

St. Paul's Hospital Millennium Medical College



Maternal and Neonatal Colonization of ESBL-Producing and Carbapenem-Resistant Enterobacterales in St. Paul's Hospital Millennium Medical College.

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Addis Ababa, Ethiopia

November, 2025

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Approval by the board of examination

As a member of the examining board of the final M.Sc. open defense, we hereby certify that we have read and evaluated the thesis entitled “ Maternal and Neonatal Colonization of ESBL-Producing and Carbapenem-Resistant Enterobacterales in St. Paul’s Hospital Millennium Medical College: A cross-sectional study” presented by Ibrahim Yesuf. The thesis has been examined and approved in its present form as fulfilling the requirements for the degree of Master of Science in Medical Microbiology.

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Declaration

I, the undersigned, declare that the thesis entitled “Maternal and Neonatal Colonization of ESBL-Producing and Carbapenem-Resistant Enterobacterales in St. Paul’s Hospital Millennium Medical College: A cross-sectional study.”Submitted for the degree of Master of Science (M.Sc.) 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

Introduction: Antibiotic-resistant Gram-negative bacteria (ABR-GNB), such as ESBL-producing and carbapenem-resistant Enterobacterales (CRE), pose a serious public health threat, particularly to pregnant women and their newborns by causing life threatening infections.

Objective: To assess maternal and neonatal colonization with ESBL-producing and carbapenem-resistant Enterobacterales (CRE) and associated factors at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.

Method: A cross-sectional study was conducted from April to August 2024, among 295 laboring mothers and their newborns by using consecutive sampling. Structured questionnaires were used to collect clinical and demographic data. Two vaginal and rectal swabs were collected from mothers and external ear swabs from newborns immediately after birth. Samples were inoculated on chromogenic agars and growth was characterized. Biochemical and Antimicrobial susceptibility testing were performed. Data were analyzed using SPSS v27. Logistic regression was used to identify associated factors and P-values < 0.05 considered as statistically significant.

Results: Maternal colonization was 23.7% for ESBL-producing Enterobacterales and 1.4% for CRE. Among the 295 newborns, 1.7% (5/295) were colonized with ESBL-producing Enterobacterales and 1.0% (3/295) with CRE. Vertical transmission was suggested, as neonates born to colonized mothers were 13 times more likely to be colonized ($P = 0.014$). *Escherichia coli* (88.4%) were the most frequent isolate. Highest resistance was seen to β -lactam antibiotics: ampicillin (100%), ceftriaxone (98.9%), and cefepime (80.0%). Resistance to carbapenems was lower (7.0–9.5%). Amikacin (96.8%) and chloramphenicol (91.6%) were the most effective drugs. Multidrug resistance was observed in 80.0% of isolates. Key predictors of maternal colonization included lower education level (AOR = 3.527; 95% CI: 1.235–10.074; $P = 0.019$) and fewer antenatal care visits (AOR = 0.804; 95% CI: 0.658–0.982; $P = 0.033$).

Conclusion and Recommendation: There is a high rate of maternal colonization with ESBL-producing Enterobacterales and concerning levels of neonatal colonization and multidrug resistance. Strengthening antenatal care, improving maternal health education, and reinforcing infection prevention strategies are crucial, particularly to protect high-risk newborns. Surveillance and stewardship programs should be prioritized in maternity care settings.

Keywords: ESBL-producing Enterobacterales, Carbapenem-resistant Enterobacterales, Maternal colonization, Neonatal colonization, Vertical transmission, Multidrug resistance, Ethiopia.

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ACRONYMS AND ABBREVIATIONS

ABR-GNB – Antibiotic Resistant Gram Negative Bacteria

APGAR – Appearance, Pulse, Grimace, Activity, and Respiration

ARB – Antibiotic Resistant Bacteria

CDC – Centers for Disease Control and Prevention

CLSI – Clinical and Laboratory Standards Institute

CRE – Carbapenem-resistant Enterobacterales

DR-GNB – Drug Resistant Gram-Negative Bacteria

EOD – Early-Onset Disease

ESBL-PE – Extended Spectrum Beta-Lactamase Producing Enterobacterales

GBS – Group B *Streptococcus*

IRB – Institutional Review Board

LOD – Late-Onset Disease

MDR-GN – Multi Drug Resistant Gram Negatives

NICU – Neonatal Intensive Care Unit

NMR – Neonatal Mortality Rate

PI – Primary Investigator

PROM – Premature Rupture of Membranes

SPHMMC – St. Paul’s Hospital Millennium Medical College

SPSS – Statistical Package for the Social Sciences

SVD – Spontaneous Vaginal Delivery

UNICEF – United Nations International Children's Emergency Fund

WHO – World Health Organization

1. INTRODUCTION

1.1 Background

Antibiotics are one of the most important classes of medications and a major medical innovation of the 20th century (1). Since their advent, millions of lives have been saved from bacterial infections, fundamentally altering the paradigm of treatment. In many impoverished and developing nations, antibiotics have been used for decades not only for medical purposes but also for various other functions, such as animal husbandry and production, as preventative measures (2).

However, the overuse and misuse of antibiotics have led to a significant rise in antibiotic resistance. This resistance occurs when microorganisms, such as bacteria, develop the ability to survive and proliferate in the presence of medications intended to eradicate them (3). As a result, antibiotics and other antimicrobial drugs lose their effectiveness, making infections harder or even impossible to treat. This increases the risk of disease transmission, serious illness, disability, and death (4).

Antibiotic-resistant bacteria (ARB) are constantly evolving and developing new resistance mechanisms to survive the effects of antibiotics. For example, bacteria like Extended Spectrum Beta-Lactamase producing Enterobacterales (ESBL-PE) produce an enzyme called extended-spectrum beta-lactamases (ESBLs), which break down and destroy commonly used antibiotics such as penicillins and cephalosporins, rendering them ineffective (5). ESBLs can hydrolyze most β -lactam antibiotics, with the exception of carbapenems (6). Alarming, some bacteria have started to resist even carbapenem antibiotics, the last line of defense against multidrug-resistant infections, by producing enzymes known as carbapenemases (7,8). These organisms are among the pathogens categorized as critical on the World Health Organization's (WHO) list of priority antibiotic-resistant pathogens (9).

The prevalence of ARB in the healthy population has garnered increasing attention in recent years. Researchers are focusing on identifying risk factors for both ARB carriage and subsequent infections, as well as understanding the long-term effects of carriage. Pregnant women are a key group under investigation in this context (10). A major concern is the potential for these bacteria

to be transmitted to newborns during vaginal delivery, potentially leading to serious infections in infants (11).

Antibiotic resistance is especially concerning in newborns, who are often infected with antibiotic-resistant Gram-negative bacteria (ABR-GNB) such as ESBL-PE. These infections are associated with increased risk of intensive care unit (ICU) admission and mortality (12). In recent years, the frequency of both early- and late-onset sepsis caused by these bacteria in newborns has risen significantly (13). The impact of these infections is especially severe in low-resource settings, where access to advanced neonatal care and effective antibiotics is limited. As a result, there are notable regional disparities in neonatal survival rates. In 2025, Sub-Saharan Africa recorded the highest neonatal mortality rate (NMR), with 42.7 deaths per 1,000 live births, and babies born in this region faced a tenfold higher risk of dying in the first month of life compared to those born in high-income countries (14–16).

Beyond the health impact, ABR imposes a substantial burden on health systems and national economies. In 2019, bacterial ABR was responsible for an estimated 4.95 million deaths worldwide, including 1.27 million directly attributable deaths (17). By the mid-21st century, experts predict this number could escalate to 10 million deaths annually. Moreover, ABR leads to significant financial costs. The World Bank estimates that by 2050, ABR could result in US\$ 1 trillion in additional healthcare expenses and annual losses in global GDP ranging from US\$ 1 trillion to US\$ 3.4 trillion by 2030 (18).

1.2 Statement of the Problem

Antibiotic resistance, particularly among Gram-negative bacteria (GNB), represents a significant global public health challenge (19). Drug resistant organisms, including ESBL-PE and CRE, have emerged as some of the most prevalent and clinically concerning pathogens worldwide. Infections caused by these bacteria often lead to severe clinical outcomes and place a substantial burden on healthcare systems (20).

According to the U.S. Centers for Disease Control and Prevention (CDC), ESBL-PE are responsible for over 197,400 infections and 9,100 deaths annually in the United States, incurring more than \$1.2 billion in healthcare costs. Similarly, CRE cause an estimated 13,100 infections and 1,100 deaths per year, with associated costs of approximately \$130 million (21).

Globally, there are more than 2.4 million newborn deaths annually, with low- and middle-income countries accounting for the majority of these deaths (22). In Ethiopia, neonatal mortality remains high at an estimated 27.2 deaths per 1,000 live births as of 2025, with neonatal sepsis accounting for approximately 20% of these deaths (16). Up to two-thirds of neonatal sepsis cases in Ethiopia are attributed to GNB (23). Over the past two decades, certain MDR-GN bacteria have become much more common in patient care environments, such as pediatric and neonatal units (24). The CDC's 2019 report highlighted MDR-GNB infections, especially those caused by ESBL-PE and CRE, as serious and urgent threats to public health (21).

Colonization of the vaginal tract by such resistant organisms is associated with increased perinatal mortality, a two-fold higher risk of preterm birth before 37 weeks of gestation, and elevated rates of postpartum maternal infections and late-term miscarriage (25,26). Consequently, maternal colonization by ABR bacteria such as ESBL-PE and CRE may lead to serious neonatal outcomes, including sepsis and other complications, particularly during the first month of life, a critical period for infant survival. In some studies approximately 80% of early-onset neonatal sepsis (EOS) cases are linked to vaginal colonization with pathogenic MDR bacteria (27).

Many studies have been conducted on this phenomenon in affluent environments and various ESBL-PE colonization rates among pregnant mothers (0.1% to 15%), new borns (0.04% to 14.8%) and vertical transmission (14% to 100%) in different countries were reported (10). As reported in different studies the incidence of ABR bacteria colonization among mothers in Africa reach from 9.7% upto 46.6% (14,28,29). However, in low- and middle-income settings including Ethiopia, virtually little is known about maternal colonization and the subsequent transmission to the neonate. There is also a shortage of up-to-date data on ABR (28,29). This phenomenon often occurs in settings where infant mortality is high, primarily due to infection. For example, the world's highest rates of newborn death (43%) are found in Sub-Saharan Africa (16,29,30).

Therefore, this study assessed the colonization of ESBL-PE and CRE among laboring mothers and their newborns. It also evaluated the phenotypic similarities between maternal and neonatal isolates and identified factors associated with bacterial colonization and vertical transmission from mother to newborn.

1.3 Rationale and significance of the Study

Treatment options for neonatal ABR-GNB, especially CRE, infections are limited, and efficacy and safety of newer antibiotics are currently extrapolated from adult data. Accordingly, surveillance and prevention of ABR-GNB infections, specifically ESBL-PE and CRE, is a research priority in healthcare-associated infections and antimicrobial stewardship (31). Studies are needed to understand the relative impacts of colonization and infection with ESBL-PE and CRE on neonatal morbidity, mortality, and longer-term outcomes compared to infections with less resistant organisms (32). Additionally, knowledge of the colonization burden in pregnant women and the extent of maternal–neonatal transmission are important for preventive strategies, including active surveillance and isolation of colonized patients (33). Furthermore, given that antimicrobial resistance is a dynamic and rapidly evolving threat, continuous monitoring and timely updates of resistance trends are essential for maintaining high-quality patient care (34).

Despite the growing body of global literature on antimicrobial resistance, there remains a paucity of data specifically focusing on maternal and neonatal colonization with ABR-GNB in resource-limited settings like Ethiopia (14). This study was aimed at addressing this gap by generating locally relevant evidence that can contribute to global efforts to combat antimicrobial resistance.

The findings of this study will therefore serve as a baseline for others and encourage further exploration of the topic. Furthermore, by focusing on this specific context, the study intends to provide actionable insights that can be implemented as focused interventions and policies to lower the prevalence of ABR-GNB in newborns. In general, the study's significance lies in its potential to enhance evidence-based practices, optimize antibiotic use, and improve maternal and neonatal health outcomes in line with national priorities and the global Sustainable Development Goal (SDG) 3, which aims to ensure healthy lives and promote well-being for all, particularly through the reduction of maternal and neonatal mortality and the containment of antimicrobial resistance (35).

2. LITERATURE REVIEW

2.1 Bacterial infection in pregnancy

Pregnancy is characterized by complex physiological, hormonal, and immunological adaptations that are essential for maintaining maternal homeostasis, preserving the maternal–fetal interface, and supporting fetal growth and development (36). These adaptations involve finely regulated immune modulation that allows tolerance of the semi-allogeneic fetus while retaining the capacity to respond to pathogens. However, such immunological and physiological changes may also increase maternal susceptibility to infections and influence the severity of infectious diseases during pregnancy (37).

Bacterial infections such as urinary tract infections (UTIs) and bacterial vaginosis are highly prevalent during pregnancy and are partly driven by immune suppression and pregnancy-associated changes in the genitourinary tract (38). Susceptibility to infection is further influenced by hormonal fluctuations, psychosocial stress, and alterations in the maternal microbiome, all of which may compromise host defense mechanisms (39). Acute bacterial infections can occur at any stage of pregnancy and are associated with increased risks of maternal morbidity and adverse obstetric outcomes, including acute antepartum pyelonephritis, chorioamnionitis, and systemic inflammatory responses (40).

Beyond maternal illness, infections during pregnancy can directly affect the developing fetus. Infectious agents, including bacteria, may invade maternal tissues or release toxins that disrupt normal cellular functions and trigger inflammatory responses (41). Pathogens may be transmitted from mother to fetus through several mechanisms. The most common routes include vertical (hematogenous) transmission, in which microorganisms cross the placenta into the fetal circulation, and ascending transmission, whereby pathogens travel from the lower genital tract into the uterine cavity. Less common routes include transperitoneal spread through the fallopian tubes, invasive obstetric procedures, or exposure during the birthing process (42).

Maternal bacterial infections have been linked to a wide range of adverse fetal and neonatal outcomes, including preterm birth, low birth weight, neonatal sepsis, meningitis, hydrocephalus,

microcephaly, and long-term neurodevelopmental impairments. These outcomes highlight the critical impact of maternal infection on fetal survival and child health (43).

Globally, infection remains a major contributor to maternal, fetal, and neonatal mortality and morbidity. Approximately 40% of maternal deaths are attributed to infection, with an additional 14.5% related to abortion, often complicated by sepsis. Among neonatal deaths, infection accounts for nearly 28%, while complications of prematurity contribute about 14% (44). Furthermore, the World Health Organization estimates that infections during pregnancy are responsible for approximately 10.7% of pregnancy-related maternal deaths worldwide (45). These figures underscore the substantial public health burden posed by infections in pregnancy and emphasize the importance of early detection, prevention, and appropriate management of bacterial infections among pregnant women.

2.2 Antimicrobial Resistance in Gram-Negative Bacteria

The advent of antibiotics revolutionized modern medicine, enabling the effective treatment of once-lethal bacterial infections such as meningitis and bacteraemia (46). However, over recent decades, the widespread misuse and overuse of these drugs, compounded by various social and economic factors, have significantly contributed to the emergence and global spread of antibiotic-resistant bacteria. As a result, many bacterial infections have become increasingly difficult to treat with conventional antibiotics, rendering previously effective treatments ineffective (47).

Antimicrobial resistance (AMR) occurs when bacteria evolve mechanisms to survive exposure to antibiotics. These resistance strategies include neutralizing the drug's action, actively expelling the antibiotic from the cell, or modifying the bacterial cell structure in ways that prevent antibiotic binding and action (48). AMR has become one of the most pressing public health threats of the 21st century. In 2021 alone, bacterial AMR was linked to an estimated 4.71 million deaths globally, with the greatest burden observed in low- and middle-income countries (49,50).

A significant portion of this threat stems from MDR-GNB, which have emerged as major pathogens responsible for both community-acquired and healthcare-associated infections (51). The intrinsic structure of Gram-negative bacteria, particularly their outer membrane, contributes

heavily to their resistance. This outer membrane acts as a barrier against a wide range of antibiotics, including β -lactams, quinolones, and colistin. Modifications in the membrane, such as altered hydrophobicity or mutations in porins and efflux systems, can enhance resistance even further (48).

Among Gram-negative pathogens, Enterobacterales are especially concerning due to their role in severe infections, particularly in immunocompromised individuals. Recognizing the urgency of the issue, WHO has identified antibiotic-resistant Enterobacterales, specifically those producing ESBLs and carbapenemases, as critical priority pathogens in its global priority list of AMR threats (51).

2.3 ESBL-Producing Enterobacterales (ESBL-PE)

Enterobacterales constitute a diverse group of GNB known to cause a wide spectrum of serious infections, both in community settings and healthcare environments. These pathogens are responsible for urinary tract infections (UTIs), bloodstream infections, gastrointestinal illnesses, and respiratory tract infections, posing significant risks to human health worldwide (7,52). Among these bacteria, strains that produce ESBLs, particularly *E. coli* and *K. pneumonia*, have gained global attention due to their high resistance to antibiotics and their contribution to escalating public health challenges (52).

Resistance to β -lactam antibiotics in Enterobacterales arises through multiple mechanisms. These include the production of β -lactamase enzymes, modification of penicillin-binding proteins (PBPs) to reduce their affinity for β -lactams, enhancement of efflux pump activity, and reduction in outer membrane permeability, all of which limit antibiotic efficacy (53). Numerous β -lactamase enzymes have been identified and classified based on their molecular properties and ability to hydrolyze different β -lactam antibiotics (54). ESBLs, a prominent class of β -lactamases, confer resistance to third-generation cephalosporins (e.g., cefotaxime, ceftriaxone, ceftazidime, cefepime) and monobactams (e.g., aztreonam). However, they do not hydrolyze cephamycins or carbapenems, and they remain susceptible to β -lactamase inhibitors like clavulanic acid, sulbactam, and tazobactam, which can restore antibiotic activity (52).

The genes responsible for ESBL production are often located on large plasmids that also carry additional AMR genes. This genetic linkage leads to co-resistance against several non- β -lactam

antibiotics commonly used in clinical settings, including aminoglycosides, quinolones, trimethoprim, sulfamethoxazole, tetracyclines, sulfonamides, and chloramphenicol. Consequently, infections caused by ESBL-producing organisms are challenging to treat, particularly in critically ill patients (55,56).

2.4 Carbapenem-Resistant Enterobacterales (CRE)

Carbapenem-resistant Enterobacterales have emerged as critical pathogens of global concern, with the United States CDC designating them as serious and urgent threats to public health (21). These bacteria are traditionally associated with infections acquired in healthcare settings; however, their increasing role in community-acquired infections has heightened global concern. Invasive infections caused by CRE are particularly worrisome due to their high mortality rates, substantial treatment costs, limited therapeutic options, and the lack of standardized treatment guidelines (57).

Before 2001, approximately 99.9% of clinical Enterobacterales isolates worldwide remained susceptible to carbapenems (58). However, resistance has escalated significantly over the past two decades, with a notable increase of approximately 60% in China alone by 2020 (59). Carbapenems are broad-spectrum antibiotics that are generally reserved for managing infections caused by MDR pathogens, including ESBL producing and AmpC β -lactamase-producing bacteria (34). The emergence of CRE has disrupted this therapeutic strategy, as these pathogens have developed diverse mechanisms to evade carbapenem activity, presenting serious challenges in both infection control and individual patient management (60).

Bacterial resistance to carbapenems can occur through multiple pathways, such as enzymatic degradation of the antibiotic, restricted entry of the drug into bacterial cells, modification of drug-binding sites, over expression of efflux systems, and changes in the composition of biofilms that protect bacteria from antimicrobial agents (61). These resistance mechanisms have contributed to a marked increase in carbapenem resistance among Enterobacterales across various regions.

A global multicenter surveillance study conducted by Sader et al. (2021), involving 8,787 Enterobacterales isolates from 64 medical centers, reported a 4.5% overall resistance rate to

carbapenems among Enterobacterales in 2019 (62). Additionally, according to the 2021 WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) report, the resistance rates for carbapenem-resistant *Klebsiella pneumoniae* (CRKP) to meropenem and imipenem were 12.34% and 10.63%, respectively. In comparison, for carbapenem-resistant *E. coli* (CREco), the resistance rates were 0.9% for meropenem and 1.3% for imipenem (63).

2.5 Colonization of ESBL-producing and Carbapenem Resistant Enterobacterales

2.5.1 Maternal colonization of ESBL-PE and CRE

Maternal colonization of ABR bacteria is the presence of resistant bacteria like *E.coli* and *Klebsiella* in the genital tract of a woman during pregnancy (28). Some of those ABR bacteria like ESBL-PE and CRE are capable of producing enzymes that break down antibiotics, making them resistant to treatment (12).

The prevalence of ESBL-PE and CRE among pregnant mothers varies widely across different literatures. According to studies conducted in Bangladesh and India, respectively, 21% and 15% of pregnant women were colonized with ESBL-PE (64). As revealed on another study conducted in south Israel 21.5% pregnant mothers were colonized with ESBL-PE (65). A study conducted in Norway shows that 2.9% of pregnant women were colonized with ESBL-producing *E.coli* at 36 weeks of pregnancy (66). Additionally, a prospective surveillance research at three Japanese perinatal facilities revealed a 5.9% rate of ESBL-PE colonization in mothers (67).

The incidence of ABR bacteria colonization was 46.6% in the Ugandan study that looked into the prevalence of clinically significant ABR bacteria in the vagina of laboring women (28). A study from Nigeria had also showed a 9.7% colonization rate of ESBL-PE among pregnant mothers (29). In a recent Ethiopian study, the carriage prevalence of ESBL-producing bacteria was 25%, among 211 pregnant mothers (14).

Regarding CRE, studies from Algeria and Uganda show colonization rates of 4.6% and 1.8% among pregnant women, respectively(28,68). A recent study conducted in Ethiopia, among pregnant women shows 3% CRE carriage rate (14).

2.5.2 Neonatal colonization of ESBL-PE and CRE

Infants are increasingly being infected with GNB that are resistant to antibiotics. The incidence of ABR bacteria leading to both early- and late-onset sepsis in newborns has gone up in the last few years (10). Furthermore, infections caused by this ABR bacteria (such as ESBL-PE and CRE) were linked to higher rates of morbidity and mortality. The colonization rates of newborns by these bacteria varied between studies, ranging from 0.04% to 14.8% (10). A study carried out in Israel revealed that 7.6% of the newborn study participants were colonized by ESBL producing Enterobacterales (10). Of the study participants in the research done in Japan, 2.4% of newborns showed ESBL-PE colonization (67). An additional investigation carried out in the prenatal center at the Germany revealed a 5.7% rate of infant colonization of ESBL-PE (69). On the other hand A study conducted in Nigerian and Ethiopia shows 4.3% and 24.5 % prevalence of ESBL-PE colonization rate among newborns respectively (14, 29).

In relation to CRE A study from Israel indicates a nil prevalence, whereas a study from Algeria estimates 1.6% carriage(10,68). Studies have not typically measured CRE in infants. Pregnant women's and their babies' CRE presence has only been assessed in a small number of studies (10). This also holds true for Ethiopia. In our efforts to look for literature on the prevalence of CRE in infants in relation to vertical transmission in Ethiopian case, we were only able find one study, which was carried out in the North Shewa Zone of Amhara. In this study which was assumed to be the first to measure asymptomatic carriage prevalence of antibiotic-resistant organisms and GBS in rural Ethiopia, the colonization prevalence estimates of CRE among neonates was reached 2.5% (14).

2.6 Transmission of ESBL-PE and CRE from Mother to Neonate

The prevalence of ABR bacteria in the healthy population is a topic of growing interest these days, with an emphasis on determining risk factors for both carriage and the development of subsequent infections, as well as comprehending the long-term effects of carriage (70). The potential for these bacteria to be passed to the newborn after vaginal delivery, which could lead to serious illnesses in the infant, is the reason for this interest (11).

In relation to the transmission rate, the rates of ABR bacteria transfer, specifically ESBL-PE, from mother to neonate have been reported to vary (from 14% to 100%) (71–74). In a study

conducted in Germany, 20% of the 184 newborns exposed to mother ABR bacteria colonization developed ABR bacteria positivity (69). In another investigation done in Austria, the maternal-to-neonatal transmission rate of ESBL-PE was 43% (73). An investigation carried out in Israel found that 48% of ESBL isolates were transmitted from the mother to the neonate (10). Despite the lack of clarity surrounding the transmission method, an Ethiopian study estimates that 38.7% of ESBL-producing organisms were shared by mothers and their infants (14).

2.7 Associated factors for colonization and transmission of ABR-GNB

Although the risk factors for ABR-GNB transmission from mother to child have not been well investigated, some literatures have categorized them as obstetric-related and infection-prevention-related factors (11).

According to a study conducted in Japan, external interventions such as artificial membrane rupture were thought to have facilitated bacterial colonization since they may have introduced rectal germs into the vagina. Additionally, the use of antibiotics during delivery was another factor associated with neonatal colonization (67,75,76). This is likely because the use of antibiotics either prior to or during childbirth may have contributed to the growth or survival of resistant bacteria in the expectant mother, which may then spread to the newborn when it passes through the birth canal. According to a study conducted in Cambodia, the majority of ABR-GNB transmission occurred at or around the time of birth (77). A further piece of evidence supporting the notion that maternal colonization of ABR bacteria increases the probability of neonatal ABR bacteria colonization comes from an odds ratio analysis of an Israeli study (10). Among other factors, low gestational age, low birth weight, and exposure and duration of hospital stay have been associated with colonization or infection of neonates with ESBL-PE (66,78,79).

As reported in a meta-analysis involving 122 studies the prevalence of neonatal colonization or infection was higher in pregnant women with risk factors, particularly PROM and preterm labor (80). Infection-prevention-related factors like suboptimal hand hygiene among mothers and healthcare workers and ineffective environmental and equipment cleaning, particularly in low-resource settings, were also stated as potential risk factors for colonization and transmission of ABR bacteria among pregnant mothers and their newborns (66,81).

In some cases, prior antibiotic use, differences in healthcare seeking behavior, infection prevalence between the woredas, climatic conditions, household flooring material, and cohabitation with domestic animals were mentioned as associated factors for ABR bacteria colonization in a study conducted in Ethiopia (14).

2.8 Impacts of ABR-GNB on pregnant mothers and their infants

The World Health Organization views MDR bacteria as a serious danger to public health because of their limited treatment potential and the high mortality of diseases they can cause. Newborns are particularly vulnerable to ABR bacteria, and long-term hospitalization and death in ICU are often associated with the presence of ESBL-PE (77). This population's weakened skin barriers and immature immune systems make infections more likely (82). Newborn infections, specifically sepsis, meningitis, and pneumonia, are responsible for over 23% of the 2.4 million newborn deaths globally during the first 28 days of life, making them one of the most common causes of death (83). Until the point of decolonization, colonization also carries the danger of MDR transfer to family members (84).

Healthcare infections, largely caused by ESBL-producing organisms, lengthen hospital stays and increase rates of morbidity and death. Particularly in individuals with severe infections like bacteremia, the presence of ESBL makes choosing an antibiotic more difficult. ESBL-producing bacteria frequently exhibit multiple resistances to different antibiotics, which is the rationale behind this (85). It is also challenging to treat ESBL-producing isolates since the majority of people in these settings cannot afford the pricey medication needed to treat these isolates. According to a study conducted in Ethiopia, antibiotic resistance and mortality were substantially correlated (86).

2.9 Antimicrobial stewardship and prevention strategy

Antimicrobial stewardship and infection-prevention strategies play a central role in reducing the acquisition and transmission of drug-resistant DR-GNB, including ESBL-PE and CRE. Restricting horizontal transmission is the most important strategy for stopping ABR bacteria colonization and infection. Good hand hygiene, the best gloving techniques, disinfection, decontamination, and sterilization techniques are examples of basic infection control treatments

(87). A key element in reducing resistance is the implementation of antimicrobial stewardship initiatives, which are linked to decreased antibiotic use (88).

Restricting cephalosporin can lower the risk of neonatal ESBL producing bacterial sepsis (89). Screening for ESBL-PE carriage in mothers and infants may enable the early identification of newborn colonization and the implementation of eradication efforts (90). Comprehensive environmental cleaning, the timely initiation of contact precautions, accurate microbiology laboratory procedures, timely notification, and the use of optimal central line infection prevention bundles are additional strategies to reduce the acquisition and transmission of ABR pathogens (87).

Conceptual framework

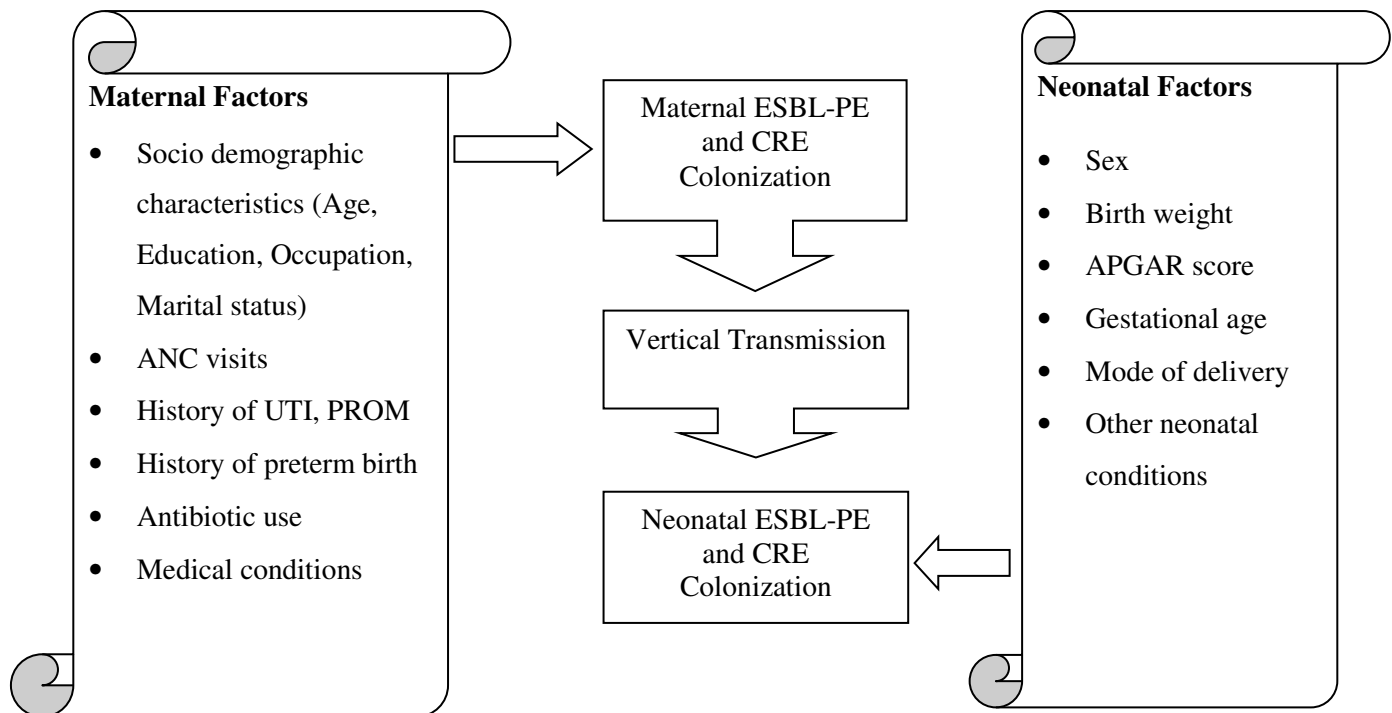


Figure 1: Conceptual framework of maternal and neonatal colonization with ESBL-PE and CRE, vertical transmission, and associated factors

3. OBJECTIVES

3.1 General Objective

- To assess maternal and neonatal colonization of ESBL-producing Enterobacterales (ESBL-PE) and carbapenem-resistant Enterobacterales (CRE), and associated factors at SPHMMC, Addis Ababa, Ethiopia.

3.2 Specific objectives

- To determine the prevalence of ESBL-PE and CRE colonization in laboring mothers and their newborns.
- To evaluate the phenotypic relatedness of ESBL-PE and CRE isolates between laboring mothers and their newborns.
- To determine the antimicrobial susceptibility patterns of ESBL-PE and CRE isolated from laboring mothers and their newborns.
- To identify factors associated with the colonization and vertical transmission of ESBL-PE and CRE among laboring mothers and their newborns.

4. METHODS

4.1 Study Setting and Period

The study was conducted at SPHMMC a major public referral hospital located in Addis Ababa, the capital city of Ethiopia. SPHMMC is among key centers for maternal and neonatal healthcare in the region, providing a wide range of medical services to a diverse population. With an inpatient capacity of over 700 beds, the hospital accommodates an average of 1,200 emergency and outpatient clients per day. The maternity unit offers 24/7 delivery services staffed by midwives, medical interns, residents, obstetricians, and gynecologists. On average, the hospital handles 850 deliveries per month, making it an ideal setting to investigate maternal and neonatal colonization of ABR-GNB. The study was conducted from April to August 2024. The combination of SPHMMC's high delivery volume and its role as a referral center provided a robust foundation for generating meaningful and generalizable findings relevant to the local and regional context.

4.2 Study Design

A cross-sectional study design was employed for this research.

4.3 Population

4.3.1 Source population

All pregnant women who attended SPHMMC labor ward during the study period and their newborns.

4.3.2 Study Population

All pregnant women who gave birth vaginally at SPHMMC during the study period and their newborns provided they consented to participate.

4.4. Inclusion and exclusion criteria

4.4.1 Inclusion criteria

- Pregnant women aged ≥ 18 years who had a spontaneous vaginal delivery at SPHMMC and provided informed consent, along with their live-born neonates, were included in the study.

4.4.2 Exclusion criteria

Pregnant women were excluded if they:

- Underwent cesarean section and assisted delivery as mode of delivery (including their babies), Experienced complications (e.g. severe preeclampsia and fetal distress), Had taken antibiotics within two weeks prior to labor, Had obstructed labor or other clinical conditions that interfered with sample collection or participation.

4.5 Sample size determination and sampling technique

4.5.1 Sample size determination

The required sample size was determined using a single population proportion formula with the following assumptions: a 95% confidence level, 5% margin of error, 10% non-response rate, and prevalence estimates from a previous study conducted in Ethiopia, which reported a prevalence of 22.3% for ESBL-PE and 0.9% for CRE (14).

The formula used for sample size calculation was:

$$n = \frac{z^2 pq}{d^2}$$

Where:

- n = sample size,
- Z = Z-score corresponding to the desired level of confidence
- P = proportion of the target population
- q = alternate proportion (1-P)

For ESBL colonization

$$n = \frac{1.96^2 * 0.223 * 0.777}{0.05^2}$$

- $n \approx 266$

Including the 10 % of non-responder rate $N = \frac{n}{1-10\%} = N = 295$

Thus, the sample size for ESBL colonization was rounded to 295

For CRE colonization

$$n = \frac{1.96^2 * 0.009 * (1 - 0.009)}{0.05^2}$$

- **n≈14**

Including the 10 % of non-responder rate $N = \frac{n}{1-10\%} = 15.89$

Thus, the sample size for CRE colonization was rounded to **16**.

To ensure the representativeness of the study population and account for both ESBL-PE and CRE colonization, the initially calculated sample size of 295 was considered.

4.5.2 Sampling technique

Study participants were recruited using a consecutive sampling technique.

4.6. Study variables

4.6.1 Dependent variables

- ✓ Maternal and neonatal colonization with ESBL-PE and CRE.
- ✓ Phenotypic relatedness between maternal and neonatal isolates.
- ✓ Antimicrobial susceptibility patterns of ESBL-PE and CRE isolates.

4.6.2 Independent variables

- **Socio-Demographic Factors** - Maternal age, marital status, education level, occupation, residence, family income, number of family members.
- **Maternal Obstetric History**- Parity, gravidity, number of antenatal care visits, previous history of (preterm delivery, abortion, PPH, PROM).
- **Maternal Medical History**- Underlying medical conditions (e.g., diabetes, hypertension, HIV/AIDS), use of IAP.
- **Neonatal-Related Obstetric Factors**- Fetal presentation (e.g., cephalic, breech), birth weight, APGAR score, multiple gestations, gestational age.

4.7 Data and sample collection procedure

4.7.1 Socio-demographic and clinical data collection

After obtaining written informed consent from each participant, demographic and clinical data were collected from both the participants and their medical records using a semi-structured questionnaire administered through the Kobo Toolbox.

4.7.2 Swab sample collection and transportation

Swab samples were collected from laboring mothers and their newborns by trained midwives. For mothers, two vaginal swabs were collected during the second stage of labor. The swabs were carefully inserted until they reached the vaginal wall, avoiding contamination from cervical mucus, gently pressed against the wall, rotated to collect the specimen and withdrawn without contacting other body parts.

Two additional sterile swabs were gently inserted approximately 2–3 cm into the rectum, taking care to minimize discomfort or injury to the mother. These swabs were rotated gently against the rectal mucosa to obtain an adequate specimen, while also avoiding contact with the perineum or other body areas.

For newborns, two swab samples were collected from the external ear immediately after delivery, prior to any routine postnatal care. The external ear was selected as the sampling site due to its proximity to the birth canal, its potential exposure to maternal flora, and its non-invasive nature. The swab was gently rotated to ensure adequate sample collection while minimizing any risk of irritation or harm.

All maternal and neonatal swabs were placed in separate Amies transport media (Oxoid, UK), clearly labeled, and immediately transported to the Microbiology Laboratory at SPHMMC for bacteriological analysis. In cases where immediate transport was not feasible the samples were stored at 2–8°C in a refrigerator and processed within 24 hours.

4.7.3 Screening and Identification Methods for ESBL-PE and CRE

Swab samples collected at delivery were processed immediately upon arrival at the microbiology laboratory. One swab from each anatomical site was inoculated onto CHROMATIC™ ESBL agar (Liofilchem, Italy) for the detection of ESBL-producing Enterobacterales, while the second swab was plated onto CHROMATIC™ CRE agar (Liofilchem, Italy) for the detection and isolation of CRE. Plates were incubated aerobically at 37°C for 24 hours. If no visible growth was observed, they were re-incubated for an additional 24 hours before being discarded.

Following incubation, the CHROMATIC™ agar plates were examined for colony morphology and characteristic color development, based on the manufacturer's color chart, to presumptively identify ESBL-PE or CRE. Suspected colonies identified on the CHROMATIC™ media were subsequently sub-cultured onto sheep blood agar plates to obtain pure colonies for further identification and antimicrobial susceptibility testing.

Bacterial species were identified primarily based on colony morphology (color, size, and shape) and conventional biochemical tests. The biochemical tests performed included indole production (Oxoid, UK), citrate utilization (Oxoid, UK), triple sugar iron test (Oxoid, UK), urease test (Oxoid, UK), lysine decarboxylase test (Oxoid, UK), lysine deaminase test (Oxoid, UK), and motility test (Oxoid, UK) (91).

4.7.4 Phenotypic confirmation of ESBL-PE and CRE

The combination disk method was used to confirm ESBL-PE. A combination disk containing 30 µg ceftazidime (Oxoid, UK) and 10 µg clavulanic acid (Oxoid, UK) was placed 15 mm away from a 30 µg ceftazidime disk on a Mueller-Hinton agar (MHA) plate. The plate was incubated at 35°C for 18-24 hours. Isolates exhibiting clear zones that were ≥ 5 mm larger around the combination disk compared to the ceftazidime disk were identified as ESBL-PE (92). Isolates that demonstrated resistance to at least one carbapenem were considered CRE (93).

4.7.5 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed for each ESBL-PE and CRE bacterial isolate using the Kirby-Bauer disk diffusion method on MHA (94). Approximately 3–5 pure colonies of the test organism were collected using a sterile wire loop and emulsified in 2 ml of normal saline

to achieve a turbidity equivalent to the 0.5 McFarland standard. A sterile cotton swab was dipped into the standardized bacterial suspension, and excess fluid was removed by squeezing the swab against the wall of the tube. The swab was then used to evenly inoculate the surface of MHA by streaking in three directions to ensure a uniform lawn of bacterial growth. After 15 minutes, Antibiotic-impregnated paper disks were placed on MHA surface, ensuring a minimum distance of 24 mm between disks to prevent overlapping zones of inhibition. The plates were incubated at 37°C for 24 hours. The diameter of the zones of inhibition was measured to the nearest millimeter using a calibrated ruler. Results were interpreted as sensitive, intermediate or resistant based on the CLSI guidelines (95).

The following antibiotic disks (Oxoid, UK) were tested: Amoxicillin-clavulanic acid (20/10 µg), Amikacin (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Trimethoprim-sulfamethoxazole (25 µg), Ertapenem (10 µg), Meropenem (10 µg), Imipenem (10 µg), Chloramphenicol (30 µg), Ampicillin (10 µg), Ceftazidime (30 µg), Cefepime (30 µg), Ceftriaxone (30 µg), and Piperacillin-Tazobactam (100/10 µg).

4.7.6 Storage of Isolates

Colonies from a 24-hour culture were picked and added to cryovials containing 1.8 ml of storage media Tryptic Soy Broth with 20% glycerol. The cryovials were then vortexed to ensure proper mixing and stored in a deep freezer (- 80°C) for further testing.

Flow chart for the identification of ESBL-PE and CRE from laboring mothers and their newborns

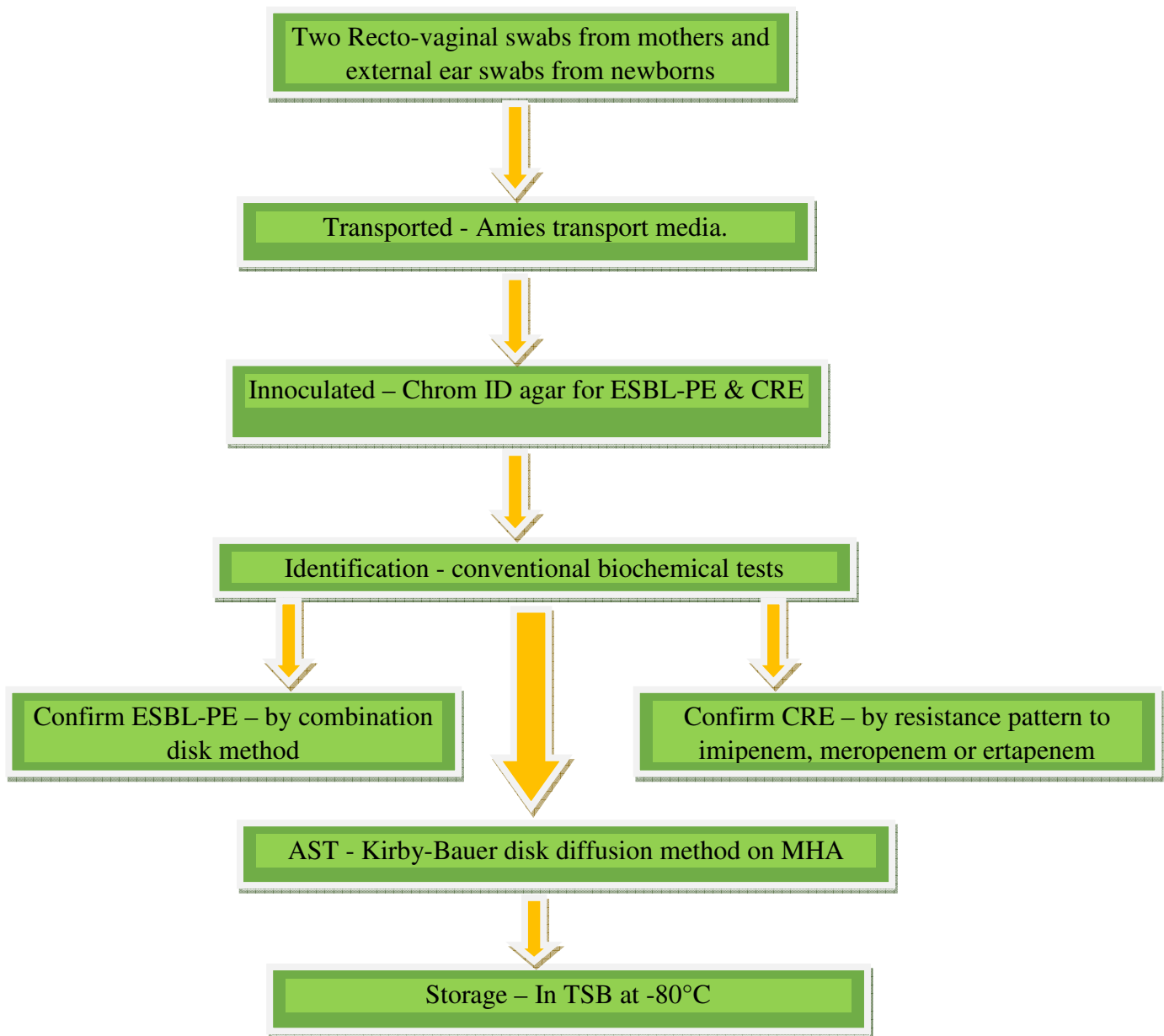


Figure 2: Flowchart of ESBL-PE and CRE screening, identification, phenotypic confirmation, and antimicrobial susceptibility testing among laboring mothers and neonates at SPHMMC, Addis Ababa, 2024

4.8 Operational Definitions

- **Maternal Colonization:** Refers to the presence of ESBL-PE and/or CRE isolated from vaginal and/or rectal swab samples collected from a laboring mother.
- **Neonatal Colonization:** Refers to the presence of ESBL-PE and/or CRE isolated from swab samples taken from the external ear of the newborn immediately after delivery, before any postnatal contact.
- **Vertical Transmission:** The acquisition of ESBL-PE and/or CRE by a newborn from a colonized mother during vaginal delivery.
- **Antibiotic Exposure:** The administration of any antibacterial agent (oral, intravenous, intramuscular, or topical) for therapeutic or prophylactic purposes during pregnancy or delivery, regardless of dosage or duration.
- **Non-married:** Refers to participants who reported being single, divorced, separated, or widowed.

4.9 Quality control and Data quality assurance

Before data collection, the data collectors received a one-day training session about collecting samples and completing the questionnaires. To ensure clarity and avoid misinterpretation, the questionnaires were translated into the local language (Amharic and Afan orommo), then back-translated into English. A 5% pretest was conducted to refine the questionnaires, with minor adjustments made based on the results.

Supervision was provided by the principal investigator to ensure consistency and accuracy in data collection. Standard operating procedures (SOPs) were strictly adhered to, and the data collection process was regularly monitored for quality control.

After collection samples were promptly transported to the SPHMMC Microbiology laboratory. In case of delays, samples were refrigerated to maintain viability. The sterility of the culture media was ensured by incubating 5% of each batch at 37°C for 24 hours. The media were also visually inspected for cracks, thickness, freezing, bubbles, and contamination before use. In addition, the expiration dates of the media, reagents, and antibiotic discs were checked before use to ensure their validity.

Quality control was done for all culture media and antimicrobial susceptibility testing. The quality of CHROMATIC™ ESBL agar was checked using *E. coli* DSM 22311 and *K. pneumoniae* ATCC® 700603 as ESBL-positive controls, *K. pneumoniae* ATCC® BAA-1144 as an AmpC control, and *E. coli* ATCC® 25922 as a susceptible control. In addition, CHROMATIC™ CRE agar was validated by using *K. pneumoniae* ATCC® BAA-1705 (KPC producer), *K. pneumoniae* ATCC® BAA-1706 (carbapenem-susceptible), *K. pneumoniae* ATCC® 700603 (ESBL producer), and *E. coli* ATCC® 25922 to verify the selectivity of CRE and inhibition of non-CRE strains. The Mueller–Hinton agar quality was verified with *S. aureus* ATCC® 25923 and *E. faecalis* ATCC® 29212. AST procedures were validated using *E. coli* ATCC® 25922 and *K. pneumoniae* ATCC® 700603.

For antimicrobial susceptibility testing, inoculums density was standardized using a 0.5 McFarland standard, ensuring consistency across tests. Internal quality control procedures, including temperature control for reagents and proper storage of culture media, were rigorously followed. Furthermore, all positive cultures and biochemical test results were verified by experienced microbiology laboratory personnel to ensure their accuracy.

4.10 Data Analysis Procedures

Data analysis was performed using SPSS version 27. Descriptive statistics were used to summarize the demographic and clinical characteristics of the study participants, as well as the prevalence of ESBL-PE and CRE colonization among laboring mothers and their newborns. Continuous variables were summarized using median and interquartile range (IQR), while categorical variables were presented as frequency distributions with corresponding percentages.

Pearson’s Chi Squared Test and Mann-Whitney U test were performed in order to compare the distribution of categorical and continues variables, respectively, between two groups (Colonized and non-colonized study participants).

To identify significant predictors of maternal ESBL-PE and CRE colonization, bivariate logistic regression was first applied to assess associations between independent variables and colonization status. Variables with p-values ≤ 0.25 were included in the subsequent multivariate logistic regression. The final model included significant variables from the bivariate analysis, along with clinically relevant factors, regardless of their p-values. Multicollinearity was assessed

using the Variance Inflation Factor (VIF), excluding variables with $VIF > 10$. Odds ratios (ORs) and 95% confidence intervals (CIs) were reported, with statistical significance set at $p < 0.05$.

For neonatal colonization, due to the small number of colonized cases ($n = 5$), multivariate analysis was not performed. Associations were evaluated using the Mann-Whitney U test for continuous variables and Chi-square test (or Fisher's Exact Test when necessary) for categorical variables.

4.11 Ethical Consideration

Ethical clearance was obtained from the Institutional Review Board of SPHMMC prior to the commencement of the study. Informed written consent was obtained from all adult participants. For newborns, informed consent was obtained from their mothers or legal guardians. The general purpose of the study was explained to each participant in their local language to ensure understanding.

Participants were informed of their right to withdraw from the study at any time without penalty. To maintain confidentiality, participants' names or other personal identifiers were not used during data collection or analysis; instead, unique codes were assigned to each participant for identification.

All research methods were conducted in accordance with the ethical principles outlined in the Declaration of Helsinki, ensuring respect for participants, beneficence, and justice throughout the study (96). Data were stored securely, and access was limited to authorized personnel only.

4.12 Dissemination of Results

Once the research will be finalized, the result will be submitted to the Department of Microbiology, Immunology, and Parasitology as part of the MSc. thesis defense process. The findings will also be presented to the SPHMMC community through the college's internal journal. The results will be shared at both local and international scientific conferences, workshops, and seminars through oral or poster presentations. Finally, the study's final document will be submitted to peer-reviewed journals for publication and further dissemination of the findings.

5. RESULTS

5.1 Demographic and Baseline Characteristics of Study Participants

5.1.1 Maternal Demographics and Baseline Data

A total of 295 laboring mothers were enrolled in the study. More than half of the mothers 171 (58.0%) were between 25-34 years of age. The majority of participants 292 (99.0%) resided in urban areas. Regarding education, 131 (44.4%) of the mothers had completed primary education, followed by 79 (26.8%) with secondary education. Most of the mothers 181(62.1%) were housewives. Nearly all participants 288 (97.6%) were married. The median family income was 10,000 ETB (IQR: 6,000–15,000) (Table 1).

Table 1. Demographic characteristics of laboring mothers at SPHMMC, Addis Ababa, 2024 (n = 295)

Variable	Category	Frequency (n) (%)
Age of the mother (Years)	18–24	91 (30.8)
	25–34	171(58.0)
	≥35	33 (11.2)
Residence of participants	Urban	292 (99.0)
	Rural	3 (1.0)
Education status of the mother	No formal education	22 (7.5)
	Primary education	131 (44.4)
	Secondary education	79 (26.8)
	Diploma and above	63 (21.3)
Occupation of the mother	Housewife	181 (62.1)
	Government worker	30 (10.2)
	Private worker	53 (18.0)
	Merchant	22 (7.5)
	Other	9 (3.1)
Marital status	Married	288 (97.6)
	Unmarried	7 (2.4)
Family income	Median (IQR)	10,000 (6,000–15,000)

5.1.2 Neonatal Demographics and Baseline Data

Among the neonates, 150 (50.8%) were male and 145 (49.2%) female. All (295) neonates were singletons. A majority 277 (93.9%) were born at term, had normal birth weight 260 (88.1%), and had normal APGAR scores (7-10) both at one minute 193 (65.4%) and five minutes 284 (96.3%) (Table 2).

Table 2. Demographic and baseline characteristics of neonates at SPHMMC, Addis Ababa, 2024 (n = 295)

Variable	Category	Frequency (n) (%)
Sex	Male	150 (50.8)
	Female	145 (49.2)
Twin Status	Singleton	295 (100)
Gestational Age	Term (37–42 weeks)	277 (93.9)
	Preterm (<37 weeks)	10 (3.4)
	Post-term (>42 weeks)	8 (2.7)
Birth Weight (grams)	LBW (<2500)	29 (9.8)
	Normal (2500-4000)	260 (88.1)
	HBW (>4000)	6 (2.0)
First Minute APGAR Score	Low (0-3)	0 (0.0)
	Fair (4-6)	102 (34.6)
	Normal (7-10)	193 (65.4)
Fifth Minute APGAR Score	Low (0-3)	0 (0.0)
	Fair (4-6)	11 (3.7)
	Normal(7-10)	284 (96.3)

Note- APGAR (Appearance, Pulse, Grimace, Activity and Respiration), LBW (Low birth weight), HBW (High Birth Weight)

5.2 Comparison of Demographic and Clinical Data Based on Colonization Status

5.2.1 Comparison of Demographic and Clinical Data of Mothers Divided According to Colonization Status

Out of 295 laboring mothers, 72 (24.4%) were found to be colonized with at least one type of ABR-GNB (ESBL-PE and/or CRE) (95% CI: 19.0%–29.0%). A significant association was observed between education status and ABR-GNB colonization ($p = 0.028$), with a higher proportion of colonized mothers having secondary education level. No significant differences were found in other demographic and clinical characteristics such as age, residence, marital status, or antenatal care visits (Table 3).

Table 3. Comparison of maternal demographic and clinical characteristics by colonization status at SPHMMC, Addis Ababa, 2024 (n = 295).

Variable	Category	Maternal ABR-GNB colonization		Total (295)	P- value
		Not Colonized (N = 223)	Colonized (N = 72)		
Age (Years)	18–24	67 (30.1)	24 (33.3)	91 (30.8)	0.534
	25–34	133 (59.6)	38 (52.8)	171 (58.0)	
	≥35	23 (10.3)	10 (13.9)	33 (11.2)	
Residence	Urban	222 (99.6)	70 (97.2)	292 (99.0)	0.149
	Rural	1 (0.4)	2 (2.8)	3 (1.0)	
Education Status	No formal education	13 (5.8)	9 (12.5)	22 (7.5)	0.028
	Primary education	109 (48.9)	22 (30.6)	131 (44.4)	
	Secondary education	55 (24.7)	24 (33.3)	79 (26.8)	
	Diploma and above	46 (20.6)	17 (23.6)	63 (21.3)	
Occupation	Housewife	139 (62.4)	42 (58.3)	181 (62.1)	0.188
	Government worker	21 (9.4)	9 (12.5)	30 (10.2)	
	Private worker	44 (19.7)	9 (12.5)	53 (18.0)	
	Merchant	13 (5.8)	9 (12.5)	22 (7.5)	
	Other	6 (2.7)	3 (4.2)	9 (3.1)	
Marital Status	Married	220 (98.7)	68 (94.4)	288 (97.6)	0.063
	Unmarried	3 (1.3)	4 (5.6)	7 (2.4)	
Family Income	Median (IQR)	10,000 (6,000–15,000)	10,000 (5,250–16,750)	10,000 (6,000–15,000)	0.796
Number of Family Members	Median (IQR)	3.0 (2.0–4.0)	3.5 (3.0–4.8)	3.0 (3.0–4.0)	0.466
History of Abortion	Yes	42 (18.8)	12 (16.7)	54 (18.5)	0.679
	No	181 (81.2)	60 (83.3)	241 (81.5)	
History of Preterm Delivery	Yes	2 (0.9)	1 (1.4)	3 (1.0)	0.569
	No	221 (99.1)	71 (98.6)	292 (99.0)	
History of PPH	Yes	25 (11.2)	4 (5.6)	29 (9.8)	0.161
	No	198 (88.8)	68 (94.4)	266 (90.2)	
History of PROM	Yes	35 (15.7)	10 (13.9)	45 (15.3)	0.711
	No	188 (84.3)	62 (86.1)	250 (84.7)	
Gravida	Median (IQR)	2 (1–3.)	2 (1–3)	2 (1–3)	0.652
Parity	Median (IQR)	2 (1–3)	2 (1–3)	2 (1–3)	0.674
Number of ANC Follow-Ups	Median (IQR)	6 (6–8)	7 (6–8)	6 (6–8)	0.083
Diabetes Mellitus	Yes	4 (1.8)	0 (0)	4 (1.4)	0.575
	No	219 (98.2)	72 (100)	291 (98.6)	
HIV	Yes	2 (0.9)	0 (0)	2 (0.7)	1.000
	No	221 (99.1)	72 (100)	293 (99.3)	
IAP	Yes	89 (39.9)	27 (37.5)	116 (39.3)	0.716
	No	134 (60.1)	45 (62.5)	179 (60.7)	
Gestational Week	Term (37–42 wk)	209 (93.7)	68 (94.4)	277 (94.0)	1.000
	Preterm (<37 wk)	8 (3.6)	2 (2.8)	10 (3.4)	
	Post-term (>42 wk)	6 (2.7)	2 (2.8)	8 (2.7)	

Note- ABR-GNB (ESBL-PE and CRE), **PROM** (Premature Rupture of Membrane), **IAP** (Intrapartum Antibiotic Prophylaxis), **PPH** (Postpartum Hemorrhage), **ANC** (Antenatal Care).

5.2.2 Comparison of Demographic and Clinical Data of Neonates Divided According to Colonization Status

Among 295 newborn participants, 5 (1.7%) neonates were colonized with at least one type of ABR-GNB (ESBL-PE and/or CRE) (95% CI: 0.0%–3.0%). No significant differences were found between colonized and non-colonized neonates in sex, birth weight, or APGAR scores (Table 4). However, gestational age differed between the two groups, with a higher proportion of preterm newborns in the colonized group compared to the non-colonized group (40.0% vs. 2.8%, respectively; $p = 0.016$).

Table 4. Comparison of neonatal demographic and clinical characteristics by colonization status at SPHMMC, Addis Ababa, 2024 (n = 295)

Variable	Category	ABR-GNB Not Colonized (N = 290)	ABR-GNB Colonized (N = 5)	Total (N=295)	P-value
Sex	Male	148 (51.0)	2 (40.0)	150 (50.8)	0.680
	Female	142 (49.0)	3 (60.0)	145 (49.2)	
Gestational Age	Term (37–42 weeks)	274 (94.5)	3 (60.0)	277 (93.9)	0.016
	Preterm (<37 weeks)	8 (2.8)	2 (40.0)	10 (3.4)	
	Post-term (>42 weeks)	8 (2.8)	0 (0.0)	8 (2.7)	
Birth Weight (grams)	LBW (<2500)	27 (9.3)	2 (40.0)	29 (9.8)	0.171
	Normal (2500-4000)	257 (88.6)	3 (60.0)	260 (88.1)	
	HBW (>4000)	6 (2.0)	0 (0.0)	6 (2.0)	
First Minute APGAR Score	Low (0-3)	0 (0.0)	0 (0.0)	0 (0.0)	0.345
	Fair (4-6)	99 (34.1)	3 (60.0)	102 (34.6)	
	Normal (7-10)	191 (65.9)	2 (40.0)	193 (65.4)	
Fifth Minute APGAR Score	Low (0-3)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
	Fair (4-6)	11 (3.8)	0 (0.0)	11 (3.7)	
	Normal (7-10)	279 (96.2)	5 (100)	284 (96.3)	

Note: ABR-GNB (ESBL-PE and CRE), APGAR (Appearance, Pulse, Grimace, Activity, and Respiration, LBW (Low Birth Weight), HBW (High Birth Weight)

5.3 Prevalence and Distribution of ESBL-PE and CRE Colonization

5.3.1 Prevalence of ESBL-PE and CRE Colonization among Study Participants

In this study, 68 (23.1%) of laboring mothers were colonized with ESBL-PE, while 2 (0.7%) were colonized with CRE. Notably, 2 (0.7%) of mothers were co-colonized with both ESBL-PE

and CRE. In contrast, among the 295 newborns, 2 (0.7%) were colonized with ESBL-PE, and 3 (1.0%) were co-colonized with both ESBL-PE and CRE (Fig 3).

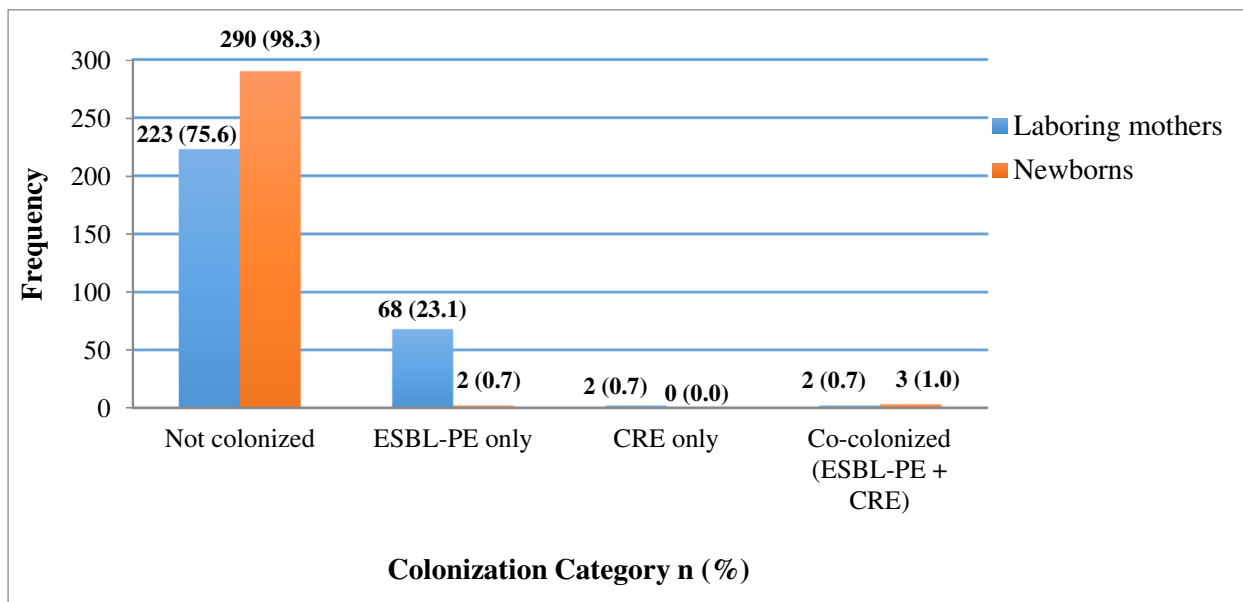


Figure 3. Prevalence of ESBL-PE and/or CRE colonization among laboring mothers and their newborns at SPHMMC, Addis Ababa, 2024 (n = 295).

5.3.2 Distribution of Maternal ESBL-PE and CRE Isolates by Sample Source

Among the 89 maternal ABR-GNB isolates, rectal samples were the source of 70/82 (85.4%) ESBL-PE and 5/7 (71.4%) CRE isolates, indicating the rectum as the primary reservoir of ABR-GNB among laboring mothers (Fig 3).

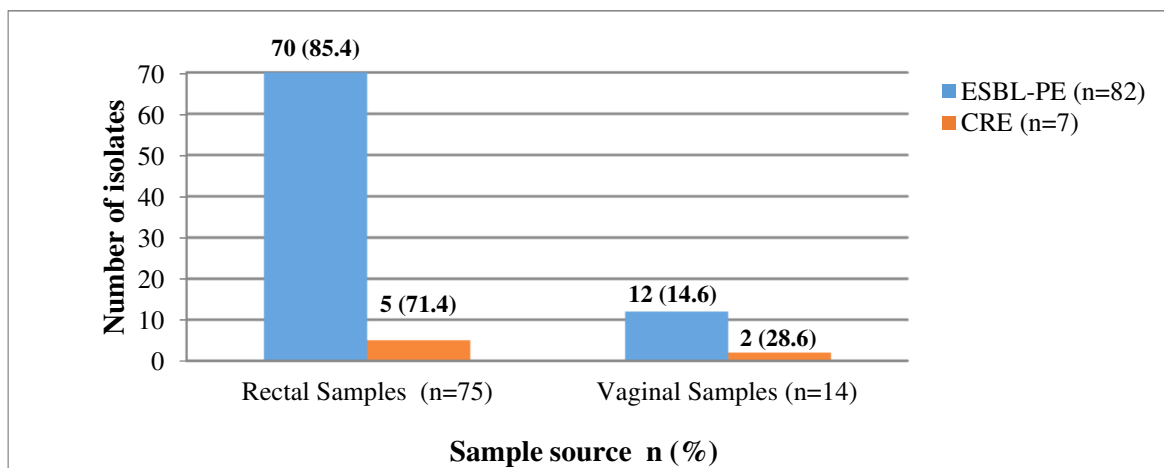


Figure 4: Distribution of Maternal ESBL-PE and CRE Isolates by Sample Source at SPHMMC, Addis Ababa, 2024 (n = 295)

5.3.3 Bacterial Profile of ESBL-PE and CRE Isolates Among Study Participants

A total of 101 Gram negative bacteria isolates were identified, with 95 (94.1%) classified as ABR GNB: 85 (84.2%) ESBL-PE and 10 (9.9%) CRE. *Escherichia coli* were the most common isolate, accounting for 90 (89.1%), while *Klebsiella spp.* accounted for 11 (10.9%) (Table 5).

Table 5. Distribution and antimicrobial resistance pattern of bacterial isolates among laboring mothers and their newborns at SPHMMC, Addis Ababa, 2024 (n = 101 isolates)

Type of Bacteria Isolated	ESBL-PE (n, %)	CRE (n, %)	Non-ESBL (n, %)	Total (n, %)
<i>Escherichia coli</i>	75 (83.3)	9 (10.0)	6 (6.7)	90 (89.1)
<i>Klebsiella pneumoniae</i>	4 (80.0)	1 (20.0)	0 (0.0)	5 (5.0)
<i>Klebsiella ozanae</i>	4 (100.0)	0 (0.0)	0 (0.0)	4 (4.0)
<i>Klebsiella oxytoca</i>	2 (100.0)	0 (0.0)	0 (0.0)	2 (2.0)
Total	85 (84.2)	10 (9.9)	6 (5.9)	101 (100.0)

5.3.4 Extent and Diversity of Maternal and Neonatal ESBL-PE and CRE Colonization

The study revealed that in most cases, a single bacterial species was isolated from colonized mothers 61 (84.7%) and neonates 4 (80%).

Table 6. Extent and Diversity of maternal and neonatal ESBL-PE and CRE colonization at SPHMMC, Addis Ababa, 2024 (n = 295)

Group	Colonized Cases n (%)	Single Species n (%)	Multiple Species n (%)
Mothers (n = 295)	72 (24.4)	61 (84.7)	11 (15.3)
Neonates (n = 295)	5 (1.7)	4 (80.0)	1 (20.0)

5.4 Association between Maternal and Neonatal Colonization Status and Bacterial Isolates

5.4.1 Association between Maternal and Neonatal ESBL-PE and CRE Colonization Status

The analysis between maternal colonization status and neonatal colonization indicated a significant association. Out of the 72 neonates born to colonized mothers, 4 (5.6%) were

colonized, whereas only 1 (0.4%) of the 223 neonates born to non-colonized mothers was colonized (OR: 13.059, 95% CI: 1.435–118.812, $p = 0.014$).

Table 7. Association between maternal and neonatal Antibiotic Resistant Gram Negative Bacteria colonization at SPHMMC, Addis Ababa, 2024 (n = 295)

Maternal ABR-GNB Colonization Status	Neonatal ABR-GNB Colonization Status		Total	OR (95% CI)	p-value
	Colonized	Not Colonized			
Colonized	4	68	72	13.059 (1.435 - 118.812)	0.014
Not Colonized	1	222	223		
Total	5	290	295		

Note: ABR-GNB (ESBL-PE and CRE)

Analysis of colonized cases also showed that 3 out of 70 neonates born to ESBL-PE-positive mothers were tested positive (Fisher's Exact Test p -value = 0.013). For CRE, 1 out of 4 neonates born to a CRE-positive mother was tested positive, which was also statistically significant, with a ($p = 0.040$).

Table 8. Association between Maternal and Neonatal Colonization by ESBL-PE and CRE at SPHMMC, Addis Ababa, 2024 (n = 295)

Maternal ESBL- PE Colonization	Neonatal ESBL-PE Colonization (n)			p-value
	+ve(n)	-ve (n)	Total	
+ve (n)	3	67	70	0.013
-ve (n)	0	225	225	
Total	3	292	295	

Maternal CRE Colonization	Neonatal CRE Colonization (n)			p-value
	+ve (n)	-ve (n)	Total	
+ve (n, %)	1	3	4	0.040
-ve (n, %)	2	289	291	
Total	3	292	295	

Note - +ve (positive / colonized), -ve (Negative/ Not colonized)

5.4.2 Phenotypic Similarities between Maternal and Neonatal Isolates

This study investigated phenotypic similarities between bacterial isolates from maternal and neonatal samples, focusing on the potential for vertical transmission during childbirth. Phenotypic associations were assessed using biochemical results and AST pattern matching.

A. Biochemical result based species similarity

Species-level analysis showed that 3 neonates born from *E. coli*-colonized mothers also carried *E. coli*, suggesting a phenotypic match. Additionally, 1 neonatal sample from a mother with both *E. coli* and *K. pneumoniae* showed the same combination, indicating possible vertical transmission. The p-value of 0.006 confirms a significant association between maternal and neonatal bacterial isolates.

Table 9. Biochemical result based association between Maternal and Neonatal Isolates at SPHMMC, Addis Ababa, 2024 (n = 295)

		The type of bacteria isolated from neonatal sample					p-value (Fisher's Exact)
		<i>E.coli</i>	<i>K.pneumonia</i>	<i>E.coli</i> & <i>K.pneumonia</i>	Negative	Total	
The type of bacteria isolated from maternal sample	<i>E.coli</i>	3	0	0	61	64	0.006
	<i>K.pneumonia</i>	0	0	0	1	1	
	<i>K.ozane</i>	0	0	0	2	2	
	<i>E.coli</i> and <i>K.pneumonia</i>	0	0	1	1	2	
	<i>E.coli</i> and <i>K.ozane</i>	0	0	0	1	1	
	<i>E.coli</i> , <i>k.oxytoca</i> and <i>K.ozanae</i>	0	0	0	1	1	
	<i>E.coli</i> and <i>K. oxytoca</i>	0	0	0	1	1	
	Negative	0	1	0	222	223	
	Total	3	1	1	290	295	

B. Antimicrobial Susceptibility Pattern based phenotypic similarity

AST analysis showed 35.7% to 100.0% matching rates between maternal and neonatal isolates. Most pairs exhibited high concordance ($\geq 80\%$) with statistical significant p-values ($p < 0.05$), supporting possible vertical transmission. (Table 10).

Table 10. Antimicrobial Susceptibility Patterns based association between Maternal and Neonatal Isolates at SPHMMC, Addis Ababa, 2024

Case	Maternal isolate (source)	Neonatal isolate	Resistance phenotype	Antibiotics tested (n)	Matched (n)	Unmatched (n)	AST concordance (%)	Fisher's exact p-value
1	<i>E. coli</i> (V)	<i>E. coli</i>	CRE	14	14	0	100.0	0.011
	<i>K. pneumoniae</i> (V)	<i>K. pneumoniae</i>	ESBL	13	11	2	84.6	<0.001
2	<i>E. coli</i> (V)	<i>E. coli</i>	ESBL	14	13	1	92.9	0.001
3	<i>E. coli</i> (R)	<i>E. coli</i>	ESBL vs	14	5	9	35.7	0.832

4			CRE					
	<i>E. coli</i> (V)	<i>E. coli</i>	ESBL	14	13	1	92.9	<0.001
	<i>E. coli</i> (R)	<i>E. coli</i>	ESBL	14	10	4	71.4	0.003

Note - V: Vaginal; R: Rectal

AST concordance (%) was calculated as the proportion of antibiotics with identical susceptibility interpretation (S/I/R) between maternal and neonatal isolates

5.5. Antibiotic Susceptibility Pattern and Multidrug-Resistant Status

5.5.1 Antibiotic Susceptibility Testing Pattern of Isolates

Antimicrobial susceptibility testing was performed on 95 maternal and neonatal isolates. The isolates showed high susceptibility to imipenem 86 (90.5%), meropenem 84 (88.4%), ertapenem 84 (88.4%), amikacin 92 (96.8%), gentamicin 84 (88.4%), and chloramphenicol 87 (91.6%). Additionally, Isolates shows lower susceptibility to ciprofloxacin 35 (36.8%), trimethoprim-sulfamethoxazole 22 (23.2%) and ceftriaxon 1 (1.1%) (Table 11).

Table 11. Antibiotic susceptibility patterns of isolates from laboring mothers and their newborns at SPHMMC, Addis Ababa, 2024 (n = 95 isolates)

Antibiotic	Sensitive (n, %)	Intermediate (n, %)	Resistant (n, %)
Ampicillin	0 (0)	0 (0)	95 (100)
Ceftriaxone	1 (1.1)	0 (0)	94 (98.9)
Cefepime	1 (1.1)	18 (18.9)	76 (80)
Ceftazidime	11 (11.6)	43 (45.3)	41 (43.2)
Piperacillin-Tazobactam	63 (66.3)	25 (26.3)	7 (7.4)
Amoxicillin-Clavulanate	62 (65.3)	14 (14.7)	19 (20)
Imipenem	86 (90.5)	2 (2.1)	7 (7.4)
Meropenem	84 (88.4)	2 (2.1)	9 (9.5)
Ertapenem	84 (88.4)	3 (3.2)	8 (8.4)
Amikacin	92 (96.8)	3 (3.2)	0 (0)
Gentamicin	84 (88.4)	0 (0)	11 (11.6)
Ciprofloxacin	35 (36.8)	21 (22.1)	39 (41.1)
Chloramphenicol	87 (91.6)	3 (3.2)	5 (5.3)
Trimethoprim-Sulfamethoxazole	22 (23.2)	0 (0)	73 (76.8)

5.5.2 Antibiotic Susceptibility Testing Pattern of Isolates by Bacterial Species

Species specific AST analysis of the study indicates that among isolates, 83 (98.8%) of *E. coli* were resistant to ceftriaxon and 66 (78.6%) to cefepime. All *k. pneumonia*, *K. Ozanae* and *K. ocytoca* isolates (100%) were resistant to ceftriaxon and cefepime. Carbapenem and aminoglycosed susceptibility was high: *E. coli* showed 75 (89.3%) susceptibility to imipenem

and 81 (96.4%) to amikacin, while all *klebsiella* species were 100% susceptible to both antibiotics (Table 12).

Table 12. Antibiotic Susceptibility Patterns by Bacterial Species at SPHMMC, Addis Ababa, 2024 (n, %)

Antibiotic	Suseptablity Pattern	<i>E. coli</i> (n = 84)	<i>K. pneumoniae</i> (n = 5)	<i>K. ozanae</i> (n = 4)	<i>K. oxytoca</i> (n = 2)
Ampicillin	S	0 (0.0)	N/A	N/A	N/A
	I	0 (0.0)	N/A	N/A	N/A
	R	84 (100.0)	N/A	N/A	N/A
Ceftriaxone	S	1 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)
	I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	R	83 (98.8)	5 (100.0)	4 (100.0)	2 (100.0)
Cefepime	S	1 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)
	I	17 (20.2)	1 (20.0)	0 (0.0)	0 (0.0)
	R	66 (78.6)	4 (80.0)	4 (100.0)	2 (100.0)
Ceftazidime	S	11 (13.1)	0 (0.0)	0 (0.0)	0 (0.0)
	I	38 (45.2)	3 (60.0)	0 (0.0)	0 (0.0)
	R	35 (41.7)	2 (40.0)	4 (100.0)	2 (100.0)
Piperacillin-Tazobactam	S	61 (72.6)	2 (40.0)	0 (0.0)	0 (0.0)
	I	16 (19.0)	3 (60.0)	4 (100.0)	2 (100.0)
	R	7 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)
Amoxicillin-Clavulanate	S	56 (66.7)	3 (60.0)	3 (75.0)	0 (0.0)
	I	14 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)
	R	14 (16.7)	2 (40.0)	1 (25.0)	2 (100.0)
Imipenem	S	75 (89.3)	5 (100.0)	4 (100.0)	2 (100.0)
	I	2 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)
	R	7 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)
Meropenem	S	74 (88.1)	4 (80.0)	4 (100.0)	2 (100.0)
	I	2 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)
	R	8 (9.5)	1 (20.0)	0 (0.0)	0 (0.0)
Ertapenem	S	73 (87.0)	5 (100.0)	4 (100.0)	2 (100.0)
	I	3 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)
	R	8 (9.5)	0 (0.0)	0 (0.0)	0 (0.0)
Amikacin	S	81 (96.4)	5 (100.0)	4 (100.0)	2 (100.0)
	I	3 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)
	R	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gentamicin	S	75 (89.3)	5 (100.0)	4 (100.0)	2 (100.0)
	I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	R	9 (10.7)	0 (0.0)	0 (0.0)	0 (0.0)
Ciprofloxacin	S	32 (38.1)	2 (40.0)	1 (25.0)	0 (0.0)
	I	17 (20.2)	2 (40.0)	2 (50.0)	0 (0.0)
	R	35 (41.7)	1 (20.0)	1 (25.0)	2 (100.0)
Chloramphenicol	S	78 (92.9)	5 (100.0)	4 (100.0)	2 (100.0)
	I	3 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)
	R	3 (3.6)	0 (0.0)	0 (0.0)	2 (100.0)
Trimethoprim-Sulfamethoxazole	S	18 (21.4)	1 (20.0)	3 (75.0)	0 (0.0)
	I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	R	66 (78.6)	4 (80.0)	1 (25.0)	2 (100.0)

Note: S (Sensitive), I (Intermediate), R (Resistant), N/A (Not Applicable)

5.5.3 Multidrug-Resistant Status of Isolates

This study reveals a high burden of MDR among isolates from laboring mothers and their newborns, with 76/95 (80%) of isolates classified as MDR. Specifically, 70/84 (83.3%) of *E. coli* and 3/5 (60%) of *K. pneumoniae* isolates exhibited MDR patterns. Alarming, all *K. oxytoca* isolates 2/2 (100%) were resistant to antibiotics from six different classes, indicating extensive drug resistance tendencies (Table 13).

Table 13. Multi-drug resistance patterns of isolates from laboring mothers and their newborns at SPHMMC, Addis Ababa, 2024 (n = 95)

Isolate number (n)	Antibiotic resistance level (n (%))*							Total MDR Isolates (R _{≥3})
	R1	R2	R3	R4	R5	R6	≥R7	
<i>E.coli</i> (84)	0 (0.0)	14 (16.7)	35 (41.7)	22 (26.2)	11 (13.1)	2 (2.4)	0 (0.0)	70 (83.3)
<i>K. pneumoniae</i> (5)	1 (20.0)	1 (20.0)	1 (20.0)	2 (40.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (60.0)
<i>K.Ozanea</i> (4)	2 (50.0)	1 (25.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)
<i>K.Oxytoca</i> (2)	0 (0.0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (100.0)	0 (0.0)	2 (100.0)
Total (95)	3 (3.2)	16 (16.8)	37 (39.0)	24 (25.3)	11 (11.6)	4 (4.2)	0 (0.0)	76 (80.0)

Note: R0: resistance to no antibiotics, R1-7: resistance to 1, 2, 3, 4, 5, 6, and 7 antibiotics; ≥R3: resistance to at least one agent in 3 or more antimicrobial classes, MDR-multi drug resistance

Antimicrobial class used to define MDR- Penicillin (Ampicillin, Amoxicillin-Clavulanate, Piperacillin-Tazobactam), Cephalosporin (Ceftazidime, Ceftriaxone, Cefepime), Carbapenem (Ertapenem, Meropenem, Imipenem), Aminoglycoside (Amikacin, Gentamicin), Sulfonamide and Trimethoprim (Trimethoprim-Sulfamethoxazole), Amphenicol (Chloramphenicol), Fluoroquinolone (Ciprofloxacin).

5.6 Factors Influencing Colonization with ESBL-PE and CRE

5.6.1 Factors Influencing Maternal Colonization with ESBL-PE and CRE

The analysis of maternal ESBL-PE and CRE colonization revealed that women with primary education had significantly higher odds of colonization compared to those with no formal education [AOR = 3.527, 95% CI: 1.235–10.074, p = 0.019]. Regarding marital status, married women had significantly higher odds of colonization compared to those categorized as "not-

married” (including divorced, widowed, separated, or single) [AOR = 9.897, 95% CI: 1.331–73.602, $p = 0.025$]. In addition, each additional ANC visit was associated with a reduced likelihood of colonization [AOR = 0.804, 95% CI: 0.658–0.982, $p = 0.033$]. No significant associations were observed for other factors, including age, residence, occupation, family income, and medical history (Table 14).

Table 14. Factors associated with maternal colonization by ESBL-PE and CRE at SPHMMC, Addis Ababa, 2024 (n = 295)

che	Category	Maternal ABR-GNB Colonization Status		Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
		Not Colonized (N = 223)	Colonized (N = 72)				
Age of the Mother	18–24	67 (73.6)	24 (26.4)	1	-	-	-
	25–34	133 (77.8)	38 (22.2)	1.250 (0.695–2.260)	0.452	-	-
	≥35	23 (69.7)	10 (30.3)	0.824 (0.343–1.980)	0.665	-	-
Residence	Urban	222 (76.0)	70 (24.0)	1	-	1	-
	Rural	1 (33.3)	2 (66.7)	0.158 (0.014–1.765)	0.134	0.160 (0.010–2.545)	0.194
Education Status	No formal education	13 (59.1)	9 (40.9)	1	-	1	-
	Primary education	109 (83.2)	22 (16.8)	3.430 (1.306–9.008)	0.012	3.527 (1.235–10.074)	0.019*
	Secondary education	55 (69.6)	24 (30.4)	1.590 (0.598–4.210)	0.354	1.326 (0.453–3.879)	0.607
	Diploma and above	46 (73.0)	17 (74.0)	1.870 (0.678–5.173)	0.226	2.147 (0.639–7.218)	0.217
	Housewife	139 (76.8)	42 (23.2)	1	-	1	0.141
Occupation	Government worker	21 (70.0)	9 (30.0)	0.705 (0.300–1.656)	0.422	1.919 (0.643–3.215)	0.428
	Private worker	44 (83.0)	9 (17.0)	1.477 (0.667–3.274)	0.337	1.860 (0.773–4.476)	0.166
	Merchant	13 (59.1)	9 (40.9)	0.436 (0.174–1.092)	0.076	0.405 (0.155–1.055)	0.064
	Other	6 (66.7)	3 (33.3)	0.604 (0.145–2.521)	0.489	1.015 (0.178–5.775)	0.987
	Married	220 (76.4)	68 (23.6)	4.314 (0.942–19.752)	0.060	9.897 (1.331–73.602)	0.025*
Marital Status	Unmarried	3 (42.9)	4 (57.1)	1	-	1	-
Family Income	Median (IQR)	10,000 (6,000–15,000)	10000 (5250 – 16750)	1.000 (1.000–1.000)	0.388	-	-
Number of Family Members	Median (IQR)	3.0 (2.0–4.0)	3.5 (3.0–4.8)	0.962 (0.805–1.150)	0.671	-	-
History of	Yes	42 (77.8)	12 (22.2)	1.160 (0.573–	0.679	-	-

Abortion				2.348)			
	No	181 (75.1)	60 (24.9)	1	-	-	-
History of Preterm Delivery	Yes	2 (66.7)	1 (33.3)	0.640 (0.057-7.192)	0.720	-	-
	No	221 (75.7)	71 (24.3)	1	-	-	-
History of PPH	Yes	25 (86.2)	4 (13.8)	2.146 (0.721-6.389)	0.170	2.961 (0.903-9.706)	0.073
	No	198 (74.4)	68 (25.6)	1	-	1	-
History of PROM	Yes	35 (77.8)	10 (22.2)	1.154 (0.540-2.466)	0.711	0.908 (0.404-2.041)	0.815
	No	188 (75.2)	62 (24.8)	1	-	1	-
Gravida	Median (IQR)	2 (1-3)	2 (1-3)	1.042 (0.853-1.274)	0.686	-	-
Parity	Median (IQR)	2 (1-3)	2 (1-3)	1.010 (0.810-1.256)	0.939	-	-
Number of ANC	Median (IQR)	6 (6-8)	7 (6-8)	0.850 (0.706-1.022)	0.084	0.804 (0.658-0.982)	0.033*
Follow-ups IAP	Yes	89 (76.7)	27 (23.3)	1.107 (0.640-1.913)	0.716	-	-
	No	134 (74.9)	45 (25.1)	1	-	-	-
Gestational Week	Term (37-42 wk)	209 (75.5)	68 (24.5)	1	-	-	-
	Preterm (<37 wk)	8 (80.0)	2 (20.0)	1.301 (0.270-6.280)	0.743	-	-
	Post-term (>42 wk)	6 (75.0)	2 (25.0)	0.976 (0.192-4.950)	0.977	-	-

Note – **ABR-GNB** (ESBL-PE and CRE), **PROM** (premature rupture of membrane), **IAP** (Intrapartum Antibiotic Prophylaxis), **PPH** – (Postpartum Hemorrhage), **ANC** (Antenatal Care).

5.6.2 Factors Influencing Neonatal Colonization with ESBL-PE and CRE

The analysis of neonatal ESBL-PE and CRE colonization reveals significant associations between neonatal colonization and maternal education status ($p = 0.049$), history of preterm delivery ($p = 0.050$), maternal colonization ($p = 0.014$) and gestational age ($p = 0.016$). Neonates born to mothers colonized with ESBL-PE and CRE, secondary education, a history of preterm delivery, or born preterm were more likely to be colonized. Other factors such as maternal age, occupation, family income, and birth weight showed no significant associations.

Table 15. Factors associated with neonatal colonization by ESBL-PE and CRE at SPHMMC, Addis Ababa, 2024 ($n = 295$)

Variable	Category	Neonatal AR-GNB Colonization status		Fisher exact test p-value
		Not Colonized (N =290)	Colonized (N = 5)	

Age of the Mother	18–24	89 (97.8)	2 (2.2)	0.503
	25–34	169 (98.8)	2 (1.2)	
	≥35	32 (97.0)	1 (3.0)	
Residence	Urban	287 (98.3)	5 (1.7)	1.000
	Rural	3 (100.0)	0 (0.0)	
Education Status	No formal education	21 (95.5)	1 (4.5)	0.049*
	Primary education	131 (100.0)	0 (0.0)	
	Secondary education	76 (96.2)	3 (3.8)	
Occupation	Diploma and above	62 (98.4)	1 (1.6)	1.000
	Housewife	177 (97.8)	4 (2.2)	
	Government worker	30 (100.0)	0 (0.0)	
	Private worker	52 (98.1)	1 (1.9)	
	Merchant	22 (100.0)	0 (0.0)	
	Other	9 (100.0)	0 (0.0)	
Marital Status	Married	283 (98.3)	5 (1.7)	1.000
	Unmarried	7 (100.0)	0 (0.0)	
Family Income	Median (IQR)	10,000 (6,000-15,250)	8,000 (3,500-10,000)	0.148
Number of Family Members	Median (IQR)	3.0 (3.0-4.3)	3.0 (2.5-4.50)	0.711
History of Abortion	Yes	52 (96.3)	2 (3.7)	0.228
	No	238 (98.8)	3 (1.2)	
History of Preterm Delivery	Yes	2 (66.7)	1 (33.3)	0.050*
	No	288 (98.6)	4 (1.4)	
History of PPH	Yes	29 (100.0)	0 (0.0)	1.000
	No	261 (98.1)	5 (1.9)	
History of PROM	Yes	44 (97.8)	1 (2.2)	0.566
	No	246 (98.4)	4 (1.6)	
Gravida	Median (IQR)	2.0 (1.0-3.0)	2.0 (1.0-3.5)	0.897
Parity	Median (IQR)	2.0 (1.0-3.0)	1.0 (1.0-2.5)	0.418
Number of ANC Follow-ups	Median (IQR)	6.0 (6.0-8.0)	6.0 (5.0-6.5)	0.320
IAP	Yes	114 (98.3)	2 (1.7)	0.975
	No	176 (98.3)	3 (1.7)	
Maternal colonization	Yes	68 (94.4)	4 (5.6)	0.014*
	No	222 (99.6)	1 (0.4)	
Sex	Male	148 (98.7)	2 (1.3)	0.680
	Female	142 (98.0)	3 (2.0)	
Gestational Age	Term (37–42 weeks)	274 (99.0)	3 (1.0)	0.016*
	Preterm (<37 weeks)	8 (80.0)	2 (20.0)	
	Post-term (>42 weeks)	8 (100.0)	0 (0.0)	
Birth Weight (grams)	LBW (<2500)	27 (93.1)	2 (6.9)	0.171

	Normal (2500-4000)	257 (98.8)	3 (1.2)	
	HBW (>4000)	6 (100.0)	0 (0.0)	
First Minute APGAR Score	Low (0-3)	0 (0.0)	0 (0.0)	0.345
	Fair (4-6)	99 (97.1)	3 (2.9)	
	Normal (7-10)	191 (99.0)	2 (1.0)	
Fifth Minute APGAR Score	Low (0-3)	0 (0.0)	0 (0.0)	1.000
	Fair (4-6)	11 (100.0)	0 (0.0)	
	Normal (7-10)	279 (98.2)	5 (1.8)	

Note – **ABR-GNB** (ESBL-PE and CRE), **PROM** (premature rupture of membrane), **IAP** (Intrapartum Antibiotic Prophylaxis), **PPH** – (Postpartum Hemorrhage), **ANC** (Antenatal Care), **APGAR** – (Appearance, Pulse, Grimace, Activity, and Respiration).

6. DISCUSSION

This study aimed to assess maternal and neonatal colonization of ESBL-producing and carbapenem-resistant Enterobacterales and to identify factors associated with colonization and vertical transmission at St. Paul Hospital Millennium Medical College. The study demonstrated that 70/295 (23.7%) laboring mothers were colonized with ESBL-PE and 4/295 (1.4%) with CRE, while among 295 newborns, 5 (1.7%) were colonized with ESBL-PE and 3 (1.0%) with CRE. Maternal colonization with ESBL-PE and CRE showed a strong association with neonatal colonization (OR = 13.06, $p = 0.014$), and four of the five colonized neonates were born to colonized mothers with matching bacterial species and similar phenotypic resistance profiles, indicating probable vertical transmission. Furthermore, several maternal-related factors were significantly associated with colonization by ESBL-PE and CRE. Collectively, these findings highlight a substantial burden of ABR-GNB colonization and emphasize the important role of maternal carriage in neonatal acquisition.

6.1 Prevalence of ESBL-PE and CRE Colonization

In this study, we observed a maternal colonization rate of 23.7% for ESBL-PE, which raises significant concerns regarding the potential for vertical transmission to neonates. This prevalence aligns with findings from a recent systematic review, which reported a pooled maternal carriage of ESBL-PE in Africa that can peak at 30% (97,98). Similarly, a study from Israel also reported a comparable maternal colonization rate of 21.5% (65). In contrast to our finding, studies from other high-income countries (HICs) such as Germany (11.1%) (69), Norway (2.9%) (66), Spain (6.7%) (74), and Austria (0.1%) (73) report relatively lower prevalence rates of ESBL-PE colonization among mothers. The comparatively lower prevalence in these countries may be attributed to better infection control practices, more judicious use of antibiotics, and enhanced surveillance systems for antimicrobial resistance.

On the other hand, studies conducted in LMICs, including those within Africa, tend to report higher levels of maternal colonization. For example, comparable rates have been observed in Benin (22.3%) (99), Madagascar (30%) (100), Nigeria (31.7%) (101), and Ethiopia (22.3%) (14). Some settings have reported even higher rates; Tanzania (64.3%) (102), which is much higher from both our study and those from HICs. This high prevalence in LMICs, including Ethiopia,

could be attributed to several factors, such as high rates of antimicrobial use, poor infection control practices in healthcare settings, overcrowding and limited health education and hygien awareness in some settings (103,104).

Neonatal colonization with ESBL-PE in our study was relatively lower, with only 1.7% of newborns found to be colonized. This finding aligns with reports from Japan, Austria and Spain (2.4%, 0.04%, 1.6%,) respectively (67,73,74). The lower prevalence in neonates is consistent with the assumption that neonates are less likely to harbor these organisms due to their immature microbiota and more limited exposure to environmental sources of colonization. However, a contrasting finding comes from Ethiopia's Amhara region, where neonatal ESBL-PE colonization was found to be 24.5% (14). This discrepancy may be due to differences in laboratory methods, sampling time and site of sample collection. In addition, variations in study setting and greater postnatal exposure could have contributed to the higher neonatal colonization observed in the Amhara study. The low neonatal colonization rate in our study (1.0%) may also reflect our sampling approach, which focused solely on external ear swabs. This method could underestimate the true colonization burden, as neonates may harbor bacteria in other body sites such as the gastrointestinal tract or skin (105).

Regarding CRE colonization, we found a relatively low maternal colonization rate of 1.4%, which is consistent with previous finding from Ethiopia (0.9%) by Amsalu *et.al* (14). Studies from Israel, Algeria and America have reported similar trends of low CRE colonization 0%, 4.6% and 2% respectively (10,68,106). Neonatal CRE colonization was also low in our study (1.0%), which is consistent with findings from previous study of Ethiopia (2.5%) (14), northern Algeria (1.6%) (68), and Israel (0%) (10). Our finding is also in line with a recent meta-analysis estimating the pooled prevalence of CRE in Ethiopia to be 4.4% (107). The consistently low rates of CRE colonization might be due to the limited use of carbapenem antibiotics in this vulnerable population pregnant women and neonates, as these agents are typically reserved for severe MDR infections (108).

6.2. Bacterial Profile and Phenotypic Relationship between Isolates

In our study, *E. coli* was the predominant gram-negative pathogen, accounting for 88.4% of isolates, followed by *K. pneumoniae* at 5.3%. This distribution consistent with a meta-analysis

by Yousef Moradi *et al.*, which reported *E. coli* as a leading pathogen (34%) among pregnant women (109). Similarly, a previous Ethiopian study found that the majority of ESBL-producing isolates among laboring mothers and their neonates were *E. coli* (80.3%) followed by *K. pneumoniae* (12.7%) (14). Comparable trends were also observed in Israel, where *E. coli* predominated in neonatal colonization, with *K. pneumoniae* as a secondary contributor (10).

The persistence of *E. coli* and *K. pneumoniae* as leading pathogens across different geographic regions highlights the need for comprehensive surveillance and effective infection control measures, particularly in healthcare settings that care for pregnant women and neonates. A review by Sands *et al.* indicated that *E. coli* is commonly implicated in neonatal sepsis in LMICs, underscoring the significant role of these pathogens in maternal-neonatal health dynamics (110). The presence of *Klebsiella* species in our findings further emphasizes the need for ongoing surveillance, as these pathogens are frequently associated with severe infections and multidrug resistance (111).

Our study revealed phenotypic similarities between bacterial isolates from mothers and their neonates, particularly *E.coli*. This finding suggests the potential for vertical transmission of resistant pathogens during delivery. Maternal colonization with resistant pathogens is widely recognized as a significant risk factor for neonatal infections (112). The observed phenotypic relationship between maternal and neonatal isolates underscores the importance of understanding the dynamics of colonization in preventing neonatal infections.

Consistent with our findings, a study conducted in rural Ethiopia found a significant association between maternal carriage of ESBL-producing organisms and neonatal carriage, highlighting the critical role maternal colonization play in transmitting resistant bacteria to their infants (14). Our study also revealed that neonates born to colonized mothers were 13 times more likely to be colonized (OR: 13.059, 95% CI: 1.435–118.812, $p = 0.014$), further supporting the hypothesis of vertical transmission. This aligns with findings from a Nigerian study, which identified maternal ESBL-PE colonization as a strong predictor of neonatal colonization (AOR 14.85; 95% CI 7.83–28.15) (29).

Moreover, a study in Norway found that 35.7% of newborns carried ESBL-producing strains identical to those found in their mothers during the first days of life (66). Similarly, in Israel, maternal colonization was shown to significantly increase the risk of neonatal colonization (113). These findings indicate that vertical transmission occurs across different healthcare settings and geographic regions. A systematic review by Bulabula *et al.* (2020) emphasized that up to 27% of MDR-GNB transmission occurs from mothers to infants, reinforcing the importance of monitoring maternal health during pregnancy (11). This body of evidence suggests that maternal colonization, particularly with MDR organisms, plays a crucial role in neonatal colonization and subsequent infection.

6.3. Antimicrobial Susceptibility Patterns of ESBL-PE and CRE Isolates

The antimicrobial susceptibility testing results from our study indicates concerning patterns of antibiotic resistance among ESBL-PE and CRE isolates. Notably, all isolates were resistant to ampicillin (100%), with markedly high rate of resistance against third-generation cephalosporins, including ceftriaxone (98.9%) and cefepime (80%). The findings reflect the growing challenge posed by resistant GNB in health system (49). Similar observations have been noted worldwide; a German study reported complete resistance to amoxicillin and ampicillin (100%) while relatively lower resistance rates against cefepime (18%) and ceftazidime (69%) among ESBL-producing isolates were recorded (71). A high resistance to penicillins and cephalosporins has also been observed across Africa. A study from Benin documented complete resistance to amoxicillin and ceftriaxone (100%) and 89.4% to cefotaxime (114). In contrast, a recent Ethiopian study illustrated a smaller number of resistant isolates, 76.5%, 44.6%, and 14% against ampicillin, ceftriaxone, and ceftazidime, respectively (27). This could be due to the specific type of bacteria targeted in the respective studies, a difference in local prescribing practices and antibiotics stewardship.

In addition to that, our study demonstrated high resistance rates to ciprofloxacin (41.1%) and trimethoprim-sulfamethoxazole (76.8%), indicating the need for careful monitoring of these agents. A much higher ciprofloxacin resistance rate of 61% was recorded in Israel, possibly reflecting more frequent use of this antibiotic and the resulting selective pressure (10). On the other hand, a study conducted in Germany found relatively low rates of 14% and 42% resistance

to ciprofloxacin and trimethoprim-sulfamethoxazole, respectively (71). Reports from African countries also indicate substantial resistance to these antibiotics; for example, in Benin, 75.8% of the isolates were resistant to ciprofloxacin, while 89.4% were resistant to trimethoprim-sulfamethoxazole (114). A recent Ethiopian study documented a comparable rate of resistance to ciprofloxacin at 47.8% but a lower rate for trimethoprim-sulfamethoxazole at 22% (27). The variability in resistance levels observed may therefore relate to differences in prescription practices, an overall antimicrobial stewardship program, and levels of regulation over over-the-counter drug accesses, which are best achieved in high-income countries. Despite this, the findings are remarkably consistent with high-level resistance to these commonly used antibiotics, raising concerns over their eventual effectiveness in clinical practice.

Despite this high level of resistance to commonly used antibiotics, the carbapenems (imipenem, meropenem, and ertapenem) retained relatively higher susceptibility rates, ranging from 88.4% to 90.5% in this study. Similarly, amikacin (96.8%) and chloramphenicol (91.6%) exhibited high efficacy, suggesting that these antibiotics may still be valuable therapeutic options. A comparable study from Israel similarly found that carbapenems retained 100% susceptibility, and amikacin (96%) and chloramphenicol (96%) remained highly effective (10). Additionally, a study conducted in Germany on antibiotic resistance in ESBL-PE from neonates reported similar trends (96-100%) to our findings.. The study also found that amikacin had high efficacy (96%), consistent with our results (71). A recent study from Ethiopia indicated a 100% Amikacin susceptibility which is in line with our finding but much lower susceptibility for meropenem (67.7%) and imipenem (71%) which is an indication for the emergence of resistance to these classes is an ever-present risk, particularly if these antibiotics are overused (27).

Furthermore, the high levels of multidrug resistance (80%) observed in our isolates is consistent with studies from other LMICs that report widespread multidrug resistance among hospitalized patients (115). For instance, a recent systematic review and meta-analysis conducted in Ethiopia spanning 15 studies indicated that the pooled estimate of MDR among extended-spectrum β -lactamase producers was 71.7% (107). The increasing MDR rates in both hospital and community settings raise urgent concerns about the sustainability of effective treatments, especially for vulnerable populations such as pregnant women and neonates.

6.4. Factors Associated with Colonization and Vertical Transmission

To investigate the factors associated with maternal and neonatal colonization by ESBL-PE and CRE, several socio demographic and clinical variables were examined. The study identified educational attainment, marital status, and antenatal care (ANC) visits as significant factors influencing maternal colonization. Notably, women with primary education showed significantly higher odds of colonization (AOR = 3.527, 95% CI: 1.235–10.074) compared to those with no formal education. This indicates that primary education may not adequately equip women with the knowledge or resources necessary to mitigate their risk of colonization. In contrast, mothers with secondary education or higher were not significantly associated with colonization, possibly because better education improves health practices and awareness. This aligns with studies showing that higher education improves health literacy, leading to more informed decisions and healthier behaviors (116,117). A comparable finding also observed from Tanzania in which the odds of carrying ESBL-PE were two times higher among participants having a low education level than high education level (AOR = 2.19, 95% CI = 1.08–4.43) (102).

In terms of marital status, the study revealed that married women had significantly higher odds of colonization compared to non-married women (AOR = 9.897, $p = 0.025$). This could be due to close household contacts and shared environments, which may increase exposure and colonization risk among married women. In contrast to this finding, marriage is generally associated with improved healthcare access, better hygiene practices, and stronger social support systems, all of which typically contribute to better health outcomes (118). However, the significant sample size disparity in the current study (288 married vs. 7 non-married participants) may limit the reliability of this conclusion. Therefore, further research with a more balanced sample distribution is necessary to better understand the potential relationship between marital status and ABR-GNB colonization.

Moreover, the study highlights the importance of ANC visits, with each additional visit associated with a reduced likelihood of colonization (AOR = 0.804, $p = 0.033$). This finding aligns with the WHO recommendations on antenatal care (119). The finding is also consistent with a study by Ayalew *et.al* that shows increased ANC visits lead to better health outcomes, including reduced rates of infection (120). Encouragingly, this suggests that enhancing access to

and utilization of ANC services could be a vital strategy in reducing ABR-GNB colonization among pregnant women.

The analysis of neonatal ESBL-PE and CRE colonization reveals significant associations between neonatal colonization and maternal education status ($p = 0.049$), history of preterm delivery ($p = 0.050$), maternal colonization ($p = 0.014$) and gestational age ($p = 0.016$). Neonates born to colonized mothers were significantly more likely to also be colonized, with an odds ratio of 13.059 (95% CI: 1.435 - 118.812, $p = 0.014$). This finding corroborates results from several other studies (29,73), highlighting the substantial role of vertical transmission in neonatal colonization. The study also indicated that preterm neonates were at a higher risk of colonization compared to term neonates ($p = 0.016$). This may be due to the underdeveloped immune systems of preterm infants, which make them more susceptible to colonization by opportunistic pathogens, as well as potential differences in delivery exposure(121,122).

In addition, maternal factors such as a history of preterm delivery ($p = 0.050$) and lower maternal education level ($p = 0.049$) were significantly associated with an increased risk of neonatal colonization. According to WHO guidelines, preterm birth and a history of infections may indicate underlying systemic healthcare challenges and disruptions in the maternal microbiome, both of which can heighten susceptibility to colonization with resistant organisms in both mothers and their newborns (123). Neonates born to mothers with lower levels of education (either no formal education or only secondary education) had a higher likelihood of colonization. This suggests that maternal education influences health behaviors, including hygiene practices, which could directly affect the likelihood of neonatal colonization (124). These findings highlight the intergenerational impact of maternal health and education on neonatal outcomes and emphasize the need for education and awareness programs targeting expectant mothers. Data on risk factors for maternal and neonatal ABR-GNB colonization are very limited (125).

7. STRENGTHS AND LIMITATIONS OF THE STUDY

7.1 Strengths of the study

- To the best of our knowledge, this is one of the few studies in Ethiopia to simultaneously assess the colonization prevalence and antimicrobial susceptibility patterns of *ESBL-PE* and CRE among laboring mothers and their newborns. This contributes valuable baseline data for local AMR surveillance and maternal-newborn health.
- Conducting the study at SPHMMC was an additional strength, as the hospital's high delivery volume and role as a referral center created a valuable setting for generating locally and regionally relevant findings.
- Another strength was the effort to collect neonatal samples immediately after birth, minimizing the risk of postnatal contamination, especially important given the lack of molecular methods to confirm vertical transmission.
- Samples were collected from both vaginal and rectal sites in mothers, providing a more comprehensive picture of maternal colonization.

7.2 Limitations of the study

Our study was also subject to several limitations

- Neonatal samples were only collected from the external ear, which may have contributed to underestimating the true rate of neonatal colonization.
- While phenotypic analysis suggested possible vertical transmission of *ESBL-PE* and CRE, molecular characterization was not performed. This limited the ability to confirm transmission mechanisms or determine the genetic relatedness of the isolates.
- Although SPHMMC is a referral center, this study was conducted in a single institution. Therefore, the prevalence and transmission rates observed may not fully represent the epidemiology of ABR-GNB in pregnant women and neonates across Ethiopia.
- Despite including most known and potential risk factors identified in the literature, some relevant exposures may not have been captured, which could influence the comprehensiveness of the associated factors analysis.

8. CONCLUSION AND RECOMMENDATION

8.1 Conclusion

The prevalence of colonization with ESBL-PE and CRE among laboring mothers in our study was considerably high, whereas neonatal colonization and vertical transmission rates remained low. *E. coli* and *Klebsiella* species were the predominant isolates, exhibiting extensive multidrug resistance. Phenotypic similarities between maternal and neonatal isolates indicated a potential for vertical transmission, particularly among neonates born to colonized mothers. Antimicrobial susceptibility testing revealed widespread resistance to commonly used antibiotics, underscoring the urgent need for effective antimicrobial stewardship. Maternal educational status, marital status, and antenatal care attendance were significantly associated with colonization, with regular ANC visits demonstrating a protective effect. These findings highlight the critical need for enhanced maternal healthcare access and targeted interventions to curb the spread of multidrug-resistant Gram-negative bacteria.

8.2 Recommendations

- The high prevalence of ESBL-PE and CRE colonization among laboring mothers, coupled with the extensive multidrug resistance observed, highlights the urgent need for routine surveillance of antimicrobial-resistant Gram-negative bacteria in maternity and neonatal wards. Hospitals should strengthen infection prevention and control (IPC) practices, including screening of high-risk patients, promoting hand hygiene, and ensuring appropriate antibiotic stewardship to limit further spread of MDR organisms.
- Given that regular ANC visits were associated with a reduced likelihood of maternal colonization, it is crucial to strengthen and promote the accessibility of quality ANC services.
- As maternal education was found to influence neonatal colonization, targeted health education programs should be implemented to raise awareness about proper hygiene practices, antibiotic use, and the risks of bacterial colonization during pregnancy.
- Preterm neonates were found to be at higher risk for bacterial colonization. Given their underdeveloped immune systems, more intensive infection control measures and closer monitoring for ARB colonization are essential in neonatal care.

- Focusing solely on the external ear sample could have led to an underrepresentation of neonatal colonization. Therefore, future studies could benefit from including additional sampling sites (such as the oral cavity, skin, or rectum) to better capture the full spectrum of neonatal colonization of ABR-GNB.
- While phenotypic similarities were observed between maternal and neonatal bacterial isolates, molecular tests are necessary to definitively confirm vertical transmission. Further studies should be conducted using molecular methods to investigate the mechanisms of vertical transmission of drug-resistant bacteria, which would provide clearer insights into the transmission dynamics and inform better prevention strategies.

9. REFERENCE

1. R Ribeiro da Cunha B, Fonseca LP, Calado CRC. Antibiotic discovery: where have we come from, where do we go? *Antibiotics (Basel)*. 2019;8(2):45. doi:10.3390/antibiotics8020045
2. Williams-Nguyen J, Sallach JB, Bartelt-Hunt S, Boxall AB, Durso LM, McLain JE, et al. Antibiotics and antibiotic resistance in agroecosystems: state of the science. *J Environ Qual*. 2016;45(2):394–406. doi:10.2134/jeq2015.07.0393
3. Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N, Rabaan AA, et al. Antimicrobial resistance: a growing serious threat for global public health. *Healthcare (Basel)*. 2023;11(13):1946. doi:10.3390/healthcare11131946
4. World Health Organization (WHO). Antimicrobial resistance [Internet]. Geneva: WHO; [Internet]. [cited 2025 May 28]. Available from: <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance>
5. Centers for Disease Control and Prevention (CDC). ESBL-producing Enterobacterales: about ESBL-producing Enterobacterales [Internet]. Atlanta (GA): CDC; 2024 [cited 2025 May 28]. Available from: <https://www.cdc.gov/esbl-producing-enterobacterales/about/index.html>.
6. Machado E, Costa P, Carvalho A, Investigators on behalf of the S. Occurrence of Healthcare-Associated Infections (HAIs) by *Escherichia coli* and *Klebsiella spp.* Producing Extended-Spectrum β -lactamases (ESBL) and/or Carbapenemases in Portuguese Long-Term Care Facilities. *Pathogens*. 2022;11(9):1019. doi:10.3390/pathogens11091019
7. Centers for Disease Control and Prevention (CDC). Enterobacterales (carbapenem-resistant): about carbapenem-resistant Enterobacterales [Internet]. Atlanta (GA): CDC; [cited 2025 May 29]. About Carbapenem-resistant Enterobacterales. Available from: <https://www.cdc.gov/cre/about/index.html>
8. Mustafai MM, Hafeez M, Munawar S, Basha S, Rabaan AA, Halwani MA, et al. Prevalence of carbapenemase and extended-spectrum β -lactamase producing Enterobacteriaceae: a cross-sectional study. *Antibiotics (Basel)*. 2023;12(1):148. doi:10.3390/antibiotics12010148
9. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis*. 2018;18(3):318–27. doi:10.1016/S1473-3099(17)30753-3
10. Matok LA, Azrad M, Leshem T, Abuzahya A, Khamaisi T, Smolkin T, et al. Mother-to-neonate transmission of antibiotic-resistant bacteria: a cross-sectional study. *Microorganisms*. 2021;9(6):1245. doi:10.3390/microorganisms9061245
11. Bulabula ANH, Dramowski A, Mehtar S. Transmission of multidrug-resistant Gram-negative bacteria from colonized mothers to their infants: a systematic review and meta-analysis. *J Hosp Infect*. 2020;104(1):57–67. doi:10.1016/j.jhin.2019.10.001
12. Maina JW, Onyambu FG, Kibet PS, Musyoki AM. Multidrug-resistant Gram-negative bacterial infections and associated factors in a Kenyan intensive care unit: a cross-sectional study. *Ann Clin Microbiol Antimicrob*. 2023;22(1):85. doi:10.1186/s12941-023-00614-2

13. Didier C, Streicher MP, Chognot D, Campagni R, Schnebelen A, Messer J, et al. Late-onset neonatal infections: incidences and pathogens in the era of antenatal antibiotics. *Eur J Pediatr*. 2012;171(4):681–7. doi:10.1007/s00431-011-1633-9
14. Amsalu G, Wen CT, Perovic O, Gebru A, Hunegnaw BM, Tadesse F, et al. Carriage of antimicrobial-resistant Enterobacterales among pregnant women and newborns in Amhara, Ethiopia. *Int J Infect Dis*. 2024;143: [Epub ahead of print]. doi:10.1016/j.ijid.2023.12.123
15. Al-Selwi AGM, Barkat A. International and national data on maternal, neonatal, and child mortality rates and policies to reduce the high in the Middle East and Morocco: a multilevel meta-analysis. *J Biosci Appl Res* [Internet]. 2023 Dec 1 [cited 2025 May 29]; Available from: https://journals.ekb.eg/article_330290.html
16. MacroTrends. Ethiopia infant mortality rate 1950-2025 [Internet]. [cited 2025 May 29]. Available from: <https://www.macrotrends.net/global-metrics/countries/eth/ethiopia/infant-mortality-rate>
17. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*. 2022;399(10325):629–55. doi:10.1016/S0140-6736(21)02724-0
18. World Bank. By 2050, drug-resistant infections could cause global economic damage on par with 2008 financial crisis [Internet]. [cited 2025 Mar 19]. Available from: <https://www.worldbank.org/en/news/press-release/2016/09/18/by-2050-drug-resistant-infections-could-cause-global-economic-damage-on-par-with-2008-financial-crisis>
19. Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N, Rabaan AA, et al. Antimicrobial Resistance: a growing serious threat for global public health [Internet]. *Healthcare (Basel)*. 2023 [cited 2025 May 29];11(13):1946. Available from: <https://www.mdpi.com/2227-9032/11/13/1946>
20. Abera D, Negash AA, Fentaw S, Mekonnen Y, Cataldo RJ, Wami AA, et al. High prevalence of colonization with extended-spectrum β -lactamase-producing and multidrug-resistant Enterobacterales in the community in Addis Ababa Ethiopia: risk factors, carbapenem resistance, and molecular characterization. *BMC Microbiol*. 2024;24(1):402. doi:10.1186/s12866-024-03218-2
21. Centers for Disease Control and Prevention (CDC). 2019 antibiotic resistance threats report [Internet]. 2019 [cited 2024 Jan 1]. Available from: <https://www.cdc.gov/drugresistance/biggest-threats.html>
22. World Health Organization (WHO). Newborns: improving survival and well-being [Internet]. [cited 2025 May 29]. Available from: <https://www.who.int/news-room/fact-sheets/detail/newborns-reducing-mortality>
23. Sorsa A, Früh J, Stötter L, Abdissa S. Blood culture result profile and antimicrobial resistance pattern: a report from neonatal intensive care unit (NICU), Asella teaching and referral hospital, Asella, south East Ethiopia. *Antimicrob Resist Infect Control*. 2019;8:42. doi:10.1186/s13756-019-0493-2
24. Kaye KS, Pogue JM. Infections caused by resistant Gram-negative bacteria: epidemiology and management. *Pharmacotherapy*. 2015;35(10):949–62. doi:10.1002/phar.1649

25. Giraldo PC, Araújo ED, Junior JE, do Amaral RLG, Passos MRL, Gonçalves AK. The prevalence of urogenital infections in pregnant women experiencing preterm and full-term labor. *Infect Dis Obstet Gynecol.* 2012;2012:878241. doi:10.1155/2012/878241
26. Abdelaziz ZA, Ibrahim ME, Bilal NE, Hamid ME. Vaginal infections among pregnant women at Omdurman Maternity Hospital in Khartoum, Sudan. *J Infect Dev Ctries.* 2014; 8(4):490–7. doi:10.3855/jidc.3060.
27. Shimeles G, Gedefie A, Motbainor H, Genet C. Vaginal colonization, vertical transmission rate, antimicrobial susceptibility profile, and associated factors of potential neonatal pathogens among pregnant women at public health facilities of Northeast Ethiopia. *Front Public Health* [Internet]. 2025 Feb 5 [cited 2025 May 29];13. Available from: <https://www.frontiersin.org/journals/public-health/articles/10.3389/fpubh.2025.1475357/full>
28. Tumuhamy J, Steinsland H, Bwanga F, Tumwine JK, Ndeezi G, Mukunya D, et al. Vaginal colonization with antimicrobial-resistant bacteria among women in labor in central Uganda: prevalence and associated factors. *Antimicrob Resist Infect Control.* 2021;10(1):37. doi:10.1186/s13756-021-00898-y
29. Neemann K, Olateju EK, Izevbogie N, Akaba G, Olanipekun GM, Richard JC, et al. Neonatal outcomes associated with maternal recto-vaginal colonization with extended-spectrum β -lactamase producing Enterobacteriaceae in Nigeria: a prospective, cross-sectional study. *Clin Microbiol Infect.* 2020;26(4):463–9. doi:10.1016/j.cmi.2019.07.004
30. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: When? Where? Why? *Lancet.* 2005;365(9462):891–900. doi:10.1016/S0140-6736(05)71048-5
31. Coffin SE, Abanyie F, Bryant K, Cantey J, Fiore A, Fritz S, et al. Pediatric research priorities in healthcare-associated infections and antimicrobial stewardship. *Infect Control Hosp Epidemiol.* 2021;42(5):519–22. doi:10.1017/ice.2020.454
32. Flannery DD, Chiotos K, Gerber JS, Puopolo KM. Neonatal multidrug resistant gram-negative infection: epidemiology, mechanisms of resistance, and management. *Pediatr Res.* 2022;91(2):380–91. doi:10.1038/s41390-021-01887-7
33. Rybczynska H, Melander E, Johansson H, Lundberg F. Efficacy of a once-a-week screening programme to control extended-spectrum beta-lactamase-producing bacteria in a neonatal intensive care unit. *Scand J Infect Dis.* 2014;46(6):426–32. doi:10.3109/00365548.2014.892059
34. Smith HZ, Hollingshead CM, Kendall B. Carbapenem-Resistant Enterobacterales. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 [cited 2025 May 29]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK551704/>
35. Sustainable Development Goal 3: Good Health and Well-being | United Nations in Ethiopia [Internet]. [cited 2025 May 29]. Available from: <https://ethiopia.un.org/en/sdgs/3>
36. Ander SE, Diamond MS, Coyne CB. Immune responses at the maternal-fetal interface. *Sci Immunol.* 2019 Jan 11;4(31):eaat6114. doi:10.1126/sciimmunol.aat6114.

37. Rac H, Gould AP, Eiland LS, Griffin B, McLaughlin M, Stover KR, et al. Common bacterial and viral infections: review of management in the pregnant patient. *Ann Pharmacother*. 2019 Jun;53(6):639–651. doi:10.1177/1060028018817935.
38. Mikhail MS, Anyaegbunam A. Lower urinary tract dysfunction in pregnancy: a review. *Obstet Gynecol Surv*. 1995 Sep;50(9):675–683. doi:10.1097/00006254-199509000-00022.
39. Chan MY, Smith MA. Infections in pregnancy. In: McQueen CA, editor. *Comprehensive Toxicology*. 3rd ed. Amsterdam: Elsevier; 2018. p. 232–49. doi:10.1016/B978-0-12-801238-3.64293-9.
40. Kumar M, Saadaoui M, Al Khodor S. Infections and pregnancy: effects on maternal and child health. *Front Cell Infect Microbiol*. 2022 Jun 8;12:873253. doi:10.3389/fcimb.2022.873253.
41. Chan MY, Smith MA. Infections in pregnancy. In: McQueen CA, editor. *Comprehensive Toxicology*. 3rd ed. Amsterdam: Elsevier; 2018. p. 232–49. doi:10.1016/B978-0-12-801238-3.64293-9.
42. Richardson AN, Pollak EA, Williams D, Smith MA. Intrauterine infection. In: McQueen CA, editor. *Comprehensive Toxicology*. Oxford: Elsevier; 2010. p. 239–258.
43. Chan MY, Smith MA. Infections in pregnancy. In: McQueen CA, editor. *Comprehensive Toxicology*. 3rd ed. Amsterdam: Elsevier; 2018. p. 232–49. doi:10.1016/B978-0-12-801238-3.64293-9.
44. George CRR, Lahra MM, Jeffery HE. The impact of infection during pregnancy on the mother and baby. In: Khong TY, Malcomson RDG, editors. *Keeling's Fetal and Neonatal Pathology*. Cham: Springer International Publishing; 2015. p. 219–256. doi:10.1007/978-3-319-19207-9_9.
45. Ribeiro-do-Valle CC, Bonet M, Brizuela V, Abalos E, Baguiya A, Bellissimo-Rodrigues F, et al. Aetiology and use of antibiotics in pregnancy-related infections: results of the WHO Global Maternal Sepsis Study (GLOSS), 1-week inception cohort. *Ann Clin Microbiol Antimicrob*. 2024;23:21. doi:10.1186/s12941-024-00681-8.
46. Christensen SB. Drugs that changed society: history and current status of the early antibiotics: salvarsan, sulfonamides, and β -lactams. *Molecules*. 2021 Oct 7;26(19):6057.
47. Mancuso G, Midiri A, Gerace E, Biondo C. Bacterial antibiotic resistance: the most critical pathogens. *Pathogens*. 2021; 10(10):1310.
48. Breijyeh Z, Jubeh B, Karaman R. Resistance of Gram-negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules*. 2020;25(6):1340. doi:10.3390/molecules25061340
49. Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, et al. Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. *Lancet*. 2024;404(10459):1199–226. doi:10.1016/S0140-6736(24)00160-6
50. Macesic N, Uhlemann AC, Peleg AY. Multidrug-resistant Gram-negative bacterial infections. *Lancet*. 2025;405(10474):257–72. doi:10.1016/S0140-6736(25)00010-1
51. World Health Organization. WHO bacterial priority pathogens list 2024: bacterial Pathogens of Public Health Importance, to guide research, development, and strategies to prevent and control antimicrobial resistance. 1st ed. Geneva: World Health Organization; 2024. 1 p.

52. Khadka C, Shyaula M, Syangtan G, Bista S, Tuladhar R, Singh A, et al. Extended-spectrum β -lactamases producing Enterobacteriaceae (ESBL-PE) prevalence in Nepal: A systematic review and meta-analysis. *Sci Total Environ*. 2023;901:166164. doi:10.1016/j.scitotenv.2023.166164
53. Thenmozhi S, Moorthy K, Sureshkumar BT, Suresh M. Antibiotic resistance mechanism of ESBL producing Enterobacteriaceae in clinical field: a review. 2014.
54. Bush K. Past and Present Perspectives on β -Lactamases. *Antimicrob Agents Chemother*. 2018;62(10):e01076-18.
55. Paterson DL, Bonomo RA. Extended-Spectrum β -Lactamases: a clinical update. *Clin Microbiol Rev*. 2005;18(4):657–86.
56. Shyaula M, Khadka C, Dawadi P, Banjara MR. Systematic review and meta-analysis on extended-spectrum β -lactamases producing *Klebsiella pneumoniae* in Nepal. *Microbiol Insights*. 2023;16:11786361221145179. doi:10.1177/11786361221145179.
57. Lowe M, Kolesnik T, Coetzee M, Moodley C, Dhansay N, Galvão B, et al. Colonisation with extended spectrum beta-lactamase-producing and carbapenem-resistant Enterobacterales in children admitted to a paediatric referral hospital in South Africa. *PLoS One*. 2020;15(11):e0242772. doi:10.1371/journal.pone.0242772.
58. Zong Z, Feng Y, McNally A. Carbapenem and colistin resistance in *Enterobacter*: determinants and clones. *Trends Microbiol*. 2021;29(6):473–6. doi:10.1016/j.tim.2020.11.006.
59. Hu F, Guo Y, Zhu D, Wang F, Jiang X, Xu Y, et al. CHINET surveillance of bacterial resistance across China: report of the results in 2016. *Chin J Infect Chemother*. 2017;481–91. doi:10.3969/j.issn.1009-7708.2017.05.001.
60. Munita JM, Arias CA. Mechanisms of Antibiotic Resistance. *Microbiol Spectr*. 2016;4(2):10.1128/microbiolspec.VMBF-0016–2015.
61. Ma J, Song X, Li M, Yu Z, Cheng W, Yu Z, et al. Global spread of carbapenem-resistant Enterobacteriaceae: epidemiological features, resistance mechanisms, detection and therapy. *Microbiol Res*. 2023;266:127249. doi:10.1016/j.micres.2022.127249.
62. Sader HS, Carvalhaes CG, Arends SJR, Castanheira M, Mendes RE. Aztreonam/avibactam activity against clinical isolates of Enterobacterales collected in Europe, Asia and Latin America in 2019. *J Antimicrob Chemother*. 2021;76(3):659–66. doi:10.1093/jac/dkaa483.
63. World Health Organization. Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report 2021. 1st ed. Geneva: World Health Organization; 2021. 1 p. <https://www.who.int/publications/i/item/9789240027336>
64. Tarana MN, Shamsuzzaman SM. Laboratory diagnosis of bacterial vaginosis and potential pathogens other than group B Streptococcus in vaginal swab of pregnant women in Dhaka Medical College Hospital. *Mymensingh Med J MMJ*. 2018;27(4):834–42.
65. Danino D, Melamed R, Sterer B, Porat N, Hazan G, Gushanski A, et al. Mother-to-child transmission of extended-spectrum-beta-lactamase-producing Enterobacteriaceae. *J Hosp Infect*. 2018; 100(1):40–6. doi:10.1016/j.jhin.2018.04.011.

66. Rettedal S, Löhr IH, Bernhoff E, Natås OB, Sundsfjord A, Øymar K. Extended-spectrum β -lactamase-producing Enterobacteriaceae among pregnant women in Norway: prevalence and maternal-neonatal transmission. *J Perinatol*. 2015;35(11):907–12. doi:10.1038/jp.2015.108
67. Koizumi A, Maruyama K, Ohki Y, Nakayama A, Yamada Y, Kurosawa H, et al. Prevalence and risk factor for antibiotic-resistant *Escherichia coli* colonization at birth in premature infants: a prospective cohort study. *Pediatr Infect Dis J*. 2020; 39(6):546–52. doi:10.1097/INF.0000000000002674.
68. Mairi A, Touati A, Ait Bessai S, Boutabtoub Y, Khelifi F, Sotto A, et al. Carbapenemase-producing Enterobacteriaceae among pregnant women and newborns in Algeria: Prevalence, molecular characterization, maternal-neonatal transmission, and risk factors for carriage. *Am J Infect Control*. 2019;47(1):105–8. doi:10.1016/j.ajic.2018.07.018.
69. Denkel LA, Schwab F, Kola A, Leistner R, Garten L, von Weizsäcker K, et al. The mother as most important risk factor for colonization of very low birth weight (VLBW) infants with extended-spectrum β -lactamase-producing Enterobacteriaceae (ESBL-E). *J Antimicrob Chemother*. 2014;69(8):2230–7. doi:10.1093/jac/dku145.
70. Yelin I, Snitser O, Novich G, Katz R, Tal O, Parizade M, et al. Personal clinical history predicts antibiotic resistance of urinary tract infections. *Nat Med*. 2019;25(7):1143–52. doi:10.1038/s41591-019-0456-8.
71. Heigl K, Zamfir M, Adler AC, Dammeyer A, Schomacher L, Karlin B, et al. Prevalence of methicillin-sensitive, methicillin-resistant *Staphylococcus aureus*, and extended-spectrum beta-lactamase-producing *Escherichia coli* in newborns: a cross-sectional study. *J Matern Fetal Neonatal Med*. 2022; 35(22):4243–9. doi:10.1080/14767058.2021.1885409.
72. Longardt AC, Piening B, Weizsäcker K von, Dame C, Bühner C, Garten L. Screening for third-generation cephalosporin-resistant bacteria reduces the incidence of late-onset sepsis and antibiotic use in neonates. *Klin Padiatr*. 2020;232(4):203–9. doi:10.1055/a-1085-5172.
73. Foessleitner P, Gasser J, Kiss H, Flunt A, Presterl E, Petricevic L, et al. Vaginal colonization of extended-spectrum beta-lactamase-producing bacteria during pregnancy: An observational study. *Eur J Obstet Gynecol Reprod Biol*. 2020;246:86–9. doi:10.1016/j.ejogrb.2020.03.027..
74. Jiménez-Rámila C, López-Cerero L, Aguilar Martín MV, Vera Martín C, Serrano L, Pascual Á, et al. Vagino-rectal colonization and maternal-neonatal transmission of Enterobacteriaceae producing extended-spectrum β -lactamases or carbapenemases: a cross-sectional study. *J Hosp Infect*. 2019;101(2):167–74. doi:10.1016/j.jhin.2018.11.005.
75. Bromiker R, Ernest N, Bar Meir M, Kaplan M, Hammerman C, Schimmel MS, Schlesinger Y. Correlation of bacterial type and antibiotic sensitivity with maternal antibiotic exposure in early-onset neonatal sepsis. *Neonatology*. 2013;103(1):48–53. doi:10.1159/000342215.
76. Giuffrè M, Bonura C, Cipolla D, Mammina C. MRSA infection in the neonatal intensive care unit. *Expert Rev Anti Infect Ther*. 2013;11(5):499–509. doi:10.1586/eri.13.28.
77. Chomkatekaw C, Thaipadungpanit J, Hearn P, Soeng S, Pol S, Neou L, et al. Detection of maternal transmission of resistant Gram-negative bacteria in a Cambodian hospital setting. *Front Microbiol*. 2023;14:1158056. doi:10.3389/fmicb.2023.1158056.

78. Shakil S, Akram M, Ali SM, Khan AU. Acquisition of extended-spectrum beta-lactamase producing *Escherichia coli* strains in male and female infants admitted to a neonatal intensive care unit: molecular epidemiology and analysis of risk factors. *J Med Microbiol.* 2010;59(Pt 8):948–54. doi:10.1099/jmm.0.019471-0.
79. Papst L, Beović B, Seme K, Pirš M. Two-year prospective evaluation of colonization with extended-spectrum beta-lactamase-producing *Enterobacteriaceae*: time course and risk factors. *Infect Dis Lond Engl.* 2015; 47(9):618–24. doi:10.3109/23744235.2015.1038221.
80. Chan GJ, Lee AC, Baqui AH, Tan J, Black RE. Prevalence of early-onset neonatal infection among newborns of mothers with bacterial infection or colonization: a systematic review and meta-analysis. *BMC Infect Dis.* 2015;15(1):118. doi:10.1186/s12879-015-0844-2
81. Giuffrè M, Geraci DM, Bonura C, Saporito L, Graziano G, Insinga V, et al. The increasing challenge of multidrug-resistant Gram-negative bacilli. *Medicine (Baltimore).* 2016;95(10):e3016. doi:10.1097/MD.0000000000003016.
82. Hooven TA, Polin RA. Healthcare-associated infections in the hospitalized neonate: a review. *Early Hum Dev.* 2014 Mar;90 Suppl 1:S4–6. doi:10.1016/S0378-3782(14)70002-7.
83. Lawn JE, Blencowe H, Oza S, You D, Lee AC, Waiswa P, et al. Every Newborn: progress, priorities, and potential beyond survival. *Lancet.* 2014;384(9938):189–205. doi:10.1016/S0140-6736(14)60496-7.
84. Löhr IH, Rettedal S, Natås OB, Naseer U, Oymar K, Sundsfjord A. Long-term faecal carriage in infants and intra-household transmission of CTX-M-15-producing *Klebsiella pneumoniae* following a nosocomial outbreak. *J Antimicrob Chemother.* 2013;68(5):1043–8. doi:10.1093/jac/dks502.
85. Shamsrizi P, Gladstone BP, Carrara E, Luise D, Cona A, Bovo C, et al. Variation of effect estimates in the analysis of mortality and length of hospital stay in patients with infections caused by bacteria-producing extended-spectrum beta-lactamases: a systematic review and meta-analysis. *BMJ Open.* 2020;10(1):e030266. doi:10.1136/bmjopen-2019-030266.
86. Seboxa T, Amogne W, Abebe W, Tsegaye T, Azazh A, Hailu W, et al. High mortality from bloodstream infection in Addis Ababa, Ethiopia, is due to antimicrobial resistance. *PLoS ONE.* 2015;10(12):e0144944. doi:10.1371/journal.pone.0144944.
87. Ramirez CB, Cantey JB. Antibiotic resistance in the neonatal intensive care unit. *NeoReviews.* 2019; 20(3):e135–44. doi:10.1542/neo.20-3-e135.
88. Prusakov P, Goff DA, Wozniak PS, Cassim A, Scipion CEA, Urzúa S, et al. A global point prevalence survey of antimicrobial use in neonatal intensive care units: The no-more-antibiotics and resistance (NO-MAS-R) study. *EClinicalMedicine.* 2021;32:100727. doi:10.1016/j.eclinm.2021.100727.
89. Murki S, Jonnala S, Mohammed F, Reddy A. Restriction of cephalosporins and control of extended spectrum beta-lactamase producing gram negative bacteria in a neonatal intensive care unit. *Indian Pediatr.* 2010;47(9):785–8. doi:10.1007/s13312-010-0075-4.
90. Stapleton PJM, Murphy M, McCallion N, Brennan M, Cunney R, Drew RJ. Outbreaks of extended spectrum beta-lactamase-producing *Enterobacteriaceae* in neonatal intensive care units: a systematic

- review. *Arch Dis Child Fetal Neonatal Ed.* 2016;101(1):F72–8. doi:10.1136/archdischild-2015-308243.
91. Temsegen W, Gorems K, Mekonnen M, Fufa D, Kassa T. Fecal carriage of extended-spectrum β -lactamase and carbapenemase-producing *Enterobacteriaceae* among oncology and non-oncology patients at Jimma Medical Center in Ethiopia: a comparative cross-sectional study. *Cancer Manag Res.* 2023 Nov 1;15:1217–31. doi: 10.2147/CMAR.S395680.
 92. Rawat D, Nair D. Extended-spectrum β -lactamases in Gram negative bacteria. *J Glob Infect Dis.* 2010;2(3):263–74. doi: 10.4103/0974-777X.68531.
 93. Rabaan AA, Eljaaly K, Alhumaid S, Albayat H, Al-Adsani W, Sabour AA, et al. An overview on phenotypic and genotypic characterisation of carbapenem-resistant *Enterobacterales*. *Medicina (Mex).* 2022 Nov 19;58(11):1675. doi: 10.3390/medicina58111675.
 94. Hudzicki J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol [Internet]. Washington (DC): American Society for Microbiology; 2009 Dec 8 [cited 2025 Nov 7]. Available from: <https://www.asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/kirby-bauer-disk-diffusion-susceptibility-test-protocol-pdf.pdf>
 95. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*. 34th ed. CLSI supplement M100. Wayne (PA): Clinical and Laboratory Standards Institute; 2024. Available from: https://www.darvashco.com/wp-content/uploads/2024/07/CLSI-2024_compressed-1.pdf
 96. World Medical Association. *Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects*. 59th WMA General Assembly, Seoul, Korea, October 2008. Available from: <https://www.wma.net/wp-content/uploads/2016/11/DoH-Oct2008.pdf>
 97. Beaumont AL, Kermorvant-Duchemin E, Breurec S, Huynh BT. Neonatal colonization with antibiotic-resistant pathogens in low- and middle-income countries: a systematic review and meta-analysis. *JAMA Netw Open.* 2024;7(11):e2441596. doi:10.1001/jamanetworkopen.2024.41596
 98. Moradi Y, Eshrati B, Motevalian SA, Majidpour A, Baradaran HR. A systematic review and meta-analysis on the prevalence of *Escherichia coli* and extended-spectrum β -lactamase-producing *Escherichia coli* in pregnant women. *Arch Gynecol Obstet.* 2021 Feb;303(2):363–79. doi: 10.1007/s00404-020-05903-w.
 99. Sintondji K, Fabiyi K, Hougbenou J, Koudokpon H, Lègba B, Amoussou H, et al. Prevalence and characterization of ESBL-producing *Escherichia coli* in healthy pregnant women and hospital environments in Benin: an approach based on Tricycle. *Front Public Health* [Internet]. 2023 Sep 28 [cited 2025 Nov 10];11:1227000. Available from: <https://www.frontiersin.org/articles/10.3389/fpubh.2023.1227000/full> doi: 10.3389/fpubh.2023.1227000
 100. Milenkov M, Proux C, Rasolofoarison TL, Rakotomalala FA, Rasoanandrasana S, Rahajamanana LV, et al. Tricycle surveillance in Antananarivo, Madagascar: circulation of both extended-spectrum beta-lactamase-producing *Escherichia coli* strains and plasmids among humans, chickens and the environment [Internet]. *medRxiv*; 2023 [cited 2025 Nov 10]. p. 2023.01.16.23284583. Available from: <https://www.medrxiv.org/content/10.1101/2023.01.16.23284583v1> doi: 10.1101/2023.01.16.23284583

101. Fortini D, Fashae K, Villa L, Feudi C, García-Fernández A, Carattoli A. A novel plasmid carrying *bla*_{CTX-M-15} identified in commensal *Escherichia coli* from healthy pregnant women in Ibadan, Nigeria. *J Glob Antimicrob Resist*. 2015 Mar 1;3(1):9–12. doi: 10.1016/j.jgar.2014.11.002.
102. Mwandigha AM, Kamori D, Kibwana UO, Masoud S, Manyahi J, Majigo M. Fecal carriage and factors associated with extended-spectrum β -lactamase-producing *Enterobacteriaceae* among pregnant women at the tertiary referral hospital, Tanzania. *Trop Med Health*. 2020 Oct 8;48(1):84. doi: 10.1186/s41182-020-00276-7.
103. Abejew AA, Wubetu GY, Fenta TG. Assessment of challenges and opportunities in antibiotic stewardship program implementation in Northwest Ethiopia. *Heliyon*. 2024;10(11):e32663. doi:10.1016/j.heliyon.2024.e32663.
104. Boltana MT, Wolde M, Hailu B, El-Khatib Z, Steck V, Woldegerima S, et al. Point prevalence of evidence-based antimicrobial use among hospitalized patients in sub-Saharan Africa: a systematic review and meta-analysis. *Sci Rep*. 2024;14(1):12652. doi:10.1038/s41598-024-40135-6.
105. Wang HY, Tseng YJ, Lin WY, Wang YC, Lin TW, Hsu JF, et al. Comparison of multiple carbapenemase tests based on an unbiased colony-selection method. *Biomedicine*. 2024; 12(9):2134. doi:10.3390/biomedicine12092134
106. Rawstron SA, Jackman JM, Serebro E, Johnson G, Cabbad M, Bromberg K, et al. Perirectal screening for carbapenem-resistant *Enterobacteriaceae* obtained from 100 consecutive healthy pregnant women in labor at a Brooklyn hospital: results and risk factors. *Infect Control Hosp Epidemiol*. 2018;39(3):369–71. doi:10.1017/ice.2017.296
107. Tadesse BT, Kebede A, Mekonnen D, Abebe T, Birhanu S, et al. Colonization with extended-spectrum β -lactamase and carbapenemase-producing *Enterobacterales* in Ethiopia: a systematic review and meta-analysis [Internet]. *ResearchGate*; 2025 Aug 4 [cited 2025 Nov 10]. Available from: https://www.researchgate.net/publication/390394875_Colonization_with_extended-spectrum_b-lactamase_and_carbapenemaseproducing_Enterobacterales_in_Ethiopia_A_systematic_review_and_meta-analysis
108. Tekele SG, Teklu DS, Legese MH, Weldehana DG, Belete MA, Tullu KD, et al. Multidrug-resistant and carbapenemase-producing *Enterobacteriaceae* in Addis Ababa, Ethiopia. *Biomed Res Int*. 2021;2021:9999638. doi:10.1155/2021/9999638
109. Moradi Y, Eshrati B, Motevalian SA, Majidpour A, Baradaran HR. A systematic review and meta-analysis on the prevalence of *Escherichia coli* and extended-spectrum β -lactamase-producing *Escherichia coli* in pregnant women. *Arch Gynecol Obstet*. 2021 Feb;303(2):363–79.
110. Sands K, Spiller OB, Thomson K, Portal EAR, Iregbu KC, Walsh TR. Early-onset neonatal sepsis in low- and middle-income countries: current challenges and future opportunities. *Infect Drug Resist*. 2022;15:933–46. doi:10.2147/IDR.S310939
111. Awoke T, Teka B, Seman A, Sebre S, Yeshitela B, Aseffa A, et al. High prevalence of multidrug-resistant *Klebsiella pneumoniae* in a tertiary care hospital in Ethiopia. *Antibiotics (Basel)*. 2021;10(8):1007. doi:10.3390/antibiotics10081007
112. Styczynski A, Amin MB, Hoque KI, Parveen S, Pervez AFM, Zeba D, et al. Perinatal colonization with extended-spectrum beta-lactamase-producing and carbapenem-resistant Gram-negative bacteria:

- a hospital-based cohort study. *Antimicrob Resist Infect Control*. 2024;13(1):13. doi:10.1186/s13756-024-01325-9
113. Peretz A, Skuratovsky A, Khabra E, Adler A, Pastukh N, Barak S, et al. Peripartum maternal transmission of extended-spectrum β -lactamase organism to newborn infants. *Diagn Microbiol Infect Dis*. 2017;87(2):168–71. doi:10.1016/j.diagmicrobio.2016.11.017
 114. Sintondji K, Fabiyi K, Hougbenou J, Koudokpon H, Lègba B, Amoussou H, et al. Prevalence and characterization of ESBL-producing *Escherichia coli* in healthy pregnant women and hospital environments in Benin: an approach based on Tricycle. *Front Public Health* [Internet]. 2023 Sep 28 [cited 2025 Nov 12];11:1227000. Available from: <https://www.frontiersin.org/articles/10.3389/fpubh.2023.1227000/full>
doi: 10.3389/fpubh.2023.1227000
 115. Gulumbe BH, AAF. Epidemiology of multidrug-resistant organisms in Africa [Internet]. *Mediterr J Infect Microbes Antimicrob*. [cited 2025 Jan 5]. Available from: <https://www.mjima.org/articles/epidemiology-of-multidrug-resistant-organisms-in-africa/doi/mjima.galenos.2019.2019.25>
 116. Berkman ND, Sheridan SL, Donahue KE, Halpern DJ, Crotty K. Literacy and health outcomes: a systematic review of the literature [Internet]. *ResearchGate*; [cited 2025 May 29]. Available from: https://www.researchgate.net/publication/8120494_Literacy_and_health_outcomes_A_systematic_review_of_the_literature
 117. Blackmon S, Avendano EE, Nirmala N, Chan CW, Morin RA, Balaji S, McNulty L, Argaw SA, Doron S, Nadimpalli ML. Socioeconomic status and the risk for colonisation or infection with priority bacterial pathogens: a global evidence map. *Lancet Microbe*. 2025 Apr 1;6(4):100993. doi: 10.1016/j.lanmic.2024.100993.
 118. Pandey KR, Yang F, Cagney KA, Smieliauskas F, Meltzer DO, Ruhnke GW. The impact of marital status on health care utilization among Medicare beneficiaries. *Medicine (Baltimore)*. 2019;98(12):e14871. doi:10.1097/MD.00000000000014871
 119. World Health Organization. New guidelines on antenatal care for a positive pregnancy experience [Internet]. Geneva: World Health Organization; 2016 Nov 7 [cited 2025 Jan 6]. Available from: <https://www.who.int/news/item/07-11-2016-new-guidelines-on-antenatal-care-for-a-positive-pregnancy-experience>
 120. Ayalew HG, Asefa KT, Liyew AM. Determinants of recommended antenatal care visits among pregnant women in Ethiopia: a generalized linear mixed-effects modeling. *BMC Pregnancy Childbirth*. 2022;22(1):867. doi:10.1186/s12884-022-05243-6
 121. Dias M, Saleem J. Surface colonization and subsequent development of infections with multidrug resistant organisms in a neonatal intensive care unit. *Ann Clin Microbiol Antimicrob*. 2019;18:12. doi:10.1186/s12941-019-0314-3
 122. Muraro SP, Pitrez PM, De Souza APD, Porto BN, Vargas JE, Ewald IP, et al. Immune response of toddlers with history of prematurity. *Allergol Immunopathol (Madr)*. 2017;45(5):425–31. doi:10.1016/j.aller.2016.11.004

123. World Health Organization. *WHO recommendations on interventions to improve preterm birth outcomes* [Internet]. Geneva: World Health Organization; 2015 [cited 2025 Jun 13]. 98 p. Available from: <https://iris.who.int/handle/10665/1830377>
124. Rezaeizadeh G, Mansournia MA, Keshtkar A, Farahani Z, Zarepour F, Sharafkhah M, et al. Maternal education and its influence on child growth and nutritional status during the first two years of life: a systematic review and meta-analysis. *eClinicalMedicine*. 2024; 71:102574. doi:10.1016/j.eclinm.2023.1025
125. Bulabula ANH, Dramowski A, Mehtar S. Maternal colonization or infection with extended-spectrum beta-lactamase-producing Enterobacteriaceae in Africa: a systematic review and meta-analysis. *Int J Infect Dis*. 2017; 64:58–66. doi:10.1016/j.ijid.2017.08.015

10. ANNEXES

Annexes (English, Amharic and Afaan Oromo versions)

Annex I: Information Sheet (English, Amharic and Afaan Oromo versions)

Annex 1.1: Information Sheet for Pregnant Women and Their Newborns (English Version)

Introduction

You are kindly invited to participate in this study, which involves mothers and their newborns from St. Paul's Hospital Millennium Medical College. The aim of this study is to assess maternal and neonatal Colonization of ESBL-Producing and Carbapenem-Resistant Enterobacterales, assess the possible associated factors of colonization, and to give recommendations to concerned bodies so as to take appropriate measures to prevent this disease. These antibiotic-resistant gram-negative bacteria are recognized as a major cause of disease among newborns in different parts of the world. Transmission of the bacteria from the colonized mother to their newborn could be vertical; it occurs during birth.

Purpose: The purpose of this study is to assess maternal and neonatal Colonization of ESBL-Producing and Carbapenem-Resistant Enterobacterales and associated factors in St. Paul's Hospital Millennium Medical College (SPHMMC), Addis Ababa, Ethiopia.

Duration: The duration of this study depends on the availability of study subjects, and it can take about five months. However, specimens from you and your child will be collected only once.

Procedure to be carried out: the procedure is easy and simple; a sample from pregnant women will be collected from the vagina and anorectal area using a sterile cotton swab by an attending midwife. A sample will also be taken from your child's ear area using a cotton swab by the attending midwife. All the samples will be transported to the laboratory for analysis.

Risk and discomfort: There will be minor discomfort during the collection of samples. During the collection of samples from you and your child, appropriate precautions will be taken, and all samples will be collected by trained health professionals. Appropriate medical care will be provided to your child.

Expected benefits: This study does not provide direct benefits to participating mothers; however, it has the potential to significantly improve neonatal health in Ethiopia. By identifying the prevalence, antimicrobial resistance patterns, and risk factors of drug-resistant Gram-negative bacteria colonization, the findings can inform infection prevention strategies, enhance neonatal care protocols, and guide antibiotic stewardship programs. These insights may contribute to reducing neonatal infections and improving newborn survival rates in Ethiopia.

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

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

Contact information:

If you have questions about the study, you can use the following address for clarification:

Primary investigator: Ibrahim Yesuf

Tel +251970227402.

Email: Ibrahimyesuf99@gmail.com

Primary Advisor: Mrs. Rozina Ambachew (Ass. Prof.)

Email: nanieyasu@gmail.com

Co-advisor: Mrs. Melkayehu Kassa (Ass. Prof.)

Email: melkayehu.kassa@sphmmc.edu.et

Annex II: Consent Form and Assent Form (English, Amharic and Afaan Oromo versions)

Annex: 2.1 Consent Form for Mother (English Version)

Serial no _____

I, _____, have been informed about the research study titled “Maternal and Neonatal Colonization of ESBL-Producing and Carbapenem-Resistant Enterobacterales in St. Paul’s Hospital Millennium Medical College: A cross-sectional study. I understand the purpose of the study, the procedures involved, and the potential risks and benefits associated with my participation.

I understand that my participation in this study is voluntary, and I have the right to withdraw at any time without any consequences. I understand that all information collected during the study will be kept confidential and used only for research purposes.

I have had the opportunity to ask questions about the study, and all my queries have been answered to my satisfaction. I agree to participate in this research study voluntarily.

Participant’s Signature: _____

Date: _____

Researcher’s Signature: _____

Date: _____

Annex 2.2 parental or guardian informed consent form:

Serial no _____

I, _____, have been informed about the research study titled “Maternal and Neonatal Colonization of ESBL-Producing and Carbapenem-Resistant Enterobacterales in St. Paul’s Hospital Millennium Medical College: A cross-sectional study.” I understand that I am being asked to participate in this study.

I understand that my participation is voluntary, and I can choose not to participate or withdraw at any time without any consequences. I understand that all information collected during the study will be kept confidential.

I have had the opportunity to ask questions about the study, and all my queries have been answered to my satisfaction. I agree to participate in this research study voluntarily.

Parent/Guardian Signature: _____

Date: _____

Annex III: Questionnaire (English, Amharic and Afaan Oromo versions)

3.1. Questionnaire (English Version)

These are questions for the study of the prevalence of ESBL-Producing and Carbapenem-Resistant Enterobacterales in St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, during the period between April to August 2024 G.C.

I am kindly request that you give an appropriate response to each question, as your answer is valuable for the study. Your response will be kept confidential.

Unique identification code (to be assigned by the data collector): _____

Data collection date: _____

Data collector name and signature: _____

Questionnaire for the Collection of Socio-Demographic and Clinical Data(pregnant women)

Directions: Answer the following questions by circling the letter of your choice or writing on the provided space accordingly..

A. Participant Identification and socio-demographic data

Serial Number: _____

1. Age (Years)_____

2. Residency

a) Urban

b) Rural

3. Marital Status

a) Single

c) Divorced

b) Married

d) Widowed

4. Educational Status

a) No formal education

d) College/TVET completed

b) Primary school

e) Higher Education

c) Secondary school

completed

5. Occupation_____

- a) House wife
- b) Farmer
- c) Government worker
- d) Private worker
- e) Merchant
- f) Other

6. Number of family members residing in the house _____

7. Total family income per month (ETB) _____

B. Study participant clinical course history (Maternal)

8. Total number of pregnancies (Gravidity) _____

9. Parity _____

10. Previous history of preterm delivery

- a) Yes
- b) No

11. Previous History of Abortion

- a) Yes
- b) No
- c) If yes, how many times? _____

12. Previous History of Stillbirth

- a) Yes
- b) No
- c) If yes, how many times? _____

13. Previous History of postpartum hemorrhage (PPH):

- a) Yes
- b) No

14. Number of Antenatal visit

- a) 1 visit
- b) 2 visits
- c) 3 visits
- d) 4 visits
- e) More than five visits
- specify _____

15. Previous History of rupture of membrane before the onset of labor

- a) Yes
- b) No
- c) If yes, duration of rupture of membrane _____

16. Any history of other chronic medical conditions

- a) Yes
- b) No

c) If yes, specify the chronic disease _____

17. Use of Intrapartum Antibiotic Prophylaxis (IAP) during labor:

a) Yes

b) No

18. Week of gestation at delivery

a) Term (37-42 week)

b) Preterm (<37 week)

c) Post term (> 42 week)

19. Date and time of recto-vaginal specimen collection _____

C. Participant Identification and clinical course history (Newborn)

Serial number _____

a) **Type of pregnancy** - a) Singleton b) Multiple gestation

b) **Gender** _____

c) **Week of birth** _____

d) **Weight of newborn on delivery (gram)** _____

e) **Fetal presentation** _____

f) **APGAR score at the**

First minute _____

Fifth minutes: _____

g) **Date and time of specimen collection from the external ear:** _____

Note: If your child is sick within 7 days after birth, please bring your baby to our hospital so that he will get medical follow-up. We really appreciate your time. Thank you very much!!!

አባሪ 1.1 - የመረጃ ቅጽ ለነፍሰ ጡር እናቶች እና ለህጻናቶቻቸው (Amharic version)

በዚህ እናቶችን እና አዳዲስ የሚወለዱ ህጻናትን በሚያካትት ጥናት እንዲሳተፉ በትህትና ተጋብዘዋል። ይህ ጥናት የሚካሄደው በቅዱስ ጳውሎስ ሆስፒታል ሚሴኒየም ሜዲካል ኮሌጅ ውስጥ ነው። የዚህ ጥናት ዋና አላማ መድሀኒት የተላመዱ ባክቴሪያዎች በእናቶች እና በህጻናት ላይ ያላቸውን ስርጭት እና መንስኤዎችን ማወቅ እና አስፈላጊውን የመከላከል እርምጃ እንዲወስድ ለሚመለከተው አካል ማሳወቅ ነው። መድሀኒት የተላመዱ ባክቴሪያዎች በአለማችን ላይ ዋና የህጻናት ህመም አምጪ እንደሆኑ ታውቋል። እነዚህ ባክቴሪያዎች በአብዛኛው ከእናት ወደ ልጅ በወሊድ ጊዜ በቀጥታ ሊተላለፉ ይችላሉ።

ሀ. አላማ: የዚህ ጥናት አላማ በቅዱስ ጳውሎስ ሆስፒታል ሚሴኒየም ሜዲካል ኮሌጅ መድሀኒት የተላመዱ ባክቴሪያዎች በእናቶች እና በህጻናት ላይ ያላቸውን ስርጭት ማወቅ እና አስፈላጊውን የመከላከል እርምጃ እንዲወስድ ለሚመለከተው አካል ማሳወቅ ነው።

ለ. የሚፈጀው ጊዜ: ይህ ጥናት የሚፈጀው ጊዜ በጥናቱ ተሳታፊ የመገኘት ሁኔታ ላይ ይወሰናል፤ ሆኖም እስከ አንድ አመት ጊዜ ሊፈጅ ይችላል። ነገር ግን ከእርሶ እና ከልጅት ናሙና የሚወሰደው አንድ ጊዜ ብቻ ነው።

ሐ. የአሰራር ሁኔታ: ከዚህ ጥናት የሚወሰደው ናሙና በቀላል መንገድ የሚከናወን ነው። ናሙና የሚወሰደው በባለሞያ ከእናቶች ሀፍረታስጋ እና ከፊንጢጣ ላይ ጥጥን በመጠቀም ነው። ከሚወሰደው ልጅ ላይ ደግሞ ከጆሮ ጥጥን በመጠቀም ናሙና ይወሰዳል።

መ. የሚያስከትለው አደጋ: ናሙና በሚወሰድበት ወቅት አነስተኛ አለመመቸት ሊኖር ይችላል። ናሙና ከልጅት በሚወሰድበት ጊዜ አስፈላጊው ጥንቃቄ ይደረጋል። እንዲሁም ሁሉም ናሙና የሚወሰደው በሰለጠኑ የጤና ባለሙያዎች ነው። አስፈላጊው የህክምና ክትትልም ለልጅት ይደረጋል።

ሠ. የሚያስገኘው ጥቅም: ይህ ጥናት ለተሳታፊ እናቶች ቀጥተኛ ጥቅም ላይሰጥ ቢችልም በኢትዮጵያ የአዲስ ተወለዱ ሕፃናት ጤናን በከፍተኛ ሁኔታ የማሻሻል አቅም አለው። የባክቴሪያዎችን ሥርጭት፣ መድሃኒት የመቋቋም ዘዴዎችን እና ተያይዘው የሚመጡ አደጋዎችን ምክንያቶቻቸውን በመለየት የኢንፌክሽን መከላከል ስልቶችን ማሳወቅ፣ የአራስ ሕፃናት እንክብካቤ ፕሮቶኮሎችን ማሻሻል እና የመድሃኒት አስተዳደር ፕሮግራሞችን ማሻሻል ያስችላል። እነዚህ ግኝቶች በኢትዮጵያ ውስጥ አዲስ በሚወለዱ ህፃናት ላይ የሚከሰቱ ኢንፌክሽኖችን ለመቀነስ እና ተያይዞ የሚመጣው የህፃናት ሞት መጠንን ለመቀነስ አስተዋፅዖ ያደርጋሉ።

ነገር ግን ወደፊት ኢትዮጵያ ውስጥ የሚወለዱ ህጻናት በሰፊው ተጠቃሚ ይሆናሉ። ምክንያቱም ይህ ጥናት ወደ ፊት ኢትዮጵያ ውስጥ ለዚህ ህመም የሚሆን ቅድመ ህክምና ብሎም ክትባት እንደመከላከያ ዘዴ እንዲታሰብ ትልቅ አስተዋጽኦ ስላለው ነው።

ፈ. ሚስጥራዊነት፡ የእርሶ እና የልጆችን ሚስጥር እናከብራለን። እርሶን እና ልጆችን የሚመለከት ማንኛውንም መረጃ ከተመራማሪዎች በስተቀር ለማንም ተላልፎ አይሰጥም። ከዚህ ጥናት በመፅሄት ላይ የሚታተም ነገር ካለ እርሶም ሆነ ልጆች በስም አይጠቀሱም። ከዚህ ጥናት ክርሶም ሆነ ከልጆች የተገኘ ማንኛውንም መረጃ ሳጥን ውስጥ ይቆላፍበታል ወይም በሚስጥር ቁጥር በተቆለፈ ኮምፒውተር ውስጥ ይቀመጣል። ይህንን መረጃ ማግኘት የሚችሉት ከምርምሩ ጋር ግንኙነት ያላቸው ሰዎች ብቻ ናቸው።

ሰ. በፈቃደኝነት የመሳተፍ እና ከጥናቱ የማቋረጥ መብት፡ በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በፈቃደኝነት ላይ የተመሰረተ ነው። ከልጆች ጋር በጥናቱ ያለመሳተፍ መብት አሎት። እርሶ እና ልጆች በማንኛውም ጊዜ የዚህ ጥናት ተሳታፊነቱን የስምምነት ቅፁንም ከፈረሙ በኋላም ቢሆን ማቋረጥ ይችላሉ። ይህን በመወሰኖችም ከልጆች ጋር አሁን የሚያገኙትም ሆነ ወደፊት የሚያገኙት የጤና አገልግሎት በምንም አይነት መልኩ አይስተጓጎልም።

ሸ. አድራሻ፡ ይህን ጥናት በተመለከተ ጥያቄ ካሎት በሚከተለው አድራሻ ተመራማሪዎችን በመጠየቅ ዝርዝር ማብራርያ ማግኘት ይችላሉ።

ዋና ተመራማሪ ኢብራሂም የሱፍ

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ኢሜል — nanieyesu@gmail.com

ተባባሪአማካሪ - መልካየሁ ካሳ (ረዳት ፕሮፌሰር)

ኢሜል - melkayehu.kassa@sphmmc.edu.et

አባሪ 2 - የስምምነት ቅጽ ለነፍስ ጡር እናቶች እና ለህጻናቶች (Amharic version)

አባሪ: 2.1 የፈቃደኝነት መጠየቂያ ቅጽ ለእናቶች (የአማርኛ ቅጂ)

መለያ ቁጥር _____

እኔ _____ በዚህ በቅዱስ ጳውሎስ ሆስፒታል ሚሊኒየም ሜዲካል ኮሌጅ ውስጥ እናቶች እና አራስ ልጆች ላይ መድሀኒት የተላመዱ ባክቴሪያዎች ያላቸውን ስርጭት እና ተያያዥ ምክንያቶችን በሚያጠና ምርምር ላይ እንድሳተፍ ተጋብዣለሁ። የጥናቱ ዓላማ፣ የአሰራሩ ሂደቶች፣ እና ከእኔ ተሳትፎ ጋር ተያይዘው ሊኖሩ የሚችሉትን ጉዳዮች እና ጥቅሞች ተረድቻለሁ።

በዚህ ጥናት ውስጥ ያለኝ ተሳትፎ በፈቃደኝነቴ ላይ የተመሰረተ እንደሆነ ተረድቻለሁ፣ እና በማንኛውም ጊዜ ያለምንም ችግር የማቋረጥ መብት እንዳለኝ አውቂያለሁ። በጥናቱ ወቅት የሚሰበሰቡት መረጃዎች በሙሉ ሚስጥራዊ ሆነው ለምርምር ዓላማ ብቻ እንደሚውሉም ተረድቻለሁ።

ስለ ጥናቱ ጥያቄዎችን ለመጠየቅ እድሉን አግኝቻለሁ። ሁሉም ጥያቄዎቼም በአግባቡ ስለተመለሱልኝ በዚህ የምርምር ጥናት ላይ በፈቃደኝነት ለመሳተፍ ተስማምቻለሁ።

የተሳታፊ ፊርማ:- _____

ቀን: _____

የተመራማሪ ፊርማ:- _____

ቀን: _____

አባሪ 2.2፣ ለህፃኑ ተሳትፎ በፈቃደኝነት መጠየቂያ ቅጽ፡ (ለወላጅ ወይም በአሳዳጊ)

መለያ ቁጥር _____

እኔ _____ በዚህ በቅዱስ ጳውሎስ ሆስፒታል ሚሊኒየም ሜዲካል ኮሌጅ ውስጥ እናቶች እና አራስ ልጆች ላይ መድሀኒት የተላመዱ ባክቴሪያዎች ያላቸውን ስርጭት እና ተያያዥ ምክንያቶችን የሚያጠና ምርምር ላይ እንድሳተፍ ተጋብዣለሁ። የጥናቱ ዓላማ፣ የአሰራሩ ሂደቶች፣ እና ከእኔ ተሳትፎ ጋር ተያይዘው ሊኖሩ የሚችሉትን ጉዳዮች እና ጥቅሞች ተረድቻለሁ።

በዚህ ጥናት ውስጥ ያለኝ ተሳትፎ በፈቃደኝነቴ ላይ የተመሰረተ እንደሆነ ተረድቻለሁ፣ እና በማንኛውም ጊዜ ያለምንም ችግር የማቋረጥ መብት እንዳለኝ አውቂያለሁ። በጥናቱ ወቅት የሚሰበሰቡት መረጃዎች በሙሉ ሚስጥራዊ ሆነው ለምርምር ዓላማ ብቻ እንደሚውሉም ተረድቻለሁ።

ስለጥናቱ ጥያቄዎችን ለመጠየቅ እድሉን አግኝቻለሁ። ሁሉም ጥያቄዎቼም በአግባቡ ስለተመለሱልኝ በዚህ የምርምር ጥናት ላይ በፈቃደኝነት ለመሳተፍ ተስማምቻለሁ።

የወላጅ/የአሳዳጊ ፊርማ፡- _____

ቀን፡ _____

አባሪ 3: ለነፍሰ ጡር እናቶች እና አዲስ ስለተወለዱ ህፃናት መጠይቅ

3.1. መጠይቅ (አማርኛ ቅጂ)

ይህ ከሚያዝያ እስከ ነሐሴ 2016 ባለው ጊዜ በቅዱስ ጳውሎስ ሆስፒታል ሚሴሪየም ሜዲካል ኮሌጅ ውስጥ መድሀኒት የተላመዱ ባክቴሪያዎች በወላድ እናቶች እና አራስ ልጆቻቸው ላይ ያላቸውን ስርጭት እና ተያያዥ መንስኤዎችን ለማጥናት የሚደረግ መጠይቅ ነው።

መልስዎ ለጥናቱ ጠቃሚ ስለሆነ ለእያንዳንዱ ጥያቄ ተገቢውን ምላሽ እንዲሰጡ በትህትና እጠይቃለሁ። ምላሽዎም በሚስጥር ይጠበቃል።

ልዩ የመታወቂያ ኮድ (በመረጃ ሰብሳቢው የሚመደብ)፡ _____

መረጃ የሚሰበሰብበት ቀን፡_____

የመረጃ ሰብሳቢ ስም እና ፊርማ፡- _____

መጠይቅ - የእናቶች ስነ-ሕዝብ እና ክሊኒካዊ መረጃን በተመለከተ

ሀ. የተሳታፊዎች መለያ (ለነፍሰ ጡር ሴቶች)

ተከታታይ ቁጥር፡ _____

1. ዕድሜ _____

2. የመኖሪያ ቦታ

a) ከተማ

b) ገጠር

3. የጋብቻ ሁኔታ

a) ያላገባች

c) የተፋታች

b) ባለትዳር

d) ባል የሞተባች

4. የትምህርት ሁኔታ

a) መደበኛ ትምህርት

d) ኮሌጅ/ቴክኒክና ሙያ

ያልተማረች

ያጠናቀቀች

b) አንደኛ ደረጃ ትምህርት

e) የከፍተኛ ትምህርት

ያጠናቀቀች

ያጠናቀቀች

c) ሁለተኛ ደረጃ ትምህርት

f) ሌላ (ይጥቀሱ)

ያጠናቀቀች

5. የሥራ ዓይነት _____

a) የቤት እመቤት

d) የግል ሰራተኛ

b) ገበሬ

e) ነጋዴ

c) የመንግስት ሰራተኛ

f) ሌላ

6. በቤቱ ውስጥ የሚኖሩ የቤተሰብ አባላት ብዛት _____

7.ጠቅላላ የቤተሰብ ገቢ በወር _____

ለ) የጥናቱ ተሳታፊ ክሊኒካዊ መረጃ

8.የእርግዝና ብዛት -----

9.የወሊድ ብዛት -----

10. ቀደም ሲል የነበረ ጊዜው ሳይደርስ የመውለድ ታሪክ አለ?

- a) አዎ b) አይ

11. ቀደም ሲል የነበረ ፅንሰ የማስወረድ ታሪክ

- a) አዎ b) አይ

c) አዎ ከሆነ ስንት ጊዜ? _____

12. ቀደም ሲል የነበረ የሞተ ፅንሰ የመውለድ ታሪክ

- a) አዎ b) አይ

c) አዎ ከሆነ ስንት ጊዜ? _____

13. ቀደም ሲል ያጋጠመ የድህረ-ወሊድ ደም መፍሰስ ታሪክ አለ?

- a) አዎን b) አይ

14. የቅድመ ወሊድ ክትትል ቁጥር

- a) 1 ጊዜ d) 4 ጊዜ
b) 2 ጊዜ e) ከ 5 ጊዜ በላይ
c) 3 ጊዜ ስንት ጊዜ? _____

15. ቀደም ሲል ያጋጠመ ምጥ ከመጀመሩ በፊት የሚከሰት የአምራቱክ ሽፋን መሰንጠቅ ታሪክ

- a) አዎ b) አይ
c) አዎ ከሆነ፣ የሽፋን መሰንጠቁ ቆይታ _____

16. ማንኛውም ሌላ ሥር የሰደደ በሽታ ታሪክ

- a) አዎ b) አይ
c) አዎ ከሆነ፣ ሥር የሰደደው በሽታ ምንነት _____

17. በወሊድ ጊዜ የኢንትራፓርተም አንቲባዮቲክ መከላከያ ተጠቃሚ ሆናለች?

- a) አዎን b) አይደለም

18. በወሊድ ጊዜ የነበረው የእርግዝና ሳምንት

- a) 37-42 ሳምንት
b) <37 ሳምንት
c) > 42 ሳምንት

19. ናሙናው የተሰበሰበበት ቀን እና ሰዓት _____

ሐ. የተሣታፊ መለያ እና ክሊኒካዊ ታሪክ (አራስ)

a) ተከታታይ ቁጥር _____

b) የእርግዝና አይነት

a) ነጠላ እርግዝና

b) ብዙ ፅንሰ እርግዝና

c) ጾታ _____

d) የትውልድ ሳምንት _____

e) በወሊድ ጊዜ የነበረ ክብደት (በግራም) _____

f) አዲስ በተወለደው ሕፃን ላይ የሚታይ ማንኛውም የአካል መዛባት _____

g) የAPGAR ውጤት

በመጀመሪያ ደቂቃ የነበረ _____

በአምስተኛው ደቂቃ የነበረ _____

h) አዲስ ከተወለደ ሕፃን ውጫዊ ጆሮ ላይ ናሙና የሚሰበሰበበት ቀን እና ሰዓት

ልጅዎ ከተወለደ በ7 ቀናት ውስጥ ከታመመ፣ እባክዎን ልጅዎን የህክምና ክትትል እንዲደረግለት ወደ ሆስፒታላችን ያምጡት። ስለተሳትፎዎ በጣም አመሰግናለሁ!!!

Dabalata I: Waraqaa Odeeffannoo Dubartoota Ulfaa fi Daa'imman Isaanii Reefuu dhalatan

Dabalata 1.1: Waraqaa Odeeffannoo Dubartoota Ulfaa fi Daa'imman Isaanii Reefuu dhalatan (Oromiffa Version).

Seensa

Qorannoon haadholii fi daa'imman isaanii reefuu dhalatan Kolleejjii Meedikaalaa Mileniyeemii Hospitaala Qulqulluu Phaawuloos irraa kan hirmaachisu kana irratti akka hirmaattan kabajaan isin afeerra. Kaayyoon qorannoo kanaa dubartoota ulfaa fi daa'imman isaanii reefuu dhalatan gidduutti baakteeriyaa giraam-negaatiivii qorichaan dandamatu, ba'aa dhukkuba daa'immanii maaykiroobii kanaan dhufu murteessuu fi qaamolee dhimmi ilaallatuuf yaada kennuudhaan tarkaanfii barbaachisaa ta'e akka fudhatan gochuudha dhukkuba kana ittisuu. Baakteeriyaa qoricha dandamatu giraam-negaatiivii kun daa'imman reefuu dhalatan biratti kutaalee addunyaa adda addaa keessatti dhukkubaaf sababa guddaa ta'uun beekamtii argateera. Dabarsi baakteeriyaa haadha koloneeffame irraa gara daa'ima isaanii reefuu dhalateetti daddarbuu dhaabbataa ta'uu danda'a; yeroo dhalootaa ni uuma.

Kaayyoo: Kaayyoon qorannoo kanaa dubartoota ulfaa fi daa'imman isaanii reefuu dhalatan gidduutti saffisa koloneeffannaa baakteeriyaa giraam-negaatiivii qorichaan dandamatu murteessuu fi akkasumas Kolleejjii Meedikaalaa Mileniyeemii Hospitaala Seent Pool keessatti sababoota balaa kanaan walqabatan murteessuudha.

Yeroo: Turtiin qorannoo kanaa gosoota qorannoo kanaa jiraachuu isaanii irratti kan hundaa'u yoo ta'u, gara waggaa tokkoo fudhachuu danda'a. Haa ta'u malee, saamuda siifi daa'ima kee irraa fudhatamu yeroo tokko qofa.

Hojimaata raawwatamuu qabu: hojimaanni salphaa fi salphaadha; saamuda dubartoota ulfaa irraa naannoo qaama saalaa fi anorectal irraa suufii sterile fayyadamuun deessiftuu hordoftuun ni fudhatama. Akkasumas saamuda naannoo gurra daa'ima keessanii haaraa irraa suufii fayyadamuun deessiftuu kunuunsaa jirtuun ni fudhatama. Saamuda hunda gara laaboraatooriitti geejjibsiifamee xiinxalaaf ni taasifama.

Balaa fi miira namaa hin tolle: Yeroo saamuda walitti qabamu miira gaarii dhabuun xiqqaan ni jiraata. Yeroo saamuda siifi daa'ima kee irraa fudhatamu of eeggannoowwan barbaachisoo ta'an ni taasifamu, saamuda hunduu ogeeyyii fayyaa leenji'aniin ni sassaabama. Kunuunsi fayyaa sirrii ta'e daa'ima keessaniif ni kennama.

Faayidaa eegamu: Qorannoon kun haadholii hirmaatan kallattiin faayidaa hin kennu; haa ta'u malee, Itoophiyaa keessatti fayyaa daa'immanii haalaan fooyyessuuf carraa qaba. Tatamsa'ina, akkaataa ittisa farra maaykiroobiyaanii, fi sababoota balaa koloneeffannaa baakteeriyaa Giraamii-negaatiivii qoricha dandamatu adda baasuudhaan, argannoowwan tooftaalee ittisa infekshinii odeeffannoo kennuu, pirootokoolii kunuunsa daa'immanii guddisuu, fi sagantaalee bulchiinsa farra baakteeriyaa qajeelchuu danda'u. Hubannoowwan kunneen Itoophiyaa keessatti dhukkuba daa'imman reefuu dhalatan hir'isuu fi lubbuun jiraachuu daa'imman reefuu dhalatan fooyyessuuf gumaachuu danda'u.

Iccitii: Iccitii fi iccitii keessanii fi mucaa keessanii ni kabajna. Odeeffannoon si ykn mucaa kee adda baasu kamiyyuu garee qo'annootiin ala nama biraatiif hin qoodamu. Barreeffamni qorannoo ykn maxxansi qorannoo kana irraa yoo argame, atii fi mucaan keessan maqaan adda hin baafamtan. Odeeffannoon akka qaama qorannichaa ta'ee siifi daa'ima kee irraa walitti qabnu kaabinee faayila cufame keessatti ni qabama ykn jecha icciitii kompiitara irratti argamuun kan eegamu ta'a, kunis hojjetoota qorannicha keessatti hirmaatan qofaaf ni argama.

Tola Ooltummaadhaan Hirmaachuu fi Qorannicha Irraa Bahuu: Hirmaannaan keessan guutummaatti fedhiidhaan kan raawwatamu yoo ta'u, qo'annoo kana irratti hirmaachuu diduudhaaf mirga qabda. Hayyama kee erga kennitee booda yeroo barbaaddetti hirmaachuu dhaabuu fi hirmaannaa daa'ima kee qo'annicha irratti dhorkuu dandeessa. Murtoon kun haala kamiinuu kunuunsa fayyaa ammaa ykn gara fuula duraa dhaabbata fayyaa keessatti keessanii fi daa'ima keessanii irratti dhiibbaa hin geessisu.

Odeeffannoo quunnamtii:

Waa'ee qorannichaa gaaffii yoo qabaattan, qulqulleessuuf teessoo armaan gadii fayyadamuu dandeessu:

Qorataan jalqabaa: Ibraahim Yesuf

Bilbila +251970227402.

Imeelii: Ibraahimyesuf99@gmail.com

Gorsituu Sadarkaa Duraa: Aadde Roozinaa Ambaachoo (Ass. Prof.)

Imeelii: nanieyasu@gmail.com irratti ergaa

Gorsituu waliin: Aadde Melkayehu Kassa (Ass. Prof.)

Imeelii: melkayehu.kassa@sphmmc.edu.et irratti ergaa

Dabalata II: Unka Hayyamaa fi Unka Hayyama

Dabalata: 2.1 Unka Hayyama Haadhaaf (Ormiffa Version).

Lakk _____.

Ani, _____, waa'ee qorannoo qorannoo mata duree “Koloneeshinii Baakteeriyaa Giraamii Negaatiivii Qoricha Haadholii fi Daa'immanii Danda'uu fi Qabxiilee Walqabatan Hospitaala Qulqulluu Phaawuloos Kolleejjii Meedikaalaa Mileniyeemii: Qo'annoo Qaxxaamuraa” jedhuun beeksiseera. Kaayyoo qorannichaa, hojimaata qorannichaa, fi balaa fi faayidaa hirmaannaa koo wajjin walqabatee dhufuu danda'u nan hubadha.

Qo'annoo kana keessatti hirmaachuun koo fedhii koo akka ta'e nan hubadha, yeroo kamiyyuu bu'aa tokko malee ofirraa baasuuf mirga qaba. Odeeffannoon yeroo qorannichaa walitti qabamu hundi iccitii ta'ee kan eegamu ta'ee qorannoo qofaaf akka oolu nan hubadha.

Waa'ee qorannichaa gaaffii gaafachuuf carraa argadheera, gaaffiin koo hundinuu deebii naaf kennameera. Qorannoon qorannoo kana irratti fedhii kootiin akkan hirmaadhuuf walii gala.

Mallattoo Hirmaataa: _____.

Guyyaa: _____

Mallattoo Qorataa: _____.

Guyyaa: _____

Dabalata 2.2 Unka Hayyama:

Lakkoofsi tartiiba_____

Ani, _____, waa'ee qorannoo qorannoo mata duree “Koloneeshinii Baakteeriyaa Giraamii Negaatiivii Qoricha Haadholii fi Daa'immanii Danda'uu fi Qabxiilee Walqabatan Hospitaala Qulqulluu Phaawuloos Kolleejjii Meedikaalaa Mileniyeemii: Qo'annoo Qaxxaamuraa” jedhuun beeksiseera. Qorannoon kun akkan hirmaadhu na gaafatamaa akka jiru naaf gala.

Hirmaannaan koo fedhii kootiin akka ta'e nan hubadha, yeroo kamiyyuu bu'aa tokko malee hirmaachuu dhiisuu ykn ofirraa baasuu dhiisuu filachuu nan danda'a. Odeeffannoon yeroo qorannichaa walitti qabamu hundi iccitii akka ta'u nan hubadha.

Waa'ee qorannichaa gaaffii gaafachuuf carraa argadheera, gaaffiin koo hundinuu deebii naaf kennameera. Qorannoon qorannoo kana irratti fedhii kootiin akkan hirmaadhuuf walii gala.

Mallattoo Warra/Guddistuu: _____.

Guyyaa: _____

Dabalata III: Gaaffii Dubartootaa Ulfaa fi Daa'imman Reefuu dhalatan

3.1 Gaaffii (Oromiffa Version).

Kunneen gaaffilee qorannoo babal'ina baakteeriyaa giraam-negaatiivii antibaayootikii dandamatu haadholii da'umsaa fi daa'imman isaanii reefuu dhalatan SPHMMC, Addis Ababa, Ethiopia keessatti, yeroo Fulbaana hanga June 2024 gidduutti.

Deebiin keessan qorannichaaf gatii guddaa waan qabuuf, tokkoon tokkoon gaaffiidhaaf deebii sirrii akka kennitan gaarummaa guddaan isin gaafadha. Deebiin keessan iccitii ta'ee ni eegama.

Koodii adda baasuu addaa (kan deetaa walitti qabuun ramadamu): _____.

Guyyaa odeeffannoon walitti qabamu: _____.

Maqaa fi mallattoo walitti qabaa odeeffannoo: _____.

Gaaffii Walitti Qabuu Ragaa Hawaas-Dimoogiraafii fi Kilinikaalaa

Questionnaire for the Collection of Socio-Demographic and Clinical Data (pregnant women) (Afan Oromo translation)

D. Eenyummaa Hirmaattotaa (dubartoota ulfaa).

Lakkoofsa Tartiiba: _____

1. Umurii _____

2. Jireenya

a) Magaalaa

b) Baadiyyaa

3. Haala Gaa'elaa

a) Tokkicha

b) Fuudhaa fi heeruma

c) Hiikkaan

d) Dubartii abbaan manaa irraa
du'e

4. Haala Barnootaa

a) Barnoota idilee hin qabu

b) Manni barumsaa sadarkaa
tokkoffaa xumurame

c) Manni barumsaa sadarkaa
lammaffaa xumurame

d) Kolleejjii/TVET xumurame

e) Barnoota Olaanoo xumurame

5. Hojii - _____

- a) haadha manaa
- b) Qonnaan bulaa
- c) Hojjetaa mootummaa
- d) hojjetaa dhuunfaa
- e) Daldalaa
- f) Kan biroo

6. Baay'ina miseensota maatii mana keessa jiraatan _____ .

7. Waliigala galii maatii ji'atti _____ .

E. Seenaa koorsii kilinikaa hirmaataa qorachuu

8. Lakkoofsa guutuu ulfaa _____

9. Lakkoofsa dhaloota _____

10. Seenaa duraanii dhaloota yeroo isaa hin guunne qaba?

- a) Eeyyee
- b) Lakk

11. Seenaa duraanii gudeeddii (abortion) qaba?

- a) Eeyyee.
- b) Lakk
- c) Yoo eeyyee ta'e, yeroo meeqa? _____?

12. Seenaa duraanii dhaloota du'aa qaba?

- a) Eeyyee.
- b) Lakk
- c) Yoo eeyyee ta'e, yeroo meeqa? _____?

13. Seenaa duraanii dhiig-ba'uu boodaa dhalootaa qaba?

- a) Yeroo 1
- b) Yeroo 2
- c) Yeroo 3
- d) Yeroo 4
- e) Yeroo shan ol

14. Lakkoofsa yeroo hordoffii ulfaa :

- a) Yeroo 1
- b) Yeroo 2
- c) Yeroo 3

- d) Yeroo 4
- e) Yeroo shan caalaa (meeqa akka ta'e ibsi) _____

15. Seenaa duraanii gogaan ilma (membrane) osoo hin da'umsin dura ciccitee qaba?

- a) Eeyyee
- b) Lakki
- c) Yoo eeyyee ta'e, yeroo gogaan ciccitee ture: _____

16. Seenaa haalawwan fayyaa yeroo dheeraa biroo kamiyyuu

- a) Eeyyee
- b) Lakk
- c) Yoo eeyyee ta'e dhukkuba yeroo dheeraa _____ ibsi.

17. Yeroo dhalootaa fayyadama qoricha ittisa dhibee qabaatte?

- a. Eeyyee.
- b. Lakk

18. Torban ulfaa yeroo da'umsaa

- a) 37-42 torban
- b) < torban 37
- c) > torban 42

19. Guyyaa fi yeroo saamuda qaama saalaa qaama saalaa (recto-vaginal specimen)

walitti qabamu_____

F. Eenyummaa Hirmaataa (daa'ima reefuu dhalate).

Lakkoofsa tartiiba_____.

- a) **Gosa ulfaa**
 - a. Ulfaa tokko
 - b) Ulfaa dachaa
- b) **Koorniyaa** _____
- c) **Torban dhalootaa** _____.
- d) **Ulfaatina daa'ima reefuu daa'ima yeroo da'umsaa** _____.
- e) **Rakkoon qaamaa daa'ima reefuu dhalate waliin mul'ate kamiyyuu**_____.
- f) **Qabxiin APGAR daqqiqaa jalqabaa fi shanaffaa irratti:** _____.

g) Guyyaa fi sa'aatii saamuda gurra alaa daa'ima reefuu dhalate irraa walitti qabamu

_____.

Daa'imni keessan erga dhalatee guyyaa 7 keessatti yoo dhukkubsate, maaloo daa'ima keessan gara hospitaala keenyatti fidaa akka inni hordoffii fayyaa argatu. Yeroo keessan baay'ee dinqisiifanna. Baay'ee galatoom.

Annex IV: Standard Operating Procedures (SOPs)

V.1. Sample collection and transportation SOP

Materials needed:

- Sterile cotton swabs
- Amies transport media (Oxoid, UK)

Safety Precautions:

- Wear gloves, a lab coat, and mask/eye protection.
- Handle all specimens as potentially infectious.

VI.1.1 Maternal Rectal Swab Collection

- ✓ Swabs were collected during the second stage of labor.
- ✓ Collected from every respondent were two sterile swabs:
- ✓ Insert swab 1 inch into the anal canal. Rotate for 10 seconds.
- ✓ Place each swab in Amies transport media, labeled.

VI.1.2 Maternal Vaginal Swab Collection

- ✓ Also performed during the second stage of labor.
- ✓ Two sterile swabs were collected
- ✓ Gently roll the swab along the vaginal wall to obtain the specimen.
- ✓ Put each swab into labeled Amies transport media.

VI.1.3 Neonatal External Ear Swab Collection

- ✓ Collected immediately after birth, before either skin-to-skin contact or exposure to the environment.
- ✓ Two sterile swabs were collected:
- ✓ Roll swab over the external ear surface.
- ✓ Place into Amies transport media, labeled:

VI.1.4 Sample Transportation

- ✓ All maternal and neonatal swabs were placed in separate Amies transport media (Oxoid, UK), clearly labelled, and immediately transported to the Microbiology Laboratory at SPHMMC for bacteriological analysis.
- ✓ In situations where immediate transportation was not possible, the specimens were kept at 2–8°C (refrigerated) until delivery to the laboratory to ensure processing within acceptable viability time.

V.2. CHROM agar ESBL media preparation SOP

Procedure

- ✓ 59.2 g of powder were suspended in one liter of distilled water.

- ✓ Boiled and shaken until completely dissolved.
- ✓ Sterilized at 121°C for 15 minutes and Cooled up to 45-50°C.
- ✓ Aseptically, rehydrated content of 2 vials (10 ml) of Chromatic™ ESB� Supplement were added
- ✓ Poured in Petri dishes.

Interpretation

- *E. coli* produces pink-reddish-mauve colonies.
- *Klebsiella* spp, *Enterobacter* spp, *Serratia* spp produce green-blue colonies.
- *Proteus* spp produces brown colonies.

V.3. CHROM agar CRE media preparation SOP

Procedure

- ✓ 56.8 g of powder were suspended in one liter of distilled water.
- ✓ Boiled and shaken until completely dissolved.
- ✓ Sterilized at 121°C for 15 minutes and Cooled up to 45-50°C.
- ✓ The contents of 2 vials (10 ml) of Chromatic™ CRE Supplement (ref. 81088) were added aseptically.
- ✓ Poured in Petri dishes.

Interpretation

- *K. pneumoniae* produces blue-violet colonies.
- *E. coli* produces red colonies.
- *Enterobacter* spp, produces blue-green colonies.
- *Citrobacter* spp, produces blue colonies with red halo.
- Non-Enterobacterales (if not inhibited) produce white to naturally pigmented colonies.

V.4 Screening for ESB�-PE and CRE SOP

Step	ESBL Screening	CRE Screening
Media	CHROMATIC™ ESB� agar (Liofilchem, Italy)	CHROMATIC™ CRE agar (Liofilchem, Italy)
Inoculation	Direct streaking from swab	Direct streaking from swab
Incubation	37°C for 24 hours (extend to 48 hours if no growth)	37°C for 24 hours (extend to 48 hours if no growth)
Interpretation	Based on manufacturer's instructions	Based on manufacturer's instructions

Suspected colonies → sub-cultured to Sheep Blood Agar to obtain pure isolates.

V.5 Identification of Isolates SOP

Tests Performed:

- Gram staining
- Indole test
- Citrate utilization test
- Triple Sugar Iron (TSI) test
- Urease test
- Lysine decarboxylase and lysine deaminase tests
- Motility test
- **Interpretation:** According to CLSI M100 (34th ed., 2024)

V.6 Phenotypic Confirmation of ESBL Production SOP

Item	Specification
Method	Combination Disk Test
Disks Used	Ceftazidime (30 µg) & Ceftazidime-Clavulanate (30/10 µg)
Disk Distance	15 mm apart on MHA
Incubation	35°C for 18–24 hours
Interpretation	≥ 5 mm increase in zone size around CAZ/CLA confirms ESBL-PE

V.7 Identification of CRE

Definition- According to CLSI M100 (34th ed., 2024):

Isolates resistant to Imipenem, Meropenem, or Ertapenem were classified as CRE.

V.8 Antimicrobial Susceptibility Testing SOP

Parameter	Description
Method	Kirby–Bauer disk diffusion
Medium	Mueller-Hinton Agar
Inoculum Standardization	0.5 McFarland
Incubation	37°C for 24 hours
Interpretation	CLSI M100-S32 breakpoints

Annex V: AST Disk Diffusion Breakpoints Used

Reference: CLSI M100 (2024)

Antibiotic	Susceptible (S, mm)	Intermediate (I, mm)	Resistant (R, mm)
Ampicillin	≥ 17	14–16	≤ 13
Ceftriaxone	≥ 23	20–22	≤ 19
Cefepime	≥ 25	19–24	≤ 18
Ceftazidime	≥ 21	18–20	≤ 17
Piperacillin-Tazobactam	≥ 25	21–24	≤ 20
Amoxicillin-Clavulanate	≥ 18	14–17	≤ 13
Imipenem	≥ 23	20–22	≤ 19
Meropenem	≥ 23	20–22	≤ 19
Ertapenem	≥ 22	19–21	≤ 18
Amikacin	≥ 17	15–16	≤ 14
Gentamicin	≥ 18	15–17	≤ 14
Ciprofloxacin	≥ 26	22–25	≤ 21
Chloramphenicol	≥ 18	13–17	≤ 12
Trimethoprim-Sulfamethoxazole	≥ 16	11–15	≤ 10