area

This study was conducted at Asella Referral and Teaching Hospital (ARTH), ARTH is a public teaching and referral hospital found in the Arsi zone of Oromia regional state, central Ethiopia. The hospital is located about 168 km South-East of Addis Ababa, Ethiopia at an altitude of 2247.25 meters above sea level. ARTH serves more than 4.6 million population in the catchment areas. The hospital has 347 beds, and 58 beds are found in Gynecology and Obstetrics departments/wards accounts for 58(16.7%) of the beds. According to the annual data of 2020 found from the Health Management and Information System (HMIS) team of the hospital about 9,240 women were attending maternity ward/outpatients of ATRH for delivery, abortion/miscarriage cases. The Gynecology and Obstetrics departments of the hospital have given service for more than 8,400 women with postpartum/aborted cases in the last 12 months.

4.2. Study design and period

A cross-sectional study was conducted from September 2020 to August 2021.

4.3. Population

4.3.1. Source population

All women attending Asella Referral and Teaching Hospital for delivery, abortion/miscarriage, or postnatal care service.

4.3.2. Study population

All women suspected of puerperal sepsis attending ARTH after delivery or abortion/miscarriage during the study period who were fulfill the inclusion criteria.

4.4. Eligibility criteria

4.4.1. Inclusion criteria

All women attending the study setting immediately after the rupture of amniotic membrane to 42ndday of delivery or abortion/miscarriage and presenting with at least two of the following

symptoms: pelvic pain, fever (i.e., the temperature of 38°C or higher), abnormal vaginal discharge (e.g. presence of pus), abnormal smell or foul odor of discharge, delay in the reduction of the uterus size (below 2 cm per day within the first eight days), visible evidence of infection in cesarean wounds, any system/organ failure and shock and those who are willing to participate in the study and gave written consent.

4.4.2. Exclusion criteria

- Exclusion criteria for this study were all women with a sign of infection before delivery or abortion/miscarriage
- Women those who cannot give both blood culture and endocervical swab samples due to different reason.

4.5. Sample size determination

The sample size was determined using a single population proportion formula as follows: n = $Z^2p(1-p)/ d^2$; where: n = the number of postpartum or aborted/miscarriage women to be involved in this study; Z = Standard normal distribution value at 95 % CI, which is 1.96; P = the Prevalence of puerperal sepsis determined at 12.9% (7); d= the margin of error, taken as 5 %. Accordingly, the sample size was, $\mathbf{n = (1.96)^2 0.13(1-0.13)/0.05^2 = 174}$. Therefore, a total sample size for this study were **174** puerperal sepsis suspected women who fulfill the WHO criteria for puerperal sepsis.

4.6. Sampling technique

A convenience sampling technique was used, and study subjects were enrolled conveniently until the desired sample size was achieved.

4.7. Study variables

4.7.1. Dependent variable

Presence of at least one bacterial species from either blood culture or endocervical swab samples.

4.7.2. Independent variable

Age, Residence, Marital status, Maternal education, Husband education, Maternal occupation, Husband occupation, Mode of delivery, Place of delivery, Pregnancy number, Antibiotic given, Duration rapture of membrane, Duration of labour, Number of ANC follow-up, Vital sign, Hospital Length of stay, History of chronic disease, History of FGM, Anemia, Major current complications, History of current infection

4.8. Operational definitions

Premature rupture of membrane (PROM): refers to a patient who is beyond 37 weeks of gestation and has presented with rupture of membrane before the onset of labor (41).

Prolonged labor: when all the stages of labor (from the onset of true labor to delivery) last for more than 18 hours (41).

Abortion: is the deliberate termination of a women's pregnancy, most often performed during the first 28 weeks of pregnancy (42).

Miscarriage: is the spontaneous loss of a women's pregnancy before the 20^{th} week of pregnancy (42).

Stillbirth: is the death or loss of a baby at or after 20 weeks of pregnancy (42).

Multi Drug Resistance Organism (MDRO): is antimicrobial resistance shown by a species of organism to at least one antimicrobial drug in three or more antimicrobial categories (10).

Women with a sign of infection: women with at least two of the following symptoms: fever (i.e., the temperature of 38.5°C or higher), pelvic pain, abnormal vaginal discharge, visible evidence of infection in cesarean wounds or any system/organ failure and shock

Susceptible: when the bacteria cannot grow around the antibiotics with the diameter recommended by EUCAST (43).

Resistant: when the organism grows around the antibiotics with the less diameter recommended by EUCAST to be susceptible (this also includes intermediate resistance) (43).

4.9. Data collection method

4.9.1. Data collection

Socio-demographic data of study participants were collected using a structured questionnaire by interviewing the study participants. Obstetric data of the participants were collected using a checklist by consultation of gynecologist and midwife and by reviewing their medical records. The questionnaire and checklist were prepared based on the objectives of the study by reviewing different literatures and by consultation of the gynecologist (7,16).

4.9.2. Sample collection and processing

Two bottles of blood samples (about 10 ml for each vial) were collected from all study participants into a separate blood culture bottle BacT/ALERT®3D aerobic and anaerobic vials) using a sterile vacutainer needle aseptically after proper disinfection. Endocervical swab samples were also collected from all study participants by experienced midwives and gynecologist following standard protocol, added into Amies transport medium (Copan Italia Spa, Italy), and transported. The specimens were delivered to the Laboratory of Hirsch Institute of Tropical Medicine (HITM), Asella Ethiopia immediately where bacterial isolation, identification, and AST were done.

Both aerobic and anaerobic blood culture bottles were incubated into BacT/ALERT® 3D automated blood culture system following the standard instructions of the manufacturer. All blood cultures positive by the machine within seven days were sub-cultured onto a blood agar plate (Oxoid Ltd Basingstoke, Hampshire, UK), chocolate agar plate (incubated at 5% CO2 atmosphere in the anaerobic incubator), and MacConkey agar (Oxoid Ltd Basingstoke, Hampshire, UK) plates and examined for growth after 24–48 hours of incubation. All blood culture bottles negative by the machine after the 7th day was discarded and the result was recorded as “no growth”.

Endocervical swab sample was also cultured onto a blood agar plate (Oxoid Ltd Basingstoke, Hampshire, UK), chocolate agar plate (incubated at 5% CO2 atmosphere in anaerobic incubator), and MacConkey agar plate (Oxoid Ltd Basingstoke, Hampshire, UK) and examined for growth after 24–48 hours of incubation.

Preliminary identification of those sub-cultured bacterial isolates was done based on cultural characteristics such as colonial morphology, hemolysis pattern on the blood agar plate, Gram’s

reaction, changes in physical appearance in selective and differential media like MacConkey. Further identification of Gram negative bacteria was carried out by performing biochemical tests like motility, indole, glucose and lactose fermentation on Triple-sugar Iron Agar (TSI), citrate, urease, gas production, H2S2 production, oxidase test, and others. For Gram positive bacteria biochemical tests like catalase, coagulase, CAMP test, and others (44).

4.10. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was performed using the Kirby-Bauer disk diffusion method after preparing homogenous colonial suspension from the pure culture that is comparable to 0.5 McFarland turbidity standards. The inoculum was spread on Mueller-Hinton agar. The antibiotic disk was placed on and incubated at 35-37 °C for 18-20 hours. Disk quality control was tested by using control strains (*E.coli* (ATCC 25922) and *S. aureus* (ATCC 25923)). All antibiotic disks used were from "Mast Group Ltd., Merseyside, UK".

For gram positive bacteria antimicrobial discs tested were Piperacillin(30µg), Cefoxitin (30µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Trimethoprim-sulphamethaxazole (25µg), Amikacin (30µg), Gentamicin (10µg), Erythromycin (15 µg) and Clindamycin (2µg). For Gram negatives, Piperacillin(30µg), Cefoxitin (30µg), Ceftazidime(10µg), Ceftriaxone (30µg), Meropenem (10µg), Aztreonam (30µg), Ciprofloxacin (5µg), Amikacin (30µg), Gentamicin (10µg), Nitrofurantoin (100µg), Trimethoprim-sulphamethaxazole (25µg), discs were tested.

Antibiotic breakpoints were defined according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, version 9.0 (43). Disks were selected from each class of antibiotics according to the EUCAST recommendation for both Gram negative and Gram positive bacteria.

4.11. Quality control

Pre-analytical, analytical, and post-analytical stages of quality assurance that are incorporated in standard operating procedures of the Medical Microbiology Laboratory were strictly followed. All materials and equipment were checked for their performance by internal quality control officers and certified annually by external officers, reagents were checked for their expiry date before use, and procedures were done according to the lab SOPs.

Culture media were tested for sterility and performance. The sterility of culture media was ensured by incubating 5% of each batch of the prepared media at 37oc for 24 hours and checked for any growth. If there is any growth all the batches will be discarded. Performance of all prepared media was also checked by inoculating international standard-strains such as *E.coli* (ATCC 25922) for Gram negative bacteria, *S. aureus* (ATCC 25923) for Gram positive bacteria (43).

4.12. Data processing and analysis

Data were entered into EpiData version 4.6 and transferred to SPSS version 25.0 for analysis. Descriptive statistics were used to analyze participants' socio-demographic, clinical characteristics, frequency of isolates, and antimicrobial susceptibility patterns. Bivariate and multivariate logistic regression analysis was used to compute the risk factors associated with puerperal sepsis to bacterial isolation. All variables with a p-value <0.25 on bivariate regression analysis were analyzed in multivariate regression analysis. Odds ratio and 95% confidence interval were computed to assess the presence and degree of association between dependent and independent variables. P-value < 0.05 was considered statistically significant for all cases.

4.13. Ethical considerations

Ethical clearance has been obtained from Jimma University institutional research ethics review committee (Ref No; IRB000153/2020) and the institutional ethical review board of Arsi University (Ref No; A/U/H/S/C/120/13084/2012). Permission was obtained from ARTH before data and biological sample collection. Written informed consent was obtained from all study participants for their willingness to participate after explaining the objective of the study. All information was kept confidential, and culture positive results and their antimicrobial resistance test results were delivered to the physicians for better management of the patients.

4.14. Dissemination of research findings.

The final research finding will be submitted to the school of Medical Laboratory Science, Jimma University as a research thesis. The finding will also be submitted to Hirsch Institute of Tropical Medicine, Arsi University. Finally, the findings of this study will be presented on the thesis defense, in different seminars, workshops and will be published in reputable journals.

CHAPTER 5: RESULT.

5.1. Socio-demographic and obstetric characteristics

A total of 174 women suspected of puerperal sepsis were enrolled in this study. The median age of the participants was 25 years (IQR: 21-30). Out of these only 11(6.3%) and 36(22.1%) of maternal and husband were educated more than secondary school, respectively. Most, 104(59.8%) and 102(62.6%) of study women and their husbands were farmers, respectively, Table 1.

Table 1: Socio-demographic status of puerperal sepsis suspected women at Asella Referral and Teaching Hospital, Central Ethiopia from September 2020 to August 2021.

Variable	Frequency	Percentage
Age group (years)		
18-24	68	39.1
25-34	89	51.1
35-42	17	9.8
Residence		
Rural	110	63.2
Urban	64	36.8
Marital status		
Single	1	0.6
Married	163	93.7
Divorced	8	4.6
Widowed	2	1.1
Maternal Education		
No education	40	23.0
Primary	67	38.5
Secondary	56	32.2
More than secondary	11	6.3
Husband education		
No education	21	12.9
Primary	51	31.3
Secondary	55	33.7
More than secondary	36	22.1
Maternal occupation		
Housewife	50	28.7
Farmer	104	59.8
Employee	20	11.5
Husband occupation		
Farmer	102	62.6
Gov't employee	37	22.7
Private employee	24	14.7
History of Female Genital Mutilation		
Yes	77	44.3
No	97	55.7

5.2. The proportion of bacterial species isolates from puerperal sepsis suspected women

From the total of 174 puerperal sepsis suspected women participated in this study, 85 of them had at least one bacterial growth from either blood culture or endocervical swab culture showing the overall positivity rate of 48.9% (95%CI 41.25-56.5). Out of these 76(89.4%) (95%CI 80.4 - 94.74) bacterial species were isolated from endocervical swabs, while 9(10.6%) (95%CI 5.26-19.6) bacterial species were isolated from blood culture.

Among the total isolates, 4(4.7%) of bacterial species were grown from both blood culture and endocervical swab samples. The proportion of Gram negative bacteria in this study was 74(87.1%) (95% CI 77.6 - 93.1). The most frequently isolated bacteria were *E. coli* 46(54.1%) followed by *Klebsiella* species 20(23.5%) and *S. aureus* 9(10.6%), Figure 2.

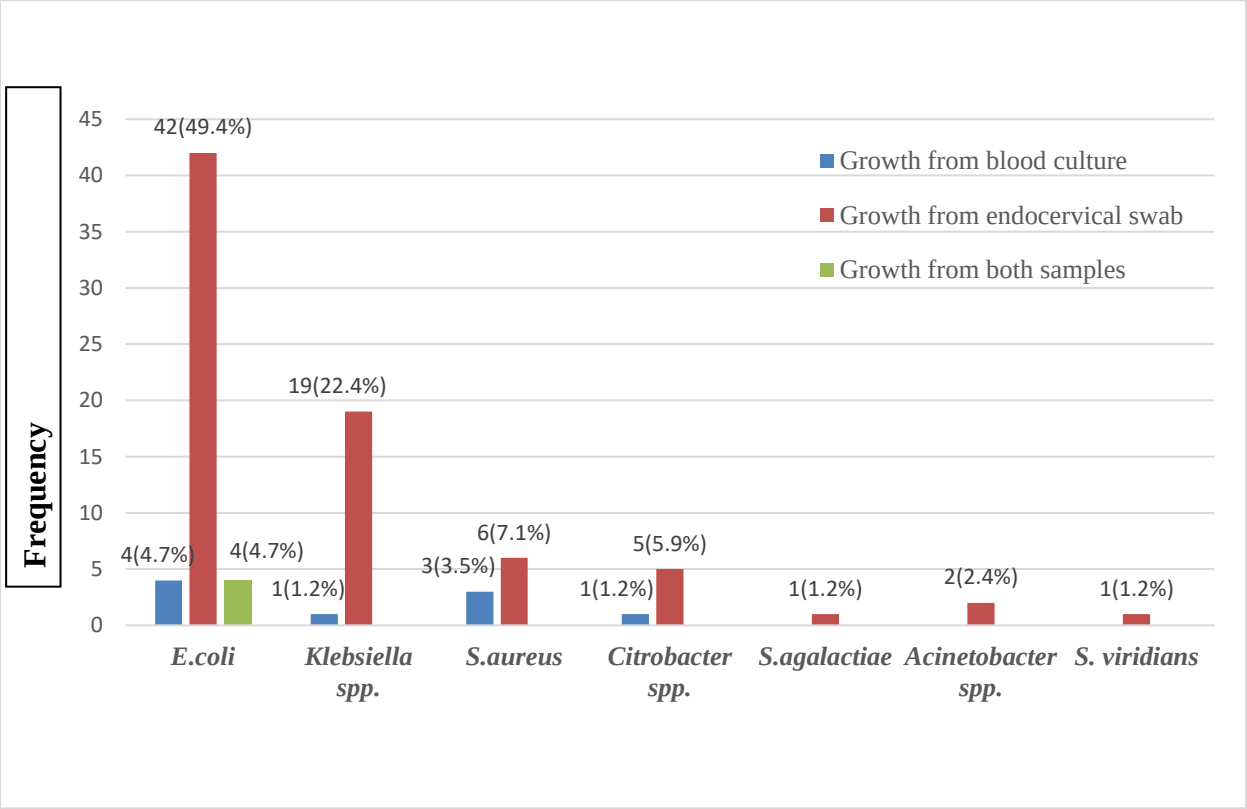

Figure 2: Frequency of bacterial isolates from blood culture and endocervical swab samples of puerperal sepsis suspected women at Asella Referral and Teaching Hospital, Central Ethiopia, from September 2020 to August 2021

5.3. Antimicrobial susceptibility profiles of bacteria isolates

5.3.1. AST profile for Gram negative bacteria

In total, 74 Gram negative isolates were available for antimicrobial susceptibility with the Kirby-Bauer disc diffusion test. Among those, a high rate of resistance was observed for Piperacillin (73%), Trimethoprim-sulfamethoxazole (60.8%), and Aztreonam (60.8%). A lower resistance rate was observed for Amikacin (12.2%) and Meropenem (20.3%). From 74 Gram negative isolates 64(86.5%) were MDR, Table 2.

Table 2: Antimicrobial susceptibility patterns of Gram negative bacterial isolates from puerperal sepsis suspected women at Asella Referral and Teaching Hospital, Central Ethiopia, from September 2020 to August 2021.

Isolates		**Antibiotics No (%)**											**MDR**
		PCR	**FOX**	**CAZ**	**CRO**	**MEM**	**ATM**	**CIP**	**AK**	**GEN**	**TS**	**NI**	
E. coli (n=46)	S	6 (13.0)	36 (78.3)	27 (58.7)	22 (47.8)	37 (80.4)	18 (39.1)	21 (45.7)	41 (89.1)	37 (80.4)	16 (34.8)	26 (56.5)	40 (87.0)
	R	40 (87.0)	10 (21.7)	19 (41.3)	24 (52.2)	9 (19.6)	28 (60.9)	25 (54.3)	5 (10.9)	9 (19.6)	30 (65.2)	20 (43.5)	
Klebsiella spp.(n=20)	S	10 (50.0)	9 (45.0)	9 (45.0)	7 (35.0)	14 (70.0)	7 (35.0)	9 (45.0)	16 (80.0)	7 (35.0)	9 (45.0)	10 (50.0)	18 (90.0)
	R	10 (50.0)	11 (55.0)	11 (55.0)	13 (65.0)	6 (30.0)	13 (65.0)	11 (55.0)	4 (20.0)	13 (65.0)	11 (55.0)	10 (50.0)	
Citrobacter spp. (n=6)	S	2 (33.3)	3 (50.0)	3 (50.0)	3 (50.0)	6 (100)	4 (66.7)	3 (50.0)	6 (100)	4 (66.7)	4 (66.7)	3 (50.0)	4 (66.7)
	R	4 (66.7)	3 (50.0)	3 (50.0)	3 (50.0)	-	2 (33.3)	3 (50.0)	-	2 (33.3)	2 (33.3)	3 (50.0)	
Acinetobacter spp. (n=2)	S	2 (100)	2 (100)	-	-	2 (100)	-	-	2 (100)	2 (100)	-	2 (100)	2 (100)
	R	-	-	2 (100)	2 (100)	-	2 (100)	2 (100)	-	-	2 (100)	-	
Total (n=74)	S	20 (27.0)	50 (67.6)	39 (52.7)	32 (43.2)	59 (79.7)	29 (39.2)	33 (44.6)	65 (87.8)	50 (67.6)	29 (39.2)	41 (55.4)	64 (86.5)
	R	54 (73.0)	24 (32.4)	35 (47.3)	42 (56.8)	15 (20.3)	45 (60.8)	41 (55.4)	9 (12.2)	24 (32.4)	45 (60.8)	33 (44.6)	

Key: PCR: Piperacillin, FOX: Cefoxitin, CAZ: Ceftazidime, CRO: Ceftriaxone, MEM: Meropenem, ATM: Aztreonam, CIP: Ciprofloxacin, NI: Nitrofurantoin, TS: Trimethoprim-sulfamethoxazole, AK: Amikacin, GEN: Gentamicin, S: Susceptible, R: Resistant, MDR: Multi Drug Resistant

5.3.2. AST profile for Gram positive bacteria

In total, 11 Gram positive isolates were available for antimicrobial susceptibility with the Kirby-Bauer disc diffusion test. Among those, a high rate of resistance was observed for Trimethoprim-sulfamethoxazole (63.6%) and Erythromycin (54.5%). A lower resistance rate was observed for Ceftriaxone (9%), Cefoxitin (9%), and Piperacillin (9%). From 11 Gram positive isolates 5(45.5%) were MDR, Table 3.

.

Table 3: Antimicrobial susceptibility patterns of Gram positive bacterial isolates from puerperal sepsis suspected women at Asella Referral and Teaching Hospital, Central Ethiopia, from September 2020 to August 2021.

Bacterial Isolates	**Resistance No (%)**										**MDR**
	AST	**PCR**	**FOX**	**CRO**	**CIP**	**AK**	**GEN**	**TS**	**E**	**CD**	
S. aureus (n=9)	S	8 (88.9)	9 (100)	9 (100)	6 (66.7)	7 (77.8)	7 (77.8)	3 (33.3)	3 (33.3)	7 (77.8)	3 (33.3)
	R	1 (11.1)	-	-	3 (33.3)	2 (22.2)	2 (22.2)	6 (66.6)	6 (66.7)	2 (22.2)	
Strep. agalactiae (n=1)	S	1 (100)	1 (100)	1 (100)	-	-	-	-	1 (100)	1 (100)	1 (100)
	R	-	-	-	1 (100)	1 (100)	1 (100)	1 (100)	-	-	
Strep. Viridians (n=1)	S	1 (100)	-	-	-	-	-	1 (100)	1 (100)	-	1 (100)
	R	-	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	-	-	1 (100)	
Total (n=11)	S	10 (91.0)	10 (91.0)	10 (91.0)	6 (54.5)	7 (63.6)	7 (63.6)	4 (36.4)	5 (45.5)	8 (72.7)	5 (45.5)
	R	1 (9.0)	1 (9.0)	1 (9.0)	5 (45.5)	4 (36.4)	4 (36.4)	7 (63.6)	6 (54.5)	3 (27.3)	

Key: PCR: Piperacillin, FOX: Cefoxitin, CRO: Ceftriaxone, CIP: Ciprofloxacin, NI: Nitrofurantoin, TS: Trimethoprim-sulfamethoxazole, AK: Amikacin, GEN: Gentamicin: E: Erythromycin, CD: Clindamycin, S: Susceptible, R: Resistant

5.4.Risk factors for acquisition of bacteria among puerperal sepsis suspected woman.

Bivariate regression analysis of risk factors showed association at p-value <0.25 for maternal age, maternal education, husband education, maternal occupation, husband occupation, and patient status. Multivariate regression analysis showed no statistically significant association between sociodemographic and obstetric variables as compared to having bacterial pathogen. However, women with age 35-42 had a higher positivity rate (70.6%) of bacterial pathogen, while women educated above secondary school had a lower positivity rate (27.3%), Table 5.

Table 4: Bivariate and multivariate regression analysis of risk factors associated with culture-positive puerperal sepsis suspected women at Asella Referral and Teaching Hospital, Central Ethiopia, from September 2020 to August 2021.

Variable	Organism isolated		Bivariate analysis		Multivariate analysis	
	Yes (%)	No (%)	COR (CI)	P-Value	AOR (CI)	P-Value
Age (years)						
18-24	30(44.1)	38(55.9)	1			
25-34	39(43.8)	50(56.2)	0.988(0.523, 1.866)	0.970	0.809(0.370, 1.767)	0.594
35-42	12(70.6)	5(29.4)	3.040(0.965, 9.580)	0.058	2.179(0.512, 9.280)	0.292
Residence						
Rural	51(46.4)	59(53.6)	0.980(0.528, 1.817)	0.948		
Urban	30(46.9)	34(53.1)	1			
Maternal Education						
No education	21(52.5)	19(47.5)	2.947(0.681, 12.753)	0.148	0.965(0.134, 6.951)	0.972
Primary	35(52.2)	32(47.8)	2.917(0.711, 11.957)	0.137	1.053(0.157, 7.053)	0.958
Secondary	22(39.3)	34(60.7)	1.725(0.412, 7.219)	0.455	0.746(0.127, 4.394)	0.746
Above secondary	3(27.3)	8(72.7)	1			
Husband education						
No education	11(52.4)	10(47.6)	2.200(0.731, 6.620)	0.161	0.689(0.132, 3.603)	0.659
Primary	29(56.9)	22(43.1)	2.636(1.086, 6.402)	0.032	1.072(0.274, 4.190)	0.921
Secondary	23(41.8)	32(58.2)	1.437(0.599, 3.452)	0.417	0.663(0.197, 2.226	0.506
Above secondary	12(33.3)	24(66.7)	1			
Maternal occupation						
Housewife	16(32.0)	34(68.0)	0.385(0.133, 1.114)	0.078	0.404(0.102, 1.600)	0.197
Farmer	54(51.9)	50(48.1)	0.884(0.338, 2.311)	0.801	0.131(0.005, 3.396)	0.221
Employee	11(55.0)	9(45.0)	1		1	
Husband occupation						
Farmer	53(52.0)	49(48.0)	1.278(0.524, 3.119)	0.590	0.572(0.124, 2.631)	0.473
Gov't employee	11(29.7)	26(70.3)	0.500(0.172, 1.456)	0.204	0.581(0.182, 1.859)	0.360
Private employee	11(45.8)	13(54.2)	1		1	
History of FGM						
Yes	40(51.9)	37(48.1)	1.477(0.809, 2.695)	0.254		
No	41(42.3)	56(57.7)	1			
Patient status						
Abortion	12(48.0)	13(52.0)	0.991(0.423, 2.322)	0.983	0.918(0.340, 2.477)	0.866
Miscarriage/Still birth	1(12.5)	7(87.5)	0.153(0.018, 1.279)	0.083	0.113(0.013, 1.011)	0.051
Live birth	68(48.2)	73(51.8)	1		1	
Mode of delivery						
SVD	39(48.8)	41(51.2)	1			
C/S	30(45.5)	36(54.5)	0.876(0.456, 1.684)	0.691		
PROM						
Yes	6(46.2)	7(53.8)	0.952(0.304, 2.985)	0.933		
No	63(47.4)	70(52.6)	1			

Place of delivery or abortion						
Home	3(50.0)	3(50.0)	1.228(0.239, 6.319)	0.806		
Gov't H/C	21(51.2)	20(48.8)	1.289(0.637, 2.610)	0.480		
ARTH	57(44.9)	70(55.1)	1			
Parity						
Primiparous	44(49.4)	45(50.6)	1			
Multiparous	37(43.5)	48(56.5)	0.788(0.434, 1.432)	0.435		
DROM						
<18 hours	56(48.3)	60(51.7)	1			
≥18 hours	13(43.3)	17(56.7)	0.819(0.365, 1.839)	0.629		
Duration of labor						
<12 hours	29(50.9)	28(49.1)	1			
12-24 hours	31(43.7)	40(56.3)	0.748(0.372, 1.506)	0.417		
>24 hours	9(50.0)	9(50.0)	0.966(0.335, 2.786)	0.948		
History of chronic disease						
Yes	2	1	2.329(0.207, 26.172)	0.493		
No	79	92	1			
ANC follow-up						
Yes	68	13	1			
No	75	18	0.797(0.363, 1.747)	0.570		

Key: ES: Endocervical swab, BC: Blood culture, FGM: Female genital mutilation, SVD, Spontaneous vaginal delivery, C/S, Caesarean section, DROM: Duration of rupture of membrane, H/C: Health Center, ARTH: Asella Referral and Teaching Hospital, ANC: Antenatal care

DISCUSSION

Puerperal sepsis is among the leading cause of preventable maternal morbidity and mortality particularly in developing countries (13), but the availability of epidemiological data about causative pathogens and distribution of antimicrobial susceptibility patterns remains limited.

In this study, we found the overall positivity rate of bacterial pathogens causing puerperal sepsis to be 48.9% (95%CI 41.25-56.5). The proportion of bacterial isolates from blood culture in this study is 10.6%. This finding is comparable with the study from Dire Dhawa, Ethiopia 12.9% (7) and Tanzania 11.2% (31). However, it is higher than the studies from Zimbabwe 2% (25) and USA 3.2% (6). In contrast, it is lower than the studies from Bahir Dar, Ethiopia 33.7% (22), India 68.65% (34) and Sudan 72.9% (45).

The proportion of bacterial isolates from an endocervical swab in this study was 89.4%. This is in line with the study done in Nigeria with a proportion of 82.7% (30) and Tanzania 90.5% (46). However its higher than the studies from Tanzania 43.6% (31), India 52.6% (47), and Zimbabwe 68.2% (25). In contrast, this is in a lower proportion as compared to the study from Nigeria 99.2% (29). Proportion differences of this study compared with other studies might be due to differences in infection prevention practice, management of laboring mothers by clinicians, and availability of microbiology laboratory facility.

In this study the most frequently isolated Gram negative bacteria causing puerperal sepsis among the study participants were *E. coli* (54.1%) followed by *Klebsiella* species (23.5%). This is supported by previous studies where *E. coli* and *Klebsiella* species were the majority bacterial isolates from puerperal sepsis patients (25,31). However, our findings differ from other findings on the most frequently isolated bacteria causing puerperal sepsis being group A streptococcus, group B streptococcus, *Bacteroides* species, *Pseudomonas aeruginosa, Proteus,* and *Enterococcus* species, (6,34,48,49).

E. coli is among the commonest bacterial pathogens causing bloodstream infections, wounds, otitis media, and other complications in humans(50). *E.coli* and *Klebsiella* species are normal flora of the gastrointestinal tract and recently, has emerged as a significant cause of hospital-acquired infections (urinary tract infection, pneumonia, and septicemia) especially among immune-compromised individuals (51).

From the Gram's positive isolates, the most frequently isolated bacteria were *S. aureus* (10.6%). This is comparable with other studies where *S. aureus* isolates were the common pathogenic bacteria isolated from puerperal sepsis patients (30,52). *S. aureus* is frequently found on the skin that can easily contaminate during vaginal delivery or by cesarian section (53).

E. coli, the most common Gram-negative isolates, showed higher sensitivity to Amikacin (89.1%), Gentamycin (80.4%), and Cefoxitin (78.3%), and the least sensitivity to Piperacillin(13%) and Trimethoprim sulfamethoxazole (34.8%). This is comparable with a study conducted in the USA where 90.5% and19% of *E. coli* were sensitive to gentamicin and ampicillin respectively (6). Penicillin resistance could be due to negative selective pressure exerted by the overuse of antibiotics.

Apart from that, it is also known that *E. coli* has resistant genes for beta-lactam agents including piperacillin (54). *Klebsiella* species which is the second common isolate showed 80% sensitivity to Amikacin, while they were 65% resistant to both Aztreonam and Ceftriaxone. The finding agreed with a study done in Bahir Dar, Ethiopia where 57.1% of *Klebsiella* species were resistant to ceftriaxone (22).

All (100%) *S. aureus* isolates were sensitive to Ceftriaxone and Cefoxitin but, showed higher resistance to Trimethoprim-sulfamethoxazole (66.6%). Another similar study reported *S. aureus* were 100% sensitive to ceftriaxone, ceftazidime, ciprofloxacin, and ofloxacin (29). Different from our study *S. aureus* was reported susceptible to clindamycin at 100% (31), while our study found susceptibility to clindamycin at 77.8%.

The overall proportion of MDR in this study was 81.2%. This is in line with the study done at Bahir Dar referral hospital, Northwest Ethiopia (84%) (22) and study from Uganda (80%) (55), while higher than a study reported from Zimbabwe,10.9% (25). A high rate of MDR was observed among *Klebsiella* species. *and E. coli.*

This might be due to enzymatic degradation of antibacterial drugs, alteration of bacterial proteins that are antimicrobial targets, and changes in membrane permeability to antibiotics. ESBL producing Enterobacteriaceae has intrinsic resistance mechanisms, most importantly, they have chromosomal and plasmid-encoded beta-lactam hydrolyzing enzymes (56).

In the current study, multivariate regression analysis did not show a statistically significant association between socio-demographic and obstetrics factors compared with having bacterial pathogen. This could probably be due to the small sample size in our study and a cross-sectional study design implemented.

However, women with ages greater than 34 years 12(70.6%) demonstrated a higher proportion of culture-positive bacterial infection. Similarly, another study reported that the majority of women admitted with puerperal sepsis were above thirty years of age 65.11% (57). This could probably be due to decreasing in immunity as age increases and having multiple deliveries.

Women educated above secondary school have a lower positivity rate (27.3%). This is comparable with another study from Bahir Dar, Ethiopia (22). This is probably as the educational status of women increases their health-seeking behavior and their standard of living increases.

LIMITATION OF STUDY

Our study has some limitations. This study focused on women attending the study hospital and suspected of puerperal sepsis and hence may not represent the community. The study also had a limitation, since most of the samples were collected after the patients have started antibiotic treatment that may decrease the bacterial isolation rate.

CONCLUSION

In conclusion, the overall bacterial positivity rate in this study was almost half. Gram negative bacteria, *E. coli,* and *Klebsiella* species were the most common bacterial pathogens isolated from women suspected of puerperal sepsis, while *S. aureus* was dominant among Gram positive bacteria.

Gram negative isolates showed a high rate of resistance for Piperacillin, Trimethoprim-sulfamethoxazole, and Aztreonam, while a lower resistance rate was observed for Amikacin and Meropenem. Gram positive isolates showed a high rate of resistance for Trimethoprim-sulfamethoxazole and Erythromycin, while a lower resistance rate was observed for Ceftriaxone, Cefoxitin, and Piperacillin.

High numbers of multidrug-resistant bacterial isolates were identified in this study. There was no statistically significant association between socio-demographic and obstetric variables as compared to having bacterial pathogens. But this study found that as the age of women increases the chance of having bacterial infection increases, while as the educational status of the women increases the chance of getting bacterial infection decreases.

RECOMMENDATIONS

The study found that the proportion of bacterial isolation causing puerperal sepsis and the level of multidrug resistance is high and thus, based on the result, the following recommendations are forwarded:

1) We recommend if health institutions to follow strict aseptic procedures and implement infection control practice during delivery.

2) We recommend health institutions to establish and strengthen microbiology laboratories for rapid diagnosis of sepsis and antimicrobial resistance and thereby proper management of septic patient's drugs.

3) We recommend health institutions to establish an antibiotic stewardship committee.

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ANNEXES

Annex I: Participants Information Sheet

My name is **AbduselamAbbiso**; I am a graduating class Medical Microbiology student at Jimma University. I want to collect information data and 10-20 ml of blood samples and endocervical swab samples for the study being conducted at your hospital. I kindly ask you to lend me your attention to explain to you about the study.

Study title: "Puerperal Sepsis: Bacterial Profile, Antimicrobial Susceptibility Pattern and Associated Factors at Asella Referral and Teaching Hospital, Central Ethiopia".

Purpose: I have planned to conduct a study to investigate bacterial profile, their antimicrobial susceptibility pattern, and associated factors among women suffering from puerperal sepsis at Asella Referral and Teaching Hospital. The knowledge gained from this work is believed to help program to reduce the morbidity and mortality of women associated with puerperal sepsis

Procedure: For this study a structured questionnaire was used to interview postpartum women to collect their socio-demographic data. Medical data was collected from patient card using checklist. From each patient 10-20 ml of blood samples was collected and transferred to two blood culture bottles. Endocervical swab samples was also collected in Amies transport medium. Willingness of study participates were requested to give their genuine response and sample to the data and sample collectors during interview.

Risk and benefits: The risk of being participated in the study is very minimal because every procedure will be aseptic by following the standardized medical procedure. But the findings from this research may reveal important information regarding puerperal sepsis on behalf of your population.

Confidentiality: The information collected in the patient chart and questionnaire will be confidential. There will be no information that will identify patients, to do so; each mother's information will have a code. The findings of the study will be general for the study population and will not reflect anything particular of individual person or patient.

Rights: This study will be done if you are voluntary to be enrolled in the study. You have the right to terminate your participation at any point that will not convene you.

Contact address: If there are any questions or enquires at any time about the study or the procedures, please contact **Abduselam Abbiso** Mob +251-924-098-238 or with email address abdabiso2012@gmail.com

Annex II: የመረጃና የስምምነት ዉል ቅጽ (Amharic version)

ስሜ **አብዱሰላም አቢሶ** ነው ፤ እኔ በጅማ ዩኒቨርሲቲ የምረቃ ክፍል የሕክምና ማይክሮባዮሎጂ ተማሪ ነኝ። በሆስፒታልዎ ውስጥ ለሚደረገው ጥናት የመረጃ እና ከ10-20 ሚሊ ሊትር የደም ናሙናዎች እና የማህጸን ጫፍ ናሙናዎችን መሰብሰብ እፈልጋለሁ። ስለ ጥናቱ ለማብራራት ትኩረትዎን እንዲሰጡኝ በትህትና እጠይቃለሁ።

የጥናቱ ርእስ- “በማዕከላዊ ኢትዮጵያ በአሰላ ሪፈራል እና ትምህርት ሆስፒታል ዉስጥ እናቶች ከወለዱ በኋላ ምን ያህሎቹ በባክተሪያ አንደተጠቁ እና ባክተሪያዉን ለመግደል የሚወለዉ መድሃኒት ከባክተሪያዉ ጋር መላመዱን ወይም አለመላመዱን መለየት እናተኳዳኝ ምክንያቶች” ነው::

የጥናቱ ዓላማ- የዚህ ጥናት አላማ በዋናነት እናቶች ከወለዱ በኋላ ምን ያህሎቹ በባክተሪያ አንደተጠቁ እና ባክተሪያዉን ለመግደል የሚወለዉ መድሃኒት ከባክተሪያዉ ጋር መላመዱን ወይም አለመላመዱን ለመለየት ነው:: ከጥናቱ የሚገኘውን ውጤት ለጤና ተቋም አስተዳዳሪዎች፣ ለሚመለከታቸው አካላት እና ለጥናቱ ተሳታፊዎች በማሳዎቅና በዚህ ጀርም ምክንያት የሚመጣዉን ችግር ለመቆጣጠር እና ለመከላከል የሚያስችሉ መሠረታዊ መረጃዎችን በመጠቆም ችግሩ እንዲቀረፍ ይረዳል::

የአሠራር ሂደት-ለዚህ ጥናት የተዋቀረ መጠይቅ ከድኅረ ወሊድ በኋላ ለሚገኙ ሴቶች ማኅበራዊ ሥነሕዝባዊ መረጃዎቻቸውን ለመጠየቅ ቃለ መጠይቅ ተደርጓል። የማረጋገጫ ዝርዝርን በመጠቀም የሕክምና መረጃ ከታካሚ ካርድ ተሰብስቧል። ከእያንዳንዱ በሽተኛ 10-20 ሚሊ ሊትር የደም ናሙና ተሰብስቦ ወደ ሁለት የደም ጠርሙሶች ተላልፈል። በአሚስ የትራንስፖርት ሚዲያዎች ውስጥ የማህጸን ጫፍ ናሙናዎችንም ተሰብስበዋል። የጥናት ተሳታፊዎች ፈቃደኛነት በቃለ መጠይቁ ወቅት እውነተኛውን ምላሽ እና ናሙና ለመረጃ እና ለናሙና ሰብሳቢዎች እንዲሰጡ ተጠይቀዋል።

አደጋ እና ጥቅሞች - በጥናቱ ውስጥ የመሳተፍ አደጋ በጣም አናሳ ነው ፣ ምክንያቱም እያንዳንዱ አሰራር ደረጃውን የጠበቀ የሕክምና ሂደትን በመከተል ይሆናል። ነገር ግን ከዚህ ምርምር የተገኙ ግኝቶች በሕዝብዎ ስም እናቶች ከወለዱ በኋላ ምን ያህሎቹ በባክተሪያ አንደተጠቁ እና ባክተሪያዉን ለመግደል የሚወለዉ መድሃኒት ከባክተሪያዉ ጋር መላመዱን ወይም አለመላመዱን መለየት እናተኳዳኝ ምክንያቶችን በተመለከተ ጠቃሚ መረጃን ሊያሳይ ይችላል።

ምስጢራዊነት - በታካሚው ሰንጠረዥ እና መጠይቅ ውስጥ የተሰበሰበው መረጃ ምስጢራዊ ይሆናል። ይህንን ለማድረግ ታካሚዎችን የሚለይ መረጃ አይኖርም ፤ የእያንዳንዱ እናት መረጃ ኮድ ይኖረዋል። የጥናቱ ግኝቶች ለጥናቱ ህዝብ አጠቃላይ ይሆናሉ እናም የግለሰቡን ወይም የታካሚውን ማንኛውንም ነገር የሚያንፀባርቅ አይሆንም።

መብቶች - በጥናቱ ውስጥ ለመመዝገብ ፈቃደኛ ከሆኑ ይህ ጥናት ይደረጋል። እርስዎን ምቾት አለመስጠት ከተሰማዎት በማንኛውም ጊዜ ተሳትፎዎን የማቋረጥ መብት አለዎት።

አድራሻ-ስለ ጥናቱ ወይም የአሠራር ሂደቶች በማንኛውም ጊዜ ጥያቄዎች ካሉ እባክዎን ለአብዱሰላም አቢሶ በእጅ ስልክ ቁጥር +251-924-098-238 እባክዎን ይደውሉ ወይም በኢሜል አድራሻ abdabiso2012@gmail.com ያግኙ

Annex III: Informed Consent Form (English version)

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information to enable the participant to make an informed decision on whether to participate or not.

Name: _____ Study No _____

Date: _____ signature: _____

Statement of person giving consent:

I have read the information on the study **"Puerperal Sepsis: Bacterial Profile, Antimicrobial Susceptibility Pattern and Associated Factors at Asella Referral and Teaching Hospital, Central Ethiopia"** or I have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction and I understand that my participation is voluntary (optional). For this study I explicitly allow the investigators delegate to obtain one pair or 10-20ml of blood sample for the investigation. Additionally, I also understand that no monitory benefits will arise by participating in this study except getting a result for my treatment option.

Name_____ Date _____ Signature _____

Witnesses Name _____ Signature _____

Annex IV: መረጃ ያለው የውል ቅጽ(Amharic version)

በመረጃ የተደገፈ ስምምነት የሚያገኝ ሰው መግለጫ;

ይህንን ምርምር ለ _____ ሙሉ በሙሉ አብራርቻለሁ እና ተሳታፊው ለመሳተፍ ወይም ላለመሳተፍ በመረጃ ላይ ውሳኔ እንዲሰጥ ለማስቻል በቂ መረጃ ሰጥቻለሁ።

ስም: _____ የጥናት ቁጥር _____

ቀን: _____ ፊርማ: _____

ፈቃድን የሚሰጥ ሰው መግለጫ;

በጥናቱ ላይ ያለውን መረጃ "በማዕከላዊ ኢትዮጵያ በአሰላ ሪፈራል እና ትምህርት ሆስፒታል ዉሰጥ እናቶች ከወለዱ በኋላ ምን ያህሎቹ በባክተሪያ አንደተጠቁ እና ባክተሪያዉን ለመግደል የሚወለዉ መድሃኒት ከባክተሪያዉ ጋር መላመዱን ወይም አለመላመዱን መለየት እናተጓዳኝ ምክንያቶች" ወይም እኔ ወደ ተረዳሁት ቋንቋ እንዲተረጎም አድርጌዋለሁ። እኔም ከቃለ መጠይቅ አድራጊው ጋር እስከ እርካታዬ ድረስ ተነጋግሬያለሁ እናም የእኔ ተሳትፎ በፈቃደኝነት (አማራጭ) መሆኑን ተረድቻለሁ። ለዚህ ጥናት መርማሪዎቹ ውክልና ለምርመራው አንድ ጥንድ ወይም 10-20ml የደም ናሙና እና የማህጸን ጫፍ ናሙናዎችን እንዲያገኙ በግልፅ እፈቅዳለሁ። በተጨማሪም ፣ እኔ በሕክምና ምርጫዬ ውጤት ከማግኘት በስተቀር በዚህ ጥናት ውስጥ በመሳተፍ ምንም የክትትል ጥቅሞች እንደማይኑ እረዳለሁ።

ስም: _____ የጥናት ቁጥር _____

ቀን: _____ ፊርማ: _____

Annex V: Data Collection Format (English Version)

Part one: Socio-demographic status

1. Hospital Card Number _____
2. Age in year? _____
3. Residence place? _____
4. Residence type? □ Rural □ Urban
5. Maternal educational level? □ Illiterate □ Read and Write □ Primary (1-8) _____ □ High school (9-12) ____ □ Higher education (>12) _____
6. Husband educational level? □ Illiterate □ Read and Write □ Primary (1-8) _____ □ High school (9-12) ____ □ Higher education (>12) _____
7. Marital status? □ Single □ Married □ Divorced □ Widowed
8. Maternal occupation? □ Farmer □ Employed/government □ Private □Housewife □Unemployed □ Other specify_____
9. Husband occupation? □ Farmer □ Employed/government □ Private □ Unemployed □ Other specify _____

Part two. Clinical and obstetric variables

1. Did you have Pregnancy follow-up (ANC)? □ Yes □ No
2. If No to Q12, why? _____
3. If Yes to Q12, when did you start? _____
4. If Yes to Q12, how many times did you visit health facility? _____
5. Is this your first pregnancy? □ Yes □ No
6. If No to Q16, pregnancy number? _____
7. History of infection during current pregnancy? □ Yes □ No
8. If yes to Q18, infection told by physician (if applicable)? _____
9. If yes to Q18, name of antibiotics given(if applicable)? _____
10. History of chronic disease? □ None □ HIV □ SLE(lupous erythro,) □ CHF □ CLD □ DM □ Other, specify? _____
11. History of female genital mutulation(FGM)? □ Yes □ No
12. Place of delivery □ Home □ ATH □ AHMC □ Referred
13. If referred on Q23, name of health facility(HF) referred from _____

14. Hospital length of stay at HF referred from in hours/days? ___:___hr _____ days
15. Hospital length of stay at current HF in hours/days? ___:___hr _____ days
16. What is your major current complain? _____
17. When did the major complain start after delivery? _____
18. Date and time of delivery ___/ ____/ ____(DD/MM/YYY) time _____:_____
19. Date and time of study inclusion ___/ ____/ ____(DD/MM/YYY) time _____:_____
20. Duration of rapture of membrane? □<18 hr □>=18 hr
21. Duration of labor? □< 12hr □12-24 hr □>24 hr
22. Mode of delivery? □ Vaginal delivery □ Emergency C/S □ Elective C/S
23. Number of vaginal examination? _____
24. Vital signs?

    Pulse rate _____/min

    Resp. rate _____/min

    BP systolic/diastolic ____/____ mmHg

    Temperature _____^{O}C
25. Antibiotics given related to delivery?□ Yes □ No
26. If yes to Q22, list of the antibiotics? _____
27. Clinical samples collected for culture □ Blood □ Wound swab □ Other _____

Annex VI: መጠየቂያ (Amharic version)

1. የሆስፒታልካርድቁጥር _____

2. ዕድሜ _____

3. መኖሪያዎ? □ገጠር□ከተማ

4. ከሆስፒታልወይምከጤናማእከል _____ ኪ.ሜርቀትያለውየቤትርቀት

5. የትምህርትደረጃዎ? □ማንበብናመጻፍ□የመጀመሪያደረጃ (1-8) ___ □ሁለተኛደረጃትምህርት (9-12) ___ □ከፍተኛትምህርት (> 12) _____

6. የጋብቻሁኔታዎ? □ነጠላ□ያገባች□ፍቺ□የትዳርጓደኛ

7. የቤተሰብወርሃዊገቢ? _____ ብር

8. የእናቶችሥራ□ተቀጣሪ / የመንግሥት□የቤትእመቤት□ሌላይግለጹ_____

9. ባልየሥራስምሪት□ተቀጣሪ / የመንግስት□ነጋዴ□የሥራአጥነት□ገበሬ□ሌላይግለጹ _____

ክፍልሁለት. ተጓዳኝምክንያቶች

1. የሚላክበትቀንእናሰዓት __ / ___ / ___ (DD / MM / YYY) ሰዓት ____ ____

2. የጥናትቀንእናሰዓት __ / ___ / ___ (DD / MM / YYY) ሰዓት ____ ____

3. የእርግዝናከትትልአለዎት (ኤኤንሲ) □አዎ□የለም

4. ቁጥር-3 የለምከሆነለምን? _____

5. ቁጥር-3 አዎከሆነመቼነውየጀመሩት? _____

6. መልስዎአዎከሆነ ለ ቁጥር-3ስንትጊዜየጤናተቋማትንየጎበኙት_____

7. ይህየመጀመሪያእርጉዝዎነው□አዎ□የለም

8. ቁጥር-7የለምከሆነ ፣ የእርግዝናቁጥር _____

9. የመላኪያቦታ□ቤት□ATH □ AHMC □ተጠቀሰ

10. ቁጥር-9 ከተጠቀሰው ከ _____ የተጠቀሰውየጤናተቋም

11. የሆስፒታልቆይታየበፊት_____

12. አሁንባለውየሆስፒታልቆይታ _____

13. የእብጠትመነሳትቆይታ□<18 ሰዓት□>= 18ሰዓት

14. የጉልበትጊዜ□<12hr □12-24 ሰዓት□> 24 ሰዓት

15. የመላኪያሁኔታ□የእርግዝናአቅርቦት□ድንገተኛ C / S □መራጭ C / S

16. የሴትብልትምርመራቁጥር _____

17. በእርግዝናወቅትበግብረሥጋግንኙነትየሚተላለፉበሽታዎችታሪክ□አዎ□የለም

18. የሴትልጅግርዛትታሪክአላት?□አዎ□የለም

19. ዋናውየአሁኑቅሬታዎምንድነው? _____

20. ከወለዱበኋላዋናቅሬታያሰማዎትመቼነበር?_____

21. ወሳኝምልክቶች

የምስልፍጥነት _____ / ደቂቃ

ቅናሽተመን _____ / ደቂቃ

BPsystolic / diastolic ___ / ___ mmHg

ሙቀት _____ ^{O}C

22. ከመውለድዎጋርበተያያዘማንኛውንምአንቲባዮቲክወስደዋል? □አዎ□የለም

23. ቁጥር-21 መልስዎአዎከሆነ ፣ አንቲባዮቲክስዝርዝር _____

24. የተሰበሰቡክሊኒካዊናሙናዎች□ደም□ቁስለት□ሌላ _____

Annex VII: Laboratory Request Form

Name_____ Card No _____

Sex: ☐Male ☐Female Age _____

Specimen: ☐Blood ☐Swab ☐Pus ☐Other Specimen _____

Clinical Diagnosis_____

Requesting Physician _____Phone no._____

Date of collection _____Time of Collection _____

REQUEST: ☐ Culture and susceptibility test

Blood culture BacT/ALERT 3D: Aerobic (FA) ☐Negative ☐Positive at _____ day/s

Blood culture BacT/ALERT 3D: Anaerobic (FN) ☐Negative ☐Positive at _____ day/s

Endocervical swab (ES)

Gram stain _____

Identification and AST results:

Bacteria Isolates		Piperacillin(PCR)	Cefoxitin(FOX)	Ceftazidime(CAZ)	Ceftriaxone(CRO)	Meropenem(MEM)	Aztreonam (ATM)	Ciprofloxacin(CIP)	Amikacin(AK)	Gentamicin(GM)	Erythromycin(E)	Nitrofurantoin(NI)	Trimethoprim-Sulfamethoxazole(TS)	Clindamycin(CD)
FA														
FN														
ES														

Lab No_____ S= Sensitive I= Intermediate R= Resistance

Performed by _____ Sig. _____ Date_____

Approved by _____ Sig. _____ Date _____

Annex VIII: Procedures

Culture media preparation

1. Read the label on a bottle of dehydrated agar media. It specifies the amount of dehydrated powder required to make 1 litter (1,000 ml) of medium. Calculate the amount needed for 1/2 litter and weigh out this quantity.
2. Place 500 ml of distilled water in an Erlenmeyer flask. Add the weighed, dehydrated agar while stirring with a glass rod to prevent lumping.
3. Set the flask on a tripod over an asbestos mat.
4. When the agar mixture is completely dissolved, remove the flask from the flame or hot plate, close it with the cotton plug or cap, and it must be sterilized in the autoclave.
5. When the flask of sterilized agar is returned to you, allow it to cool to about 50°C (the agar should be warm and melted, but not too hot to handle in its flask). Remove the plug or cap with the little finger of your right hand and continue to hold it until you are sure it won't have to be returned to the flask. Quickly pour the melted, sterile agar into a series of Petri dishes. The Petri dish tops are lifted with the left hand and the bottoms are filled to about one-third capacity with melted agar.
6. Replace each Petri dish top as the plate is poured. When the plates are cool (agar solidified), invert them to prevent condensing moisture from accumulating on the agar surfaces.
7. Place inverted agar plates in the 35°C incubator. 5% of the batch should be incubated for at least 24 hours to ensure they are sterile (free of contaminating bacteria) before you use.

Gram stain procedure

- Prepare a thin smear of the culture and allow to air-dry
- Fix the dried smear
- Cover the fixed smear with crystal violet stain for 30–60 seconds.
- Rapidly wash off the stain with clean water.

- Tip off all the water and cover the smear with Lugol's iodine for 30–60 seconds.
- Wash off the iodine with clean water.
- Decolorize rapidly (few seconds) with acetone–alcohol. Wash immediately with clean water.
- Cover the smear with Safranin for 30-60 seconds minutes.
- Wash off the stain with clean water.
- Wipe the back of the slide clean and place it in a draining rack for the smear to air-dry.
- Examine the smear microscopically, first with the 40x objective to check the staining and to see the distribution of material, and then with the oil immersion objective to report the bacteria and cells

Results

Gram positive bacteria ……. Dark purple

Yeast cells ……. Dark purple

Gram negative bacteria ……. Pale to dark red

Nuclei of pus cells …… Red

Epithelial cells ……. Pale red

A. Biochemical testing procedure

1. For Gram Positive Bacteria

Catalase

Catalase test is used to identify organisms that produce the enzyme, catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas. The *Staphylococcus* spp. and the *Micrococcus* spp. are catalase positive. The *Streptococcus* and *Enterococcus spp. are* catalase negative.

Procedure

- Pour 2-3 ml of the hydrogen peroxide solution into a test tube.
- Using a sterile wooden stick or a glass rod, remove several colonies of the test organism and immerse in the hydrogen peroxide solution.
- Look for immediate bubbling. Bubbles resulting from production of oxygen gas clearly indicate a catalase positive result.

Coagulase

Coagulase is an enzyme that clots blood plasma. This test differentiates *Staphylococcus aureus* from other coagulase negatives.

Procedure

- Place a drop of distilled water on each end of a slide or on two separate slides
- Emulsify a colony of the test organism (previously checked by Gram staining) in each of the drops to make two thick suspensions.
- Add a loopful (not more) of plasma to one of the suspensions and mix gently.
- Look for clumping of the organisms within 10 seconds.
- No plasma is added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping

2. **For Gram negative bacteria**
    - Prepare a suspension of the test organism with nutrient broth. 3-4 colony of test organism in 5 ml nutrient broth.
    - A loop full of the bacterial suspension is inoculated into indole, citrate agar, triple sugar iron agar, lysine decarboxylase agar, urea agar and motility medium.
    - Incubate at 35-37$_o$c for 18-24 hours.
    - Look for color change (turbidity for motility) of the medium.
    - Identify the test organism by considering the result of the six biochemical tests

B. Antimicrobial susceptibility testing procedure

1. Using a sterile wire loop, touch 3 – 5 well – isolated colonies of similar appearance to the test organism and emulsify in 3 – 4 ml to sterile physiological saline or nutrient broth.
2. In a good light match the turbidity of the suspension to the turbidity standard (mix the standard before use)
3. Using a sterile swab, inoculate a plate of Muller Hinton agar. Remove excess fluid by pressing and rotating the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions rotating the plate approximately 60^o to ensure even distribution.

4. With the Petri dish lid in place, allow 3 – 5 minutes for the surface of the agar to dry.
5. Using sterile forceps or multi disc dispenser, place the appropriate antimicrobial discs evenly distributed on the inoculated plate.

   -The discs should be about 15mm from the edge of the plate and no closer than 25mm from disc to disc. No more than 6 discs should be applied in 90mm dish.

   - Each disc should be lightly pressed down to ensure its contact with the agar. It should not be moved once in place.
6. Within 30 minutes of applying the discs, invert the plate and incubate it aerobically at 35°C for 16 – 18 hours.
7. After overnight incubation, examine the control and the test plates. Using a ruler measure the diameter of each zone of inhibition in mm on the underside of the plate. the end point of inhibition is where growth starts.

Interpretation of Zone Size

Using the interpretative chart, interpret the zones sizes of each antimicrobial and report the organisms as 'Resistant', Intermediate (moderately sensitive) or 'Sensitive' (susceptible). ***Resistant***: -A pathogen reported as 'resistant' implies that the infection it has caused will not respond to treatment with the drug to which it is resistant irrespective of dose or site of infection.

Intermediate:- A pathogen reported as intermediately sensitive suggests that the infection it has caused is likely to respond to treatment when the drug is used in larger doses than normal or when the drug is concentrated at the site of infection.

Sensitive (Susceptible): - A pathogen reported as sensitive suggests that the infection it has caused is likely to respond to treatment when the drug is used in normal recommended doses.

Annex IX: Ethical Approval

Ref.No: IRB0001553/2020
Date: 8/7/2012

Institute of Health
Jimma University
Tel : +251 917762109
E-mail: konetsanet@gmail.com

To: **Abduselam Abbiso**

Subject: **Ethical Approval of Research Protocol**

The IRB of Institute of Health has reviewed your research project titled,

"Puerperal sepsis: Bacterial Profile Antibiotics Resistance Pattern And Associated Factors at Selected Hospitals In Central Ethiopia."

Thus, this is to notify that this research protocol has presented to the IRB meets the ethical and scientific standards outlined in national and international guidelines. Hence, we are pleased to inform you that your research protocol is ethically cleared.

We strongly recommend that any significant deviation from the methodological details indicated in the approved protocol must be communicated to the IRB before it has been implemented.

With Regards!

Dr Netsanet Workneh

IRB Chairperson

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DECLARATION

The research work in this thesis entitled **"Puerperal Sepsis: Bacterial Profile, Antimicrobial Susceptibility Pattern and Associated Factors at Asella Referral and Teaching Hospital, Central Ethiopia"** was carried out by me under the supervision of **Prof. Getinet Beyene, Mr. Mulatu Gashaw, Mr. Kedir Abdella and Mr. Tafese Beyene** at Jimma University, Institute of Health, Faculty of Health Sciences, School of Medical Laboratory Sciences, for the award of MSc Degree in Medical Microbiology. I declare that this work is original and has not been submitted to any other University or institution

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Place: Jimma, Ethiopia Date. _____ Signature _____

Name of the advisors:

Prof. Getinet Beyene Date. _____ Signature _____

Mr. Mulatu Gashaw Date. _____ Signature _____

Mr. Kedir Abdella Date. _____ Signature _____

Mr. Tafese Beyene Date. _____ Signature _____

Name of the examiners:

Dr. Kassu Desta Date. _____ Signature _____

Mr. Lule Teshager Date. _____ Signature _____