Antimicrobial Susceptibility Profile and Associated Factors with Fecal Carriage of Extended Spectrum Beta-Lactamase Producing *Escherichia coli* and *Klebsiella* species among Pregnant Women Attending Antenatal Care at Government Health Centers in Addis Ababa, Ethiopia

Yordanos Kefyalew Tesfaye

A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Applied Biology (Specialization in Applied Microbiology)

Office of Graduate Studies

Adama Science and Technology University

August 2023

Adama, Ethiopia

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Declaration

I hereby declare that this Master Thesis entitled **"Antimicrobial Susceptibility Profile and Associated Factors with Fecal Carriage of Extended Spectrum Beta-Lactamase Producing *Escherichia coli* and *Klebsiella* species among Pregnant Women Attending Antenatal Care at Government Health Centers in Addis Ababa, Ethiopia"** is my original work. That is, it has not been submitted for the award of any academic degree, diploma, or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

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Name of the Student Signature Date

Recommendation

We, the advisors of this thesis, hereby certify that we have read the revised version of the thesis entitled **"Antimicrobial Susceptibility Profile and Associated Factors with Fecal Carriage of Extended Spectrum Beta-Lactamase Producing *Escherichia coli* and *Klebsiella* species among Pregnant Women Attending Antenatal Care at Government Health Centers in Addis Ababa, Ethiopia"** prepared under our guidance by Yordanos Kefyalew submitted in partial fulfillment of the requirements for the degree of Masters of Science in Applied Microbiology.

Therefore, we recommend the submission of the revised version of the thesis to the department following the applicable procedures.

_____	_____	_____
Major Advisor	Signature	Date
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Co-advisor	Signature	Date

Approval sheet

We, the advisors of the thesis entitled **"Antimicrobial Susceptibility Profile and Associated Factors with Fecal Carriage of Extended Spectrum Beta-Lactamase Producing *Escherichia coli* and *Klebsiella* species among Pregnant Women Attending Antenatal Care at Government Health Centers in Addis Ababa, Ethiopia"** and developed by Yordanos Kefyalew, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

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We, the undersigned, members of the Board of Examiners of the thesis by Yordanos Kefyalew have read and evaluated the thesis entitled **"Antimicrobial Susceptibility Profile and Associated Factors with Fecal Carriage of Extended Spectrum Beta-Lactamase Producing *Escherichia coli* and *Klebsiella* species among Pregnant Women Attending Antenatal Care at Government Health Centers in Addis Ababa, Ethiopia"** and examined the candidate during the open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Applied Microbiology.

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Final approval and acceptance of the thesis are contingent upon the submission of its final copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and School Graduate Committee (SGC).

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Office of Postgraduate Studies, Dean	Signature	Date

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ACRONYMS AND ABBREVIATIONS

AmpC Ampicillinase C

AMR Antimicrobial Resistance

ANC Antenatal care

AOR Adjusted odd ratios

AST Antimicrobial susceptibility testing

ATCC American Type Culture Collection

BD Becton, Dickinson

CI Confidence interval

CLSI Clinical and Laboratory Standards Institute

COR Crude odd ratio

DGC Office of Postgraduate Studies

DNA Deoxyribonucleic acid

EPHI Ethiopia Public Health Institute

ESBL Extended-spectrum beta-lactamases

ESBLM Miscellaneous extended-spectrum beta-lactamases

GES Guiana extended spectrum beta-lactamase

H_2S Hydrogen sulfide

HGT Horizontal gene transfer

HIV Human immunodeficiency viruses

MDR Multidrug resistance

MIC Minimum inhibitory concentration

ml Millimeter

NGO Non-governmental organization

OXA Oxacillinase

PBPs Penicillin-binding proteins

PER Pseudomonas extended resistance

RNA Ribonucleic acid

SFO Serratia fonticola

SHV sulfhydryl variable

SPSS Statistical Package for the Social Sciences

TSB Tryptic Soy Broth

UTI Urinary tract infection

VEB Vietnam extended spectrum beta-lactamase

WC Water closet

WHO World Health Organization

β Beta

µg Microgram

ABSTRACT

Antimicrobial resistance is a condition when bacteria, viruses, fungi, and parasites change and no longer respond to antimicrobial agents. The β-lactams are antibiotics that consist of carbapenems, cephalosporins, monobactams, and penicillins. They all have a β-lactam ring, which can be hydrolyzed by β lactamases. β-lactamases are enzymes that can hydrolyze a β-lactam ring. ESBLs are enzymes that confer resistance to broad-spectrum cephalosporins, oxyimino-monobactam aztreonam, and penicillin. It is a common resistance mechanism for E. coli and K. pnemoniae Studying the antimicrobial susceptibility profile and associated factors with fecal carriage of ESBL producing E. coli and Klebsiella species among pregnant women attending ANC in Addis Ababa aimed to determine the prevalence, risk factors, and antimicrobial susceptibility pattern associated with the fecal carriage. This prospective cross-sectional study was conducted from March 08-May 23, 2022.Thestool samples were collected from pregnant women attending ANC at the Addis Ababa, Ethiopia government health center. Stool samples were collected in test tubes and stool cups with Cary-Blair transport media from volunteers at the government health center's restroom. Bacterial identification was performed using Chromatic ESBL agar and standard biochemical test methods. Kirby-Bauer's diffusion method was used to perform AST. Data were analyzed and organized using SPSS version 23. AST results were analyzed in WHONET version 2021. Out of 353 pregnant women enrolled, 51% (180) carried ESBL-producing bacteria in their feces. A total of 193ESBL-producingorganisms were isolated of which 171 and 22 were E. coli and Klebsiella species respectively. ESBL-producing isolates were 100% resistant to cephalosporins. Both strains were sensitive to meropenem and chloramphenicol. Gestation age, use of water, hand washing trend after toilet use, usual food source and type of toilet are factors that are associated with ESBL fecal carriage. Gestation age p=0.002 (aOR=0.950, 95% CI= 0.920-0.981) and hand washing after toilet visit (aOR=2.219, 95% CI= 1.385-3.554) p= 0.001 were factors independently associated with ESBL fecal carriage. In general, this study necessitates increased surveillance and monitoring of ESBL-resistant colonization among pregnant women. In addition, improved sanitation and hygiene practices in the community can also be effective in preventing fecal colonization with ESBL-producing bacteria.

Keywords: Antimicrobial resistance, extended-spectrum beta-lactamase, carriage,

CHAPTER ONE

1. INTRODUCTION

1.1 Background of the Study

Antibiotics are low molecular weight (a molecule with a defined chemical structure having a relative mass) microbial metabolites that at low concentrations inhibit the growth of other microorganisms (Aminov, 2010). Antimicrobial substance discovery, marketing, and regular administration to treat infections transformed modern medicine and altered the medical approach. Developing complex medical approaches is impossible without antibiotics. The rapid rise in antimicrobial resistance among common bacterial pathogens has jeopardized this therapeutic accomplishment(Munita & Arias, 2016).

Antimicrobial resistance is a condition when bacteria, viruses, fungi, and parasites change and no longer respond to antimicrobial agents, making infections more difficult to treat and raising the risk of disease spread, severe illness, and mortality(World Health Organization, 2021).WHO declared AMR a top 10 global public health threat. Misuse and overuse of antimicrobials are the main drivers in the development of antimicrobial-resistant pathogens(WHO, 2021).

Multiple biochemical pathways can often lead to resistance to one class of antimicrobials. Antibiotics may be able to be resisted by a bacterial cell using a variety of resistance mechanisms(C Reygaert, 2018). AMR mechanism can limit the drug's uptake, modify a drug target, inactivate a drug, and active drug efflux; β-lactamases are enzymes that hydrolyze β-lactam drugs and inactivate the drug (Reygaert, 2018).

Beta-lactam antibiotics are among the most frequently prescribed drug classes, with a wide range of clinical indications(Thakuria & Lahon, 2013).The β-lactams are antibiotics that consist of four major groups: carbapenems, cephalosporins, monobactams, and penicillins. They all have a β-lactam ring, which can be hydrolyzed by β lactamases (Samaha-Kfoury & Araj, 2003).The*β*-lactams execute their antibacterial activity by inhibiting bacterial cell walls, peptidoglycan, and synthesis by preventing the precise functioning of penicillin-binding

protein(PBP), inhibition of PBPs produces an imbalance in cell wall metabolism, resulting in growth inhibition or lysis (Zapun *et al*., 2008).

β-lactamases are enzymes that can hydrolyze a β-lactam ring and are the most common mechanism of resistance for Gram-negative bacteria (Bush & Jacoby, 2010). Extended-spectrum beta-lactamases (ESBL) are enzymes that confer resistance to penicillins, broad-spectrum cephalosporins with an oxyimino side chain (cefotaxime, ceftriaxone, and ceftazidime), and the oxyimino-monobactam aztreonam but not cephamycin such as cefoxitin and carbapenems comprising doripenem, ertapenem, imipenem, and meropenem (Zhao & Hu, 2013).

ESBL-producing *Enterobacteriaceae* are among the most problematic multidrug resistance (MDR) and are increasingly causing serious infections (Thapa *et al*., 2015). The two most common ESBL producers are *E. coli* and *K. pneumoniae* (Umadevi *et al*., 2011). ESBL-producing *E. coli* and *K. pneumoniae* are important reservoirs of resistance genes, leading to ESBL-producing *Enterobacteriaceae* dissemination in communities (Kariuki & Hart, 2001).Pathogenic bacteria may acquire ESBL-encoding genes or commensal ESBL-producing bacteria might become pathogenic, which may result in hospital and community infections (Rolain, 2013; Zhang *et al*., 2015).Colonization by ESBL-producing *Enterobacteriaceae* is one of the major risk factors for antibiotic-resistant bacterium infection (Thenmozhi *et al*., 2014).

The two recent studies in Ethiopia on the prevalence of fecal carriage with ESBL-producing *E.coli* and *K. pnemoniae* showed that the prevalence of ESBL-producing *E. coli* and *K. pnemoniae* fecal carriage among children under age 5 was 17.1% (Tola *et al*., 2021). The other is the fecal carriage rate of ESBL-producing *E. coli* and *K. pnemoniae* among apparently healthy food handlers in the Dila University student cafeteria showed that the prevalence was 66.4% (Diriba *et al*., 2020).

There is a high prevalence of ESBL-producing *E. coli* and *K. pnemoniae* among adults in Ethiopia but there is limited data on the prevalence and factors associated with ESBL fecal carriage among pregnant women in Addis Ababa, Ethiopia. Therefore, this study was aimed at determining the prevalence and factors associated with fecal carriage of ESBL-producing *E.*

coli and *Klebsiella* species among pregnant women and the antimicrobial resistance patterns of ESBL-producing *E. coli* and *Klebsiella* species.

1.2 Statement of the Problem

World Health Organization reported that over 700,000 people die due to antimicrobial resistance-related diseases(World Health Organization, 2019). AMR has a significant negative impact on the economy, death disability, prolonged illness, longer hospital stays, and expensive medicines (WHO, 2021). Because of this WHO declared AMR among the top 10 global public health threats facing humanity (WHO, 2021).

Extended-spectrum β-lactamases (ESBLs), a class of diverse, complex, and quickly evolving enzymes mediated by plasmids present a significant therapeutic challenge in the case of hospitalized and outpatient patients today. ESBL producers can cause illnesses ranging from minor urinary tract infections to sepsis, which can be fatal(Rawat & Nair, 2010).Due to the plasmid location of ESBL genes, there is a risk of gene transfer to another resistant strain and the development of plasmid-mediated multidrug resistance, which makes antibiotic therapy with extended-spectrum cephalosporins harder (Będzichowska *et al.*, 2019).

Worldwide, it has been reported that the fecal carriage rate of ESBL-*Enterobacteriaceae* in pregnant women is estimated to be 15.6%. The biggest concern with ESBL-*Enterobacteriaceae* fecal carriage in pregnancy is the increased risk of transmission of these resistant strains to newborn babies, which could lead to neonatal sepsis (Denkel *et al*., 2014; Rettedal *et al*., 2015).

Furthermore, ESBL-*Enterobacteriaceae* carriage raises the danger of transmission to medical personnel and other hospitalized patients. Furthermore, pregnant women are among the immunocompromised individuals who are vulnerable to various bacterial illnesses. The colonization of ESBL-*Enterobacteriaceae* in pregnant women's urinary tracts as a consequence of fecal-vaginal transmission frequently leads to urinary tract infection (UTI) (Obata-Yasuoka *et al*., 2002; Watt *et al*., 2003).

1.3 Research Questions

1. What is the magnitude of ESBL-producing *E. coli* and *Klebsiella* species fecal carriage among pregnant women attending antenatal care at government health centers in Addis Ababa, Ethiopia?
2. What are the antimicrobial susceptibility profiles of ESBL-producing *E. coli* and *Klebsiella* species recovered from the stool of pregnant women?
3. What are the major risk factors associated with fecal colonization of ESBL-producing *E. coli* and *Klebsiella* species among pregnant women attending ANC in government health centers in Addis Ababa, Ethiopia?

1.4 Objectives of the Study

1.4.1 General Objective

To determine the antimicrobial susceptibility profile and associated factors with fecal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella* species among pregnant women attending antenatal care at government health centers in Addis Ababa, Ethiopia.

1.4.2 Specific Objectives

- To determine the magnitude of ESBL-producing *E. coli* and *Klebsiella* species fecal carriage among pregnant women attending antenatal care at government health centers in Addis Ababa, Ethiopia.
- To determine the antimicrobial susceptibility profiles of ESBL-producing *E. coli* and *Klebsiella* species recovered from the stool of pregnant women.
- To determine the major risk factors associated with fecal colonization of ESBL-producing *E. coli* and *Klebsiella* species among pregnant women attending ANC in government health centers in Addis Ababa, Ethiopia.

1.5 Scope of the Study

The sampling area for this study was eight health centers (Woreda 09, Bole 17, Semen, Saris, Weyzero Beletshachew, Addis Raey, Shegole, and Yeka health centers) with ANC services in Addis Ababa. Samples were processed in EPHI, National Clinical Bacteriology and Mycology

Reference Laboratory. This research mainly focused on screening ESBL-producing *E. coli* and *K. pnemoniae* and their antimicrobial susceptibility pattern and factors associated with a fecal carriage of ESBL-producing *E. coli* and *Klebsiella* species among pregnant women.

1.6 Significance of the Study

Research has shown that pregnant women are at a higher risk of ESBL fecal carriage compared to the general population. Several factors may contribute to this increased risk, including hormonal and immunological changes during pregnancy and exposure to antibiotics during pregnancy or before conception.

ESBL fecal carriage among pregnant women can lead to maternal infection, such as urinary tract infections or bacteremia, which can have adverse effects on fetal health. Additionally, ESBL-producing bacteria can be transmitted from mother to child during delivery, potentially causing infection in the neonate.

Studying the prevalence and risk factors for ESBL fecal carriage among pregnant women can inform prevention and control strategies to reduce the risk of maternal and neonatal infection. This may include promoting appropriate antibiotic use during pregnancy and improving infection prevention and control practices in healthcare settings. Overall, understanding the significance of ESBL fecal carriage among pregnant women can help inform clinical practice and public health strategies to reduce the burden of antibiotic-resistant infections during pregnancy and improve maternal and fetal health outcomes.

1.7Delimitations and Limitations of the Study

Limitation

- Molecular characterization of ESBL genes was not performed.

Delimitations

- Only fecal samples from pregnant women were used.
- Only ESBL-producing *E. coli* and *Klebsiella* species were used.
- Convenient sampling techniques were used.
- Enzymes of other ESBL such as carbapenemase and AmpC are not screened.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 β-lactams

Antibiotics are low molecular weight (a molecule with a defined chemical structure having a relative mass) microbial metabolites that at low concentrations inhibit the growth of other microorganisms (Aminov, 2010). Penicillin was the first antibiotic discovered by Alexander Fleming in 1929, and the culture of *Penicillium notatum* produced a substance that inhibited the growth of *Staphylococcus aureus*(Wright, 1999).

Different groups of antibiotics act differently. Vancomycin and β-lactam antibiotics interfere with the synthesis of the bacterial cell wall. Quinolones inhibit bacterial enzymes involved in the replication of DNA. Rifampicin inhibits transcription by binding to bacterial RNA-polymerase. Macrolides, tetracyclines, and aminoglycosides inhibit 70S-ribosomal function thereby inhibiting protein synthesis. Sulphonamide and trimethoprim inhibit the biosynthesis of tetrahydrofolic acid and also interfere with DNA synthesis (Henriques Normark & Normark, 2002).

The β-lactams consist of four major groups: penicillins, cephalosporins, monobactams, and carbapenems. They all have a β-lactam ring, which can be hydrolyzed by β-lactamases. The groups differ from each other by additional rings (thiazolidine ring for penicillins, cephem nucleus for cephalosporins, none for monobactams, double-ring structure for carbapenems) (Samaha-Kfoury & Araj, 2003). β-lactam drugs are the most frequently prescribed and used antimicrobials that inhibit cell wall synthesis resulting in the lysis of bacterial cells (Bui & Preuss, 2023).

The β-lactams execute their antibacterial activity by inhibiting bacterial cell walls, peptidoglycan, and synthesis by preventing the precise functioning of penicillin-binding protein, which is also known as transpeptidases which are involved in peptidoglycan metabolism(Bui& Preuss, 2022). Inhibition of PBPs produces an imbalance in cell wall metabolism, resulting in growth inhibition or lysis (Zapun *et al*., 2008).

Bacteria evolved numerous mechanisms of resistance to β-lactams. These are the four major ways

(i) Production of β-lactamase breaks the β-lactam ring and makes the antibiotic inactive before it reaches the PBP target. This mode of mechanism is very common in Enterobacteriaceae such as *Escherichia coli* and *Klebsiella*(Tooke *et al*., 2019).

(ii) Expressing altered and mutated PBPs. This mechanism is responsible for resistance to penicillin in *Pneumococci* and methicillin resistance in *Staphylococci* (Fedarovich *et al.*, 2012).

(iii) Absence or reduced expression of outer membrane proteins (OMPs) in Gram-negative bacteria (Delcour, 2009).

(iv) Efflux pumps, a system that ejects the antibiotics out of the cell with energy expenditure, then decreases intracellular concentrations of the antibiotic. The efflux of antimicrobials is the most common mode of acquired resistance to tetracycline; it has been involved in developing resistance to other drugs including β-lactams (Eton & Lepore, 2009).

2.2 Extended Spectrum β-lactamase

β-lactamases are enzymes that can hydrolyze a β-lactam ring and are the most common mechanism of resistance for Gram-negative bacteria (Bush & Jacoby, 2010). β-lactamases can differ from one another in their substrate profiles, inhibitor profile, and sequence homology (Bush & Jacoby, 2010).Extended-spectrum beta-lactamases (ESBLs) are enzymes that confer resistance to most beta-lactam antibiotics, including penicillins, cephalosporins, and the monobactam aztreonam. Infections with ESBL-producing organisms have been associated with poor outcomes.

ESBLs are β-lactamase enzymes that confer resistance to penicillins, broad-spectrum cephalosporins with an oxyimino side chain (cefotaxime, ceftriaxone, and ceftazidime), and the oxyimino-monobactam aztreonam (Zhao & Hu, 2013)but not cephamycin such as cefoxitin and carbapenems comprising meropenem, imipenem, ertapenem, and doripenem (Ur Rahman *et al*., 2018).

Two classification systems for these enzymes are currently in use (Bush & Jacoby, 2010).The first is Ambler molecular classification or molecular classification which is based on the conserved motifs and protein sequence that further categorizes these enzymes into four such classes A, B, C, and D enzymes. These enzymes utilize serine for β-lactam hydrolysis and class B metalloenzymes that require divalent zinc ions (metal ions) for substrate hydrolysis.

The second categorization is named Bush, Jacoby, and Medeiros functional classification, which groups different β-lactamases according to their substrate and inhibitor profiles. This classification is comprised of three groups: Group 1 (class C), cephalosporinases; Group 2 (classes A and D), broad-spectrum, inhibitor-resistant, extended-spectrum β-lactamases, and serine carbapenemases; and Group 3 (class B), Metallo-β-lactamases.

A recent definition by (Ur Rahman *et al*., 2018)divides ESBL into three main groups.

(i) ESBLA (class A ESBLs) comprises the most frequently found ESBL and the CTX-M, as well as SHV and TEM enzymes. These enzymes are mainly horizontally transferable and can be inactivated or inhibited by clavulanic acid.

(ii) ESBLM (miscellaneous ESBLs) are sectioned into ESBLM-C (class C, plasmid-mediated AmpC) and ESBLM-D (class D). Acquired AmpC is the most frequently found ESBL in this class.

(iii) ESBLCARBA-B (ESBLs that degrade carbapenems) are divided into ESBLCARBA-A, ESBLCARBA-B, and ESBLCARBA-D. ESBLs are often found carried on large plasmids in addition to other resistance genes that confer resistance to antimicrobials such as aminoglycosides and sulphonamides.

2.3 Types of Extended-spectrum β-lactamases (ESBLs)

ESBL-producing encoding genes are common among *Enterobacteriaceae*. At present, there are more than 300 different ESBL variants clustered into nine different structural and evolutionary families, based on their amino acid sequence (Bush & Jacoby, 2010). In previous years the predominant ESBLs reported are TEM and SHV (Yagi *et al*., 2000). SHV-5 and SHV-12 are the most prevalent ESBL variants found in Enterobacterales worldwide (Castanheira *et al*., 2021). Among nine ESBLs CTX-M, TEM, and SHV types are the most disseminated genes (Rahman *et al*., 2018).

TEM (Temoneira)-TEM-1 is the most commonly encountered β-lactamase in gram-negative bacteria and they can hydrolyze penicillin (Ur Rahman *et al*., 2018). These enzymes are also responsible for the ampicillin and penicillin resistance that is seen in *H. influenza* and *N. gonorrhea* (Castanheira *et al*., 2021).

SHV (Sulfhydryl Variable)-These types of enzymes are mostly found in *Klebsiella* species (Ur Rahman *et al*., 2018). SHV is the first ESBL, identified in a clinical isolate of *Klebsiella ozaena*(Zhao & Hu, 2013).

CTX-M (Cefotaximase) CTX-M-type enzymes constitute a distinct lineage of molecular class A β-lactamase and are a rapidly growing group (Rossolini *et al*., 2008). They are plasmid-based encoded cefotaimases that contribute to the fast-growing family of ESBLs (Ur Rahman *et al*., 2018). CTX-M has been detected in at least 26 bacterial species but they are most prevalent in *E. coli* and *K. pnemoniae* (Zhao & Hu, 2013).

OXA (Oxacillin-hydrolyzing). They were among the earliest β-lactamases detected. OXA ESBLs are very common class A enzymes, particularly in conferring resistance to penicillins (Evans & Amyes, 2014). These enzymes have been first discovered and are mainly found in *P. aeruginosa* (Amirkamali *et al.*, 2017).

Minor Extended Spectrum β-Lactamases

During the last ten years, class A β-lactamases have been designated, including SFO, BES, BEL, TLA, GES, PER, and VEB types. Some of these minor ESBLs are infrequently identified or are very restricted; others are becoming locally prevalent or are progressively isolated globally (Rahman *et al*., 2018).

2.4 Detection of ESBLs

Screening for ESBL producers

(I) Disk diffusion methods

Clinical and Laboratory Standards Institute (CLSI) recommended disk diffusion methods for screening for ESBL production by *Klebsiella, Escherichia coli*, and *Proteus mirabilis.* Using disk diffusion methods for antibiotic susceptibility testing can screen for ESBL production by

specific zone diameters which indicates a high level of indication for ESBL production. Cefpodoxime, ceftazidime, aztreonam, cefotaxime, or ceftriaxone is used (Clinical and Laboratory Standards Institute, 2022).

(II) Screening by dilution antimicrobial susceptibility tests

The dilution method is another screening method for ESBL production for *Klebsiella* and *Escherichia coli*. Ceftazidime, aztreonam, cefotaxime, or ceftriaxone can be used at a screening concentration of 1μg/ml (Paterson & Bonomo, 2005). MIC for the cephalosporin is ≥2μg/ml which is an indicator of ESBL production and is an indication for the organism to be tested by a phenotypic confirmatory test (Paterson & Bonomo, 2005).

Phenotypic Confirmatory Tests for ESBL Production

Cephalosporin/clavulanate combination disks

CLSI suggests the use of cefotaxime (30μg) or ceftazidime (30μg) disks with or without clavulanate (10μg) for phenotypic confirmation of ESBLs in *Klebsiella* and *Escherichia coli* (Abdelmoktader and El Far, 2019). CLSI recommends that the disk tests be performed with the help of growth on Mueller-Hinton agar. A difference of ≥5 mm between the zone diameters of either of the cephalosporin disks alone or cephalosporin with clavulanate disk is taken to be a phenotypic confirmation of ESBL production (Clinical and Laboratory Standards Institute, 2022).

Non-ESBL-producing *Escherichia coli* ATCC 25922 and an ESBL-producing organism *Klebsiella pneumoniae* ATCC 700603 are used as negative and positive controls, respectively (Clinical and Laboratory Standards Institute, 2022).

Broth microdilution

This is another method for phenotypic confirmatory testing performed by broth microdilution assays using ceftazidime (0.25 to 128μg/ml), ceftazidime plus clavulanic acid (0.25/4 to 128/4μg/ml), cefotaxime (0.25 to 64μg/ml), and cefotaxime plus clavulanic acid (0.25/4 to 64/4μg/ml) (Clinical and Laboratory Standards Institute, 2022). Phenotypic confirmation is considered as a ≥3-twofold-serial-dilution decrease in MIC of either cephalosporin in the

presence of clavulanic acid compared to its MIC when tested alone. Implications of positive phenotypic confirmatory tests From the CLSI guidelines isolates that are ESBL positive phenotypically, the confirmatory test should be reported as resistant to all cephalosporins but not to (cephamycins, cefoxitin, and cefotetan) and aztreonam (Clinical and Laboratory Standards Institute, 2022).

2.5 Epidemiology of ESBL-producing bacteria in Ethiopia

Globally, rates of ESBL-producing Enterobacteriaceae are rising. Explanations for these changes can be attributed to horizontal gene transfer (HGT) of plasmids, successful *Escherichia coli* clones, ESBLs in food animals, the natural environment and human migration, and access to basic sanitation(Bevan *et al.*, 2017).

Genes encoding ESBL enzymes can be transmitted by emerging bacterial clones or by horizontal gene transfer. Plasmids containing resistance genes are spread between bacteria of the same and/or different species (Brolund, 2014).

The movement of IS-mobilized genes between chromosomes and plasmids greatly increases the opportunity for a resistance determinant to become transferable. A particularly conjugative plasmid is one of the most important mechanisms for intra-species, inter-species, and inter-genus species (Zhao & Hu, 2013). Due to the plasmid location of ESBL genes, there is a risk of gene transfer to another resistant strain and the development of plasmid-mediated multidrug resistance, which limits the therapeutic possibilities (Będzichowska *et al.*, 2019).

The global fecal carriage rate of ESBL-E among pregnant women has been estimated to be 15.6% (Jalilian *et al*., 2019).In Africa, ESBL- *Enterobacteriaceae* fecal carriage average is 17% among pregnant women (Bulabula *et al*., 2017).The prevalence of fecal colonization of ESBL-producing *E. coli* and *K. pneumoniae* in Ethiopia ranged from 17% to 47.3 (Bayleyegn *et al*., 2021; Tola *et al*., 2021;Amare *et al*., 2022; Shenkute *et al*., 2022)

A study on the fecal carriage of ESBL-producing Enterobacteriaceae among HIV-infected children at the University of Gondar, Ethiopia showed from 161 stool samples the ESBL fecal carriage was 19.9% (Bayleyegn *et al*., 2021). The other study that showed the overall prevalence of ESBL-producing *E. coli* and *K. pnemoniae* was 17% from 269 fecal/rectal

swabs. The study was conducted among children under five years in Addis Ababa, Ethiopia (Tola *et al*., 2021)

A study on the high prevalence of fecal carriage of ESBL and Carbapenemase-producing *Enterobacteriaceae* among food handlers at the University of Gondar, northwest Ethiopia showed that from 290 stool samples, 21.7% of stool samples were confirmed as ESBL and Carbapenemase-producing *Enterobacteriaceae*(Amare *et al*., 2022). The highest prevalence was shown in the study of the high magnitude of fecal carriage of ESBL-producing *Enterobacteriaceae* at Debre Berhan Comprehensive Specialized Hospital, Ethiopia. The overall ESBL colonization rate was 47.3% from 383 stool/rectal swabs (Shenkute *et al*., 2022).

2.6 Pathogenesis of *Escherichia coli* and *Klebsiella pnemoniae*

Escherichia coli, a group of bacteria commonly found in the digestive tracts of humans and animals, participate in digestion and the synthesis of certain vitamins. Currently, over 160 serological types of *E. coli* have been identified, with 171 somatic (O), 55 flagellar (H), and 80 capsular (K) antigens. *E. coli* can cause various infections, including urinary tract infections (UTIs), hospital-acquired pneumonia (HAP), sepsis, surgical site infections (SSIs), gastrointestinal tract infection, hemolytic-uremic syndrome (HUS), meningitis, and inflammation of the meninges. Mobile genetic elements of *E. coli* can be horizontally transferred to related bacteria or bacteria from different families, enabling their colonization in various environments (Sarowska *et al*., 201).

K. pneumoniae is an opportunistic pathogen that can be found in the mouth, skin, and intestines, as well as in medical settings and devices. It primarily affects individuals with compromised immune systems or those who are already weakened by other infections.

Opportunistic *K. pneumoniae* usually colonizes the gastrointestinal tract. It can also be found in the urinary, respiratory, and blood. *K. pneumoniae* infections tend to be chronic for two main reasons. Firstly, biofilms formed by *K. pneumoniae* in the host's body protect the bacteria from the immune system and antibiotics. Secondly, nosocomial isolates of *K. pneumoniae* often exhibit multidrug-resistant phenotypes because of the presence of extended-spectrum β-lactamases or carbapenemases. This makes it challenging to select appropriate antibiotics for treatment(Li *et al*., 2016).Numerous virulence factors have been identified in *Klebsiella* spp. One of the most important of these factors is the extracellular capsule, which is essential for

virulence. This capsule consists of thick bundles of fibrillous structures that cover the surface of the bacterium in massive layers. The capsule protects the bacterium from phagocytosis by polymorphonuclear granulocytes and prevents killing by bactericidal serum factors via the complement-mediated cascade. There are currently around 80 different capsular (K) antigens known. In addition to the capsule, several other virulence factors have been identified in *Klebsiella* spp. These include about five somatic or O antigens, as well as fimbrial and nonfimbrial adhesins. The fimbriae or pili are filamentous projections on the bacterial surface that mediate the attachment of the organism to respiratory, gastrointestinal, and urinary tract mucosal cells(Gupta *et al*., 2003).

2.7 Infections due to ESBL-producing bacteria

ESBL infections are observed in both community and hospital-acquired infections (Będzichowska *et al*., 2019).Clinical infections with ESBL are associated with increased morbidity including prolonged hospital stays, increased healthcare costs, and higher mortality rates. ESBL-E is frequently implicated in urinary tract infections and a blood stream infections among neonates, children, and pregnant/ post-partum women (Bulabula *et al*., 2017).

Neonatal sepsis is one of the commonest causes of neonatal mortality in developing counties. Gram-negative bacteria are commonly isolated and resistant to drugs like penicillins, cephalosporins, aminoglycosides. ESBL-producing gram-negative sepsis is a substantial problem in neonatal infections. Maternal exposure to cephalosporins and ESBL carrier are important risk factors for ESBLs (Sharma *et al*., 2013).

Urinary tract infections (UTIs) caused by ESBL-producing bacteria have become a worldwide problem because ESBL producers cause complicated UTIs (Sharma *et al*., 2013). UTIs are the second most prevalent cause of community-acquired infection and nosocomial infection (Sharma *et al*., 2013).

ESBL-producing *P. aeruginosa* is one of the most prevalent pathogens in burn and post-operative wound infections. It is also associated with a higher mortality rate and antibiotic costs (Ullah *et al.,* 2009). It also causes urinary tract infections, dermatitis, soft tissue infections, gastrointestinal infections, and systemic infections particularly in patients with

severe burns, cancer, and AIDS (Acquired Immunodeficiency Syndrome) (Amirkamali *et al*., 2017).

2.8Treatments for infections caused by ESBL producers

ESBL-producing Enterobacterales are generally susceptible to carbapenems (ciprofloxacin, trimethoprim-sulfamethoxazole, and gentamicin). Possible medications used to treat ESBL infection includes carbapenems, Fosfomycin, beta-lactamase inhibitors, non-beta-lactam antibiotics, and colistin, which is prescribed in rare cases when other medications have failed to stop the ESBL infection (Fournier *et al*., 2013). Nitrofurantoin and trimethoprim-sulfamethoxazole are preferred treatment options for uncomplicated cystitis caused by ESBL-E. Ertapenem, meropenem, imipenem-cilastatin, ciprofloxacin, levofloxacin, or trimethoprim-sulfamethoxazole are preferred treatment options for pyelonephritis and UTIs caused by ESBL-E. A carbapenem is preferred for the treatment of infections outside of the urinary tract caused by ESBL-E (Tamma *et al*., 2022).

2.9 Risk factors associated with ESBL infection

Maternal-neonatal transmission of ESBL-E from mother to child is an important risk factor for colonization of very low birth weight infants (Denkel *et al*., 2014). Risk factors for neonatal sepsis caused by ESBL-E include maternal infection (UTI and chorioamnionitis) and prolonged rupture of membranes, maternal bacterial colonization of the vaginal tract and poor ANC (less than four antenatal visits per pregnancy) (Bulabula *et al*., 2017).

Different studies showed numerous risk factors associated with ESBL infections. For instance, a study on community-acquired UTIs in low-prevalence countries showed that traveling to Asia, the Middle East, or Africa up to 2 years in the past, recreational swimming, and eating dinner at restaurants and close occupational contact with humans were identified as significant risk factors for ESBL UTI.

The other study by Francisco Tuon *et al*.,(2011)on bacteremia by ESBLs in Brazilian hospitals showed that the factors associated with ESBL included invasive procedures such as mechanical ventilation, the use of the central venous catheter, elective surgery, male sex, diabetes mellitus, and admission to the intensive care unit, previous use of fourth-generation cephalosporin as a risk factor.

A study by Rettedal *et al*., (2015)among pregnant women in Norway showed that traveling and nationality risk factors. African or Asian nationality was a risk factor this is due to high ESBL prevalence in Africa and Asia and also lack of self-hygiene, toilet sharing, crowded community, and poverty. The prevalence of ESBL producers varies in continents, with high prevalence in Southeast Asia, Africa, and Central America, and lower prevalence in Europe (Tschudin-Sutter *et al.*, 2012).

Use of antibiotics; self-prescribed antibiotics, sharing toilets; poor toilet cleanliness, and self-hygienic practices had an increased chance of ESBL infections among pregnant women in Tanzania (Mwandigha *et al*., 2020).

2.10 Prevention of ESBL Infections

To prevent the spread of ESBL-producing strains of bacteria, it is important to implement isolation measures. This can include placing patients in a private room with a private bathroom and using contact isolation procedures, such as wearing gloves and gowns, to prevent direct contact with the patient's bodily fluids. Maintaining good hand hygiene compliance and using dedicated equipment, such as thermometers and stethoscopes, is also important for each patient to prevent cross-contamination. Patient charts should be marked to identify patients who may be colonized with ESBL-producing bacteria so that contact isolation can be considered on subsequent admissions. Additionally, the environment of care should be thoroughly decontaminated with routine low-level disinfectants, such as quaternary ammonium or phenolic disinfectants. To reduce the selective pressure for ESBL-producing strains of bacteria, antibacterial use protocols should be established to eliminate the indiscriminate use of antibacterials(Rupp *et al*., 2003).

Preventative measures for controlling the spread of infections, such as barrier nursing and cohorts of patients and nurses, as well as contact precautions including the use of disposable gloves, gowns, and rigorous hand washing, are crucial. Establishing an infection control policy and a hospital antibiotic prescribing guide is important. Providing educational materials, such as handouts, posters, and meetings, to medical and nursing staff, patients, visitors, and medical students can also be helpful(Bhattacharya, 2006).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Description of the study area

The stool samples were collected from pregnant women attending antennal care at the government health centers in Addis Ababa, Ethiopia. The city's Global position is between 8º55' and 9º05'N Latitude and 38º40' and38º50'E Longitude. The average annual temperature is about 19.6ºC. The city has an average annual rainfall of 1200mm. The size of the city covers around 540km^2 with the highest elevation point of 2800m oversea level (Tassie *et al*., 2019).

There are 11 sub-cities in the city and each sub-city has government health centers that have antenatal care services. Data from the City Government of Addis Ababa health bureau in March 2022 showed that: the Kolfe Keranyo sub-city has 7,the Kirkos sub-city has 8, the Bole sub-city has 6, the Arada sub-city has 10, the Akaki Kality sub-city has 9, the Lideta sub-city has 8,the Yeka sub-city has 11,the Nifas silk sub-city has 8,the Lemi Kura sub-city has 9,the Addis Ketema sub-city has 14 and the Gullele sub-city has 10 government health centers. A total of 100 government health centers are there in Addis Ababa and of which 91 of them were providing ANC service.

Eight health centers were selected for the study. Woreda 09 health center from the Kolfe Keranyo sub-city, Bole 17 health center from the Bole sub-city, Semen health center from the Arada sub-city, Saris health center from the Akaki Kality sub-city, Weyzero Beletshachew health center from Lideta sub-city, Addis Raey health center from Addis Ketema sub-city, Shegole health center from Gullele sub-city and Yeka health center from Yeka sub-city.

A custom map for the location of sample collection areas (health centers) and sample processing laboratory (EPHI) was created using Arc map; software Arc GIS version 10.4, United States, California.

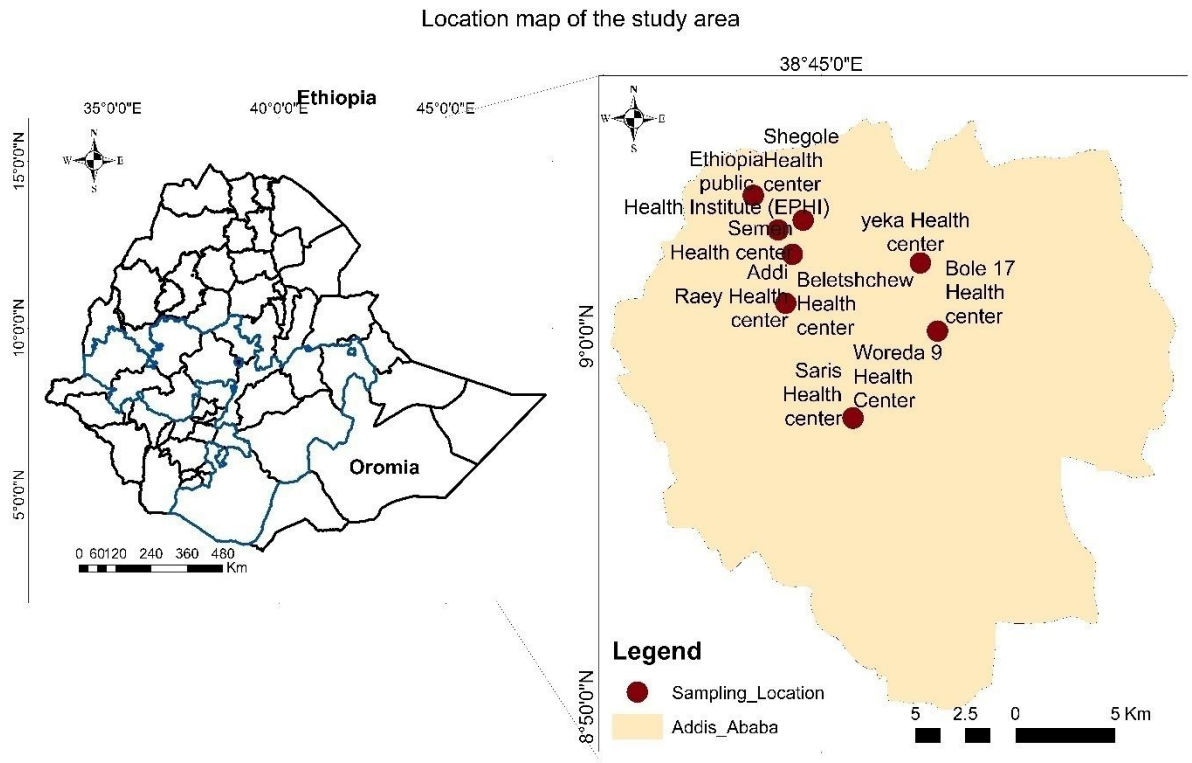

Figure 1. Location of the study area

3.2 Study design

A prospective cross-sectional study design was used in this study. Data and stool samples were collected after obtaining written informed consent from pregnant women.

3.3 Study Samples and sampling techniques

Stool samples were collected from willing pregnant women attending ANC at government health centers at any gestation stage. Samples and a questionnaire were collected by health professionals at the government health center. A convenient sampling technique was used to select pregnant women attending ANC at the government health center. A willing pregnant woman at any gestation age attending ANC at the government health center fulfilled the inclusion criteria. The sample size was calculated using the Kish Lisle formula$\frac{z^2p(1-p)}{e^2}$" (Mwandigha*et al*., 2020)"n" stands for the required minimum sample size, "z" stands for the level of confidence (1.96) at a 95% confidence interval, and "p" stands for the expected proportion in the previous studies (for the prevalence of ESBL-producing

Enterobacteriaceae among pregnant women was estimated 64.3% from a study conducted in Dares Salaam, Tanzania (Mwandigha*et al*., 2020) and "e" stands for the margin of error 5%.

Samples were collected from volunteer pregnant women at any gestation stage. The inclusion criteria for sample collection were pregnant women able and willing to provide consent and stool sample for testing. Exclusion criteria for sample collection were pregnant women without informed consent to participate in the study, documented use of antibiotics in the previous three months, and hospitalization in the previous three months.

3.3.1 Collection of socio-demographic, clinical, and hygiene practice data

Data for the sociodemographic, clinical, and hygiene practice for the pregnant women were collected using a structured, simple and short 14 questions questionnaire.

3.3.2 Stool sample collection

A total of 353 stool samples were collected from pregnant women attending antenatal care at government health centers in Addis Ababa. The stool samples were collected from eight sub city government health centers.

Stool samples were collected using a sterile stool cup and test tubes. Samples were transported by Cary-Blair (Biomark, India Ref: 03170780) transport medium and brought to EPHI clinical Bacteriology and Mycology National Reference Laboratory within the same day of collection.

Table 1. Number of stool sample collected from pregnant women in government health centers

Sub-city (Health Center)	Number of stool samples
Addis Ketema (Addis Raey health center)	62
Akaki (Saris health center)	44
Arada (Semen health center)	47
Bole (Bole 17 health center)	33
Gullele (Shegole health center)0	47
Kolfe keranyo (Woreda 09 health center)	35
Lideta (W/ro Beletshachew health center)	35
Yeka (Yeka health center	50
Total	353

3.3.3 Bacterial culture, isolation, and identification

Three hundred fifty-three stool samples were collected from volunteer pregnant women attending ANC in government health centers between a period of March 08, 2022, to May 23, 2022.Stool samples were collected in Cary-Blair transport media in sterile stool cups from volunteers at the government health center's restroom. Stool samples were brought to EPHI National Clinical Bacteriology and Mycology Reference Laboratory for culture and antimicrobial susceptibility testing.

Stool samples were inoculated on Chromatic ESBL agar (Chromatic ESBL, Liofilchem, Italy Ref:610629; Chromatic ESBL Supplement, Liofilchem, Italy Ref: 81089) and then incubated aerobically at 37°C overnight. According to the instructions of the manufacturer, pink-reddish-lilac with good growth colonies were evaluated as *E. coli* ,and green-blue colonies were evaluated as *Klebsiella* species.

Quality control strain: *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603were used as negative and positive control strains respectively for the checking of the quality of chromatic ESBL agar.

3.3.4 Biochemical Characterization of the Isolates

ESBL-positive *E. coli* and *Klebsiella* species colonies from chromatic ESBL agar were inoculated on MacConkey (Accumix, India Ref: 101130130500)and 5% sheep blood agar and incubated aerobically overnight at 37ºC.Both *E. coli* and *Klebsiella* species were lactose fermenters on MacConkey agar.

Further identification of the positive isolates was performed using standard biochemical tests(Amy L, 2016). Suspensions were prepared for biochemical tests. Colonies from sheep blood agar were used to prepare a suspension. Using normal saline bacterial suspension equivalent to 0.5 McFarland's turbidity standard was prepared using a BD Phoneix densitometer.

Urease test

A urease enzyme production test was performed using urea agar(Accumix, India Ref: 201210010500). *E. coli* was urease negative and *Klebsiella* species was urease positive.

Citrate utilization test

A sodium citrate utilization test was performed using Simmons citrate agar (Liofilchem, Italy Ref: 610046). *E. coli* was citrate negative and *Klebsiella* species was citrate positive.

Triple sugar iron test

Carbohydrate fermentation, gas production, and H_2S production tests were performed using triple sugar iron agar (Oxoid, UK Ref: 2495767). Both *E. coli* and *Klebsiella* species were positive for carbohydrate fermentation and gas production.

Lysine decarboxylation test

Lysine decarboxylation or lysine deamination test was performed using lysine iron agar (Liofilchem, Italy Ref: 013020510). Both *E. coli* and *Klebsiella* species were positive for the test. Both can decarboxylate lysine.

Sulfide, indole and motility test

Sulfide formation, indole formation, and motility tests were performed using sulfide indole motility agar (Oxoid, UK Ref: 2849596). Indole test was performed using an additional regent, Kovac's. Both *E. coli* and *Klebsiella* species did not produce H_2S. *E. coli* was motile and indole positive. *Klebsiella* species was non-motile and indole negative.

ESBL-positive *E. coli* and *Klebsiella* species were stored in 20% TSB (Tryptone Soya Broth Liofilchem, Italy Ref: 610053) with glycerol at -80°C.

3.3.5 Antimicrobial Susceptibility Testing

The Kirby-Bauer disk diffusion method was used for the antimicrobial susceptibility test. An antimicrobial susceptibility testing was done for positive ESBL isolates. Colonies from sheep blood agar(Liofilchem, Italy Ref: 630005) were used to prepare a suspension. Using normal saline bacterial suspension equivalent to 0.5 McFarland's turbidity standard was prepared using a BD Phoneix densitometer. The prepared suspensions were inoculated on Muller Hinton Agar (Muller Hinton Agar Liofilchem, Italy Ref: 610627).

CLSI M100 edition 32nd guideline was used to select an antibiotic disk and for the interpretation of AST results. The following antibiotics were used for the test. Ampicillin/ sulbactam (SAM) (20µg Oxoid, UK), chloramphenicol (CHL) (30µg Oxoid, UK), cefuroxime (CXM) (30µg Oxoid, UK), amoxicillin/clavulanic acid (AMC) (30µg Oxoid, UK), ciprofloxacin (CIP) (5µg Oxoid, UK), trimethoprim/ sulfamethoxazole (SXT) (25µg Oxoid, UK), aztreonam (ATM) (30µg Oxoid, UK), piperacillin/ tazobactam (TZP) (110µg Oxoid, UK), meropenem (MEM) (10µg Oxoid, UK), ceftriaxone (CRO) (30µg Oxoid, UK), and cefazolin (CZO) (30µg Oxoid, UK).

The selected antibiotic disks were placed on inoculated Muller Hinton agar. The prepared plate was incubated aerobically at 37ºC overnight. The inhibition zone diameter was measured and interpreted.

3.4Data analysis

The collected data from the questionnaire were organized and analyzed using SPSS version 23. Continuous variables were summarized as the median and interquartile range (IQR), whereas proportions were used to describe categorical variables. Group differences were examined by using Fisher's exact test for categorical variables and a t-test for continuous variables. Binary logistic regression was performed to identify factors associated with ESBL fecal carriage among pregnant women depending on the crude odds ratio (COR) with a 95% confidence interval (CI). Multivariable logistic regression was performed to examine the associations between the outcome variable and independent variables after adjustment of all the factors that showed significance in the bivariate analysis, Associations in the multivariable logistic models were presented as adjusted odds ratios (AOR) with 95% CI. A two-sided p-value< 0.05 was considered significant. Antimicrobial susceptibility test results were organized and analyzed in WHONET version 2021 with 95% CI. Zone diameter was measured and interpreted as resistant, intermediate, and susceptible based on CLSI guidelines.

3.5Ethical Considerations

An official letter requesting for ethical considerations was written from the Department of Applied Biology, Adama Science and Technology University to the City Government of Addis Ababa health bureau. Then the research was conducted after ethical clearance approval of the City the Government of Addis Ababa health bureau was secured. Following this, written

informed consent was obtained from voluntary participants during data collection. Data was collected and handled confidentially throughout this study. No name of the respondents was used and only numerical codes were used in the questionnaire.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Socio-demographic characteristics

A total of 353 pregnant women participated in the study. The Socio-demographic characteristics of the participants showed that out of the participants the minimum age was 18, the maximum age was 43, and the median age was 25. The median gestational age was 26 weeks. Four and 38 weeks were the minimum and maximum gestational weeks of the participants, respectively. The maximum and minimum family number of the participants was 8 and 2 respectively. Two hundred seventy-nine (79%) participants had a former pregnancy. Out of the participants, 62 (17.6%) were from Addis ketema, 44 (12.5%) were from Akaki, 47 (13.3%) were from Arada, 33 (9.3%) were from Bole, 47 (13.3%) were from Kolfe keranyo, 35 (9.9%) were from Lideta and 50 (14.2) % were from Yeka sub city. The participants were involving as cashiers6 (1.7%), chefs2 (0.6%), government employe 2 (0.6%), housewives205 (57.8%), janitors15 (4.3%), laborer75 (21.2%), merchant 11 (3.1%), Non-Governmental Organization employee 6 (1.7%), private 13 (3.7%), security 11 (3.1%), storekeeper 2 (0.6%), student 3 (0.8%) and waitress 2 (0.6%) for a living (Table 2).

Concerning their health issues 1 (0.3%) was type 2 diabetic and 1 (0.3%) was HIV seropositive, 1 (0.3%) was HIV seropositive and type 2 diabetic, and 350 (98.9%) of participants do not have any comorbidities, 2 (0.6%) pregnant women were on ART and 1 (0.3%) was on cimetidine and 1 (0.3%) were on iron sulfate supplement. Only 11 (3.1%) of pregnant women reported self-prescribed antibiotics, 3 (0.8%) had longer hospitalization in their life (Table 2).

Concerning hygiene practices, 122 (34.6 %) had no practice of hand washing trend after toilet visit, 207 (58.6 %) pregnant women use hole toilets, and 146 (41.4 %) pregnant women use WC toilet (Table 2).

Concerning feeding habits 285 (88.1%) usually use homemade made foods and 68 (19.3%) use restaurants (street-vended foods, bistro and bar) as a usual food source, 238 (67.4%) use treated water, and 115 (32.6%) use untreated water (Table 2).

Table 1. Distribution of socio-demographic, clinical, feeding and hygienic practice variables among pregnant women (N=353).

Variable		**Total number**	**Percentage (%)**	***p-value***
Age	18-23	90	25.5	0.569
	24-29	218	61.8	
	30+	45	12.7	
Occupation	Government and NGO employee	49	13.9	0.165
	Housewife	205	58.1	
	Laborer	3	20.7	
	Private	23	6.5	
	Student	3	0.8	
Number of families	2-4	220	62.3	0.434
	5-7	131	37.1	
	8	2	0.6	
Gestation age	4-16	58	16.4	0.004
	17-33	256	72.5	
	34-38	39	11.1	
Former pregnancy	Yes	279	79	0.361
	No	74	21	
Comorbidities	Type 2 diabetes	2	0.57	0.617
	HIV seropositive	2	0.57	
	No comorbidities	350	99.2	
Current Medication	ART	2	0.57	1.000
	Cimetidine	1	0.28	
	Iron sulfate	1	0.28	
	No current medication	349	98.8	
Longer hospitalization	Yes	3	0.8	1.000
	No	350	99.2	
Self-prescribed antibiotics	Yes	11	3.1	0.220
	No	342	96.9	
Hand washing after a toilet visit	Yes	231	65.4	0.002
	No	122	34.6	
Type of toilet	Hole	207	58.6	0.041
	WC	146	41.4	
Use of water	Treated	238	67.4	0.023
	Untreated	115	32.6	
Food source	Home	285	80.7	0.003
	Restaurant	68	19.3	

4.2 Prevalence of ESBL-producing *E. coli* and *K. pneumoniae*

Out of 353 pregnant women enrolled,180 (51%) were positive for ESBL-producing bacteria. Of these,167 (92.8%) carried at least one ESBL-producing *E. coli* and *Klebsiella* species. Thirteen (7.2%) were positive for both ESBL *E. coli* and *Klebsiella* species.

A total of 193 ESBL-producing organisms were isolated of which 171 and 22 were *E. coli* and *Klebsiella* species, respectively. In the current study, *E. coli*88.6% was the dominant ESBL-producing bacteria isolated from the stool of pregnant women followed by *Klebsiella* species11.4%.

The findings of this study agrees with the results in Tanzania 84.6% (Mwandigha*et al*., 2020), and Cambodia 94.0% (de Lauzanne *et al*., 2022)*E. coli* was the dominant ESBL producing bacteria isolated among pregnant women.

Several studies have reported prevalence rates of ESBL among pregnant women in different countries. The finding of this study is consistent with a study by Oktaviani *et al*., (2020) which showed that the prevalence of ESBL fecal carriage among pregnant women in primary health care center in Indonesia showed that it is 49.5%. The other study on Madagascar showed the prevalence of ESBL fecal carriage among pregnant women were 34% (Milenkov *et al*., 2021) which is lower than the results of the current study. The observed disparity may be caused by the fact that the collected data for this study was from a single city, whereas the data for the previous study was collected from three cities and higher sample size was collected.

Predictors of ESBL fecal carriage

In this study gestation age, hand washing after a toilet visit, type of toilet and, use of, water, and food source are significantly associated with fecal carriage associated ($p< 0.05$) with ESBL producing *E. coli* and *Klebsiella* species among pregnant women (Table2).

The bivariate and multivariate analysis showed that as one week increases in gestation age of the participants, it was 0.950 times more likely to ESBL fecal carriage or one week increases in gestation age of the participants ESBL fecal carriage decreases in 5%, (aOR=0.950, 95% CI= 0.920-0.981). Participants that use treated were observed 0.648 times less likely vulnerable to ESBL fecal carriage and participants that use treated water as 35.2% less likely

vulnerable to ESBL fecal carriage as compared to participants that use untreated water (aOR= 0.648, 95% CI= 0.398-1.057). Participants that use hole type toilet were 0.661 times less likely to be vulnerable to ESBL fecal carriage or participants that use hole type of toilet is 33.9% less likely to be vulnerable to ESBL fecal carriage as compared to participants that use WC type of toilet (aOR= 0.661, 95%CI= 0.422-1.036). Participants that wash their hands after toilet visit is 2.219 times likely vulnerable to ESBL fecal carriage than participants that don't wash their hands before cooking (aOR=2.219, 95% CI= 1.385-3.554). Participants that use restaurants (street foods, bistro and bar) as usual food source are 1.968 times more likely vulnerable to ESBL fecal carriage as compared to participants that use home as a usual food source (aOR= 1.968, 95% CI= 1.082-3.579) (Table 3).

Table 2. Multivariate logistic regression for the factors associated with ESBL fecal carriage among pregnant women.

Variable		Fecal carriage n(%)	95% CI	cOR	*p*-value	95% CI	aOR	*p*-*value*
Gestation age	4-16	36 (20)	0.928-0.986	0.957	0.004	0.920-0.981	0.950	0.002
(*p*=0.004)	17-33	129 (71.7)						
	34-38	15 (8.3)						
Use of water	Treated	134 (74.4)	0.0371-0.915	0.583	0.019	0.398-1.057	0.648	0.082
(*p*=0.023)	Untreated	46 (25.6)	Ref			Ref		
Hand washingafter	No	69 (38.7)	Ref		0.002	Ref		0.001
toilet visit(*p*=0.002)	Yes	111 (61.7)	1.315-3.214	2.056		1.385-3.554	2.219	
Food source	Home	84 (46.7)	Ref		0.003	Ref		0.027
(*p*=0.003)	Restaurant	96 (53.3)	1.348-4.120	2.356		1.082-3.579	1.968	
Type of toilet	Hole	132 (74.4)	0.417-0.978	0.638	0.039	0.422-1.036	0.661	0.071
(*p*=0.041)	WC	46 (25.6)	Ref			Ref		

aOR- adjusted odd ratios cOR- crude odd ratios Ref- References

The multivariate analysis showed that gestation age p=0.002and hand washing after toilet visit p= 0.001were factors independently associated (p< 0.05) with ESBL fecal carriage.

In the present study, gestation age, the use of untreated water for drinking, WC type of toilet, hand washing trend after toilet visits, and using restaurants as a usual food source are identified as factors associated with ESBL fecal carriage among pregnant women.

Studies on ESBL fecal carriage among pregnant women showed that self-prescription of antibiotic medication during pregnancy, low educational level, and toilet sharing as factors associated with ESBL fecal carriage among pregnant women in Tanzania (Mwandigha *et al*., 2020). The other study in Cambodia showed that low income, nutation, multiparity, and regulated consumption of pork, dried, meat, and raw vegetables as risk factors for ESBL fecal carriage among pregnant women(de Lauzanne *et al*., 2022). The other study showed that adjusted for traveling, African or Asian nationality is a risk factor for ESBL fecal carriage among pregnant women in Norway (Rettedal *et al*., 2015).

The observed disparity is due to the life style among individuals, this study collected stool samples from urban area, Addis Ababa whereas a study by de Lauzanne *et al.*, (2022) used both rural and urban area in southeast-Asia to collect samples. Geographical differences, feeding habits Norway, Tanzania and Southeast-Asia (Rettedal *et al*., 2015; Mwandigha *etal*., 2020; de Lauzanne *et al*., 2022), , are also factors for the disparity. The other factors for the disparity may be a selected population Tanzania gestation age above 37 weeks (Mwandigha *etal*., 2020), Southeast-Asia peripartum women (de Lauzanne *et al*., 2022)and Norway 36 weeks of pregnancy and during delivery (Rettedal *et al*., 2015).

4.3 Antimicrobial resistance profile of ESBL-producing *E. coli*

In this study, the antimicrobial resistance profile of ESBL-producing *E. coli* was screened among the carriers using the Kirby-Bauer disk diffusion method (Table 4). Overall, ESBL-producing *E. coli* were 100% resistant to cefazolin, cefuroxime, ceftriaxone, and aztreonam. The resistance rates were 83.9%, and 98.8% to amoxicillin/clavulanic acid and trimethoprim/sulfamethoxazole, respectively. ESBL-producing *E. coli* showed lower resistance rates to ampicillin/sulbactam with resistance rates of 73.1% susceptibility and 99.4% susceptibility to meropenem and 83.6% susceptibility to chloramphenicol. (Table 4).

Table 3. Antimicrobial resistance profile of ESBL-producing *E. coli*

Organism	Antibiotic name	Breakpoints (mm)	%R	%I	%S	%R95%C.I.	%S 95%C.I.
E. coli	Amoxicillin/Clavulanic acid	14 – 17	83.6	14.1	2.3	77.3-89.0	0.5-5.5
	Ampicillin/Sulbactam	12 – 14	9.4	17.5	73.1	5.7-15.3	65.7-79.6
	Piperacillin/Tazobactam	18 – 20	56.7	0.0	43.3	0.0-2.8	97.2-100
	Cefazolin	S >= 15	100.0	0.0	0.0	97.2-100	0.0-2.8
	Cefazolin	20 – 22	100.0	0.0	0.0	97.2-100	0.0-2.8
	Cefuroxime	15 – 17	100.0	0.0	0.0	97.2-100	0.0-2.8
	Cefuroxime	15 – 22	100.0	0.0	0.0	97.2-100	0.0-2.8
	Ceftriaxone	20 – 22	100.0	0.0	0.0	97.2-100	0.0-2.8
	Aztreonam	18 – 20	100.0	0.0	0.0	97.2-100	0.0-2.8
	Meropenem	20 – 22	0.6	0.0	99.4	0.0-2.8	97.2-100
	Ciprofloxacin	22 – 25	46.8	32.2	21.0	38.8-54.3	15.6-28.6
	Trimethoprim/Sulfamethoxazole	11 – 15	98.8	1.2	0.0	95.3-99.8	0.0-2.8
	Chloramphenicol	13 – 17	16.4	0.0	83.6	11.0-22.7	77.3-89.0

R- resistant I- intermediate S- susceptible

Antimicrobial resistance profile of ESBL-producing *Klebsiella* species

The antimicrobial resistance profile of extended-spectrum beta-lactamase-producing *Klebsiella* species was 100% resistant to cefazolin, cefuroxime, ceftriaxone, and aztreonam, respectively (Table 4). However, showed lower resistance to meropenem, chloramphenicol, ampicillin/sulbactam, piperacillin/tazobactam, and ciprofloxacin with resistance rates of 0%,9.1%,9.1%, 36.4%, and 54.5% respectively. Moreover, the *Klebsiella* species isolates were sensitive to meropenem, chloramphenicol, ampicillin/sulbactam and piperacillin/tazobactam, with sensitivity rates of 100%, 90.9%, 72.7% & 63.6%, respectively (Table 5).

Table 4. Antimicrobial resistance profile of ESBL-producing *Klebsiella* species

Organisms	Antibiotic name	Breakpoints(mm)	%R	%I	%S	%R 95%C.I.	%S 95%C.I.
	Amoxicillin/Clavulanic acid	14 – 17	68.2	27.3	4.5	47.0-85.9	0.2-24.0
	Ampicillin/Sulbactam	12 – 14	9.1	18.2	72.7	1.5-29.5	51.3-88.9
	Piperacillin/Tazobactam	18 – 20	36.4	0.0	63.6	0.0-17.8	82.2-100
	Cefazolin	S >= 15	100.0	0.0	0.0	82.2-100	0.0-17.8
	Cefazolin	20 – 22	100.0	0.0	0.0	82.2-100	0.0-17.8
	Cefuroxime	15 – 17	100.0	0.0	0.0	82.2-100	0.0-17.8
	Cefuroxime	15 – 22	100.0	0.0	0.0	82.2-100	0.0-17.8
	Ceftriaxone	20 – 22	100.0	0.0	0.0	82.2-100	0.0-17.8
	Aztreonam	18 – 20	100.0	0.0	0.0	82.2-100	0.0-17.8
	Meropenem	20 – 22	0.0	0.0	100.0	0.0-17.8	82.2-100
	Ciprofloxacin	22 – 25	54.5	31.8	13.7	34.9-76.1	3.4-34.7
Klebsiella	Trimethoprim/Sulfamethoxazole	11 – 15	100.0	0.0	0.0	82.2-100	0.0-17.8
species	Chloramphenicol	13 – 17	9.1	0.0	90.9	1.5-29.5	70.5-98.5

R- resistant I- intermediate S- susceptible

In this study, the antimicrobial resistance profile of extended-spectrum beta-lactamase-producing isolates of *E. coli* &*Klebsiella* species was carried out. Both *E. coli* and *Klebsiella* species demonstrated lower rates of resistance to meropenem. This is in line with a study conducted in Malaysia (Mahdi Yahya Mohsen *et al*., 2016),South Korea (Cho *et al*., 2015), Iran (Gharavi *etal*., 2021),Saudi Arabia (Almana *et al.*, 2005), Israel (Aila *et al*., 2023) and Uganda (Najjuka *etal*., 2016). However, both organisms showed 100% resistance to first to third-generation cephalosporins and very high resistance to trimethoprim/sulfamethoxazole, amoxicillin/clavulanic acid, and ciprofloxacin (Tables4&5).

This finding is consistent with the findings from Nigeria (Mofolorunsho *et al*., 2021), and Uganda (Najjuka *et al*., 2016).In our study, both organisms showed lower resistance rates to chloramphenicol which agrees with findings from the study in Uganda (Najjuka *et al*., 2016). However disagrees with a retrospective review from 11 Canadian hospitals which reported

lower resistance rates of ESBL-producing *E.coli* and *K.pneumoniae* to ciprofloxacin and trimethoprim/sulfamethoxazole(Lowe *et al*., 2012).

The observed difference might be associated with the retrospective nature of the compared study, the specific strain of bacteria and the local prevalence of antibiotic resistance, the geographic location, and local prescribing practices.

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study shows that the prevalence of ESBL fecal carriage is 51% among pregnant women attending antenatal cate at government health centers in Addis Ababa. *E. coli* was the predominant ESBL producing isolate. Gestation age, use of water, hand washing trend after toilet visits, type of toilet, and usual food source were significantly associated ($p<0.05$) with ESBL fecal carriage among pregnant women. From these gestation age (aOR= 0.648, 95%CI 0.398-1.057) and hand washing trend after toilet visit (aOR= 2.219, 95% CI 1.385-3.554) were identified as independent factors associated with ESBL fecal carriage among pregnant women. ESBL-producing *E. coli* and *Klebsiella* species demonstrated 100% resistance to cephalosporins (cefazolin, cefuroxime, and ceftriaxone) and aztreonam. Both ESBL isolates were very sensitive to meropenem.

5.2 Recommendations

To reduce the burden of ESBL fecal colonization among pregnant women in Ethiopia, there should be a need for increased surveillance and monitoring for ESBL-resistant colonization among pregnant women. Additionally, promoting appropriate use of antibiotics and improved infection prevention and control measures in healthcare facilities including frequent hand washing and proper disposal of contaminated materials can help to reduce the spread of these bacteria. Improved sanitation and hygiene practices in the community can also be effective in preventing fecal colonization with ESBL-producing bacteria.

Molecular analysis for the ESBL-producing *E. coli* and *Klebsiella* species should be conducted for further identification of ESBL carrier genes among other groups with larger sample sizes.

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Appendices

Appendix 1

Participant Information Sheet

The study entitled **"Antimicrobial susceptibility profile and associated factors with fecal carriage of extended spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella* species among pregnant women attending antenatal care at government health centers in Addis Ababa, Ethiopia"** aims to study the major risk factors associated with ESBL producing *E. coli* and *Klebsiella* species among pregnant women, determine the magnitude and characterize ESBL producing *E. coli* and *Klebsiella* species. Being a participant in this study will require fresh stool samples during your ANC visit and all the laboratory results would be kept confidential. Samples will be collected individually by self in the government health center restroom. Appropriate instructions will be given by health professionals at the health centers.

Appendix 2

Consent form

PIN ----------

Name of study participant ---------- Age---------- Sex----------

Researcher Name---------- Site ----------

I have been informed about a study that plans to investigate the magnitude and factors associated with fecal carriage of ESBL-producing *E. coli* and *Klebsiella* species among pregnant women in public health centers in Addis Ababa, Ethiopia entitled **"Antimicrobial susceptibility profile and associated factors with fecal carriage of extended spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella* species among pregnant women attending antenatal care at government health centers in Addis Ababa, Ethiopia".**

For the study, I have been requested to give fresh stool samples with a sterilized stool cup. Based on this, I have agreed to continue the examination. The investigator also informed me that if I want all the laboratory results would be kept confidential.

I have been given enough time to think over it before I signed this informed consent. It is, therefore, with a full understanding of the situation that I gave my informed consent and cooperates at my will in the course of the conduct of the study.

Name (participant) ----------Signature ----------Date ----------

Name (investigator) ----------Signature ----------Date ----------

Name (witness) ----------Signature ----------Date ----------

Appendix 3

Questionnaire

I am conducting a research study on the magnitude of fecal carriage by Extended-Spectrum Beta-Lactamase (ESBL) producers *E. coli* and *Klebsiella* species. Your participation in this research project is entirely voluntary and your responses will be kept confidential. The survey questionnaire is composed of 14 questions. Thank you for your time and effort.

Please do not leave any questions unanswered.

1. Age_____
2. Address, Sub-City_____
3. Occupation_____
4. Number of family members_____
5. Gestation age_____
6. Former pregnancy Yes/No
7. Comorbidities_____
8. Current medications if any_____
9. Longer hospitalization in a lifetime _____
10. History of self-prescribed antibiotics_____
11. Use of treated/untreated water_____
12. Hand washing trend after toilet visit Yes/No
13. Usual food source home/restaurant
14. Type of toilet WC/ hole

መጠይቅ

በሰገራውስጥበሚገኙኤክስቴንድድስፔክትረምቤታላክታሜስበሚያመርቱኢኮላይእናክሌብሴላኒሞኔስር ጭትላይጥናታዊምርምርእያደረኩእገኛለሁ። ለዚህም ጥናት የእርሶ ተሳትፎ ያስፈልገኛል። በዚህ መጠይቅ ላይ የሚሰጡት ምላሽ ፍፁም ሚሥጥራዊ ነው። መጠይቁ በአጠቃላይ 14 ጥያቄዎችን ይዟል። ስለሰጡኝ ጊዜ እና አገልግሎት በቅድሚያ አመሰግናለሁ።

1. ዕድሜ_____
2. አድራሻ/ ክፍለከተማ_____
3. የተሰማሩበት ሥራ_____
4. የቤተሰብአባላት ቁጥር_____
5. የፅንሡ ዕድሜ_____
6. ከዚህ በፊት የተከሰተ እርግዝና አለ? አዎ/አይ
7. ተጓዳኝ ህመሞች_____
8. አሁን ላይ ያለ ሕክምና_____
9. ረዘም ያለ የሆስፒታል ቆይታ አለ? አዎ/አይ
10. ከሐኪም ትዕዛዝ ውጪ የፀረ-ተዋህሲ መድሃኒት ወስደው ያውቃሉ? አዎ/ አይ
11. የሚጠቀሙት ውሃ የታከመ/ያልታከመ
12. መፀዳጃ ከተጠቀሙ በኋላ እጅ የመታጠብ ልምድ አለ?አዎ/አይ
13. አዘወትረው ከየት ይመገባሉ? በቤት የተሰራ/የሬስቶራንት
14. ምን አይነት መፀዳጃ ይጠቀማሉ? የጉድጓድ/ባለመቀመጫ

Appendix 4

Antimicrobial susceptibility testing procedural steps

1. Touch the top of at least 4 to 5 well-isolated colonies.
2. Transfer the growth to the tube of Normal saline
3. Emulsify the inoculum on the inside of the tube to avoid clumping of the cells.
4. Adjust the inoculum standard to a 0.5 McFarland
5. Compare turbidity to that in the 0.5 McFarland and standard using a Nephelometer or paper with black lines or use a photometric device. Adjust the turbidity of the inoculum to match that standard.
6. Visually examine the Mueller Hinton agar plates before use or other media according to the organism, organism, ensure plates are: free of visible contamination, poured to a uniform depth of approximately 4mm, not excessively wet, not cracked or dry.
7. Dip a sterile cotton swab into the inoculum. Rotate the swab several times and press firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab.
8. Streak the swab over the entire surface of the Mueller Hinton agar plate or other media according to the organism.
9. Rotate the plate approximately 60 o then repeat the streaking motion.(3x)
10. Complete inoculation by running the swab around the rim of the agar.
11. Allow any excess moisture on the agar surface to be absorbed before applying the antimicrobial disks.
12. Dispense disks to the agar surface with sterile forceps (Disk dispenser) or E- test /MIC strip. As per the CLSI guideline
13. After application, ensure that the disk E-test /MIC strip has made complete contact with the agar surface by touching the top of the disk with forceps.
14. Incubate plates inverted at 36±1 ^{o} C for 16 to 18 hours in ambient air/ under 5% CO 2 depending on the air requirement.
15. After incubation with appropriate time, check that the growth is a confluent lawn.
16. Check that zones are round; not oval, deformed, or have jagged edges.

NB: Sometimes when disks are placed closely together, the interaction between antimicrobials may produce distortion of inhibition zones

17. Measure the diameter of inhibition zones. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye (Naked eye)
18. If no inhibition is present (confluent growth is present up to the border of the disk), the diameter of the disk should be recorded (6mm)
19. For interpretation of results refer to CLSI M100 guideline

Procedural Note

- Avoid using over-heated forceps; Do not relocate any disks after contact with the agar.
- These assays are highly sensitive to variations in inoculum density, media formulation, media pH, and incubation conditions.
- In addition, agar diffusion methods are strongly influenced by agar depth, The diffusion rate of the antimicrobial agent, and growth rate of the specific bacteria.
- Filter paper disks impregnated with antimicrobial agents are placed on the agar.

Appendix 5

Standard Operating Procedure for Culture Media Reading, Interpreting, and Reporting

Note:

- Read all cultures daily, ideally early in the morning, and always consider the inoculation time.
- Ensure that all reagents and supplies are available on the workbench before starting.
- Record and initial all observations and workup on the specimen worksheet.
- Day 1 (after overnight incubation)

Step Action

1. Remove culture plates from the incubator.
2. Arrange cultures in numerical order.
3. Read cultures in numerical order. Read new cultures (24 hours) before old cultures (48- 72-hour).
4. Perform an initial examination of growth on each media type. Record observations on the culture worksheet.
5. Set up definitive identification tests (e.g., Biochemical tests) and
6. Antimicrobial susceptibility testing (AST), when appropriate from a pure culture. If there is insufficient growth for testing, sub-culture colony to plated media (label plate as purity plate or pp). Record workup on the worksheet.
7. Re-incubate all primary and subculture plates for an additional 24 or 48 hours, as appropriate.

Day 2 and succeeding days:

8. Follow-up identification and susceptibility testing results until all relevant isolates

have been identified. Record all results on the worksheet.

9. Freeze isolates at minus 80 ^{C}.

Appendix 6

Media Preparation General Guidelines

- Presently, a wide range of culture media are available commercially in the form of dehydrated media. These media are simply reconstituted by weighing the required quantities and by adding distilled water, as per the manufacturer's instructions.
- When preparing media from dehydrated materials, the manufacturer's instructions should be followed closely, as indicated on the label of the dehydrated media powder.
- Clean glassware and distilled or demineralized water should always be used unless specified otherwise. Containers of dehydrated media used to prepare in-house should be labeled with the date of receipt date and the date first opened. Below are the steps to follow when preparing media:

Rehydration

- Weigh out the appropriate amount of dehydrated medium quickly and accurately. Avoid creating dust. Do not inhale the powder. Close the container as soon as possible to prevent contamination.
- Add half of the required volume of water in the flask (preferably an Erlenmeyer flask that is at least 2-3× the volume of medium), followed by the weighed quantity of powdered media.
- Agitate briskly for a few minutes until a homogenous suspension is obtained.
- Pour the rest of the distilled water down the sides of the vessel to wash any adherent medium back into the solution.

Sterilization:

- The recommended steam sterilization cycle is 15 minutes at 121°C for 1 liter or less.
- After sterilization, remove the media from the autoclave as soon as the pressure has fallen to zero.
- Hastening the opening of the autoclave before zero pressure is reached can result in the ejection of media from the sterilization vessels with considerable loss of contents

Adding Enrichments and Supplements

- Sterile blood used for the preparation of blood agar should be fresh and stored at 2 - 8°C.
- Warmblood in an incubator (35 - 37°C) before adding it to a sterile medium.
- Adequate mixing in a large head-space vessel is essential to ensure aeration of the blood.
- Poorly oxygenated blood plates are purplish in color whereas properly aerated blood agar is cherry-red.

Dispensing of Prepared Media

- Condensation is minimized if agar is cooled to 50°C before pouring (preferably in a water bath). Agar media should be dispensed into 15 x 100-mm or 15 x 150-mm Petri dishes to a uniform depth of 3–4 mm; approximately 20 ml of liquid agar medium will achieve this depth in a 15 x 100-mm plate.
- Ensure gentle mixing during dispensing and dispense quickly
- After pouring, the plates should be kept at room temperature for several hours to prevent excess condensation from forming on the covers of the dishes.
- Another means by which condensation will be reduced is if plates are stacked so that they cool more slowly.

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City Government of Addis Ababa Health Bureau

Ref.N.o. AAHB/12354/227
Date 19/7/2014

TO:

- NEFAS SILK LAFTO SUB-CITY HEALTH OFFICE
- KIRKOS SUB-CITY HEALTH OFFICE
- YEKA SUB-CITY HEALTH OFFICE
- LIDETA SUB-CITY HEALTH OFFICE
- ARADA SUB-CITY HEALTH OFFICE
- ADDIS KETEMA SUB-CITY HEALTH OFFICE
- BOLE SUB-CITY HEALTH OFFICE
- AKAKI KALITY SUB-CITY HEALTH OFFICE
- LEMI KURA SUB-CITY HEALTH OFFICE
- GULELLE SUB-CITY HEALTH OFFICE
- KOLFE KERANYO SUB-CITY HEALTH OFFICE

Subject: Request to access Facilities to conduct approved research

This letter is to support **Yordanos Kefyalew Tesfaye** conduct research which is entitled as "FACTORS ASSOCIATED WITH FECAL COLONIZATION OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE AT GOVERNMENT HEALTH CENTERS IN ADDIS ABABA, ETHIOPIA." The study proposal was duly reLviewed and approved by Addis Ababa Health Bureau procedures and submit an activity progress report to the Ethical Committee as required. Therefore we request the facility and staffs to provide support to the principal investigator.

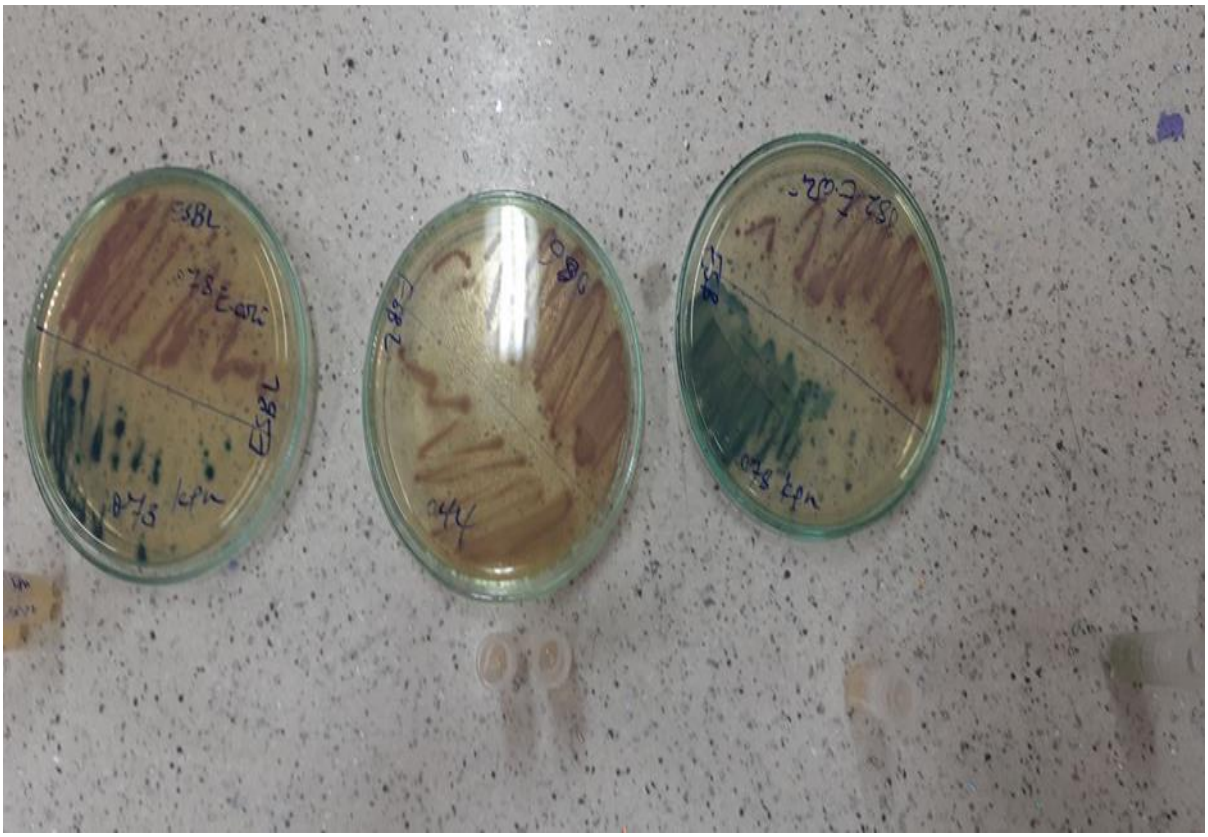
With Regards

Ethical Clearance Committee

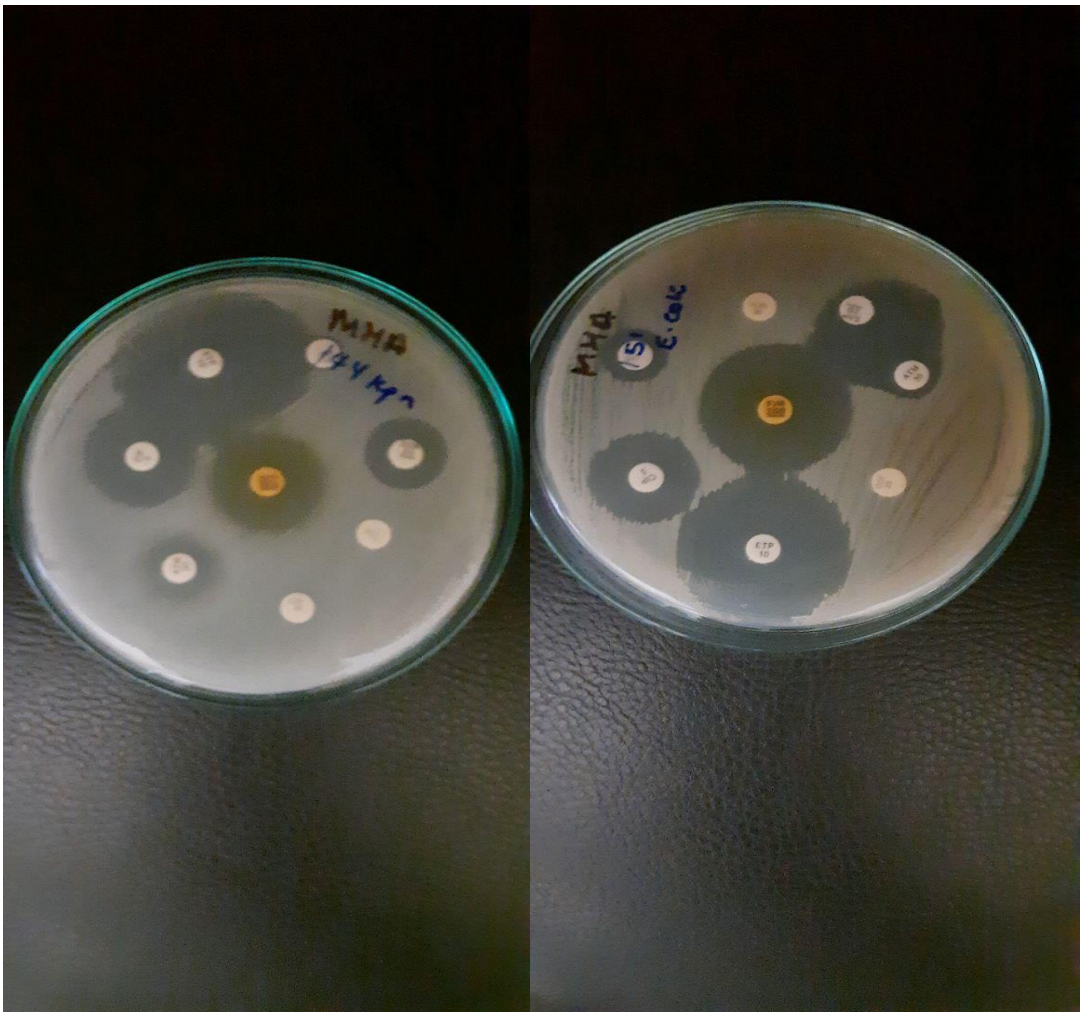
Cc

- Yordanos Kefyalew Tesfaye
- To Ethical Clearance Committee

Appendix 7



Picture of ESBL producing *E. coli* and *Klebsiella* species isolates from pregnant women's stool. (Picture taken by Yordanos Kefyalew, EPHI, Addis Ababa, Ethiopia (26/04/2022GC).



Pictures of antimicrobial susceptibility test of ESBL producing *Klebsiella* species and*E. coli* on Muller Hinton agar respectively(Picture taken by Yordanos Kefyalew, EPHI, Addis Ababa, Ethiopia (29/05/2022GC).