



St. Paul's Hospital Millennium Medical College School of Medicine

Department of Microbiology, Immunology, and Parasitology

Magnitude, Bacteriological Profile, and Antimicrobial Susceptibility Pattern of Neonatal Sepsis in Neonates Admitted to NICU of Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia.

Investigator: Mulugeta Girma

November, 2025



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Declaration

I, the undersigned, declare that the thesis entitled “Magnitude, Bacteriological Profile and Antimicrobial Susceptibility Patterns of Neonatal Sepsis in the NICU of Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia.” submitted for the degree of Master of Science (MSc) to St. Paul’s Hospital Millennium Medical College is my own work and has not previously been submitted at another university. The materials obtained from other sources have been duly acknowledged and cited their sources.

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Abstract

Background:

Neonatal sepsis is a systemic infection caused by bacterial, viral, or fungal pathogens, characterized by hemodynamic instability and clinical signs in infants younger than 28 days of age. It remains a significant cause of morbidity and mortality among infants, particularly in middle- and lower-income countries. Neonatal sepsis can be classified into early-onset sepsis (which occurs within the first 0 to 3 days after birth) and late-onset sepsis (which arises after the first three days of life). Effective treatment for neonatal sepsis, therefore, requires identifying pathogens, including both gram-positive and gram-negative bacteria, and understanding the patterns of antimicrobial resistance associated with these organisms.

Objective: The overall objective of this study is to determine the magnitude, bacteriological profile, and antimicrobial susceptibility patterns of neonatal sepsis, and to assess clinical outcomes and associated factors among neonates admitted to the NICU of Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia.

Methods: An institution-based cross-sectional study was conducted among 251 neonates admitted to the NICU between June and December 2024 using a consecutive sampling technique. Blood samples (1-2 ml) were collected for bacterial culture and antimicrobial susceptibility testing. Data were analyzed using descriptive statistics, bivariate, and multivariable analyses to identify significant associations. Associations were reported with odds ratios and 95% confidence intervals; $p < 0.05$ was considered statistically significant.

Result: A total of 251 neonates clinically suspected of sepsis were included in the study, with a mean age of 5.43 (SD $\pm$ 6.41) days, ranging from 1 to 28 days. Of these, 59% were due to early-onset sepsis and 41% to late-onset sepsis. The overall culture confirmed neonatal sepsis was 114(45.4%) (95% CI: 39.4, 51.4), with 59.6% by Gram-negative bacteria and 40.4% caused by Gram-positive. Gram-negative bacteria showed high susceptibility to Piperacillin-Tazobactam (100%), Amikacin (90.8%), Meropenem (80%), and Imipenem (78.5%), but high resistance to Ceftriaxone (62%) and Trimethoprim-Sulfamethoxazole (48.2%). Notably, Gram-positive bacteria were highly resistant to Oxacillin (76%) and Penicillin (77%). Regarding clinical outcomes, 78.1% neonates were discharged home while the overall mortality rate was 13.5% (34/251; 95% CI: 9.7–

17.9). The majority of deaths (79.4%) occurred in early-onset cases. Among the neonates who died, 18 (52.9%) had culture-confirmed sepsis; of these, 88.9% were caused by Gram-negative bacteria, and 33.3% were attributable to *Klebsiella* spp. Late neonatal age (AOR 2.19) (95% CI: 1.25, 3.82), neonates experiencing respiratory distress (AOR 3.44) (95% CI: 1.19, 9.93), and co-symptoms (AOR 5.22) (95% CI: 1.69, 16.10) were significantly associated with higher neonatal sepsis.

Conclusion:

The study revealed a high prevalence of neonatal sepsis, with Gram-negative bacteria being the dominant etiology and demonstrating alarming resistance to first-line antibiotics. A considerable mortality rate was observed, particularly among cases of early-onset sepsis and Gram-negative infections. Therefore, urgent revision of current empirical antimicrobial guidelines and continuous, active surveillance are critical to improve clinical outcomes and reduce neonatal mortality.

Keywords: neonatal sepsis, antimicrobial susceptibility, bacterial profile

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Abbreviations

APH: Antepartum Hemorrhage

AMR: Antimicrobial resistance

ANC: Antenatal care

APGAR: Activity, pulse, Grimace, appearance, and respiration

BA: Birth asphyxia

EDHS: Ethiopia Demographic and Health Survey

EONS: Early onset neonatal sepsis

IPC: Infection prevention committee

LBW: Low birth weight

LONS: Late-onset neonatal sepsis

MCH: Maternal to child health

MDG: Millennium Development Goal

MSAF: Meconium-stained amniotic fluid

NICU: Neonatal intensive care unit

NMR: Neonatal mortality rate

PROM: Premature Rupture of Membranes

UTI: Urinary Tract Infection

1. Introduction

1.1 Background

Neonatal sepsis is a systemic infection caused by bacterial, viral, or fungal pathogens, characterized by hemodynamic instability and clinical signs in infants younger than 28 days of age(1). It remains a leading cause of morbidity and mortality among neonates and encompasses systemic infections, including meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infections (2,3). It is a life-threatening condition that can be triggered by microorganisms entering the bloodstream, mainly bacteria. Neonatal sepsis is classified into early-onset sepsis (EOS) and late-onset sepsis (LOS). EOS refers to sepsis that occurs within the first 3 days of birth, while LOS occurs after 3 days of birth (4). Early onset infection is usually due to vertical transmission by ascending contaminated amniotic fluid or during vaginal delivery from bacteria in the mother's genital tract (5,6). Whereas late-onset sepsis is caused by bacteria acquired from the healthcare environment, community, and vertical transmission after initial neonatal colonization that evolves into a later infection (7,8).

Neonatal sepsis is responsible for approximately 25% of the neonatal deaths in the world (9), and 30–50% of these occur in developing countries (10). It is the third most common cause of death among neonates, accounting for 225,000 deaths globally every year (11,12). Globally, it was reported that every year, about 4 million children die in the first 4 weeks of life, of which 99% of the deaths occur in low- and middle-income countries (13). In Ethiopia, approximately 42% of under-five mortality is attributable to neonatal deaths (14).

It has been reported that various gram-positive and gram-negative bacteria cause neonatal sepsis (15). The knowledge of the bacterial etiology and its antibiotic sensitivity patterns is of immense help in saving the lives of neonates with septicemia (16). Antibiotics are the standard therapy for high-risk patients with neonatal sepsis, including preterm infants and those who have early rupture and low body weight. However, the diversity of organisms causing sepsis changes over time, which poses a challenge to antibiotic selection for empirical treatment (17,18). Additionally, prolonged antibiotic exposure leads to a poor prognosis in neonates and to antimicrobial resistance (19). However, data on neonatal sepsis in Ethiopia remain fragmented. Hence, the objective of this study was to assess the magnitude, bacteriological profile, and antimicrobial susceptibility patterns

of neonatal sepsis, and to assess clinical outcomes and associated factors among neonates admitted to the NICU of Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia.

1.2 Statement of the problem

The distribution of microorganisms causing neonatal sepsis changes over time and varies from region to region (20,21). The World Health Organization (WHO) reported that about 6.5–38/1000 live births in the hospital developed neonatal sepsis globally in 2017 (22). In high- and middle-income countries, it has been estimated that neonatal sepsis occurs in 2,200 neonates per 100,000 live births, equating to 3 million cases of neonatal sepsis annually, with a mortality rate of 11% to 19% (23). The incidence of neonatal sepsis in middle-income countries has been reported to be up to 40 times higher than in high-income countries (23). A systematic review spanning four decades (1979–2019) and covering nearly 2.8 million live births from 14 countries reports neonatal sepsis as an urgent global health challenge, with a staggering 99% of sepsis-related mortality occurring in low- and middle-income countries (LMICs) (24). Another study by the Developing Societies reported the incidence rates of 166.0 per 1000 livebirths for clinically suspected sepsis, 46.9 per 1000 livebirths for laboratory-confirmed sepsis, and 0.83 per 1000 neonate days for all-cause mortality (25). Although sepsis is a common and frequently fatal condition affecting neonates, there are fragmented studies done in Ethiopia to estimate the magnitude of neonatal sepsis and associated factors. Evidence from a 2019 systematic review highlights a significant burden of disease, reporting a pooled sepsis prevalence of 45%, with individual studies showing a range between 17% and 78% (26). Another study from Ethiopia also reported that the prevalence of neonatal sepsis ranges from 78.6% in Shashemene, Oromia region (27) to 23.8% in Bahir Dar (28).

The previous study identified several risk factors that contribute to neonatal sepsis, which include prolonged rupture of the membrane (29), the mode of delivery (30), preterm birth, lower gestational age, urinary tract infection, and high intrapartum fever (31,32). Additionally, newborns with a birth weight of less than 2.5 kg (31) and maternal age between 30 and 34 years (33) They are found to have a higher association with neonatal sepsis.

Antibiotic therapy is the primary choice to prevent neonatal sepsis-related mortality. However, antibiotic prescribing practices in neonatal and pediatric sepsis have been shown to significantly diverge from the WHO recommendations in LMICs such as Ethiopia (34). For that matter, the

emergence of Antimicrobial resistance (AMR) in bacteria imposes further challenges in their treatment (35,36). For instance, A review conducted in Ethiopia from 2016 to 2020 showed high antimicrobial resistance in *Klebsiella pneumoniae* and *Staphylococcus aureus*, with gram-negative organisms exhibiting >50% resistance to third-generation cephalosporins, fluoroquinolones, and sulfamethoxazole-trimethoprim. (37). However, although there are sporadic studies in some regions of Ethiopia, data are still lacking in most parts of the country, including the current study setting. Therefore, the current study will address the gap by assessing the magnitude, bacteriological profile, and antimicrobial susceptibility patterns of neonatal sepsis, and assessing clinical outcomes and associated factors among neonates admitted to the NICU of Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia.

1.3 Rationale and Significance of the Study

The overall prevalence of neonatal sepsis was high in sub-Saharan Africa in 2022. Therefore, neonatal sepsis and antimicrobial drug susceptibility need urgent attention and proper interventions. In many settings, management mainly depends on clinical diagnosis without routine blood culture and antimicrobial susceptibility testing, which can lead to inappropriate antibiotic use and poor treatment results. Additionally, no previous study has documented the scope of neonatal sepsis or the bacterial profile in this study setting

In Ethiopia, data on neonatal sepsis and antimicrobial susceptibility patterns remain limited, and no evidence is available for the study area. Consequently, this study aims to address this gap by determining the magnitude, bacteriological profile, and antimicrobial susceptibility patterns of neonatal sepsis and assessing clinical outcomes and associated factors.

The findings of this study will provide baseline data to inform future research and support the development of targeted interventions. In addition, the results will guide clinicians in selecting appropriate first- and second-line antibiotics and assist hospital infection-prevention and antimicrobial-resistance committees in designing evidence-based strategies. Ultimately, this study contributes to improved medication practices, enhanced neonatal care, and the reduction of neonatal morbidity and mortality, while supporting national and global efforts to combat antimicrobial resistance in resource-limited settings.

2. Literature review

2.1 Global overview of the magnitude of neonatal sepsis

Neonatal sepsis remains one of the most critical challenges in global neonatal health, contributing significantly to morbidity and mortality. Nearly 50% of deaths in children younger than 5 years occurred in neonates (aged < 28 days), and the share continues to rise. Globally, the incidence of neonatal sepsis increased by 14.33% from 5.18 million in 1990 to 5.92 million in 2019, and the incidence varies from one country to another (38). For instance, the study conducted in Nepal reported that 16.9% cases showed a positive blood culture in suspected cases of neonatal sepsis (15). The other Nepal study finding, specifically at Kanti Children's Hospital in Kathmandu, indicated that among neonates admitted to the NICU, 20.5% had culture-positive sepsis. The majority were early-onset sepsis, 78.3% (15,18). In addition, in the other South Asian study, which was done in Bahawal Victoria Hospital, Bahawalpur, Pakistan, from patients admitted with sepsis, 8.9% had bacteriologic culture-confirmed neonatal sepsis (39).

In Yangon, Myanmar, among those who had blood culture tests in two tertiary care hospitals for all clinically suspected neonatal septicemia cases, 42% were bacteriologically confirmed. Neonates with late-onset sepsis (LOS) were more likely to have bacteriologically confirmed sepsis (45%) compared to early-onset sepsis (38%)(40). Another study done in northern India showed that out of suspected cases of neonatal sepsis (15.3%), cases showed a positive blood culture. Of these, the majority (69.8%) had late-onset neonatal sepsis (41). A similar but separate study in India found that, specifically in Vadodara, Gujarat, 32.89% were positive blood cultures. According to this study, early bacteremia was detected in 64% of the patients, and late bacteremia was detected in 36% of the patients (42).

A prospective cross-sectional study conducted in the Neonatal Unit of the National Hospital in Bhutan, South Asia, found that the incidence of culture-positive neonatal sepsis was 19 per 1000 admissions, with a blood culture positivity rate of 14%, and 54.5% had culture-positive early-onset sepsis (EOS) (1). In Shiraz, Iran, out of 90 neonates diagnosed with sepsis, 50 (55.5%) had early-onset sepsis(43).

In a retrospective analytical study done in the NICU of Lagos State University Teaching Hospital (LASUTH), Lagos, Nigeria, among neonates, 32% were blood culture-positive. According to this report, a total of 78.6% infected neonates had early-onset sepsis (44). A similar but separate retrospective cohort study in Muhimbili National Hospital, Tanzania, found that in total, 45.4% of mortality resulted from 238 neonates who had sepsis, exclusively based on blood culture positivity (45). Two cross-sectional studies in East Africa examined neonatal sepsis and its etiology. In Dar es Salaam, Tanzania, a study aimed to identify the causes of neonatal sepsis and patterns of antimicrobial resistance, revealed that approximately 72% of samples tested positive for bacterial cultures(46). In Uganda, a cross-sectional study at the pediatric emergency care unit of Mulago National Referral Hospital focused on neonates with signs and symptoms of sepsis. Out of these, 12.8% to 16.7% had positive blood cultures, indicating the presence of sepsis(47).

2.2. Overview of neonatal sepsis in Ethiopia

In Ethiopia, studies conducted at different times in several areas also reported interesting findings. The cross-sectional study, which was conducted between March 2017 and September 2018, among 317 suspected septicemia-susceptible neonates at the Ayder Comprehensive Specialized Hospital, Mekelle, North Ethiopia, found that 116 (36.6%) had culture-proven septicemia (14).

A cross-sectional study conducted on 368 study participants in referral hospitals in East and West Gojam Zones in 2019 reported early-onset neonatal sepsis in 34% of the babies (48). The other study finding from Felege-Hiwot Referral Hospital showed that 41.3% were positive for blood culture (30). An institution-based cross-sectional study conducted in Woldia and Dessie Comprehensive Specialized Hospitals on 344 randomly selected neonates who visited the hospital found that the prevalence of neonatal sepsis was 79.4% (49). Another hospital-based cross-sectional prospective study conducted at Hawassa University Comprehensive Specialized Hospital (HUCSH) Neonatal Intensive Care Unit reported a prevalence of 36.5% sepsis among newborns (13).

A study at the Wachemo University Comprehensive Specialized Teaching Hospital in southern Ethiopia found that out of 205 neonates studied, there was an overall prevalence rate of neonatal sepsis of 39.5% (50). A separate study in NICUs of two governmental hospitals in Shashemene

town showed that out of 244 neonates, the prevalence of neonatal sepsis was 77.9%. Within this group, 65% developed early-onset neonatal sepsis, and 35% developed late-onset neonatal sepsis (51). Another study conducted at four hospitals in Northern Oromia, including 378 neonates, reported an overall prevalence of neonatal sepsis of 52.27%. Of these, 81.12% developed early-onset neonatal sepsis, and 18.88% developed late-onset neonatal sepsis (52). Lastly, a study conducted at St. Paul's Hospital Millennium Medical College, with 400 suspected cases of neonatal sepsis, found an overall prevalence of 21% (53).

2.3.Bacteriological profile of neonatal sepsis

Different groups of bacteria cause neonatal sepsis, and the etiology varies from one region to the other. Early-onset sepsis is most commonly caused by *Group B Streptococci*, *Escherichia coli*, *Coagulase-negative Staphylococci*, *Haemophilus influenzae*, and *Listeria monocytogenes*, while coagulase-negative *Staphylococci*, *Staphylococcus aureus*, *Klebsiella species*, *Pseudomonas species*, *Acinetobacter spp*, and anaerobes commonly cause LOS (54).

Studies conducted in Asian countries have shown varying prevalence of different bacterial species causing neonatal septicemia. A study in Nepal's tertiary hospital identified *Staphylococcus aureus* and *coagulase-negative Staphylococcus* as the most common isolates (54). Similarly, in North India, Gram-positive bacteria were found to be more prevalent (60%) compared to Gram-negative species (40%) (55). In other north India cases showed among positive blood culture, *Klebsiella pneumoniae* (37.5%) was the most predominant pathogen in both early-onset (23.1%) and late-onset (46.7%) sepsis (41). Additionally, a study performed in China highlighted *Staphylococcus epidermidis* (47.17%) and *Escherichia coli* (49.09 %) as prominent pathogens among Gram-positive and Gram-negative bacteria, respectively (56). In Pakistan, the most commonly identified bacteria were *Klebsiella spp* (36.5%), followed by *Escherichia coli* (28.5%) and *Staphylococcus aureus* (15.4%) (39).

In Africa, studies have revealed insights into the variation in the etiology of neonatal sepsis. In Lagos State University Teaching Hospital, Nigeria, Gram-positive bacteria, particularly *Staphylococcus aureus*, 44.6%, were predominant (44). Similarly, in Dar es Salam, Tanzania, Gram-positive bacteria were predominantly isolated, with *coagulase-negative Staphylococci* (81%) leading the isolates (46). In Muhimbili National Hospital, Tanzania, high neonatal mortality

was associated with Gram-negative bacteria (60%), with *Staphylococcus aureus* and *Klebsiella spp* being major contributors to neonatal deaths. *Staphylococcus aureus* contributed to the largest proportion (40%), while *Klebsiella spp*, *Escherichia coli*, *Pseudomonas*, *Acinetobacter*, and *Streptococcus* were responsible for 38%, 14%, 5%, 4%, and 2%, respectively (45). Studies from Uganda and Kenya examined bacterial isolates in neonatal sepsis. In Uganda, *Staphylococcus aureus* was the predominant bacterium, isolated in 29 out of 46 cases (63.0%), followed by *Escherichia coli* in seven cases (15.2%), and *Klebsiella pneumoniae* in five cases (10.9%). (47). In Kenya, among 151 bacterial isolates identified, *Klebsiella spp* were the most common, accounting for 46.4% of isolates. *Coagulase-negative Staphylococcus (CoNS)* was the second most frequent, representing 27.8% of isolates(57).

In Ethiopia, various studies conducted across different hospitals consistently identify Gram-negative bacteria as the predominant etiologic agents of neonatal sepsis, accounting for majority of the cases. In a study conducted at Ayder Comprehensive Specialized Hospital in Mekelle, Tigray, *Klebsiella spp* were identified as the primary causative agents (14). Similarly, Felege-Hiwot Referral Hospital study reported that *Klebsiella pneumoniae* was the prevalent isolate, comprising 28.2%, followed closely by *Staphylococcus aureus* at 24.7% (30). Hawassa University Comprehensive Specialized Hospital's study highlighted *Klebsiella spp* as the predominant bacteria at 42.65%, with non-lactose fermenting Gram-negative bacteria and *Enterococcus species* following at 18.88% and 18.18%, respectively (13). Similarly, a study at St. Paul's Hospital Millennium Medical College revealed that gram-negative bacteria accounted for 79.8%, with *Klebsiella spp* leading at 44%, followed by *Escherichia coli* at 21.6%, and *Coagulase-negative Staphylococci* at 15.47%. Overall, *Klebsiella spp* is a significant contributor to neonatal sepsis across multiple Ethiopian hospital settings (53).

2.4. Antimicrobial susceptibility pattern in neonatal sepsis

Antimicrobial resistance, as per the WHO, is described as the ability of a microorganism to withstand the effects of an antimicrobial drug up to the minimal inhibitory concentration (MIC), which signifies the endpoint of antimicrobial susceptibility testing on notable bacterial isolates (58). Susceptibility testing aims to predict the probable treatment outcome for a patient's infection with a specific antimicrobial drug that has previously been effective in combating infections caused by known microorganisms. The precision of this testing method is generally considered to be within a plus or minus one two-fold concentration range.

Studies done across the globe indicated an alarming increase in antimicrobial resistance (AMR). In a comprehensive analysis across Asian regions, studies conducted at Kanti Children's Hospital in Kathmandu, Nepal, and the Maternal and Child Health Hospital of Hubei Province, Tongji Medical College, China, shed light on antimicrobial sensitivities in neonatal care. At Kanti Children's Hospital, Gram-positive isolates exhibited notable sensitivity to gentamicin (93%), whereas ampicillin faced resistance at 78%. Among Gram-negative isolates, meropenem and imipenem showcased a remarkable 100% effectiveness, contrasting with the low sensitivity of cefotaxime at 28% (15). In the study conducted in Hubei Province, China, Gram-positive bacteria displayed higher susceptibility to vancomycin, tigecycline, and linezolid. *Escherichia coli* exhibited 100% susceptibility to piperacillin, cefotetan, meropenem, and imipenem (56).

Moreover, findings from a Tertiary Hospital in Kathmandu Valley, Nepal, indicated that a significant proportion of bacteria were resistant to at least one first-line antibiotic used for neonatal sepsis treatment (54). This study revealed that *Klebsiella spp* demonstrated high resistance to commonly used antibiotics, including Cefotaxime (90.5%), Gentamicin (75%), Ciprofloxacin (76.2%), Ofloxacin (72.2%), and Chloramphenicol (65%). However, the bacterium exhibited good susceptibility to Carbapenems (100%), Colistin (88.8%), and Tigecycline (81.8%). *Coagulase-negative staphylococci (CONS)* showed notable resistance to Oxacillin (80%), Cefotaxime (66.7%), and Meropenem (80%), while maintaining good susceptibility (100%) to Vancomycin and Linezolid. The prevalence of multidrug-resistant strains was recorded at 73.9% (18). In Vadodara, Gujarat, effective drugs against gram-positive bacteria included linezolid, vancomycin, and ciprofloxacin, while tigecycline, imipenem, and amikacin were noted as effective against

gram-negative bacteria. *Coagulase-negative staphylococci* and gram-negative organisms, such as *Klebsiella spp*, *Acinetobacter*, and *Staphylococcus aureus*, were primarily responsible for neonatal septicemia. Almost all isolates displayed resistance to various antibiotics, indicating widespread antimicrobial drug resistance (42).

Across various regions in Africa, distinct studies conducted in healthcare institutions reveal concerning patterns of antibiotic resistance among bacterial isolates. In Lagos, Nigeria, at Lagos State University Teaching Hospital (LASUTH), *Staphylococcus aureus* isolates displayed resistance rates of 36%, 48%, 52%, 0%, and 15% against cefotaxime, ciprofloxacin, gentamicin, linezolid, and meropenem, respectively. The predominant Gram-negative bacterial isolate, *Klebsiella pneumoniae*, showed complete resistance to cefotaxime, ceftazidime, ciprofloxacin, and gentamicin, but 100% susceptibility to meropenem and 66% to amikacin (44).

A study in DareSalam, Tanzania, reported antibiotic resistance rates ranging from 23% (Chloramphenicol) to 93% (Penicillin) for Gram-positive pathogens and from 24.7% (amikacin) to 91% (ampicillin) for Gram-negative bacteria. Multidrug resistance (MDR) was observed in about 69% of Gram-positive and 75% of Gram-negative bacteria, with an overall MDR proportion of approximately 70%, slightly higher in Gram-negative pathogens (46). In another study in Tanzania's Muhimbili National Hospital, isolated organisms exhibited overall resistance rates of 84% for ampicillin and 71.3% for gentamicin. Resistance patterns for recommended second-line antibiotics were 76.2% for ceftriaxone, 35.9% for vancomycin, and 17.5% for amikacin (45). In Kenya, *Klebsiella spp* was sensitive to meropenem, amikacin, and cefepime but resistant to ceftriaxone, gentamycin, and cefotaxime(57). These findings collectively underscore the urgent need for effective strategies to address antibiotic resistance in African healthcare settings.

In Ethiopia, various studies across health facility settings revealed an alarming trend of AMR. Studies at Ayder Hospital, Mekele; Felege Hiwot Hospital; Hawassa University Comprehensive Specialized Hospital; and St. Paul's Hospital Millennium Medical College highlighted the severity of AMR. A study at Ayder Hospital, Mekele, showed high drug resistance rates among common bacterial isolates. *Klebsiella spp*, *Escherichia coli*, *Enterobacter*, and *Citrobacter spp* exhibited resistance ranging from 57% to 100% against ceftazidime, ceftriaxone, gentamicin, amoxicillin-clavulanic acid, and ampicillin. All *Staphylococcus aureus* isolates (9/9, 100%) were resistant to

oxacillin, ampicillin, erythromycin, and gentamicin. Additionally, 55.6% of *Staphylococcus aureus* isolates were identified as Methicillin-Resistant *Staphylococcus aureus* (MRSA) (12). Findings from Felege Hiwot Hospital indicated a notably high overall proportion of multidrug resistance at 78.2% (29). Hawassa University Comprehensive Specialized Hospital reported varying rates of antimicrobial resistance among Gram-negative bacteria, ranging from 10.2% to 99.1%. Notably, all *Klebsiella spp* were resistant to ceftriaxone (13). At St. Paul's Hospital Millennium Medical College, ciprofloxacin and amikacin proved to be the most effective antibiotics for both Gram-negative and Gram-positive bacteria. Alarming, 84% of bacterial isolates showed multidrug resistance, underscoring the severity of the antimicrobial resistance problem (52).

2.5.Factors associated with neonatal sepsis

2.5.1 Sociodemographic-related factors

At Kanti Children's Hospital in Kathmandu, Nepal, positive blood cultures among neonates revealed distinctive patterns associated with various risk factors. The highest prevalence of positive bacterial growth was observed in males, accounting for 52.3%. Furthermore, neonates aged three days or more exhibited a notable positive bacterial growth rate of 71.2% (15). In a study conducted in Bhutan, South Asia, early-onset sepsis (EOS) was prevalent, with 54.5% of cases showing culture-positive results (1). Among study findings in Kenya, the study enrolled 141 neonates, the majority (57.4%) of whom were female(57).

In Ethiopia, various studies conducted in different regions have identified several sociodemographic factors as significant contributors to neonatal sepsis. At Ayder Comprehensive Specialized Hospital in Mekelle, Tigray, North Ethiopia, Neonates who stayed for greater than or equal to three days in the hospital were about 3.7 times more at risk of developing sepsis compared to those who stayed for less than three days (AOR = 3.65 (14). In Hawassa, the sex of newborns (female) was linked to culture-confirmed neonatal sepsis (13). In Shashemene, neonatal age was found to have significant associations with neonatal sepsis (51). At Wachemo University Comprehensive Specialized Teaching Hospital, a significant association was found between neonatal sepsis onset and neonates born to unmarried mothers (50).

2.5.2 Obstetrics-related factors

From neonates at Kanti Children's Hospital, Kathmandu, Nepal, the prevalence of positive blood culture with risk factors in the cesarean delivery mode was 63.3% (15). Among study findings in Uganda, Cesarean section delivery was strongly associated with culture-positive sepsis (adjusted odds ratio 3.45, 95% CI: 1.2, 10.1)(47).

Various studies conducted in different regions of Ethiopia have identified several obstetric factors as significant contributors to neonatal sepsis. At Felege-Hiwot Referral Hospital, statistically significant associations were found between bacteriologically confirmed neonatal sepsis and factors such as prolonged rupture of membranes (≥ 18 hours) and cesarean section delivery modes (30). Referral hospitals in East and West Gojam Zones revealed that nulliparity, labor duration exceeding 18 hours after membrane rupture, gestational age of 32–37 weeks, and the need for

neonatal resuscitation at birth were significantly linked to early-onset neonatal sepsis (48). Wachemo University Comprehensive Specialized Teaching Hospital identified maternal fever during delivery and premature rupture of membranes as variables increasing the odds of neonatal sepsis (50). A study in Shashemene highlighted significant associations between neonatal sepsis and factors such as maternal history of urinary tract infection (UTI) and meconium-stained amniotic fluid (51). The findings at Hawassa University Comprehensive Hospital indicated associations between premature rupture of membranes, mode of delivery (especially instrumental vaginal delivery and cesarean section), and culture-confirmed neonatal sepsis(13). Woldia and Dessie Comprehensive Specialized Hospitals identified a history of maternal premature rupture of membranes (PROM) as a significant risk factor for neonatal sepsis (49). Hospitals in Northern Oromia determined that multiple per digital vaginal examinations, a history of urinary tract infection, meconium-stained amniotic fluid (MSAF), and intrapartum high fever were independent determinants of neonatal sepsis (52).

2.5.3 Clinical-related factors

In the neonatal unit of Kanti Children's Hospital in Kathmandu, Nepal, positive blood cultures revealed a correlation with distinct neonatal risk factors. Notably, low birth weight was prevalent, accounting for 62.7% of positive blood cultures, and preterm gestational age was a significant factor, contributing to 31.4% of positive cases (15).

A parallel study conducted in North India identified prominent risk factors associated with septicemia. Nasal cannula use constituted a notable risk at 54%, followed by birth asphyxia at 48%, and prematurity at 38% (55). In Pakistan, the factors that continue to be significantly linked to neonatal sepsis are preterm birth, low birth weight, and the length of hospital stay(39).

In the Tanzanian context, neonatal mortality in sepsis cases was further influenced by factors such as low birth weight, early-onset sepsis, and the utilization of mechanical ventilation (45). Among the studies enrolled in Kenya, gestational age and birth weight were associated with neonatal sepsis(57).

Various studies conducted across different hospitals in Ethiopia have shed light on diverse risk factors associated with neonatal sepsis. Ayder Hospital in Ethiopia reported that the length of hospital stay and low birth weight were key factors associated with neonatal sepsis (14). A separate

study conducted in Mekele identified several potential risk factors for neonatal sepsis, including maternal history of urinary tract infection or sexually transmitted infection, prolonged rupture of membranes, delivery at a health center, intrapartum fever, an Apgar score less than 7 at five minutes, and the neonate not crying immediately at birth. Each of these factors was associated with a substantially increased likelihood of neonatal sepsis (29). At Wachemo University Comprehensive Specialized Teaching Hospital, maternal fever during delivery and premature rupture of membranes were variables that increased the odds of developing neonatal sepsis (50). In Northern Oromia hospitals, factors such as the age of neonates and neonatal birth weight less than 2.5 kg were determined as independent determinants of neonatal sepsis (52). In Hawassa, premature rupture of membrane (AOR = 12.7), absence of respiratory support (AOR = 3.53), sex of newborns (AOR = 2.10) and reason for admission (AOR = 3.17) were significantly associated with culture-confirmed neonatal sepsis(13). A systematic review and meta-analysis in Ethiopia found that neonates born to mothers with antenatal urinary tract infections had a 3.55 times higher risk of sepsis, and neonates whose mothers experienced intra-partum fever were 3.63 times more likely to develop sepsis (32). At St. Paul's Hospital Millennium Medical College, low birth weight and preterm birth emerged as factors associated with neonatal sepsis, emphasizing the consistency of these risk factors across diverse healthcare settings in Ethiopia (53).

2.5.4 Conceptual framework

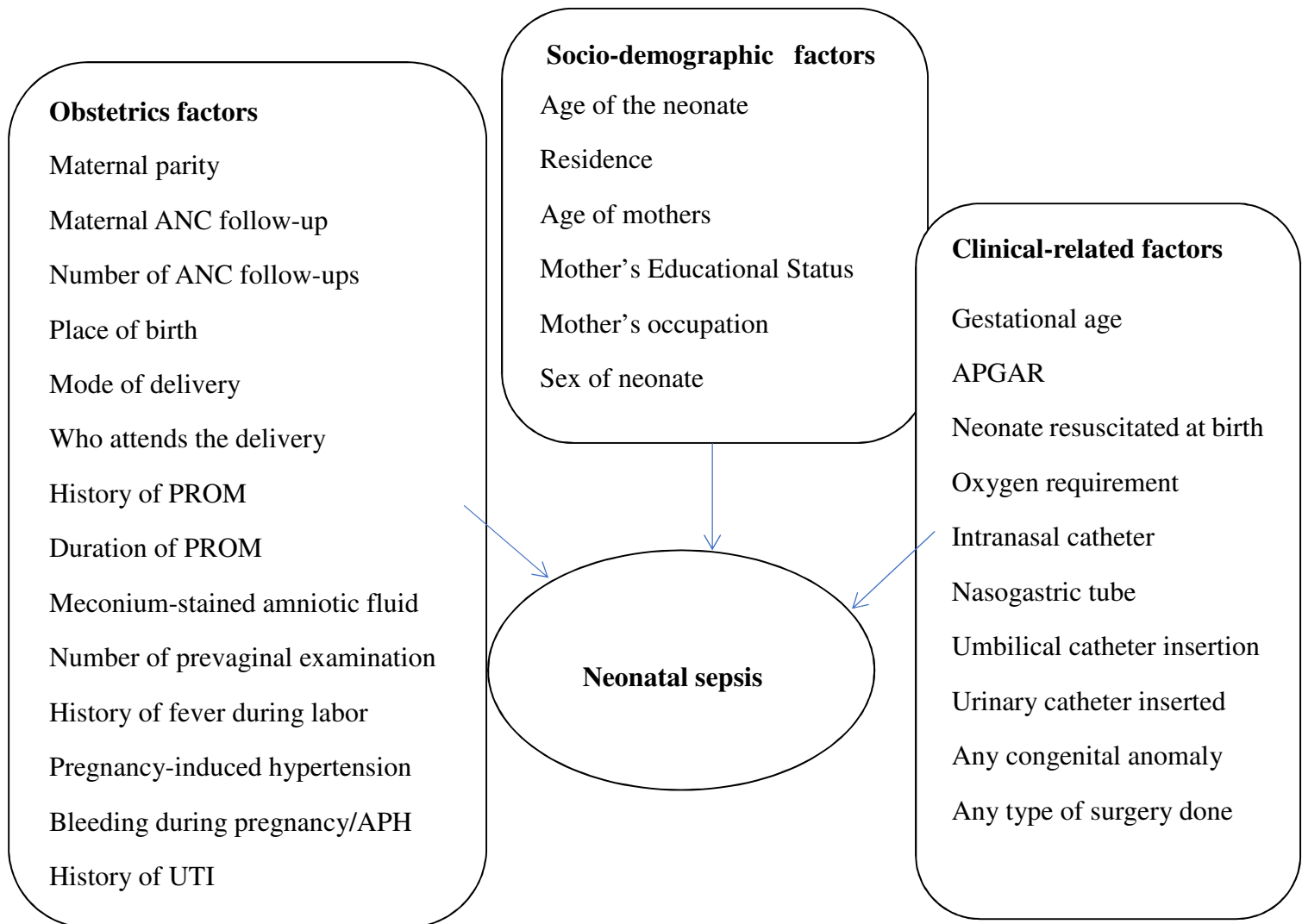


Figure 1: Conceptual Framework for Factors associated with neonatal sepsis attending Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia, 2025

3. Objective

3.1. General objective

To determine the magnitude, bacteriological profile, and antimicrobial susceptibility patterns of neonatal sepsis, and to assess clinical outcomes and associated factors among neonates admitted to the NICU of Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia.

3.2. Specific objectives

- To determine the magnitude of neonatal sepsis among neonates admitted to the NICU
- To determine bacteriological profile responsible for neonatal sepsis in the NICU
- To determine the antimicrobial susceptibility pattern of bacteria isolated from neonatal sepsis in the NICU
- To assess the clinical outcomes of neonates with sepsis in the NICU
- To identify factors associated with neonatal sepsis in the NICU

4. Methods and Materials

4.1. Study design and period

A cross-sectional institution-based study was conducted between June and December 2024.

4.2 Study setting

The study was carried out at Menelik II Comprehensive Specialized Hospital, a referral hospital located in Addis Ababa, Ethiopia, that falls under the jurisdiction of the Addis Ababa Health Bureau. Established in 1902 E.C. by Emperor Menelik II, it is the first government hospital in the country. Originally conceived to cater to injured soldiers, the hospital's mission has evolved since then.

At present, Menelik II Comprehensive Specialized Hospital is dedicated to providing a range of high-quality services. These services encompass the treatment and care of tuberculosis and HIV, forensic investigations, rehabilitation, surgery, gynecology, orthopedics, ophthalmology, pediatrics, the adult intensive care unit (ICU), the neonatal intensive care unit (NICU), and various other infectious diseases (59). The study setting recently initiated microbiology laboratory services for bacterial culture and antimicrobial susceptibility testing (AST) on February 09, 2024.

4.3 Source and Study Population

4.3.1 Source population

All neonates admitted to the NICU of MIICSH

4.3.2 Study population

All neonates who were admitted to the neonatal ward, presenting with clinical features suggestive of sepsis during the study period.

4.4 Inclusion and Exclusion Criteria

4.4.1 Inclusion criteria

All neonates who were admitted to the NICU during the study period and who showed signs and symptoms of sepsis

4.4.2 Exclusion criteria

- Newborns without family members/caregivers who can provide background information

- Neonates who had received systemic antibiotic therapy for more than 48 hours before blood sample collection.
- Neonates with inadequate or contaminated blood samples that were unsuitable for culture

4.5. Sample size determination and sampling procedures

4.5.1. Sample size determination

The maximum number of samples required for this study is determined by using the Single population proportion formula, considering the following assumptions

$$ni = \frac{Z\alpha / 2^2 pq}{d^2}$$

Where

n = minimum sample size required for the study

Z= standard normal distribution with a confidence interval of 95%, Z=1.96 and $\alpha=0.05$

d = Absolute precision or tolerable margin of error. d=0.05

P is the anticipated population proportion with neonatal sepsis

As per our review of related literature, the study done at St. Paul's Hospital Millennium Medical College revealed that the overall prevalence of neonatal septicemia was 21% (54). Hence, the population proportion of neonates with sepsis was 21%.

Therefore $ni = \frac{(Z\alpha/2)^2 pq}{d^2}$

$$ni = \frac{(1.96)^2 0.21(1-0.21)}{(0.05)^2} = 254$$

$$q=1-p$$

Then, by considering of 10% non-response rate, the final sample size will be $(254+25) = 279$

4.5.2. Sampling technique

A consecutive sampling technique was used. We included consecutively all the suspected neonates who were sent to the laboratory during the study period.

4.6. Variables of the study

4.6.1. Dependent variable

- Neonatal sepsis
- Bacterial profile of neonatal sepsis
- Antimicrobial susceptibility pattern of isolates

4.6.2. Independent variables

▪ Socio-demographic variables

- ✓ Age of the neonate (in days)
- ✓ Residence
- ✓ Age of mothers (in Years)
- ✓ Mother's Educational Status
- ✓ Mother's occupation
- ✓ Sex of neonate

▪ Clinical-related factors

- ✓ Gestational age
- ✓ APGAR
- ✓ Neonate resuscitated at birth
- ✓ Oxygen requirement
- ✓ Intranasal catheter
- ✓ Nasogastric tube
- ✓ Umbilical catheter insertion
- ✓ Urinary catheter inserted
- ✓ Any congenital anomaly
- ✓ Any type of surgery done

▪ Obstetrics factors

- ✓ Maternal parity
- ✓ Maternal ANC follow-up
- ✓ Number of ANC follow-up
- ✓ Place of birth, health institution
- ✓ Mode of delivery
- ✓ Who attends the delivery
- ✓ History of PROM
- ✓ Duration of PROM
- ✓ Meconium-stained amniotic fluid
- ✓ Number of prevaginal examination
- ✓ History of fever during labor
- ✓ Pregnancy-induced hypertension
- ✓ Bleeding during pregnancy/APH
- ✓ History of UTI

4.7. Operational definitions

- Neonatal sepsis: Neonates presented with any one of the systemic manifestations of danger signs:- not feeding well, convulsions, drowsy or unconscious, the movement only when stimulated or no movement at all, fast breathing (60 breaths per min), grunting severe chest in-drawing, raised temperature $> 38^{\circ}\text{C}$, hypothermia $< 35.5^{\circ}\text{C}$, central cyanosis or could be severe jaundice, severe abdominal infection and distension or localizing signs of infection were diagnosed as having neonatal sepsis (60,61).
- Early onset of sepsis: If sepsis occurs from birth to 3 days of age.
- Late onset of sepsis: If sepsis occurs between 4 and 28 days of age

4.8. Data collection procedures

4.8.1. Data collection instrument

A structured questionnaire was used to collect data on socio-demographic characteristics (age of the neonate, sex, and marital status of the mother, etc.), clinical characteristics, and obstetric factors. In addition, a medical records review was done for maternal information, such as chronic disease, indwelling medical devices, birth weight, and other relevant information, such as

intrapartum fever, premature rupture of membranes (PROM), gestational age, mode of delivery, fever, failure to suck, respiratory distress, and jaundice.

4.8.2 Specimen collection, handling

About 1-2 ml of blood was drawn aseptically before starting antimicrobial therapy and directly inoculated into a tryptone soya broth (1:10 ratio) (Oxoid Ltd, UK) and immediately transported to the MIICSH Medical Microbiology Laboratory for culture and antimicrobial susceptibility test (AST).

4.8.3 Blood culture and bacterial identification

The blood culture bottles were quickly placed in an incubator set at 37°C for 24 hours and then subcultured onto MacConkey agar, blood agar, and chocolate agar. The MacConkey agar plates were incubated aerobically, while the blood agar and chocolate agar plates were kept in a CO₂-enriched, humid environment using a candle jar at 37°C for 24-48 hours. If no growth is observed on the subcultured plates after 7 days of incubation, the blood culture bottles are considered negative.

All the collected blood samples were subjected to culture and isolation procedures following standard microbiological methods. Once pure colonies are obtained, further identification is performed using standard microbial techniques, including Gram staining, observation of colony morphology, and biochemical tests. Presumptive gram-negative bacteria were identified through biochemical tests like Kligler iron agar, citrate utilization, lysine decarboxylase, urease, motility, indole, and oxidase. Gram-positive bacteria were identified through assessments such as hemolytic activity on sheep blood agar, catalase, coagulase, and bileesculin tests.

4.8.4 Antimicrobial Susceptibility Testing (AST)

Antibacterial susceptibility testing was carried out using the disc diffusion method following the guidelines set by the Clinical and Laboratory Standards Institute (58). To begin, 3–5 selected colonies of bacteria were transferred into a tube containing 5 ml of sterile normal saline and gently mixed until a homogenous suspension of McFarland 0.5 turbidity was achieved. Subsequently, a sterile cotton swab will be used to evenly inoculate a suspension of bacteria onto Mueller-Hinton agar. After a brief incubation period at room temperature, antibiotic discs were placed on the surface of the inoculated plates and then incubated at 37°C for 18–24 hours. Tested antibiotics

included a range of commonly used antibiotics such as ampicillin, amoxicillin and clavulanic acid, Ceftazidime, Ceftriaxone, Piperacillin-Tazobactam, Cefepime, ciprofloxacin, Trimethoprim-Sulfamethoxazole, Imipenem, Meropenem, Amikacin, Gentamicin, Penicillin, and oxacillin.

4.8.5 Quality Control Measures

The culture media were meticulously prepared following the manufacturer's guidelines. To ensure sterility, a sample equivalent to 5% of the prepared media underwent a 48-hour incubation at 37°C every time a new batch was made. Assessing the media's ability to support the growth of suspected organisms involves inoculating the medium with a standard stock culture. To gauge the biochemical response of reagents and test kits, both negative and positive controls were incorporated. Quality control for culture media, discs, biochemicals, antimicrobial susceptibility testing (AST), and incubation conditions was maintained using reference strains, including standardized reference strains of *P. aeruginosa* (ATCC-27853), *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922), and others.

4.8.6. Data quality control issues

Training was provided to the data collectors and supervisors on specimen collection and handling, transportation of the data collection tool (Kobo Toolbox version 11), and sampling techniques by the researcher. A total of four data collectors and one supervisor were recruited. Supervision was conducted regularly during the data collection period by the researcher, co-investigators, and supervisor. The collected data were checked daily for completeness and consistency. The questionnaire was translated into an Amharic version, and necessary feedback was given to the data collectors at the end of each collection. Data was collected over a period of 7 months.

4.8.7. Data processing and analysis

All questionnaires collected through the Kobo Toolbox V11 were reviewed for completeness and consistency. After data cleaning, the dataset was exported to SPSS version 27 for analysis. Descriptive statistics, including frequencies and percentages, were used to summarize the study variables. Bivariate and multivariable analyses were performed to identify statistically significant associations between independent variables and the magnitude of neonatal sepsis. The strength of associations was reported using odds ratios and 95% confidence intervals. A p-value of <0.05 in multivariable analysis was considered statistically significant.

4.9 Ethical considerations

Ethical approval was obtained from the Institutional Review Board (IRB) of SPHMMC (PM23/1239) and from the Addis Ababa city administration, Health Bureau, Public Health Research and Emergency Management Directorate. The objectives and procedures of the study were clearly explained to parents or legal caregivers, and written informed consent was obtained before participation. Official permission to conduct the study was granted by Menelik II Comprehensive Specialized Hospital. Parents or legal caregivers were informed of their right to decline participation or to skip any questions they found uncomfortable without any consequences. To ensure confidentiality, participants' names were not recorded on the questionnaires. All interviews were conducted privately, and relevant findings were communicated to the responsible physicians for the benefit of the participants.

4.10 Dissemination of Results

After the data was analyzed based on the findings obtained, conclusions and recommendations were made. The results of the study were submitted to the SPHMMC and publicly defended. The result was presented and will be published in peer-reviewed journals.

5. Result

5.1 Socio-demographic characteristics of the neonates and mothers

A total of 251 neonates were included in the study, yielding a response rate of 98.8% from the targeted sample size of 254, with three non-respondents. The mean age of the neonates was 5.43 (SD $\pm$ 6.41) days, ranging from 1 to 28 days. The demographic data showed that 149 (59.4%) belonged to clinically suspected early-neonates and 40.6% to late-neonates. The majority, which accounts for 63.3% (159), were males. The majority of mothers (n= 80) were in the 25-29-year-old group. Those mothers with a Grade 1-8 educational background held 31.9% (n= 80) of the participants. The finding also showed that the majority of 55.8% (n=140) mothers were housewives, whereas more than three-fourths of them, which accounts for 80.0% (n=221), were from urban areas (Table 1).

Table 1: Socio-demographic characteristics of neonates with suspected Neonatal Sepsis and their mothers attending Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia, 2025

Categorical Variables		Frequency n= 251	Percent (%)
Age in days	Early Neonate (0-3)	149	59.4
	Late neonate (4-28)	102	40.6
Sex of Neonates	Male	159	63.3
	Female	92	36.7
Age of mothers	<20	34	13.5
	20-24	51	20.3
	25-29	80	31.9
	30-34	51	20.3
	35-39	28	11.2
	>40	7	2.8
Mothers' Educational Status	Unable to read and write	42	16.7
	Grade 1-8	80	31.9
	Grade 9-12	56	22.3
	College and above	73	29.1
Mothers' Occupational Status	Housewives	140	55.8
	Civil servants	35	13.9
	Private employed	44	17.5
	Merchant	5	2.0
	Students	27	10.8
Mothers' Residence	Rural	30	12.0
	Urban	221	88.0

5.2 Obstetric and gynecologic characteristics of mothers

More than half of the mothers whose neonate participated in this study, 135 (53.8%), were primipara. The majority, 237 (94.4%), had antenatal care follow-up, and 239 (95.2%) delivered in a health facility. Most deliveries, 148 (59.0%), were by spontaneous vaginal delivery, and 239 (95.2%) were attended by health professionals. A history of premature rupture of membranes (PROM) was reported in 37 (14.7%) mothers, of which 14 (5.6%) lasted for 18 hours or more. Meconium-stained amniotic fluid was observed in 32 (12.7%) deliveries. Frequent vaginal examinations (≥ 3 times) were noted in 163 (64.9%) mothers. Intrapartum fever occurred in 36 (14.3%) cases, pregnancy-induced hypertension in 29 (11.6%), antepartum hemorrhage in 7 (2.8%), and urinary tract infection during pregnancy in 20 (8.0%) (Table 2)

Table 2: Obstetric and gynecological characteristics of mothers of neonates with suspected neonatal sepsis (Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia, 2025)

Variables		Frequency, n=251	Percent (%)
Maternal parity category	Primipara (1)	135	53.8
	Multipara (2-4)	111	44.2
	Grand multipara (>4)	5	2.0
Maternal ANC follow-up	No	14	5.6
	Yes	237	94.4
Place of birth	Health institution	239	95.2
	Home	12	4.8
Mode of delivery	Cesarean	99	39.4
	Instrumental	4	1.6
	Spontaneous vaginal delivery	148	59.0
Who attends the delivery	Skilled birth attendant	239	95.2
	Unskilled birth attendant	12	4.8
History of PROM	No	214	85.3
	Yes	37	14.7
	<18	23	9.2

Duration of PROM in hours	≥18	14	5.6
Presence of Meconium-stained amniotic fluid	No	219	87.3
	Yes	32	12.7
Number of prevaginal examination	No	39	15.5
	Once	14	5.6
	2-3 times	35	13.9
	More than 3 times	163	64.9
History of fever during labor	No	215	85.7
	Yes	36	14.3
Pregnancy-induced hypertension	No	222	88.4
	Yes	29	11.6
Bleeding during pregnancy	No	244	97.2
	Yes	7	2.8
History of UTI	No	231	92.0
	Yes	20	8.0

5.3 Clinical characteristics of the neonates

The underlying clinical characteristics of neonates were presented in Table 3. The study found that neonates with respiratory distress made up the highest proportion of neonatal sepsis, accounting for 88 (35.1%). The majority 60.6% (152) of them reported being born at term gestation age. Regarding APGAR score conditions, 161 (64.1%) neonates had a normal APGAR score, while 38 (15.1%) had neonatal resuscitation at birth. Most of the neonates, 177 (70.5%), required oxygen, and 152 (60.6%) were intranasally catheterized. Additionally, 196 neonates (78.1%) had a nasogastric tube inserted, and congenital anomalies were present in 27 (10.8%) neonates (Table 3). In addition, three (1.2%) neonates underwent surgery, and one (0.4%) neonate each had an umbilical catheter and a urinary catheter inserted, respectively.

Table 3: Description of clinical characteristics of neonates with suspected Neonatal sepsis attending Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia, 2025

Variables	Clinical characteristics	Frequency, n=251	Percent (%)
Chef compliant	Jaundice	23	9.2
	Respiratory distress	88	35.1
	Unable to suck	40	15.9
	Fever	18	7.2
	Co-symptom	52	20.7
	Other	30	12.0
Gestational age in weeks	Preterm (<37)	89	35.5
	Term (37-42)	152	60.6
	Post term (>42)	10	4.0
APGAR score	Low (0-6)	90	35.9
	Normal (7-10)	161	64.1
Neonatal resuscitation at birth	No	213	84.9
	Yes	38	15.1
Oxygen requirement	No	74	29.5
	Yes	177	70.5
Intranasal catheter used	No	99	39.5
	Yes	152	60.5
Nasogastric tube inserted	No	55	21.9
	Yes	196	78.1
Congenital anomalies present	No	224	89.2
	Yes	27	10.8

Other*- Meconium aspiration syndrome, Preterm, Vomiting

Co-symptom*-presence of two or more chief complaints occurring simultaneously

5.4. Magnitude of neonatal sepsis

Among the 251 neonates admitted with suspected sepsis, 114 had blood culture-confirmed neonatal sepsis, resulting in an overall culture positivity rate of 45.4% (95% CI: 39.4–51.4) (Fig. 2). Of these confirmed cases, 59 (51.8%) were early-onset neonatal sepsis. In contrast, 55 (48.2%) were late-onset. Male neonates accounted for 76 (66.7%) of neonatal sepsis cases. The most common maternal age group was 25–29 years, comprising 40 (35.1%) mothers. Clinically, respiratory distress was observed in 46 (40.4%) neonates, and 49 (43.0%) were born preterm. A low APGAR score was recorded in 42 (36.8%) neonates, and 16 (14.0%) required resuscitation at birth. The majority of culture-positive neonates required oxygen therapy (84, 73.7%), and 71 (62.3%) received intranasal catheterization. Additionally, 26 (22.8%) neonates had a nasogastric tube inserted, and congenital anomalies were identified in 13 (11.4%) cases. Regarding obstetric characteristics, most neonates were delivered by spontaneous vaginal delivery (70, 61.4%). Premature rupture of membranes was reported in 16 (14.0%) mothers, meconium-stained amniotic fluid in 11 (9.6%) deliveries, intrapartum fever in 13 (11.4%) cases, and maternal urinary tract infection during pregnancy in 10 (8.8%) cases (Table 4).

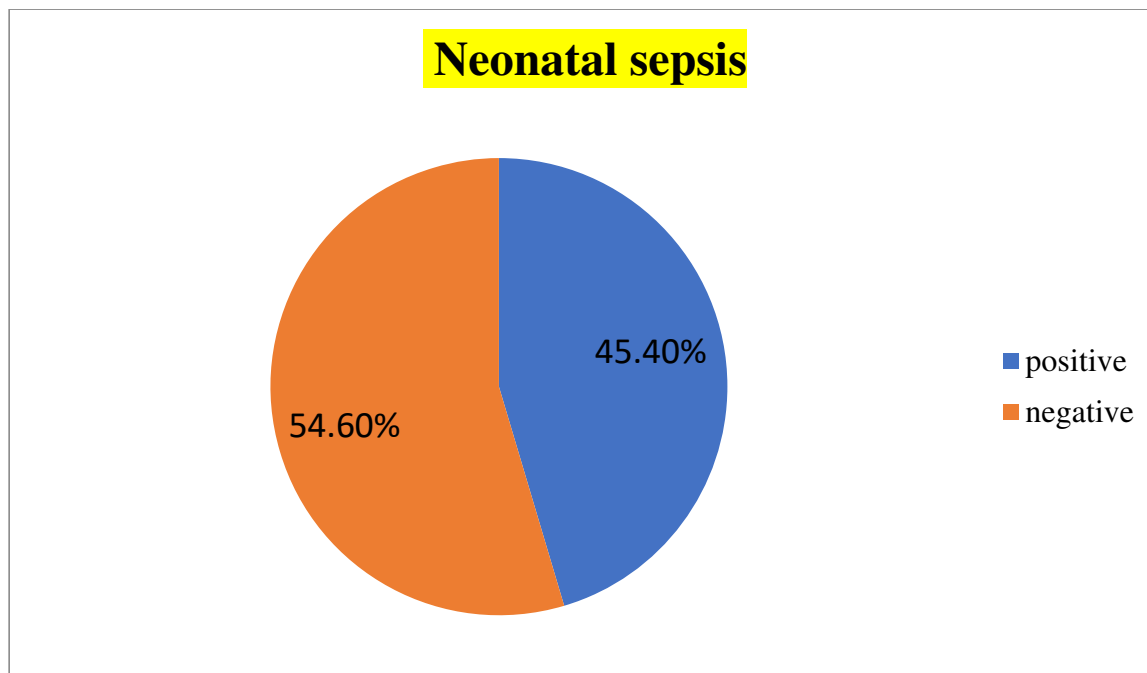


Figure 2: of neonatal sepsis among neonates attending Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia, 2025.

Table 4 characteristics of neonates with culture-confirmed neonatal sepsis admitted to Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia, 2025

Variables		Neonatal sepsis N= 114(100.0%)		
Age of Neonates	days	Early Neonate (0-3) 59 (51.8%)	Late neonate (4-28) 55(48.2%)	Total
Sex of Neonates	Male	37(32.45%)	39(34.21%)	76(66.7%)
	Female	22(19.29%)	16(14.03%)	38(33.3%)
Age of mothers	<20	6(5.26%)	11(9.64%)	17 (14.9%)
	20-24	13(11.40%)	8(7.01%)	21(18.4%)
	25-29	21(18.42%)	19(16.66%)	40(35.1%)
	30-34	14(12.28%)	9(7.89%)	23(20.2%)
	35-39	5(4.38%)	5(4.38%)	10(8.8%)
	>40	0	3(2.6%)	3(2.6%)
Chief complaint	Jaundice	3(2.63%)	3(2.63%)	6(5.3%)
	Respiratory distress	27(23.68%)	19(16.66%)	46(40.4%)
	Unable to suck	8(7.1%)	6(5.26%)	14(12.3%)
	Fever	1(0.87%)	4(3.50%)	5(4.4%)
	Co-symptom	15(13.15%)	18(15.78%)	33(28.9%)
	Other	5(4.38%)	5(4.38%)	10(8.8%)
	Preterm (<37)	27(23.68%)	22(19.29%)	49(43.0%)

Gestational age in weeks	Term (37-42)	31(29.19%)	32(28.07%)	63(55.3%)
	Post term (>42)	1(0.87%)	1(0.87%)	2(1.8%)
APGAR score	Low (0-6)	28(24.56%)	14(12.28%)	42(36.8%)
	Normal(7-10)	31(27.19%)	41(35.96%)	72(63.2%)
Neonatal resuscitation at birth	No	47(41.22%)	51(44.73%)	98(86.0%)
	Yes	12(10.52%)	4(3.50%)	16(14.0%)
Oxygen requirement	No	11(9.64%)	19(16.66%)	30(26.3%)
	Yes	48(42.10%)	36(31.57%)	84(73.7%)
Intranasal catheter used	No	20(17.54%)	23(20.17%)	43(37.7%)
	Yes	39(34.21%)	32(28.07%)	71(62.3%)
Nasogastric tube inserted	No	12(10.52%)	14(12.28%)	26(22.8%)
	Yes	47(41.22%)	41(35.96%)	88(77.2%)
Congenital anomalies present	No	53(46.49%)	48(42.10%)	101(88.6%)
	Yes	6(5.26%)	7(6.14%)	13(11.4%)
Mode of delivery	Cesarean	27(23.68%)	16(14.03%)	43(37.7%)
	Instrumental	1(0.87%)	0	1(0.9%)
	Spontaneous vaginal delivery	31(27.19%)	39(34.21%)	70(61.4%)

History of PROM	No	49(42.98%)	49(42.98%)	98(86.0%)
	Yes	10(8.77%)	6(5.26%)	16(14.0%)
Presence of Meconium-stained amniotic fluid	No	50(43.85%)	53(46.49%)	103(90.4%)
	Yes	9(7.89%)	2(1.75%)	11(9.6%)
History of fever during labor	No	51(44.73%)	50(43.85%)	101(88.6%)
	Yes	8(7.01%)	5(4.38%)	13(11.4%)
History of UTI	No	54(47.36%)	50(43.85%)	104(91.2%)
	Yes	5(4.38%)	5(4.38%)	10(8.8%)

Other*- Meconium aspiration syndrome, Preterm, Vomiting

Co-symptom*-presence of two or more chief complaints occurring simultaneously

5.5. Bacterial profile of Neonatal sepsis

The blood culture results from a total of 251 samples collected from neonates clinically suspected of having neonatal sepsis revealed that 114 (45.4%) were blood culture-positive. Out of these, 68 (59.6%) were Gram-negative bacteria and 46 (40.4%) were Gram-positive bacteria. (42.1%), *Klebsiella pneumoniae* was identified in 30 isolates (26.3%), followed by *Klebsiella ozaenae* in 18 isolates (15.8%). Other significant Gram-negative bacteria included *Pseudomonas aeruginosa* (7 isolates, 6.1%) and *Citrobacter spp* (5 isolates, 4.4%). Additionally, *Escherichia coli* comprised 4 isolates (3.5%), while *Enterobacter spp* comprised 3 isolates (2.6%). *Providencia spp* was identified in a single sample (0.9%). Among Gram-positive isolates, *Staphylococcus aureus* was the most commonly identified organism, accounting for 25 isolates (21.9%), followed by *coagulase-negative staphylococci (CONS)* with 20 isolates (17.5%), and *Enterococcus* with 1 isolate (0.9%). (Table 5).

Gram-negative bacteria predominated in both early-onset and late-onset neonatal sepsis. *Klebsiella pneumoniae* was the most common pathogen in both groups, accounting for 25.7% of early-onset and 27.3% of late-onset cases. *Klebsiella ozaenae* was more frequent in late-onset sepsis (18.2%) than in early-onset sepsis (14.3%). *Pseudomonas aeruginosa* and *Citrobacter spp.* were also more common in late-onset sepsis (6.8% each) than in early-onset sepsis (5.7% and 2.9%, respectively). In contrast, *Escherichia coli* was slightly more prevalent in early-onset sepsis (4.3%) than in late-onset sepsis (2.3%). *Enterobacter spp.* occurred at similar low proportions in both groups (2.9% early vs. 2.3% late), while *Providencia spp.* were identified only in late-onset sepsis (2.3%). Among Gram-positive organisms, *Staphylococcus aureus* was common in both early-onset (24.3%) and late-onset sepsis (18.2%), followed by *Coagulase-negative staphylococci (CONS)*, which accounted for 20.0% of early-onset and 13.6% of late-onset cases. *Enterococcus spp.* were identified only in late-onset sepsis (2.3%). (Figure 3).

Table 5: Description of bacterial isolates among Neonatal sepsis attending Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia, 2025

Variables Category	Sub-category	Frequency, N=114	Percent (%)
Bacterial Gram reaction	Gram-positive	46	40.4
	Gram-negative	68	59.6

Gram-positive bacterial isolates	<i>Staphylococcus aureus</i>	25	21.9
	<i>Cons</i>	20	17.5
	<i>Enterococcus</i>	1	0.9
Gram-negative bacterial isolates	<i>Klebsiella pneumoniae</i>	30	26.3
	<i>Klebsiella ozaenae</i>	18	15.8
	<i>Pseudomonas Aeruginosa</i>	7	6.1
	<i>Citrobacter spp</i>	5	4.4
	<i>Escherichia coli</i>	4	3.5
	<i>Enterobacter spp</i>	3	2.6
	<i>Providencia spp</i>	1	0.9

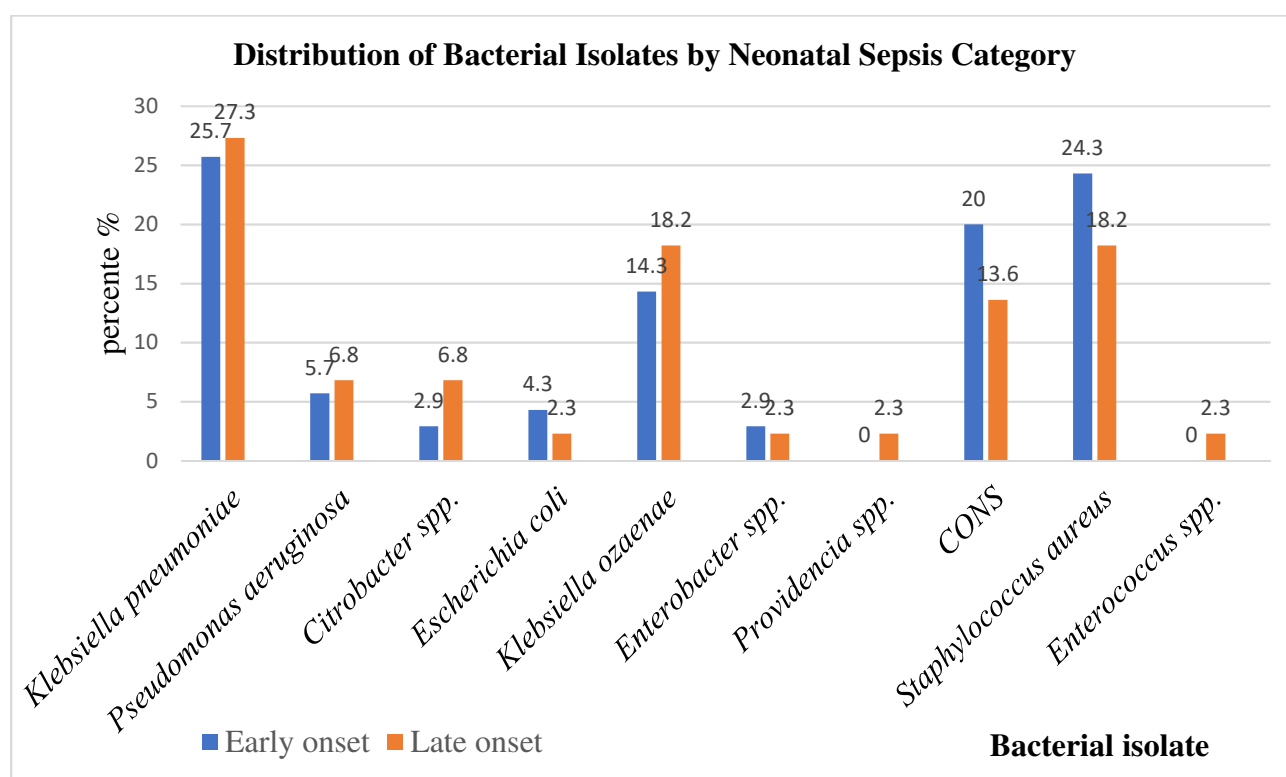


Figure 3 Distribution of bacterial isolates among early- and late-onset neonatal sepsis at Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia, 2025.

5.6 Antimicrobial susceptibility patterns of bacterial isolates

Among the Gram-negative isolates, *Klebsiella pneumoniae* showed the highest resistance to ceftriaxone and cefazolin (63.3%), while retaining strong susceptibility to amikacin (90%) and imipenem/meropenem (73.3%). *Klebsiella ozaenae* demonstrated a similar profile, with high

resistance to cefazolin (66.7%) and ceftriaxone (72.2%), but high susceptibility to amikacin (83.3%) and moderate susceptibility to imipenem (61.1%). *Citrobacter spp* showed marked resistance to cefazolin (60%) and ceftriaxone (40%), yet remained fully susceptible to ceftazidime (100%) and highly susceptible to ciprofloxacin (80%). *Escherichia coli* displayed full susceptibility to imipenem and meropenem (100%), with moderate resistance to cefazolin and ceftriaxone (50% each). *Pseudomonas aeruginosa* was fully susceptible to carbapenems, ceftazidime, and amikacin (all 100%), while showing complete resistance to cefazolin. *Enterobacter spp* showed high resistance to ceftriaxone (72.2%) and cefazolin (61.1%), but maintained full susceptibility to amikacin (100%) and moderate susceptibility to imipenem (66.7%). *Providencia spp* showed resistance to ampicillin and cefazolin but remained fully susceptible to carbapenems and amikacin. Gentamicin remained the most effective drug, with 80% susceptibility. Ciprofloxacin showed moderate susceptibility, with 60% and 30% resistance (Table 6). Among the Gram-positive isolates, *Staphylococcus aureus* showed very high resistance to Penicillin and Oxacillin (76%), suggesting a high proportion of MRSA.

Table 6: Description of Antimicrobial Susceptibility Pattern of Gram-negative Bacteria isolated among neonates with neonatal sepsis attending Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia, 2025

Drugs name	AS T	<i>Klebsiella pneumoniae</i> N=30	<i>Klebsiella ozaenae</i> N=18	<i>Citrobacter</i> <i>spp</i> N=5	<i>Escherichia coli</i> N=4	<i>Pseudomonas Aeruginosa</i> N=7	<i>Enterobacter spp</i> N=3	<i>Providencia spp</i> N=1
Ampicillin	S I R	NT*	NT	3(60.0) 2(40.0) 0.0	3(75.0) 0.0 1(25.0)	NT	1(33.3) 0.0 2(66.7)	1(100) 0.0 0.0
Cefazolin	S I R	9(30.0) 2(6.7) 19(63.3)	5(27.8) 1(5.6) 12(66.7)	1(20.0) 1(20.1) 3(60.0)	3(75.0) 1(25.0) 0.0	NT	0.0 0.0 3(100)	0.0 0.0 1(100)
Amoxa+clav	S I R	13(43.3) 1(3.3) 16(53.3)	9(50.0) 1(5.6) 8(44.4)	4(80.0) 0.0 1(20.0)	2(50.0) 0.0 2(50.0)	NT	2(66.7) 0.0 1(33.3)	1(100) 0.0 0.0
Ceftazidime	S I R	17(56.7) 4(13.3) 9(30.0)	7(38.9) 1(5.6) 10(55.6)	5(100) 0.0 0.0	3(75.0) 1(25.0) 0.0	7(100) 0.0 0.0	3(100) 0.0 0.0	1(100) 0.0 0.0
Ceftriaxone	S I R	11(36.7) 0.0 19(63.3)	4(22.2) 1(5.6) 13(72.2)	2(40.0) 1(20.0) 2(40.0)	2(50.0) 0.0 2(50.0)	NT	2(66.7) 0.0 1(33.3)	1(100) 0.0 0.0

Cefuroxime	S	12(40.0)	7(38.9)	4(80.0)	4(100)	NT	2(66.7)	1(100)
	I	3(10.0)	0.0	0.0	0.0		0.0	0.0
	R	15(50.0)	11(61.1)	1(20.0)	0.0		1(33.7)	0.0
Piperacillin-Tazobactam	S	NT	NT	NT	NT	7(100)	NT	NT
	I					0.0		
	R					0.0		
Cefepime	S	20(66.7)	7(38.9)	5(100)	4(100)	7(100)	3(100)	1(100)
	I	4(13.3)	3(16.7)	0.0	0.0	0.0	0.0	0.0
	R	6(20.0)	8(44.4)	0.0	0.0	0.0	0.0	0.0
Ciprofloxacin	S	18(60.0)	7(38.9)	5(100)	3(75.0)	7(100)	2(66.7)	1(100)
	I	3(10.0)	0.0	0.0	0.0	0.0	0.0	0.0
	R	9(30.0)	11(61.1)	0.0	1(25.0)	0.0	1(33.3)	0.0
Trimethoprim-Sulfamethoxazole	S	12(40.0)	8(44.4)	3(60.0)	3(75.0)	NT	3(100)	1(100)
	I	2(6.7)	0.0	0.0	0.0		0.0	0.0
	R	16(53.3)	10(55.6)	2(40.0)	1(25.0)		0.0	0.0
Imipenem	S	22(73.3)	11(61.1)	5(100)	4(100)	7(100)	2(66.7)	1(100)
	I	1(3.3)	0.0	0.0	0.0	0.0	0.0	0.0
	R	7(23.3)	7(38.9)	0.0	0.0	0.0	1(33.3)	0.0
Meropenem	S	22(73.3)	12(66.7)	5(100)	4(100)	7(100)	2(66.7)	1(100)
	I	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	R	8(26.7)	6(33.3)	0.0	0.0	0.0	1(33.3)	0.0
Gentamicin	S	15(50.0)	8(44.4)	4(80.0)	4(100)	NT	2(66.7)	1(100)
	I	1(3.3)	0.0	0.0	0.0		0.0	0.0
	R	14(46.7)	10(55.6)	1(20.0)	0.0		1(33.3)	0.0
Amikacin	S	27(90.0)	15(83.3)	5(100)	4(100)	7(100)	3(100)	1(100)
	I	1(3.3)	0.0	0.0	0.0	0.0	0.0	0.0
	R	2(6.7)	3(16.7)	0.0	0.0	0.0	0.0	0.0

NT* Not Tested S*susceptible I* intermediate R* resistance

5.7 Description of Total Antimicrobial Susceptibility Pattern

Gram-negative isolates were tested against multiple antimicrobial classes, and significant variability was observed in their susceptibility profiles. Carbapenems remained the most effective agents, with imipenem and meropenem showing high susceptibility rates of 78.5% and 80%, respectively, and only 20% resistance each. Aminoglycosides also performed well, particularly amikacin, with a susceptibility of 90.8% and a resistance of 7.7%, while gentamicin had a lower susceptibility rate of 58.6% and substantially higher resistance at 39.7%. Among β -lactams, piperacillin-tazobactam showed 100% susceptibility, whereas ampicillin and amoxicillin-clavulanate demonstrated moderate activity with 61.5% and 51.7% susceptibility, respectively, but also notable resistance patterns. Cephalosporins showed variable results. Cefepime and

ceftazidime showed moderate susceptibility (69.2% and 61.5%, respectively), while ceftriaxone had the highest resistance rate at 58.6%, indicating reduced effectiveness of extended-spectrum cephalosporins. Ciprofloxacin showed moderate susceptibility at 69.4%, and trimethoprim-sulfamethoxazole demonstrated lower activity with a high resistance rate of 44.8%. Minimal intermediate responses were observed across all drugs (0–15.4%) (Table 7). Similarly, among the tested antimicrobial agents for Gram-positive bacteria, Gentamicin showed the highest effectiveness with 80% susceptibility, followed by Ciprofloxacin with moderate activity (64% susceptibility). In contrast, Penicillin and Oxacillin demonstrated poor performance, with high resistance levels of 76.9% and 76%, respectively.

Table 7: Distribution of Antimicrobial Susceptibility Pattern Among Gram-Negative Bacterial Isolates among neonates with neonatal sepsis attending Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia, 2025.

<i>Drug name</i>	<i>pattern</i>	<i>Frequency</i>	<i>Percent (%)</i>
Ampicillin	<i>S</i>	8	61.5
	<i>I</i>	2	15.4
	<i>R</i>	3	23.1
Amoxa+clav	<i>S</i>	30	51.7
	<i>I</i>	1	1.7
	<i>R</i>	27	46.6
Ceftazidime	<i>S</i>	40	61.5
	<i>I</i>	7	10.8
	<i>R</i>	18	27.7
Ceftriaxone	<i>S</i>	22	37.9
	<i>I</i>	2	3.4
	<i>R</i>	34	58.6
Piperacillin-Tazobactam	<i>S</i>	7	100
	<i>I</i>	0	0
	<i>R</i>	0	0
Cefepime	<i>S</i>	45	69.2

	<i>I</i>	7	10.8
	<i>R</i>	13	20.0
Ciprofloxacin	<i>S</i>	43	69.4
	<i>I</i>	0	0
	<i>R</i>	19	30.6
Trimethoprim-Sulfamethoxazole	<i>S</i>	30	61.7
	<i>I</i>	2	3.4
	<i>R</i>	26	44.8
Imipenem	<i>S</i>	51	78.5
	<i>I</i>	1	1.5
	<i>R</i>	13	20
Meropenem	<i>S</i>	52	80
	<i>I</i>	0	0
	<i>R</i>	13	20
Gentamicin	<i>S</i>	34	58.6
	<i>I</i>	1	1.7
	<i>R</i>	23	39.7
Amikacin	<i>S</i>	59	90.8
	<i>I</i>	1	1.5
	<i>R</i>	5	7.7

*S**susceptible *I** intermediate *R** resistance

5.8. Clinical outcome of neonatal sepsis

Out of 251 neonates, 196 (78.1%) were discharged home. Early-onset neonatal sepsis was more common among those discharged, accounting for 115 (58.7%). The overall mortality was 34 (13.5%; 95% CI: 9.7–17.9), with most deaths, 27 (79.4%), occurring among early-onset cases. Among the neonates who died, 18 (52.9%) had blood culture-confirmed sepsis, with 16 (88.9%) caused by Gram-negative organisms and 6 (33.3%) attributable to *Klebsiella spp.* (Table 8).

Table 8. Distribution of clinical outcomes of neonatal sepsis by onset age, culture status, and bacterial profile among neonates attending Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia, 2025

Clinical outcome	Neonates n=251	Early-onset			Late-onset		
		Culture positive		Culture negative	Culture positive		Culture negative
		Gram negative	Gram-positive		Gram negative	Gram positive	
Sent home	196 (78.1%)	25(12.8%)	21(10.7%)	67(34.2%)	26(13.3%)	16(8.2%)	41(20.9%)
Died	34 (13.5%)	10(29.4%)	0(0.0%)	15(44.1%)	6(17.6%)	2(5.9%)	1(2.9%)
Leave against medical advice*	5 (2.0%)	0(0.0%)	1(20%)	1(20%)	0(0.0%)	2(40%)	1(20%)
Refer out to other health facilities	16(6.4)	0(0.0%)	2(12.5%)	7(43.8%)	1(6.3%)	2(2.5%)	4(25%)

Leave against medical advice* Neonatal sepsis patient who left the ward without completing the treatment due to assorted reasons, such as financial issues, or not satisfied with the treatment or hospital environment

5.9. Factors associated with the magnitude of Neonatal Sepsis

Bivariate analysis was performed for all independent variables, including socio-demographic characteristics (age of the neonate in days, residence, maternal age in years, mother's educational status, mother's occupation, and sex of the neonate), clinical-related factors (gestational age, APGAR score, neonatal resuscitation at birth, oxygen requirement, intranasal catheter insertion, nasogastric tube insertion, umbilical catheter insertion, urinary catheter insertion, presence of

congenital anomalies, and history of surgery), and obstetric factors (maternal parity, ANC follow-up, number of ANC visits, place and type of health institution for delivery, mode of delivery, birth attendant, history and duration of PROM, meconium-stained amniotic fluid, number of per vaginal examinations, maternal fever during labor, pregnancy-induced hypertension, antepartum hemorrhage, and history of UTI) (Supplementray Table 1). Four variables met the criteria for further multivariate analysis, with p-values < 0.05, indicating a significant association with the outcome variable (Table 9). These variables included socio-demographic factors, such as the neonate's age, as well as clinical factors. The clinical factors specifically encompassed Chef-compliant respiratory distress of the neonate and Chef-compliant co-symptoms, as well as the gestational age of the neonate. These variables fulfilled the requirement for further multivariate logistic analysis.

The multivariate logistic regression analysis identified several factors that were significantly linked to a higher likelihood of a neonatal sepsis diagnosis (Table 9). Specifically, a statistically significant increase in the chance of being diagnosed with neonatal sepsis was observed in participants with late neonatal age, neonates experiencing respiratory distress, and neonates exhibiting co-symptoms.

Using multivariate logistic regression, patients in late neonatal age had an AOR of 2.19 (95% CI: 1.25, 3.82), while neonates experiencing respiratory distress had an AOR of 3.44 (95% CI: 1.19, 9.93). This indicates more than three times the odds of presenting with other chief complaints and co-symptoms. The factor was associated with an AOR of 5.22 (95% CI: 1.69, 16.10) (Table 9).

Table 9: Factors associated with neonatal sepsis among neonates attending Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia, 2025

Explanatory Variables	Blood Culture		COR,95%(CI)	AOR,95%(CI)	p-value
	Positive	Negative			
Age of the neonate					
Early (0-3)	59	90	1	1	1
Late (4-29)	55	47	1.78 (1.07,2.97) *	2.19 (1.25,3.81) **	0.006
Gestational age					
Term	63	89	1	1	1
Preterm	49	40	1.73 (1.02,2.93) *	1.69 (0.95,3.02)	0.07
Post term	2	8	0.35 (0.073,1.79)	0.36 (0.69,1.93)	0.23
Chef compliant					
Jaundice	6	17	1	1	1
Respiratory distress	46	42	3.10(1.11,8.60) *	3.44 (1.19,9.93) **	0.02
Unable to suck	14	26	1.52 (0.49,4.74)	1.75 (0.53,5.70)	0.35
Fever	5	13	1.09(0.27,4.37)	1.10(0.26, 4.66)	0.89
Co symptoms	33	19	4.92(1.65,14.61) *	5.22(1.69,16.10) **	.004
Other	10	20	1.41(0.42,4.70)	1.41 (0.41,4.86)	0.58

* Significant association (p-value < 0.05 in bivariate)

**Significant association (p-value <0.05 in multivariate analysis)

Other*- Meconium aspiration syndrome, Preterm, Vomiting

6. Discussion

The overall objective of this study was to assess the magnitude of neonatal sepsis, bacteriologic etiology, antimicrobial susceptibility patterns, clinical outcome, and associated factors of neonatal sepsis at Menelik II Comprehensive Specialized Hospital. Our findings reveal a high sepsis prevalence of 45.4%, dominated by Gram-negative bacteria (59.6%) over Gram-positive bacteria. Clinical outcomes were concerning, with an overall mortality rate of 13.5%. Therapeutically, while Amikacin (90.8%) and Carbapenems remain effective, we observed significant resistance to commonly used first-line antibiotics, including Trimethoprim-Sulfamethoxazole and Ceftriaxone (58.6%). These antimicrobial resistance patterns, alongside significant associations with respiratory distress, necessitate a revision of the current empiric treatment to reduce mortality and better manage high-risk neonates.

This result aligns with findings from previous studies conducted in various countries. For instance, a report from two tertiary care hospitals in Yangon, Myanmar, indicated that 42% were bacteriologically confirmed (40). In Felege-Hiwot Referral Hospital, Bahir Dar, northwest Ethiopia, 41.3% were positive for blood culture (48). The findings are also comparable with results from a study at Wachemo University Comprehensive Specialized Hospital, Southern Ethiopia, which reported a neonatal sepsis prevalence of 39.5% (50). Notably, the prevalence of culture-confirmed neonatal sepsis observed in our study is higher than rates reported in other similar and comparable tertiary settings of low and middle-income countries. In Asia, for instance, lower positivity rates were recorded in Pakistan, 8.9% (39), Bhutan, 14% (1), Northern India, 15.3% (41), and Nepal, between 16.9% and 20.5% (15,18).

Even when compared to high-burden areas, our findings remain higher. Reports from Vadodara, India (42) and Lagos, Nigeria (44) showed rates of approximately 32%. Within Ethiopia, similar variability exists; while St. Paul's Hospital in Addis Ababa reported a 21% prevalence (53), other regions such as Northwest Ethiopia (48), Hawassa (13), and Tigray (14) reported rates ranging from 34% to 36.6%, all of which are lower than the current findings. A possible reason for the discrepancy may be the differences in study design, setting, and methodologies used. Unlike retrospective studies in Nepal and India (15,18,40) that rely on potentially incomplete records, our prospective design captured all eligible symptomatic cases. Furthermore, as a comprehensive specialized hospital, we likely manage a higher burden of critical cases compared to the general

population sampled in Pakistan(39). Diagnostic methodology also plays a role; for instance, the use of advanced MALDI-TOF spectrometry in Northern India (40,41) offers different sensitivity levels than the conventional culture and biochemical techniques used in our setting. Variations in the study population further contribute to the difference, as studies in Northwest Ethiopia and Nigeria (44,48) focused solely on early-onset sepsis, whereas our inclusion of both early- and late-onset cases results in a higher cumulative prevalence. The inclusion criteria regarding antibiotic history varied, as in the study in Hawassa, Ethiopia (13), which excluded neonates with recent antibiotic use, while our study screened all neonates presenting with signs of sepsis. Finally, operational challenges likely contributed to the higher prevalence. Specifically, significant overcrowding in our NICU, combined with variations in infection prevention and control (IPC) practices, created an environment prone to cross-contamination and increased rates of septicemia.

In contrast, higher prevalence rates of neonatal sepsis have been reported in other settings. In Dar es Salaam, Tanzania, 72% of samples were culture-positive for neonatal sepsis(46). Within Ethiopia, significantly higher rates were observed in Woldia and Dessie (79.4%)(49) and Shashemene Town (77.9%)(51). Similarly, hospitals in Northern Oromia, Ethiopia, reported a prevalence of 52.27% (52), all of which exceeded the findings of the current study. The difference between our results and those reported in studies with higher prevalence is likely attributable to differences in diagnostic techniques, case definitions, and study scope. For example, the study in Dar es Salaam, Tanzania (46) used automated blood culture systems, which have much higher sensitivity than the traditional biochemical methods used in our setting. Likewise, the significantly higher rates in Woldia and Dessie (79.4%) (49) and Shashemene (77.9%) (51) likely result from including clinical sepsis cases based on symptoms rather than only culture-confirmed cases, as well as relying on retrospective document reviews that combine all suspected cases. In contrast, the Northern Oromia study (52.27%) (52) was a multicenter survey covering a wide area; our study was limited to a single referral center with a smaller sample size.

Bacterial culture growth profile was observed in 45.4% of samples, with a notable predominance of Gram-negative bacteria (59.6%) over Gram-positive bacteria (40.4%). Notably, Gram-negative bacteria are predominant in late-onset sepsis, while Gram-positive bacteria are in early-onset sepsis. This finding is comparable with several studies conducted in various countries, including

Northern India (58.1%) (40), Pakistan (39), Kenya (57), as well as multiple Ethiopian settings, including Mekele (14), Northwest Ethiopia (30), Hawassa (13), and St. Paul's Hospital (79.8%) (53). In our study, *Klebsiella* spp. was the predominant pathogen causing neonatal sepsis (42.1%), a finding that comparable with reports from Northern India (58.1%) (40), Pakistan (36.5%) (39), Kenya (46.4%) (57), and Ethiopian hospitals such as Ayder Hospital in Mekele (62.4%) (14), Hawassa (42.65%) (13), Felege Hiwot Hospital (28.2%) (30), and St. Paul Hospital (44%) (53). Other Gram-negative bacteria, including *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterobacter* spp., were also isolated in this study. Our study showed a moderate proportion of Gram-positive isolates compared to findings from several other countries. Studies from Nepal, where *Staphylococcus aureus* and *CONS* were the leading pathogens (54), China, where *Staphylococcus epidermidis* accounted for 47.17% of Gram-positive isolates (56), Nigeria, which reported 84% Gram-positive bacteria with *S. aureus* at 44.6% (44), and Tanzania, where 81% of isolates were Gram-positive, predominantly CoNS (46), All documented a higher Gram-positive burden. In contrast, although *S. aureus* (21.9%) and *coagulase-negative Staphylococci* (17.5%) were the main Gram-positive isolates in our study, their overall proportion remained comparatively low.

The antimicrobial susceptibility testing in our study revealed that Gram-negative isolates showed high resistance to commonly used antibiotics, especially extended-spectrum cephalosporins such as ceftriaxone and cefazolin. This pattern agrees with the global problem of antimicrobial resistance, defined by the WHO as the ability of microorganisms to survive antimicrobial action up to the minimum inhibitory concentration (58). Similar findings have been reported in Nepal, where Gram-negative isolates showed high resistance to cefotaxime and other first-line drugs (15,54). A study from China also found reduced sensitivity to cephalosporins, while carbapenems such as meropenem and imipenem remained highly active against *E. coli* (56). Another study in Nepal reported that *Klebsiella* spp. had high resistance to cefotaxime, gentamicin, and ciprofloxacin but remained susceptible to carbapenems (18). Studies from Nigeria showed comparable resistance in *K. pneumoniae*, which was highly resistant to cephalosporins and ciprofloxacin, while still sensitive to meropenem and amikacin (44). Similar resistance patterns were reported in India, where most isolates were resistant to many commonly used antibiotics (41). Evidence from Tanzania also demonstrated high resistance to ampicillin and gentamicin, with high

levels of multidrug-resistant strains (46). In Kenya, *Klebsiella* spp. remained susceptible to carbapenems and amikacin, but resistant to ceftriaxone and gentamicin (57). The situation is similar in Ethiopia, where Gram-negative bacteria such as *K. pneumoniae*, *Enterobacter*, *Citrobacter*, and *E. coli* showed resistance ranging from 57% to 100% to cephalosporins and other first-line agents (13,14,30). Consistent with our results, studies from St. Paul's Hospital in Ethiopia also reported amikacin and ciprofloxacin as the most effective drugs, although the overall rate of multidrug resistance remained high (53).

In our study, Gram-positive isolates showed the highest susceptibility to Gentamicin (80%), followed by Ciprofloxacin (64%), while Penicillin and Oxacillin exhibited high resistance (87% and 76%). Similar findings were reported in Nepal, where Gram-positive isolates showed high susceptibility to gentamicin (15). In Nigeria, *S. aureus* showed substantial resistance to several antibiotics, including ciprofloxacin and gentamicin (44). Likewise, studies in Tanzania reported very high resistance to Penicillin and an MDR rate of 69% among Gram-positive organisms (46). Ethiopian data also confirmed high β -lactam resistance, including 100% oxacillin resistance and MRSA detection (13).

In the present study, we assessed the clinical outcome of clinically suspected as well as culture-proven neonatal sepsis. Among the neonates included in this study, 196 (78.1%) were discharged home, with early-onset neonatal sepsis (EONS) accounting for 115 (58.7%) of those discharged. The overall neonatal mortality rate was 13.5% (95% CI: 9.7–17.9), with most deaths occurring in early-onset cases (79.4%). This mortality rate is comparable to findings from Felege Hiwot Hospital (13.29%) (28) and Ayder Comprehensive Specialized Hospital (10.7%) (14), but lower than reports from Pakistan (20%) (39), Tbilisi, Georgia (30%) (20), and Tanzania (45.4% in culture-confirmed cases) (46). A global systematic review estimated neonatal sepsis mortality at 17.6% (24), indicating that the current figure falls within the global range. Furthermore, multicountry studies from low- and middle-income settings show that Gram-negative sepsis is a major contributor to neonatal mortality, with adverse outcomes linked to maternal illness, prematurity, perinatal complications, and overcrowded neonatal units, as reported in a WHO-supported multisite cohort (25). Globally, neonatal mortality remains a critical public health issue, with 2.3 million newborn deaths in 2022, accounting for 47% of under-five deaths (9),

emphasizing the urgent need to improve early diagnosis, timely treatment, and sepsis prevention strategies.

Concerning the associated factors, the current study found that late neonatal age increased the likelihood of sepsis by 2.19 times compared to early neonatal age, a finding consistent with reports from Nepal (15), Bhutan (1), and Ethiopia, including Shashemene (51). Neonates presenting with respiratory distress were 3.44 times more likely to develop sepsis than those with other chief complaints, similar to evidence from Nepal (15), North India (41), and Tanzania(46), where respiratory complications were important risk factors. Additionally, neonates with multiple co-symptoms were 5.22 times more likely to develop sepsis, aligning with studies from Pakistan (39), Nepal (15), Kenya (57), and Ethiopian settings (13,14,30,50,51,53), which consistently identified overlapping clinical features such as prematurity, low birth weight, maternal fever, and PROM as major contributors.

7. Strengths and limitations of the study

7.1 Strength

The strength of this study lies in its use of a cross-sectional design, which allows for the determination of the magnitude of the current research problem within the study area during a specific period. Additionally, the use of direct microbiological lab diagnosis, including culture, helps to accurately identify the magnitude of neonatal sepsis, the bacterial profile, and antimicrobial susceptibility. To our knowledge, this study was the first to report neonatal sepsis in the study setting.

7.2 limitation

The study included only clinically and laboratory-confirmed cases of neonatal sepsis; therefore, potential environmental sources of infection and circulating hospital-acquired pathogens were not evaluated. Additionally, the study was conducted in a single hospital, which may limit the generalizability of the findings. Furthermore, the microbiology laboratory contamination rate was not assessed.

8. Conclusion

The study revealed a high prevalence of neonatal sepsis (45.4%), with Gram-negative bacteria predominating over Gram-positive isolates. *Klebsiella* spp. was the leading cause of neonatal sepsis, followed by *Staphylococcus aureus*. Antibiotic susceptibility testing showed high effectiveness for Amikacin, Imipenem/Meropenem, and Gentamicin, whereas Ceftriaxone and Trimethoprim-Sulfamethoxazole had high resistance rates. Mortality from neonatal sepsis was significant, with most deaths occurring among early-onset infections. Late gestational age, respiratory distress, and comorbidities were associated with neonatal sepsis.

9. Recommendation

Based on the findings of this facility-based study, several actions are recommended to reduce the burden of neonatal sepsis and antimicrobial resistance. First, antibiotic stewardship programs should be strengthened to ensure rational antibiotic use and to minimize dependence on highly resistant agents such as Ampicillin and Ceftriaxone. Second, environmental hygiene should be improved through routine cleaning and, when necessary, scheduled terminal disinfection to reduce microbial contamination. Enhancing neonatal care infrastructure and strengthening the Infection Prevention and Control (IPC) and Antimicrobial Stewardship (AMS) committees are essential steps. All mothers and staff should consistently wear gowns and protective barriers inside the NICU, following clear entry and exit protocols. Maternal hygiene, sanitation, and proper hand-washing practices should be emphasized. Additionally, regular staff training on aseptic care, early sepsis recognition, and safe handling of neonates is strongly recommended. Future studies incorporating NICU environmental cultures and surface contamination assessments are needed to understand transmission pathways and reinforce infection prevention strategies.

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11. Annexes

Annex I. Participant's information sheet and assent form (English version)

Information sheet

Introduction: My name is -----, and I am presently participating as a data collector in a study entitled “Magnitude, Bacteriological Profile and Antimicrobial Susceptibility Patterns of Neonatal Sepsis in the NICU of Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia. Before your involvement, it is essential to comprehend and sign the agreement form. Following this, you will undergo an interview with the data collector. Your identity need not be disclosed, and all responses and findings will be treated confidentially through a coding system.

Purpose: The study aims to establish the prevalence of neonatal sepsis, determine the bacteriological profile, assess antibiotic susceptibility, and identify factors associated with neonatal sepsis at Menelik II Comprehensive Specialized Hospital.

Participant criteria: Your child is eligible for participation based on being admitted to the Neonatal Intensive Care Unit (NICU) during the study period.

Procedure and preparation:

For the success of this study, we require the active participation of your child. We kindly request your voluntary cooperation in involving your child in this study. If you choose to voluntarily enroll your child in this study, please signify your approval by signing below, indicating your agreement to participate in this study. Subsequently, you will be asked to provide socio-demographic and clinical details about your neonate and some information about you through a questionnaire. Additionally, a blood sample will be gathered for laboratory analysis, conducted by skilled professionals in the Neonatal Intensive Care Unit (NICU).

Confidentiality: The confidentiality of all personal information provided by your child and the data obtained from laboratory analysis will be strictly maintained.

Benefits:

If your child takes part in this research project, there might not be a direct advantage for your child. Nevertheless, their participation is likely to contribute significantly to achieving the research

objectives. Positive outcomes from laboratory analyses, particularly after generating bacterial susceptibility data, will be shared with your child's physician. In specific instances, this information may directly benefit patients by guiding treatment choices for those with appointments or returning for retreatment. It's crucial to understand that, aside from these particular circumstances, there may be no personal benefit to you or your child.

Right to refuse or withdraw:

Your child has the option to withdraw from or discontinue participation in the study at any time. Your child's involvement in this study is entirely voluntary, and you have the right to cease participation at any point. Additionally, you may choose not to respond to certain questions if they make your child uncomfortable, and there will be no repercussions, penalties, or loss of medical service benefits for your child as a result of such decisions.

Person to contact:

The research project will undergo review and receive approval from the Institutional Review Board (IRB) at Saint Paul's Hospital Millennium Medical College. If you have any questions, feel free to contact any of the following individuals, and you may inquire at any time.

1.Contact address of principal investigator: -

Mulugeta Girma (BSc,)

Tell: 0922866893

Email: mullergirma23@gmail.com

2.Adviser

Gadissa Bedada (MSc, PhD)

Tell 0911833184

Email: gadissa.bedada@sphmmmc.edu.et

3.Co Adviser Kasahun Gorms (BSc, MSc)

Tell 0925494531 Email: KasahunGorms63@gmail.com

Annex II Participants information sheet and assent form (Amharic version)

የጥናቱ ተሳታፊዎች መረጃ ቅጽ መግቢያ፡

ስሜ ----- አሁን ላይ ሙሉጌታ ግርማ በሚመራው የምርምር ጠናት በመረጃ ሰብሳቢነት በመሳተፍ ላይ ነኝ። ጥናታችን ነው። በዳግማዊ ምንጊክ ኮምፕሪኬንዲቭ ስፔሻላይዝድ ሆስፒታል የሚገኙ የጨቅላ ህጻናት ኢንፌክሽን አምጭ ባክቴሪያዎች ስርጭት እና ይዘት ለማወቅ የሚካሄድ ጥናት ነው። ከመሳተፍዎ በፊት የስምምነቱን ቅጽ መረዳት እና መፈረም አስፈላጊ ነው። ይህንን ተከትሎ ከመረጃ ሰብሳቢው ጋር ቃለ መጠይቅ ያደርጋሉ። ማንነትዎ መገለጽ የለበትም፣ እና ሁሉም ምላሾች እና ግኝቶች በሚስጥር የሚስተናገዱት በኮዲንግ ሲስተም

የጥናቱ አላማ፡- ዓላማው፡ ጥናቱ በዳግማዊ ምንጊክ ኮምፕሪኬንዲቭ ስፔሻላይዝድ ሆስፒታል የጨቅላ ህጻናት አምጭ ባክቴሪያዎች ስርጭትን ለማወቅ፣ ምን ያህል በባክቴሪያው ይደካል እና በባክቴሪያው ለመያዝ ምክንያት የሆኑ ነገሮችን ለማወቅ፣ መድሃኒት የተቋቋሙ ባክቴሪያ የተባለትን ረቂቅ ህዋሳት ስርጭታቸውን ለማጥናትና ለህዋሳቱ ተመራጭ የሆኑትን መድሃኒቶች ለመምረጥ ነው።

የተሳትፎ መመዘኛዎች፡ ልጅዎ በጥናቱ ወቅት ወደ የጨቅላ ህጻናት እንክብካቤ ክፍል (NICU) በመግባቱ መሰረት ለመሳተፍ ብቁ ነው።

ሂደት እና ዝግጅት፡

ለዚህ ጥናት ስኬት የልጅዎን ተሳትፎ እንፈልጋለን። በዚህ ጥናት ውስጥ ልጅዎን ለማሳተፍ የበጎ ፈቃድ ትብብርዎን በአክብሮት እንጠይቃለን። በዚህ ጥናት ውስጥ ልጅዎን በፈቃደኝነት ለማስመዝገብ ከመረጡ፣ እባክዎን ከዚህ በታች በመፈረም ማጽደቂያዎን ያመልክቱ። በዚህ ጥናት ላይ ለመሳተፍ ያለዎትን ስምምነት ያሳያል። በመቀጠል፣ ስለ እርስዎ አራስ ልጅ እና ስለእርስዎ አንዳንድ መረጃዎችን በመጠይቅ በኩል ማህበረሰባዊ-ሕዝብ እና ክሊኒካዊ ዝርዝሮችን እንዲያቀርቡ ይጠየቃሉ። በተጨማሪም፣ በአራስ ሕፃናት ከፍተኛ እንክብካቤ ክፍል (NICU) ውስጥ በተካኑ ባለሙያዎች የሚመራ የደም ናሙና ለላቦራቶሪ ምርመራ ይሰበሰባል።

ሚስጥራዊነት፡-

የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኋላ ባለት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናሉ።

ጥቅሞች፡-

ልጅዎ በዚህ የምርምር ፕሮጀክት ውስጥ ከተሳተፈ፣ ለልጅዎ ቀጥተኛ ጥቅም ላይኖረው ይችላል። ይሁን እንጂ የእነርሱ ተሳትፎ የምርምር አላማዎችን ለማሳካት ከፍተኛ አስተዋፅኦ ይኖረዋል። የላብራቶሪ ትንታኔዎች አወንታዊ ውጤቶች፣ በተለይም የባክቴሪያ ተጋላጭነት መረጃን ካመነጩ በኋላ፣ ከልጅዎ ሐኪም ጋር ይጋራሉ። ከእነዚህ ልዩ ሁኔታዎች በተጨማሪ ለእርስዎ ወይም ለልጅዎ ምንም አይነት የግል ጥቅም ላይኖረው እንደሚችል መረዳት በጣም አስፈላጊ ነው።

እምቢ የማለት ወይም የመውጣት መብት፡-

ልጅዎ በማንኛውም ጊዜ በጥናቱ መሳተፍን የማቋረጥ ወይም የማቋረጥ አማራጭ አለው። በዚህ ጥናት ውስጥ ያላቸው ተሳትፎ ሙሉ በሙሉ በፈቃደኝነት ላይ የተመሰረተ ነው፣ እና በማንኛውም ጊዜ የእነሱን ተሳትፎ የማቆም መብት አለዎት። በተጨማሪም፣ ለአንዳንድ ጥያቄዎች ልጅዎን የማይመቹ ከሆነ ምላሽ ላለመስጠት ሊመርጡ ይችላሉ፣ እና እንደዚህ ባሉ ውሳኔዎች ምክንያት ለልጅዎ ምንም አይነት መዘዝ፣ ቅጣት ወይም የህክምና አገልግሎት ጥቅማጥቅሞችን አያጡም።

ማግኘት የሚችሉት ሰው፡-

የምርምር ፕሮጀክቱ ተገምግሞ ከተቋማዊ ግምገማ ቦርድ (IRB) በሴንት ጳውሎስ ሆስፒታል ሚሊኒየም ሜዲካል ተቀባይነት አግኝቷል። ማንኛውም ጥያቄ ቢኖርዎት ከሚከተሉት ውስጥ አንዱን ለማነጋገር ነፃነት ይሰማዎ እና በማንኛውም ጊዜ ሊጠይቁ ይችላሉ።

1. የዋናው አጥኝ አድራሻ፡-

ሙሉጌታ ግርማ (ቢኤስሲ፣)

0922866893

ኢሜል፡ mullergirma23@gmail.com

2.የአጥኝዉ አማካሪዎች

ጋዲሳ በዳዳ (ኤም.ኤስ.ሲ፣ ፒኤችዲ)

0911833184

ኢሜል: gadissa.bedada@sphmmc.edu.et

3.ካሳሁን ጎርምስ (ቢ.ኤስ.ሲ፣ ኤም.ኤስ.ሲ)

0925494531

ኢሜል: kasahungorms63@gmail.com

Annex III. Assent from the English version

As the parent of the participant, I have been fully informed about the purpose of the study titled "Magnitude, Bacteriological Profile and Antimicrobial Susceptibility Patterns of Neonatal Sepsis in the NICU of Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia." My child has the autonomy to decide whether to participate in the study. I understand that the procedures involve, specifically sample collections, may cause little discomfort but pose no harm. The results will be anonymized through coding for confidentiality. I acknowledge that there may be no direct personal benefit to me, except for the clinical service derived from the results. I've had the opportunity to ask questions and receive satisfactory answers. I understand that participation, for both me and my child, is entirely voluntary, and I may decline without consequences. My child's participation is contingent on their approval. By signing below, I provide consent for my child to participate in this research study.

Participant Parent Signature: _____ Date: _____

Name of data collector _____ signature _____ date _____

Annex IV. Assent form (Amharic version)

የድጋፍ ቅጽ

የተሳታፊው ወላጅ እንደመሆኑ፣ በዳግማዊ ምኒልክ አጠቃላይ ስፔሻላይዝድ ሆስፒታል “Bacteriological Profile of Neonatal Sepsis፣ Antibiotic Susceptibility, and Associated Risk Factors” በሚል ርዕስ የጥናቱ ዓላማ ሙሉ በሙሉ ተረድቻለሁ። ልጄ በጥናቱ ለመሳተፍ የመወሰን የራስ ገዝ አስተዳደር አለው። የተካተቱት ሂደቶች በተለይም የናሙና ስብስቦች ትንሽ ምች ሊያስከትሉ እንደሚችሉ ተረድቻለሁ ነገር ግን ምንም ጉዳት የላቸውም። ውጤቶቹ ምስጢራዊነትን በኩድ በማድረግ ስማቸው እንዲገለጽ ይደረጋል። ከውጤቶቹ ከሚገኘው ክሊኒካዊ አገልግሎት በስተቀር ለእኔ ምንም አይነት ቀጥተኛ የግል ጥቅም ላይኖረው እንደሚችል አምናለሁ። ጥያቄዎችን የመጠየቅ እድል አግኝቻለሁ እናም አጥጋቢ መልሶች አግኝቻለሁ። ለእኔም ሆነ ለልጄ ተሳትፎ ሙሉ በሙሉ በፈቃደኝነት እንደሆነ ተረድቻለሁ፣ እናም ያለምንም መዘዝ እምቢ ማለት እችላለሁ። የልጄ ተሳትፎ በእነሱ ፈቃድ ላይ የተመሰረተ ነው። ከዚህ በታች በመፈረም ልጄ በዚህ የምርምር ጥናት ላይ እንዲሳተፍ ፍቃድ ሰጥቻለሁ።

የተሳታፊ ወላጅ ፊርማ: _____ ቀን: _____

የመረጃ ሰብሳቢው ስም _____ ፊርማ _____ ቀን _____

Annex V. Questionnaire

Thank you for consenting to participate in this interview. The purpose of this study is to evaluate the occurrence of neonatal sepsis, analyze the bacteriological profile, assess antibiotic susceptibility, and identify factors associated with the neonatal sepsis issue in our country. Above all, our goal is to gain a comprehensive understanding of the situation. I sincerely appreciate your patience throughout this process.

Part 1: Socio-demographic information

Identification code -----

Serial no	Questionnaires	Coding categories

1.1	Age of the neonate	Age in days
1.2	Age of mothers	Age in years
1.3	Sex of neonates	Male
		Female
1.4	Mothers' occupation	1 Merchant 2 Civil servants 3 Private employed 4 students 5 house wives 6 jobless 7 others specify
1.5	Residence	Urban Rural
1.6	Mothers' Educational status	1 unable to read and write 2 grade 1-8 3 grade 9-12 4 having certificate and above

Part 2 clinical related factor

questionnaire related to clinical factors

Serial No	Questionnaires	Coding categories
2.1	Gestational age at birth:	
2.2	APGAR score at birth:	
2.3	Was the neonate resuscitated at birth?	1. Yes 2. No
2.4	Oxygen requirement:	1. Yes 2. No
2.5	Was an intranasal catheter used?	1. Yes 2. No
2.6	Was a nasogastric tube inserted?	1. Yes 2. No
2.7	Was an umbilical catheter inserted?	1. Yes 2. No
2.8	Was a urinary catheter inserted?	1. Yes 2. No
2.9	Are any congenital anomalies present?	1. Yes 2. No

2.10	Has the neonate undergone any type of surgery?	1.Yes 2.No
------	--	-------------------

Part 3 Obstetric related factor

Serial No	Questionnaires	Coding categories
3.1	Maternal parity	Number of previous deliveries
3.2	Maternal ANC (Antenatal Care) follow-up	Yes/No
3.3	Number of ANC follow-Up	
3.4	Place of birth	Health institution Home Other
3.5	Mode of delivery	Vaginal Cesarean Instrumental
3.6	Who attends delivery	Doctor Nurse Midwife Family member Other
3.7	History of PROM	Yes/No

3.8	If yes Duration of PROM	in hours
3.9	Presence of Meconium-stained amniotic fluid	Yes/No
3.10	Number of prevaginal examination	1.No 2.Once 3. 2-3 times 4. More than 3 times
3.11	History of fever during labor	Yes/No
3.12	Pregnancy-induced hypertension	Yes/No
3.13	Bleeding during pregnancy/APH	Yes/No
3.14	History of UTI/STI	Yes/No

4 Neonatal sepsis related question

Q.no	Questionnaire	Categories/alternative
4.1	blood culture result	1. Positive 2. Negative

5. Bacteriological profile-related question

Q.no	Questionnaire	Categories/alternative
5.1	identified bacteria	1. Gram -Positive Specific bacteria----- 2. Gram-Negative Specific bacteria-----

		Others----
--	--	------------

6. Antibiotic susceptibility related question

Q.no	Questionnaire	Categories/alternative
6.1	Antibiotic susceptibility	1.name of the drug under resistance----- 2.name of the drug under intermediate ----- 3.name of the drug under susceptible -----

መጠይቅ (Amharic version questionnaire)

በዚህ ቃለ መጠይቅ ላይ ለመሳተፍ ፈቃደኛ ስለሆኑ እናመሰግናለን። የዚህ ጥናት ዓላማ የአራስ ሴፕሲስን ክስተት ለመገምገም፣ የባክቴሪያውን ፕሮፋይል ለመተንተን፣ የአንቲባዮቲክን ተጋላጭነት ለመገምገም እና በአገራችን ውስጥ ከአራስ ሕፃናት ሴፕሲስ ችግር ጋር ተያያዥነት ያላቸውን ምክንያቶች መለየት ነው። ከሁሉም በላይ ግባችን ስለ ሁኔታው አጠቃላይ ግንዛቤ ማግኘት ነው። በዚህ ሂደት ውስጥ ትዕግስትዎን ከልብ አደንቃለሁ።

ክፍል 1: የግለሰብ ማህበራዊ እና ኢኮኖሚያዊ መረጃ

የተሳታፊው መለያ ቁጥር: -----

ተከታታይ ቁጥሮች	መጠይቆች	የኮድ ምድቦች
1.1	የህጻኑ እድሜ	በቀን
1.2	የህጻኑ እናት እድሜ	በአመት
1.3	የህጻኑ ጾታ	ወንድ
		ሴት
1.4	የእናት ስራ	1 ነጋዴ 2 የመንግስት ሰራተኛ 3 የግል 4 ተማሪ 5 የቤት እመቤት 6 ስራ አጥ 7 ሌላ ካለ ይጠቀስ
1.5	የመኖሪያ አድራሻ	ከተማ ገጠር
1.6	የእናት የትምህርት ደረጃ	1. መጻፍና ማንበብ የማያችሉ 2. የመጀመሪያ ደረጃ ት/ት 3. የሁለተኛ ደረጃ ት/ት 4. ኮሌጅ እና ከዚያ በሊይ

ከክሊኒካዊ ሁኔታዎች ጋር የተያያዘ መጠይቅ

ተከታታይ ቁጥሮች	መጠይቅ	የኮድ ምድቦች
2.1	ህጻኑ በስንት ሳምንቱ ተወለደ	
2.2	ሲወለድ የነበረው 1. እንቅስቃሴ 2. የልብ ምቹ 3. ለቅጽበት ያለው ምላሽ ምሳሌ እገሩን ሲኮረኩሩት የሚሰበስብ ከሆነ 4. አጅ እና አግሩን ያንቀሳቅሳል 5. በደንብ አልቅሶ ነበር.	
2.3	ሲወለድ በራሱ መተንፈስ ይችል ነበር	1.አዎ 1. 2.አይ
2.4	አክስጅን አስፈልጎት ነበር	
2.5	በአፍንጫ በሚገባ የአክስጅን ቱቦ ተጠቅሞ ነበር	1.አዎ 2.አይ
2.6	ምግብ እና መድሃኒት በአፍንጫ በኩል በ ቱቦ ተሰጥቶ ነበር	1.አዎ

		2.አይ
2.7	በእንብርት የሚገባ ቱቦ ተጠቅሞ ነበር	1.አዎ 2.አይ
2.8	የሽንት ቱቦ ገብቶ ነበር	1.አዎ 2.አይ
2.9	በተፈጥሮ የመጡህ ያልተለመዱ ችግሮች አሉ?	1.አዎ 2.አይ
2.10	ህጻኑ ማንኛውንም ዓይነት ቀዶ ጥገና አድርጓል	1.አዎ 2.አይ

ከወሊድ ሁኔታዎች ጋር የተያያዘ መጠይቅ

ተከታታይ ቁጥሮች	መጠይቅ	የኮድ ምድቦች
3.1	ከዚህ ቀደም የተወለዱ ልጆች	የተወለዱ ልጆች ብዛት-----
3.2	ቅድመ ወሊድ እንክብካቤ ክትትል ነበር	1.አዎ 2.አይ
3.3	ቅድመ ወሊድ እንክብካቤ ክትትል በዛት	

3.4	የትውልድ ቦታ	1. በጤና ተቋም 2. ቤት 3. ሌላ
3.5	የተወለደበት መንገድ	1. በም 2. በቀዶ ጥገና 3. በመሳሪያ
3.6	ማን አዋለደው	1. ሀኪም 2. አዋላጅ ነርስ 3. ነርስ 4. ቤተሰብ 5. ሌላ
3.7	የእንሽርት ውሃ ቀድሞ የመፍሰስ ችግር ገጥሞ ነበር	1.አዎ 2.አይ
3.8	የእንሽርት ውሃ ቀድሞ የመፍሰስ ምንያክል ቆየ	በ ሠዓት
3.9	የእንሽርት ውሃ የደፈረሰ ነበር	1. ደረጃ 1 2. ደረጃ 2 3. ደረጃ 3

3.10	ሁለት ጣትን በመጠቀም የሚደረግ የማህጸን ምርመራ ስንት ጊዜ ተሰጠ	
3.11	በወሊድ ጊዜ ትኩሳት ነበር	1.አዎ 2.አይ
3.12	በእርግዝና ምክንያት የተከሰት የደም ግፊት ነበር	1.አዎ 2.አይ
3.13	በማህጸን ደም መፍሰስ ከ28 ሳምንት ቡኃላ እና ልጅ ከ መወለዱ በፊት አጋጥሞ ነበር	1.አዎ 2.አይ
3.14	ከዚ ቀደም የሽንት ባንባ ኢንፌክሽን ወይም የአባላዘር ኢንፌክሽን ነበር	1.አዎ 2.አይ

Supplimentary table 1: Factors associated with neonatal sepsis among neonates attending Menelik II Comprehensive Specialized Hospital, Addis Ababa, Ethiopia, 2025

Variables	Category	Neonatal sepsis (251)		COR,95%(CI)	AOR,95%(CI)	p-value
		NO	Yes (114)			
Age of the neonate	Early	90	59	1	1	0.006
	Late	47	55	1.78(1.07,2.97)*	2.19(1.25,3.81)**	
Sex of Neonates	Female	54	38	1	1	
	Male	83	76	1.301(0.77,2.186)		
Age of mothers	<20	17	17	0.70(0.34,1.42)		
	20-24	30	21	1.00(0.44,2.23)		

	25-29	40	40	1	1	
	30-34	28	23	0.82(0.40,1.66)		
	35-39	18	10	0.55(0.22,1.35)		
	>40	4	3	0.75(.15,3.56)		
Mother's Educational Status	Unable to read and write	26	16	0.95(0.43,2.08)		
	Grade 1-8	39	41	1.70(0.79,3.65)		
	Grade 9-12	26	30	1.87(0.83,4.23)		
	College and above	46	27	1	1	
Mothers' occupation	Housewives	72	68	1.81(0.83,3.92)		
	Civil servants	23	12	1	1	
	Private employed	25	19	1.45(0.58,3.64)		
	Merchant	3	2	1.27(0.18,8.72)		
	Students	14	13	1.78(0.63,4.97)		
Residence	Rural	18	12	1.28(0.59,2.79)		
	Urban	119	102	1	1	
Maternal parity	Primipara (1)	70	65	1	1	
	Multipara (2-4)	63	48	8.21(0.49,1.36)		
	Grand multipara (>4)	4	1	0.26(0.02,2.47)		
Maternal ANC follow-up	No	7	7	0.82(0.28,2.42)		
	Yes	137	107	1	1	
Place of birth	Health institution	130	109	1	1	
	Home	7	5	0.85(0.26,2.76)		
Mode of delivery	Cesarean	56	43	0.85(0.51,1.42)		
	Instrumental	3	1	0.37(0.03,3.65)		

	Spontaneous vaginal delivery	78	70	1	1	
Who attends the delivery?	Skilled birth attendant	131	108	1	1	
	Unskilled birth attendant	6	6	1.21(0.38,3.86)		
History of PROM	No	116	98	1	1	
	Yes	21	16	0.90(0.44,1.82)		
Duration of PROM in hours	<18	13	10	1	1	
	≥18	8	6	0.97(0.25,3.73)		
Presence of Meconium-stained amniotic fluid	No	116	103	1	1	
	Yes	21	11	0.59(0.27,1.28)		
Number of prevaginal examination	No	18	21	1	1	
	Once	7	7	0.85(0.25,2.91)		
	2-3 times	19	16	0.72(0.28,1.80)		
	More than 3 times	93	70	0.64(0.32,1.30)		
History of fever during labor	No	114	101	1	1	
	Yes	23	13	0.63(0.30,1.32)		
Pregnancy-induced hypertension	No	120	102	1	1	
	Yes	17	12	0.83(0.37,1.82)		
History of UTI	No	127	10	1	1	
	Yes	104	10	1.22(0.49,3.04)		
Chef compliant	Jaundice	17	6	1	1	
	Respiratory distress	42	46	3.10(1.11,8.60)*	3.44(1.19,9.93)**	0.02
	Unable to suck	26	14	1.52(0.40,4.74)	1.75(0.53,5.70)	0.35
	Fever	13	5	1.09(0.27,4.37)	1.10(0.26,4.66)	0.89

	Co-symptom	19	33	4.92(1.65,14.61)*	5.22(1.69,16.10)**	0.004
	Other	20	10	1.41(0.42,4.70)	1.41(0.41,4.86)	0.58
Gestational age	Term	89	63	1	1	
	Preterm	40	49	1.73(1.02,2.93)	1.69(0.953,02)	0.07
	Post term	8	2	0.35(0.07,1.71)	0.36(0.69,1.93)	0.23
APGAR score	Low (0-6)	48	42	1.08(0.64,1.81)		
	Normal (7-10)	89	72	1	1	
Neonatal resuscitation at birth	No	115	98	1	1	
	Yes	22	16	0.853(0.42,1.71)		
Oxygen requirement	No	44	30	1	1	
	Yes	93	84	1.32(0.76,2.29)		
Congenital anomalies present	No	123	101	1	1	
	Yes	14	13	1.13(0.50,2.51)		

* Significant association (p-value < 0.05 in bivariate)

**Significant association (p-value <0.05 in multivariate analysis)

Other*- Meconium aspiration syndrome, Preterm, Vomiting

Dedication

I dedicate this thesis to my family, friends, and school principals. I want to give special recognition to the staff of the Menelik II Comprehensive Specialized Hospital Laboratory and the Microbiology Department for their support, including providing laboratory facilities, reagents, and materials. Their encouragement and dedication to excellence have greatly influenced me. I am especially grateful to my mother, Tejinesh Tufa, whose strength and resilience have inspired me to stay strong. My brother, Tesfamicheal Mekonnen, and my sister, Yenework Girma, have been tremendous sources of support and have always stood by me. I also sincerely thank my friends for their unwavering support and encouragement throughout this journey.

