

**MOLECULAR CHARACTERIZATION OF EXTENDED-
SPECTRUM BETA-LACTAMASE-PRODUCING
ENTEROBACTERIACEAE FROM STOOL OF PATIENTS
WITH GASTROINTESTINAL INFECTIONS AT KISII
TEACHING AND REFERRAL HOSPITAL**

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**Molecular Characterization of Extended-Spectrum Beta-Lactamase-
Producing *Enterobacteriaceae* from Stool of Patients with
Gastrointestinal Infections at Kisii Teaching and Referral Hospital**

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**A Thesis Submitted in Partial Fulfillment of the Requirements for the
Degree of Master of Science in Medical Microbiology of the Jomo
Kenyatta University of Agriculture and Technology**

2026

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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DEDICATION

I dedicate this work to my best friend, Eden Humus, whose unwavering support, encouragement, and inspiration have been instrumental throughout this journey.

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ACRONYMS AND ABBREVIATIONS

AMC	Amoxicillin-clavulanate
AMP	Ampicillin
AMR	Antimicrobial resistance
AST	Antimicrobial Susceptibility Testing
ATCC	American Type Culture Collection
AWaRe	Access, Watch, Reserve (WHO classification of antibiotics)
AZM	Azithromycin (mentioned in timeline figure)
CAZ	Ceftazidime
CHL	Chloramphenicol
CIP	Ciprofloxacin
CLSI	Clinical Laboratory Standards Institute
CRE	Carbapenem Resistant <i>Enterobacteriaceae</i>
CRO	Ceftriaxone
CTX	Cefotaxime
DNA	Deoxyribonucleic acid
ESBL	Extended-spectrum beta-lactamase
FEP	Cefepime
GIT	Gastrointestinal Tract
GLASS	Global Antimicrobial Resistance and Use Surveillance System

IPM	Imipenem
IPR	Intellectual Property Rights
ISERC	Institutional Scientific and Research Ethics Committee
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KAN	Kanamycin
KEMRI	Kenya Medical Research Institute
KONSERVE-AB	Kenyan (Kisii) One Health Research in Environment, Veterinary and human Antibiotic
KTRH	Kisii Teaching and Referral Hospital
LF	Lactose Fermenter
MDR	Multi-drug resistant
MDDST	Modified Double Disc Synergy Test
MGE	Mobile genetic elements
MHA	Mueller Hinton Agar
MR	Methyl Red
MU	Moi University
NACOSTI	National Commission for Science, Technology, and Innovation
NAP	National Action Plan
NLF	Non-Lactose Fermenter
OPD	Outpatient department
PCR	Polymerase Chain Reaction

SDG	Sustainable Development Goals
SERU	Scientific Ethics Review Unit
SSA	Sub-Saharan Africa
SXT	Sulphamethoxazole-trimethoprim
TSI	Triple Sugar Iron
TZP	Piperacillin-tazobactam
VP	Voges–Proskauer
WGS	Whole Genome Sequencing
WHO	World Health Organization
XDR	Extensively drug-resistant

ABSTRACT

Antimicrobial resistance (AMR) is a significant global health challenge, with Extended-Spectrum Beta-Lactamase (ESBL)-producing *Enterobacteriaceae* contributing notably to multidrug-resistant infections. ESBLs were prioritized in this study given their central role in beta-lactam resistance and their disproportionate contribution to treatment failure in resource-limited settings, where third-generation cephalosporins remain a mainstay of empirical therapy. Despite this burden, no prior study had characterized the prevalence, susceptibility patterns, or molecular epidemiology of ESBL-producing *Enterobacteriaceae* at Kisii Teaching and Referral Hospital (KTRH) or within Kisii County more broadly. This study assessed the prevalence, antimicrobial susceptibility patterns, and molecular characteristics of ESBL-producing *Enterobacteriaceae* isolated from stool samples of patients with gastrointestinal infections at KTRH, southwestern Kenya. In this cross-sectional study, stool specimens were collected from 200 patients presenting with symptoms of gastrointestinal infection, including diarrhoea, fever, vomiting, and mucoid/bloody stool, and cultured to identify bacterial isolates. Antimicrobial susceptibility was determined using the Kirby-Bauer disk diffusion method, with phenotypic ESBL confirmation via the Modified Double-Disk Synergy Test (MDDST) and molecular detection of *bla*TEM, *bla*SHV, and *bla*CTX-M variants by PCR. Associations between ESBL phenotype and participant characteristics were assessed using Chi-square and Fisher's exact tests, with significance set at $p < 0.05$. A total of 187 isolates were recovered from the 200 samples analyzed, predominantly *Escherichia coli* ($n=139/187$, 74.3%), followed by *Salmonella* spp. ($n=23/187$, 12.3%), *Klebsiella* spp. ($n=16/187$, 8.6%), and *Shigella* spp. ($n=9/187$, 4.8%). Resistance was highest to Ampicillin (74.9%), Piperacillin-tazobactam (73.3%), and Amoxicillin-clavulanate (54.5%), and comparatively lower to Imipenem (25.7%) and Amikacin (24.6%). No participant characteristic, including age, sex, occupation, or water source, was significantly associated with ESBL phenotype (all $p > 0.05$), though occupation approached significance ($p=0.083$). Of the 187 isolates, 84 (44.9%) were confirmed ESBL producers by MDDST. Molecular analysis revealed *bla*TEM (63.1%, $n=53/84$) and *bla*CTX-M-15 (48.8%, $n=41/84$) as the most prevalent genes, followed by *bla*SHV (38.1%), *bla*CTX-M-8 (28.6%), and *bla*CTX-M-9 (9.5%), with prevalence differing significantly across genes ($\chi^2=36.75$, $p < 0.001$). Eighteen distinct co-carriage patterns were identified, and *bla*TEM and *bla*CTX-M-15 co-occurred significantly more often than expected by chance (OR=8.10, $p < 0.001$), consistent with shared carriage on mobile genetic elements. These findings demonstrate a substantial, genetically diverse ESBL burden at KTRH, broadly distributed across the patient population rather than confined to an identifiable risk group, underscoring the need for heightened AMR surveillance, strengthened antimicrobial stewardship, and targeted infection control in resource-limited settings.

CHAPTER ONE

INTRODUCTION

1.1 Introduction

This chapter discusses the background information for the study. It also discusses the genetic basis for the emergence of resistance and the transmission of antimicrobial resistance genes between animals, the environment, and humans. Since gastrointestinal infections are known to be a significant niche for the development of resistance, this chapter emphasises the need to understand the prevalence of resistant enteric bacteria and the genetic basis behind such resistance. The chapter thus points out the problem that exists and discusses the objectives and justification for this study.

1.2 Background Information

Enterobacteriaceae are a large family of Gram-negative bacteria that reside in the gastrointestinal tract (GIT) of humans and animals as part of the normal flora, although some members are naturally pathogenic. As opportunistic pathogens, *Enterobacteriaceae* cause both intestinal and extraintestinal disease, including diarrhoea, urinary tract infections, pneumonia, and sepsis. Naturally pathogenic members of this family, such as *Salmonella* spp. and *Shigella* spp., are major global causes of morbidity and mortality. *Enterobacteriaceae* pose a rising public health threat, as increasing antimicrobial resistance (AMR) exacerbates the morbidity, mortality, and cost of treating otherwise common infections (Kluytmans-van den Bergh et al., 2022). An estimated 1.27 million deaths annually are now directly attributable to resistant hospital-acquired *Enterobacteriaceae*, particularly *Escherichia coli* and *Klebsiella pneumoniae* (Murray et al., 2022), with resistant bacterial infections estimated to account for a substantial proportion of the broader global burden of disease attributable to AMR (Cassini et al., 2019). In Africa and Kenya specifically, high rates of multidrug-resistant infection are

driven by Extended-Spectrum Beta-Lactamase (ESBL)-producing organisms, which pose a specific threat to effective therapy (Maina et al., 2017; Omulo et al., 2021), with *E. coli* and *K. pneumoniae* consistently identified among the leading AMR pathogens of clinical concern in Kenya (Maina et al., 2021). Diarrhoeal illness remains a leading cause of childhood death in Kenya (Birhan et al., 2023), though limited surveillance likely underestimates the true burden of *Enterobacteriaceae*-related GIT disease (Paulson et al., 2021), a pattern consistent with findings from other resource-limited settings where enteric antimicrobial resistance among children remains under-characterized (Moharana et al., 2019). In remote referral facilities such as Kisii Teaching and Referral Hospital (KTRH), which serve large populations, no studies have documented the prevalence, susceptibility profiles, or molecular characteristics of local clinical isolates, hindering informed treatment and containment decisions (van Boeckel et al., 2019).

ESBLs are enzymes produced by Gram-negative bacteria, particularly members of *Enterobacteriaceae*, that confer resistance to most beta-lactam antibiotics, including penicillins and cephalosporins (Lob et al., 2016), rendering many first-line drugs ineffective against common *Enterobacteriaceae* infections. The World Health Organization (WHO, 2017) designated ESBL- and carbapenemase-producing *Enterobacteriaceae* as critical priorities for new antibiotic development, given dwindling treatment options as resistance emerged among clinical isolates. Over a third of *E. coli* and over half of *K. pneumoniae* hospital infections in Africa exhibit ESBL-mediated resistance to third-generation cephalosporins (Lester et al., 2020), with a continent-wide pooled prevalence of ESBL-producing Enterobacterales estimated at 28% (95% CI: 25–31%) across clinical infections, and 29% specifically within East Africa (Abay et al., 2025), consistent with WHO African-region estimates attributing a substantial share of the regional AMR mortality burden to resistant Enterobacterales (Sartorius et al., 2023). Regional and Kenyan studies mirror this trend, with ESBL production among *Enterobacteriaceae* reaching up to 67% (Mutua et al., 2023; Maina et al., 2017), and Kenya specifically ranking among the highest-burden countries in sub-Saharan Africa for

ESBL-producing *K. pneumoniae*, at approximately 23% (Olaitan et al., 2025). The rapid intra- and interspecies spread of resistance genes such as *bla*CTX-M between pathogens and commensals, frequently carried on conjugative plasmids with high horizontal transmission potential (Yartey et al., 2025), facilitates this dissemination (Onduru et al., 2021).

As van Boeckel et al. (2019) noted, despite this clinical severity, local molecular epidemiology data remain scarce, particularly from Kenyan referral facilities such as KTRH. Several studies in Kenya and the wider East African region have nonetheless attempted to investigate ESBL prevalence and the specific genes mediating this resistance. In Uganda, Ampaire et al. (2017) found that 35 of 261 clinical isolates tested (13.4%) were ESBL producers, with *E. coli* predominating [18 (51.4%)], followed by *K. pneumoniae* [10 (28.5%)], *Proteus mirabilis* [4 (11.4%)], *Enterobacter* sp. [2 (5.7%)], and *Acinetobacter baumannii* [1 (2.8%)]. In a meta-analysis of ESBL-producing *Enterobacteriaceae* in Ethiopia, Kiros et al. (2021) reported a pooled prevalence of 18%, with *E. coli* and *Klebsiella* spp. accounting for half of all isolates identified; a more recent Ethiopian meta-analysis similarly estimated ESBL colonization at 28.5% (95% CI: 16.4–40.5%), alongside a 4.4% carbapenemase-producer colonization rate (Tigabie et al., 2025). In Kenya, Henson et al. (2017) found that more than half of isolates examined were ESBL-positive, encoding CTX-M-15, SHV-2, SHV-12, and SHV-27, with 79% classified as multidrug-resistant (MDR), conferring resistance to at least three antimicrobial classes. Edwards et al. (2023) further reported 89% ESBL colonization among GIT *Enterobacteriaceae*, with a median time to colonization of 7 days, and the majority of genes detected belonging to the CTX-M-1 (36/38; 95%) and CTX-M-9 (2/36; 5%) groups. Notably, none of these studies collected specimens from southwestern Kenya.

The GIT represents the largest microbial biosphere in the body (Biohaz et al., 2021) and is considered a critical ecological niche for the development of AMR, given the broad diversity of interacting bacterial species it hosts in both humans and animals (Biohaz et al., 2021). Oral antibiotic use is believed to significantly promote AMR emergence within

this niche (Apisarnthanarak et al., 2020), with *Enterobacteriaceae*, as the most common GIT residents in both humans and animals, disproportionately affected (Maina et al., 2017; Aklilu et al., 2020). A parallel pattern of resistance is observed in animals, driven by increasing antibiotic use for prophylaxis and treatment to improve yields (Van et al., 2020), raising concern that resistance emerging in animals may spread to humans via mobile genetic elements (MGEs) (Biohaz et al., 2021). This underscores the need to proactively characterize the prevalence and molecular profile of resistant *Enterobacteriaceae* to inform an effective response.

ESBL production in *Enterobacteriaceae* is primarily mediated by *bla*CTX-M, *bla*TEM, *bla*SHV, and *bla*OXA genes, characterized extensively across the region (Ghanavati et al., 2017; Ndukui et al., 2022). The globally disseminated CTX-M subtype appears most prevalent in Kenya, conferring severe resistance profiles (Sonda et al., 2016; Onduru et al., 2021). Characterizing circulating genotypes is essential, as these genes transfer between bacterial strains and species via MGEs, accelerating resistance spread (Onduru et al., 2021). Despite this clinical severity, molecular epidemiology data remained limited from county referral hospitals such as KTRH, which serve millions of patients (van Boeckel et al., 2019). Without facility-specific evidence delineating prevalence and genotype, appropriate empirical therapy, containment interventions, and regionally targeted antibiotic development could not be effectively implemented. This study therefore determined the prevalence, phenotypic resistance, and prevailing ESBL genotypes among ESBL-producing *Enterobacteriaceae* isolated from stool specimens of patients presenting with gastrointestinal infections at KTRH, using antimicrobial susceptibility testing, ESBL screening, and PCR.

KTRH is a major referral facility serving approximately 5 million people across Kisii, Nyamira, Homa Bay, Migori, and Bomet counties. This 650-bed facility records bed occupancy of approximately 150% and around 200 outpatient visits daily. Essential medicines selection at such a facility should ideally be guided by a data-informed antibiogram, since not all patients requiring antibiotic treatment can await culture and

sensitivity results. However, the facility lacked local data to guide such empiric drug selection, as no recent study had delineated the susceptibility profiles and resistance patterns of *Enterobacteriaceae* isolates there. Surveillance is key to detecting emerging resistance trends, strengthening stewardship programs, and providing evidence to support clinical guideline adjustments around judicious antibiotic use (Laxminarayan et al., 2016; Kluytmans-van den Bergh et al., 2022), with laboratory-generated surveillance data specifically identified as central to effective antimicrobial stewardship (Morency-Potvin et al., 2017) and to the design of AMR surveillance systems appropriate for resource-limited African settings more broadly (Okolie et al., 2022).

This study elucidated the prevalence, susceptibility profiles, and ESBL molecular characteristics of *Enterobacteriaceae* isolated from the stool of patients presenting with gastrointestinal infections at KTRH. The findings offer local data to strengthen resistance containment and empiric therapy selection at KTRH, while contributing to the wider understanding of circulating resistant bacteria in southwestern Kenya. This work also strengthens regional AMR surveillance capacity and supports antimicrobial stewardship programs, informed policy decisions, and improved clinical outcomes. This study was conducted under the framework of the Kenyan (Kisii) One Health Research in Environment, Veterinary and Human Antibiotic (KONSERVE-AB) study, and administered in accordance with the Intellectual Property Rights (IPR) agreements established within the mother project.

1.3 Statement of the Problem

Antimicrobial resistance is a major and growing threat to healthcare delivery in Kenya, with gastrointestinal *Enterobacteriaceae* infections increasingly complicated by ESBL-mediated resistance that undermines the effectiveness of routinely used antibiotics. Existing AMR surveillance in Kenya, however, remains heavily concentrated around Nairobi and national referral facilities, leaving peripheral county hospitals largely unrepresented in the available evidence base. KTRH exemplifies this gap: despite serving

as the principal referral facility for over 5 million people across five counties, no prior study had established the prevalence, antimicrobial susceptibility patterns, or molecular characteristics of ESBL-producing *Enterobacteriaceae* among patients presenting with gastrointestinal infections at the facility. In the absence of this facility-specific evidence, clinicians at KTRH have had little choice but to rely on empirical, non-data-driven antibiotic selection for gastrointestinal infections, an approach that risks poor treatment outcomes and may itself accelerate further resistance. This study therefore addressed the specific problem of the absent local data on the prevalence, susceptibility profiles, and resistance genetics of *Enterobacteriaceae* at KTRH.

1.4 Justification

Resistant bacterial infections are, in part, driven by patients self-medicating with antibiotics to avoid the cost of formal treatment, a practice that further propagates resistance. Robust local data remain central to any evidence-based response to this problem, yet in Kenya, as in many developing countries, such data are lacking or scanty across most peripheral health facilities. At the time of this study, no known research had investigated the prevalence, susceptibility patterns, or ESBL genetics of gastrointestinal *Enterobacteriaceae* isolates at KTRH, a gap that has directly hindered empiric antibiotic selection and stewardship efforts at the facility. Setting-specific surveillance, generating current resistance rates alongside the genes mediating that resistance, is essential both for guiding appropriate patient treatment and for containing further ESBL spread. By characterizing local resistance patterns, this study aimed to contribute the essential prevalence and genetic data needed to inform infection control measures and improve patient care outcomes at KTRH.

This work carries direct implications for AMR management at KTRH and across the wider region it serves. By identifying the prevalence of ESBL-producing *Enterobacteriaceae* and their associated resistance genes, the study generated data suited to tailoring more effective empiric treatment regimens for gastrointestinal infections at the hospital, while

supporting the development of targeted AMR control strategies and strengthened infection control practices on-site. The anticipated longer-term impact of this work is a strengthening of regional AMR surveillance capacity in Kenya, contributing to the evidence base guiding antimicrobial stewardship programs, informing policy decisions, and ultimately improving clinical outcomes by addressing the burden of antimicrobial-resistant infection.

1.5 Research Questions

- i. What is the prevalence of *Enterobacteriaceae* pathogens in stool specimens of patients presenting with gastrointestinal infection symptoms at KTRH?
- ii. What are the antimicrobial susceptibility patterns of *Enterobacteriaceae* isolated from stool specimens of patients presenting with gastrointestinal infection symptoms at KTRH?
- iii. What proportion of the *Enterobacteriaceae* pathogens isolated from stool specimens of patients with gastrointestinal infection symptoms at KTRH are ESBL producers?
- iv. What are the most common ESBL genes detected in *Enterobacteriaceae* pathogens isolated from stool specimens of patients with gastrointestinal infection symptoms at KTRH?

1.6 Study Objectives

1.6.1 Broad Objective

To determine the molecular characteristics of ESBL-producing *Enterobacteriaceae* isolated from stool samples of patients with gastrointestinal infections at Kisii Teaching and Referral Hospital.

1.6.2 Specific Objectives

- i. To estimate the prevalence of *Enterobacteriaceae* isolated from stool specimens of patients presenting with symptoms of gastrointestinal infection at KTRH.
- ii. To assess the antimicrobial susceptibility patterns of *Enterobacteriaceae* isolated from stool specimens of patients presenting with symptoms of gastrointestinal infection at KTRH.
- iii. To determine the proportion of ESBL producers among *Enterobacteriaceae* pathogens isolated from stool specimens of patients presenting with symptoms of gastrointestinal infection at KTRH.
- iv. To characterize the molecular profile of common ESBL genes in *Enterobacteriaceae* pathogens isolated from stool specimens of patients presenting with symptoms of gastrointestinal infection at KTRH.

1.7 Thesis Scope

This thesis focuses on an investigation of the molecular characteristics of ESBL-producing *Enterobacteriaceae* isolated from the stool of patients diagnosed with gastrointestinal infections at KTRH. The study specifically focused on identifying the prevalence of these bacteria, their antimicrobial resistance profiles, and the molecular characteristics of the ESBL genes (*blaCTX-M*, *blaTEM*, and *blaSHV*) they harbored. The research was conducted over the period from June to August 2024, with stool specimens collected from patients presenting with gastrointestinal symptoms. The study utilized culture methods, antimicrobial susceptibility testing, and molecular techniques such as PCR to analyze the collected specimens. The findings contributed valuable data to the understanding of local antimicrobial resistance patterns, particularly regarding *Enterobacteriaceae* in Kisii County, and further support efforts to formulate local antibiograms at the facility to serve the population it offers services to, including residents of Kisii County and surrounding counties. This thesis is organized in six chapters: Introduction, literature review, methodology, results, discussion, and conclusions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter is a comprehensive review of published literature on Enterobacteriaceae infections, especially those of the gastrointestinal tract (GIT), and the occurrence of antimicrobial resistance (AMR) among members of the Enterobacteriaceae family. Focus is placed on the clinical burden of GIT infections by Enterobacteriaceae, the prevalence of resistance, especially Extended-Spectrum Beta-Lactamase (ESBL) production, and the molecular determinants of AMR among these bacteria. Each section below builds progressively from the global burden of the problem, through its regional and Kenyan manifestation, down to the specific evidence gap this study addresses at Kisii Teaching and Referral Hospital (KTRH). This structure is intended to make explicit, at every stage, how the reviewed literature connects to this study's specific objectives. This was done to determine the extent of the existing literature and the questions that remain to be answered.

2.2 Antibiotic Discovery

The term "antibiotic" can be traced back to early publications in the late 19th century and is attributed to Paul Viollemin, who used it to describe the antagonistic action observed between different microorganisms, such as bacteria and fungi (Nicolaou & Rigol, 2018). This was in the absence of a clear understanding of the exact mechanisms of action of the antagonism observed. Later, the term was used to describe naturally occurring biological substances that can either inhibit or kill bacteria (Mohr, 2016). The term has, however, evolved over time, especially with the discovery of specific antibiotic extracts from these naturally occurring organisms.

The discovery of antibiotics is one of the most important discoveries of all time in the healthcare field. Before specific antimicrobial compounds were extracted and formulated

as medicine for the treatment of infections, ancient populations relied on plant concoctions and other poorly understood formulations to treat disease (Mohr, 2016). Historical records show that antibiotics have been used since ancient times, when civilisations such as Egypt, Greece, and China applied mouldy bread or plant extracts to infected wounds (Durand et al., 2019). The first documented use of moulds for treatment was by John Parkinson in the 17th century; however, the first specific antibiotic drug was penicillin. Modern-day penicillin was discovered by Alexander Fleming in 1928, and its widespread use revolutionised medicine in the 20th century (Hutchings et al., 2019). From this first antibiotic, several other agents were discovered and manufactured, including the cephalosporins, now considered hospital workhorses, alongside lincosamides, chloramphenicol, macrolides, mupirocin, monobactams, and polymyxins (Figure 2.1). All these have continually gained usage in the clinical setting over the years. According to Hutchings et al. (2019), microorganisms manufacture antibiotics as chemical weapons against competition or as signalling molecules. Figure 2.1, adapted from Hutchings et al. (2019), visualizes the timeline of antibiotic discovery and the emergence of resistance to common antibiotics.

This same biological arms race between antibiotic-producing organisms and their competitors is, as the following sections show, the same fundamental dynamic now playing out at population scale between clinical antibiotic use and the emergence of resistant Enterobacteriaceae, the specific problem this study investigates.

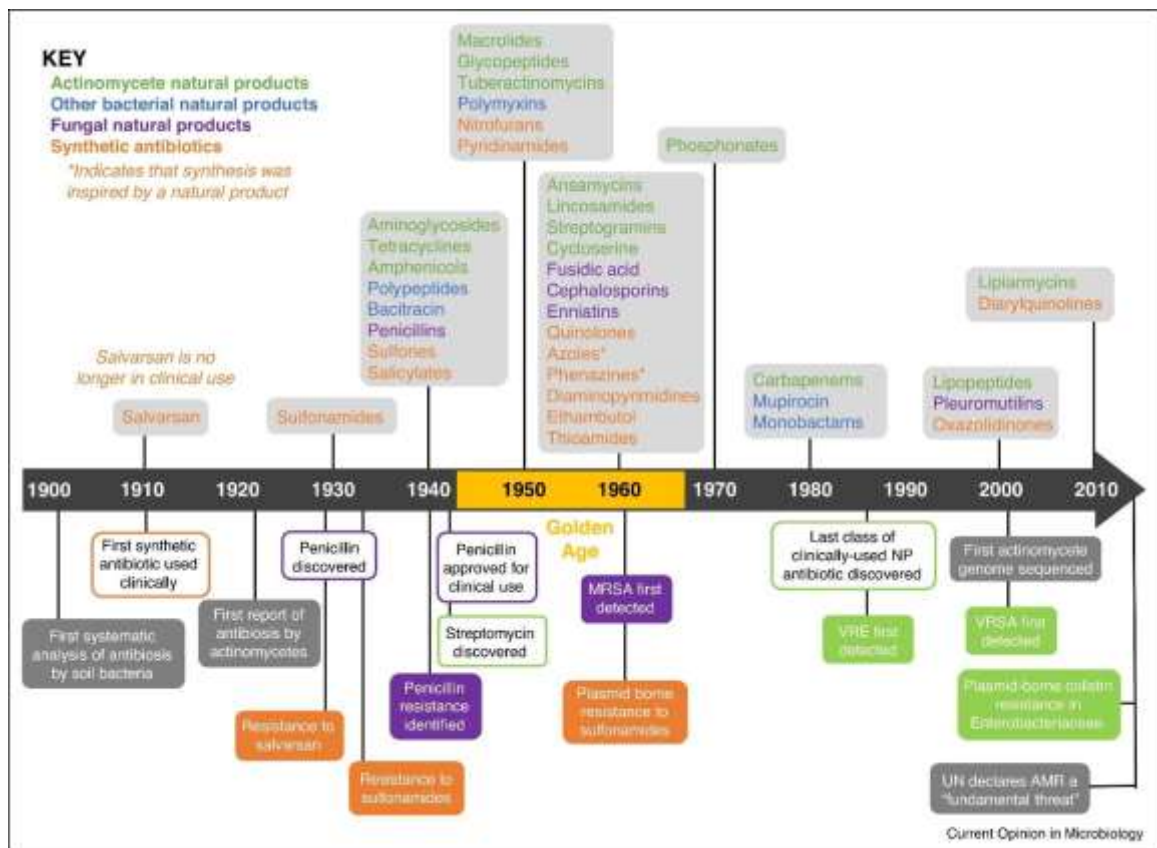


Figure 2.1: Timelines of Discovery of Antibiotics and the Emergence of Resistance

2.3 Need for Judicious Use of Antibiotics

The concern about the rise of highly resistant bacteria causing difficult-to-treat infections is emphasised by Devasahayam et al. (2010). A report by O'Neill (2016) indicated that by 2050, the world could reach an antibiotic-free era characterised by extensive resistance and the total ineffectiveness of conventional treatments. This highlights the urgent need to develop new antibacterial drugs with novel mechanisms of action that could overcome the resistance mechanisms used by bacteria. However, Brown and Wright (2016) note that despite efforts to formulate new antibiotics, none of the candidates explored over the past few decades have merited approval as new antibiotics, meaning no new drugs are available to control infection in the era of resistance. Bueno et al. (2016) further underscore the

critical need to address this issue by calling for new pharmacological strategies to prevent resistance and restore susceptibility in multidrug-resistant strains. Overall, antibiotic resistance trends continue to increase, creating an escalating public health concern, while the discovery of new antibiotic classes has stalled. There is an urgent need to develop innovative approaches to discover novel antibiotics and prevent further development of resistance. In support of this, the WHO (2017) has published a list of ESBL- and carbapenemase-producing Enterobacteriaceae among the bacterial infections for which research and development of new antibiotics are urgently needed.

Antibiotics are valuable resources that must be used judiciously. The fact that many antibiotics are synthetic derivatives of compounds originally produced by microorganisms highlights a key biological paradox: the very organisms from which these therapeutic agents are derived also possess the innate capacity to develop resistance through genetic adaptation and enzyme modification (Hutchings et al., 2019). This necessitates using antibiotics only when clearly justified. Contrary to expectation, however, antibiotic use has continually increased over the years. This may be partly explained by the recent upsurge in infectious diseases, especially those caused by bacteria, or by unjustified use resulting from failure to adhere to antimicrobial stewardship (AMS) guidelines (Blaser et al., 2021; Vaughn et al., 2022). A lack of clear policies on antimicrobial use in many countries has been the primary reason behind the ever-increasing use of antibiotics, most of which are unjustified (Akhtar et al., 2020; Mathibe & Zwane, 2020). Gaffin and Spellberg (2023) analysed antimicrobial use guidelines and observed that it would be possible to limit antimicrobial use through proper policy, even in the community setting.

The overuse of antimicrobial agents, coupled with the inherent genetic features of disease-causing organisms, has been postulated as a primary reason behind the emergence of resistance (Blaser et al., 2021). AMR results from pathogens changing so that they are no longer responsive to medicines to which they were previously sensitive. This makes infections harder to treat and increases the overall risk of spread and severity (World Health Organization, 2011). AMR is a critical global health threat requiring urgent,

multisectoral action to accelerate progress toward the Sustainable Development Goals (SDGs), particularly SDG 3, which aims to ensure good health and well-being for all (United Nations, 2015). While some bacteria possessed intrinsic resistance to certain antibiotics even at the time of their discovery, acquired resistance has expanded markedly, now including pathogens that were previously susceptible. The WHO has noted that AMR levels are rising alarmingly worldwide (World Health Organization, 2014), and projections suggest that without effective intervention, humanity could face a post-antibiotic era by 2050 (O'Neill, 2016). Novel resistance mechanisms are emerging and proliferating worldwide, threatening the ability to treat widespread infectious diseases. As antibiotic medications become less effective, a growing variety of ailments become increasingly difficult, if not impracticable, to treat.

AMR worsens when antibiotics are obtained without prescription for human or animal use (Vaughn et al., 2022). Similarly, in countries without set treatment standards, antibiotics are commonly over-prescribed by physicians and veterinarians, and over-used by the general population (Sulis et al., 2023). There have been minimal to no discoveries of antibiotics in the 21st century, and the looming problem of AMR may soon become a top cause for alarm (Hoffman, 2020; Salam et al., 2023). The WHO has identified an urgent need to develop new antibiotics against three categories of infection. ESBL-producing and carbapenem-resistant Enterobacteriaceae, alongside carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, sit in the critical category requiring the most urgent research and drug development (WHO, 2017).

To address this urgent need, contemporary research has turned toward artificial intelligence (AI)-driven drug discovery as an innovative approach. These methods employ deep learning, graph neural networks, and generative chemistry to navigate expansive chemical libraries, identify synthesizable molecular scaffolds, and predict bacterial permeability and efflux constraints (Vamathevan et al., 2019; Stokes et al., 2020; Jin et al., 2023). This approach has yielded preclinical candidates, including narrow-spectrum agents like abaucin, which target specific critical pathogens such as *Acinetobacter*

baumannii through non-traditional mechanisms designed to minimize resistance selection pressure (Jin et al., 2023). While AI significantly accelerates the initial screening of millions of compounds, reducing costs and time to candidate identification, the transition from promising in silico hits to clinically viable novel antibiotic classes remains a major bottleneck (Stokes et al., 2020; WHO, 2023). Consequently, the pipeline's alignment with the WHO's 2024 Bacterial Priority Pathogens List remains only partial; although AI efforts increasingly focus on critical Gram-negative threats like carbapenem-resistant Enterobacterales, *A. baumannii*, and *P. aeruginosa*, a persistent deficit of innovative agents in advanced development stages remains (WHO, 2023; WHO, 2024). Therefore, while AI offers a strategic response to the innovation gap described by Brown and Wright (2016) by reviving neglected chemical scaffolds and designing efflux-aware compounds, its ultimate success depends on overcoming translational challenges, including data quality and funding for clinical development. For AI-driven discovery to have a durable public health impact, it must be integrated with robust antimicrobial stewardship and infection prevention frameworks, coupling novel agents with rapid diagnostics, stewardship-linked deployment policies, and access models that preserve efficacy (WHO, 2022; WHO, 2024).

At the population level at which such interventions must eventually operate, the burden falls disproportionately on the gastrointestinal tract, where Enterobacteriaceae both reside as commensals and emerge as leading agents of resistant infection. This is the subject of the following section. The reported prevalence of Enterobacteriaceae causing GIT infection specifically within Kenya, and Kisii County in particular, is addressed directly in Section 2.3. This responds more specifically to the identified gap in facility-specific baseline data motivating this study.

2.4 Prevalence of Gastrointestinal Infections Caused by Members of the *Enterobacteriaceae* Family

Gastrointestinal infections caused by Enterobacteriaceae are highly prevalent globally. A systematic analysis by Stanaway et al. (2019) estimated roughly 14.3 million cases of typhoid and paratyphoid fever in 2017, with the highest incidence in low- and middle-income countries. Beyond infections caused by *Salmonella* spp., Stanaway, Parisi et al. (2019) reported an estimated 535,000 cases of non-typhoidal *Salmonella* invasive disease in 2017, with the highest incidence in sub-Saharan Africa and among children younger than five years. In addition, Kann et al. (2020) reported that carriage rates of multidrug-resistant Enterobacteriaceae isolates in the gut remain substantial across regions. Taken together, Enterobacteriaceae pose a major threat as gastrointestinal pathogens, with infection estimates likely underestimated owing to limited surveillance in some developing areas.

Narrowing this global picture to the African and Kenyan context that directly informs this study, Africa and other low- and middle-income regions bear the greatest burden of GIT infection caused by *E. coli*, though a deficiency of data again proves to be a major challenge to surveillance. According to Paulson et al. (2021), diarrhoeal disease is the third leading cause of mortality among children under five years of age globally, with 1 in 10 deaths attributed to diarrhoea in 2019, and the greatest burden borne by children in South Asia and sub-Saharan Africa. One Ethiopian study determined the prevalence of diarrhoeal disease among under-five children to be 29.0% (Birhan et al., 2023). Directly addressing the specific gap in Kenya-level prevalence data for Enterobacteriaceae-associated GIT infection, a Kenyan study examining the prevalence of GIT bacterial infections in children found that diarrhoeagenic *E. coli* (36.4%) was the leading species, followed by *Shigella* spp. (3.2%), *Salmonella* spp. (2.4%), *Campylobacter* spp. (1.6%), *Yersinia* spp. (1.3%), and *Aeromonas* spp. (1.1%) (Webale et al., 2020). Similarly, among children hospitalized for gastroenteritis in Nairobi, Kimathi et al. (2022) reported bacterial pathogens, predominantly diarrhoeagenic *E. coli* and *Salmonella* spp.,

in a substantial proportion of stool cultures, reinforcing that Enterobacteriaceae remain the dominant bacterial cause of paediatric GIT infection within Kenya specifically, not merely across the wider region. Given the similarities in clinical presentation across causative agents, it is often challenging to initiate specific treatment based solely on clinical suspicion (Sell & Dolan, 2018). This high prevalence of diarrhoeal disease, especially in children, necessitates further research into how it may be controlled. With resistance emerging as a major threat to the treatment of GIT infections, research into the determinants of such resistance, and how they may be tackled, remains paramount, as recommended by the WHO (2017).

Globally, GIT infections are reported as the second most common infectious cause of mortality and morbidity, after respiratory tract infections (Kann et al., 2020). These infections, often caused by a wide variety of infectious pathogens in food, water, and the environment, are promoted by factors such as age, community or nosocomial acquisition, a country's development status, and immune status (Zama et al., 2022). They commonly present as acute gastroenteritis with high fever, elevated heart rate, and diarrhoea (Sell & Dolan, 2018). In an analysis of common GIT infections, Kann et al. (2020) observed that up to 41% of GIT infections in Colombia were bacterial. Since diagnostic isolation of causative agents may be delayed by the long turnaround times of microbiology diagnostics, empirical therapy is mostly initiated to save lives. Nonetheless, upon laboratory diagnosis, it has been established that most bacterial GIT infections are reported in adults, whereas viruses are the main cause of infection in children. Common bacterial strains fall within the Enterobacteriaceae family and may include *E. coli*, *Campylobacter* spp., *Clostridioides difficile*, *Salmonella* spp., and *Shigella* spp., among others. The high resistance observed among gastrointestinal bacterial infections makes GIT infections a major threat to patient outcomes (Kariuki et al., 2021).

2.5 Risk Factors Associated with Gastrointestinal Infections

Beyond the pathogen-level epidemiology above, a distinct body of literature has examined the demographic, environmental, and behavioural factors that predispose individuals to GIT infection specifically, which is directly relevant to this study's assessment of participant-level exposure characteristics. Children are believed to be most affected by GIT infections caused by Enterobacteriaceae (Mbuthia, 2019; Rashid et al., 2019). Environmental factors have been documented as correlated with the occurrence of bacterial GIT infections, in addition to other demographic and lifestyle factors. A study in Murang'a County (Mbuthia, 2019) found that children were mainly infected by enterotoxigenic *E. coli*, enteropathogenic *E. coli*, enteroaggregative *E. coli*, *Salmonella* spp., *Shigella* spp., and *Vibrio* spp. While female children were more affected, children under one year of age, those exclusively breastfed, and those of normal birth weight were less likely to be affected by GIT infections (Mbuthia, 2019); however, the use of piped water, storing water in covered containers, and handwashing after toilet use were protective. In a similar study examining GIT bacterial infections among HIV-infected children in Kenya, the overall prevalence of pathogenic *E. coli*, *Shigella* spp., and *Salmonella* spp. was 44 (28.0%), 31 (19.7%), and 0 (0.0%), respectively (Rashid et al., 2019). Food contamination, especially of cow's milk, is a major risk factor for enteric pathogens in Kisumu County (Tsai et al., 2019), whereas poor availability and type of drinking water source are connected to a high occurrence of diarrhoeal infection (Berendes et al., 2023). According to Berendes et al. (2023), household sanitation and human-animal interaction were associated with a higher likelihood of moderate to severe diarrhoeal infection among children, pointing to a potential human-environment-animal transmission pathway.

Beyond the Kenyan context, risk factor evidence from other settings reinforces these same patterns and extends them to the resistance dimension specifically. In Nigeria, Omotade et al. (2023) found that among children under five presenting with acute gastroenteritis, at least one enteric pathogen was detected in 60.6% of cases, with diarrhoeagenic *E. coli* the

most common bacterial cause, and identified household income and nutritional status as significantly correlated with diarrhoeal risk. At the level of resistant organisms specifically, a systematic review and meta-analysis of international travellers found that inflammatory bowel disease (OR 2.1), antibiotic use (OR 2.4), travellers' diarrhoea itself (OR 1.7), and contact with a healthcare system overseas (OR 1.5) were each independently associated with acquisition of multidrug-resistant Enterobacterales, underscoring that the same behavioural and clinical exposures driving GIT infection risk also drive resistant-organism acquisition specifically (Furuya-Kanamori et al., 2020). With this in mind, the potential for resistance genes to spread through a similar human-environment-animal chain cannot be overlooked, as discussed by multiple studies (Baquero et al., 2019; McEwen & Collignon, 2018; Van Boeckel et al., 2019).

2.6 Burden of Antimicrobial Resistance among GIT *Enterobacteriaceae*

Infections caused by members of the Enterobacteriaceae family are proving among the most stubborn to treat, owing to their high prevalence and high rates of AMR. According to epidemiological data, diarrhoeal infections remain a leading cause of global mortality and morbidity, ranking second after pneumonia among causes of childhood death (Suleiman et al., 2022). One analysis of the global burden of these diseases found that up to 300 million cases and about 1.6 million deaths are reported globally, making diarrhoeal disease the fifth leading cause of death in 2016 (Webale et al., 2020). These infections were mainly associated with *E. coli*, *Campylobacter* spp., *Shigella* spp., *Vibrio* spp., and *Salmonella* spp. (Webale et al., 2020). Amidst this very high burden of enteric bacterial infection, it has been established that a significant proportion of Enterobacteriaceae infections are resistant to extended-spectrum antibiotics, such as the cephalosporins. As reported by Lester et al. (2020), resistance to third-generation cephalosporins among bloodstream isolates was recorded at 18.4% for *E. coli* and 54.4% for *Klebsiella* spp. Among urinary tract infections, an ESBL prevalence rate of 20.5% has been established in the United States (Talan et al., 2021). A study in Saudi Arabia found that 60% of Enterobacteriaceae isolates from an intensive care unit were either multidrug-

resistant (MDR) or extensively drug-resistant (XDR) (Alkofide et al., 2020). The WHO has recognised the problem posed by these bacterial species and their high resistance to commonly used antibiotics, highlighting them as focus areas for both antibiotic development and prevention interventions (WHO, 2017). As documented by the WHO, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and various Enterobacteriaceae have developed resistance to many commonly used antibiotics, necessitating research to develop new treatments (WHO, 2017), with specific attention directed toward ESBL-producing and carbapenem-resistant Enterobacteriaceae (CRE).

The threat posed by ESBL-producing Enterobacteriaceae remains the most urgent research focus in clinical microbiology today. A descriptive study in Malaysia found a high prevalence of Enterobacteriaceae infection alongside high resistance among isolates; *Klebsiella pneumoniae* (53.6%) and *E. coli* (40.9%) were the most prevalent pathogens, resistant to most penicillins and cephalosporins, with carbapenem resistance above 99% (Abubakar et al., 2022). These figures are broadly consistent with findings from other studies globally (Piewngam et al., 2019; van den Bunt et al., 2020; Moghnia et al., 2021; Onduru et al., 2021; Romandini et al., 2021).

Within Kenya specifically, the prevalence of ESBL- and CRE-producing organisms has been extensively reported in hospital settings and confirmed as a major public health threat requiring attention. Mutua et al. (2023) reported an infection prevalence of 40.8% among a hospitalised cohort, the majority attributable to ESBL producers (67.3%), predominated by *K. pneumoniae* (45.5%). Within Kenya specifically, the prevalence of ESBL- and CRE-producing organisms has been extensively reported in hospital settings and confirmed as a major public health threat requiring attention. Mutua et al. (2023) reported an infection prevalence of 40.8% among a hospitalised cohort, the majority attributable to ESBL producers (67.3%), predominated by *K. pneumoniae* (45.5%). Similarly, Rwigy et al. (2024) documented a high prevalence of multidrug-resistant Enterobacteriaceae among hospitalised children in Western Kenya, with many isolates carrying multiple beta-lactamase genes simultaneously, while Drobish et al. (2023) reported comparably poor

susceptibility to third- and fourth-generation cephalosporins among Gram-negative clinical isolates in Kenya more broadly. Studies among animals in Kenya have also shown high rates of ESBL, CRE, and fluoroquinolone-resistant *Enterobacteriaceae*, even at the genetic level (Ndukui et al., 2022), a pattern corroborated in poultry-associated *E. coli* isolates by Ndukui et al. (2021), reinforcing the plausibility of a shared human-animal resistance reservoir within Kenyan farming communities. Overall, the problem of ESBL cannot be understated; it is a prevailing issue requiring sustained investment in understanding its extent and designing comprehensive solutions.

There is a high prevalence of AMR among clinical specimens in Kenya more broadly. In one retrospective study, Drobish et al. (2023) found that Gram-negative organisms were less than 50% susceptible to third- and fourth-generation cephalosporins, 67% susceptible to piperacillin-tazobactam, 87% susceptible to amikacin, and 93% susceptible to meropenem. According to Kariuki et al. (2022), the precise burden associated with diarrhoeagenic *E. coli* remains unknown in many countries, including Kenya, though the authors approximated AMR prevalence ranging from 14.7% to 82%. Kariuki et al. (2021) further reported enteric bacterial resistance rates to commonly available antimicrobials, including ampicillin, chloramphenicol, cotrimoxazole, and tetracycline, ranging between 65% and 80%, with 15-20% of recently studied isolates showing reduced susceptibility to fluoroquinolones and emerging CTX-M-15-mediated resistance to extended-spectrum beta-lactams. According to the Institute for Health Metrics and Evaluation (2022), 4.95 million people who died in 2019 had a drug-resistant infection, with AMR directly causing 1.27 million of those deaths; about 1 in 5 occurred among children under five years old. These statistics present AMR as a major public health issue in Kenya.

Having established the substantial burden of both GIT infection and AMR at the global, regional, and Kenyan levels, the following section turns to the specific behavioural and environmental risk factors that drive resistance emergence. This sets the stage for Section 2.6, which addresses the risk factors associated with AMR among *Enterobacteriaceae* and the molecular mechanisms underlying that resistance, which are discussed in Section 2.7.

The analysis of the carriage of genes mediating such resistance determines common genes and informs the selection of genes of interest in this study.

2.7 Risk Factors Associated with Antimicrobial Resistance

Many clinical, environmental, and behavioural risk factors have been implicated in the emergence of AMR in both humans and animals; these risk factors are the primary focus of antimicrobial stewardship programmes. Indiscriminate antimicrobial use without proper diagnostic justification, excessive community use following unrestricted over-the-counter access, and failure to adhere to prescribed regimens have been identified as major drivers of AMR (Schuts et al., 2016). Failure to adhere to stewardship guidelines has also been highlighted as a major issue by the WHO (WHO, 2021). Global antimicrobial stewardship guidelines and AMR action plans published by the WHO continually urge governments to contribute to the fight against AMR by formulating policies that protect existing antibiotics from resistance development, through frameworks termed National Action Plans (NAPs) on AMR (WHO, 2021). Kenya was among the first countries in Africa to develop a NAP, but adherence to it remains questionable to date (Harant, 2022; Wesangula et al., 2020).

Aside from antimicrobial use factors, excessive environmental use has also been shown to be a major issue. The use of antibiotics in human and veterinary medicine, particularly the large-scale use of antibiotics in livestock to promote food animal growth, is thought to contribute to the current trend of emergence and spread of antibiotic-resistant bacteria (Michael et al., 2014; Vidovic & Vidovic, 2020). To better regulate the formation and spread of AMR, numerous Western European nations have banned the use of antibiotics for growth promotion in food animals (Vidovic & Vidovic, 2020). With extensive agricultural antibiotic use, AMR may develop in plants and animals and subsequently spread to humans (Thanner et al., 2016). Nonetheless, overuse in humans remains an equally significant concern.

Having identified the behavioural and environmental drivers of resistance generally, the following section addresses the molecular mechanisms through which this resistance is actually mediated within Enterobacteriaceae. It begins, deliberately, with the narrow-spectrum beta-lactamases from which ESBLs themselves evolved, to properly situate this study's specific molecular focus.

2.8 Molecular Basis and Classification of Beta-Lactamase-Mediated Resistance, and Common Genes Mediating ESBL Resistance

2.8.1 The Beta-Lactamase Phenotype Spectrum

AMR among Enterobacteriaceae is largely mediated at the genomic level, not purely phenotypically, and beta-lactamase enzymes exist along a spectrum of increasing resistance breadth that is important to establish before narrowing to this study's specific focus on ESBLs. The earliest and simplest of these enzymes are the narrow-spectrum penicillinases, exemplified by TEM-1 and SHV-1, which hydrolyse penicillins such as ampicillin but confer little activity against extended-spectrum cephalosporins. Notably, even this apparently simple baseline enzyme is not phenotypically uniform: Matlock et al. (2025), analysing 377 diverse *E. coli* isolates, demonstrated that *bla*TEM-1 exhibits substantial phenotypic heterogeneity depending on the surrounding bacterial phylogenetic background, such that genotype alone poorly predicts the resulting co-amoxiclav resistance phenotype, an important caution against assuming a simple, direct genotype-to-phenotype relationship even for the most well-characterised beta-lactamase genes.

From this narrow-spectrum baseline, point mutations in the TEM and SHV enzymes gave rise to two further, clinically important but comparatively narrower phenotypic categories before the emergence of true ESBLs. Inhibitor-Resistant TEM (IRT) variants carry mutations that reduce susceptibility to beta-lactamase inhibitors such as clavulanic acid, without necessarily extending the enzyme's substrate range to cephalosporins; this is clinically significant because it can produce a phenotype resistant to amoxicillin-

clavulanate despite retaining susceptibility to unprotected cephalosporins, a pattern that can confound phenotypic ESBL screening methods, such as the Modified Double-Disk Synergy Test used in this study, if not properly distinguished. Complex Mutant TEM (CMT) variants combine both the inhibitor-resistance mutations of IRTs and the extended-spectrum substrate mutations characteristic of ESBLs, producing enzymes resistant to both cephalosporins and clavulanate simultaneously, and posing a particular diagnostic challenge for double-disk synergy-based phenotypic detection.

A separate, chromosomally or plasmid-encoded lineage, the AmpC beta-lactamases, further complicates this landscape. Although Enterobacteriaceae may carry chromosomal AmpC genes with limited, inducible expression, plasmid-borne AmpC variants in *E. coli* (e.g., *bla*CMY, *bla*FOX, *bla*DHA, *bla*ACC, *bla*ACT, *bla*MIR, *bla*MOX) are constitutively expressed and clinically detectable on antibiotic susceptibility testing (Tamma et al., 2019). Critically, AmpC enzymes are not inhibited by clavulanic acid, meaning isolates harbouring only an AmpC mechanism will typically test negative on ESBL-specific phenotypic confirmatory tests despite exhibiting cephalosporin resistance, a distinction directly relevant to interpreting any phenotypically ESBL-negative but cephalosporin-resistant isolates identified in this study's own results. Mohamed et al. (2020) reported *bla*DHA as the predominant AmpC gene in Egypt (42.3%), with lower detection of *bla*CIT (14.2%) and *bla*MOX (11.4%), frequent coexistence of multiple AmpC genes in *K. pneumoniae*, and no detection of the *bla*FOX gene. In Kenya, Musila et al. (2021) detected clinically important AmpC genes in *Acinetobacter baumannii* (*bla*ADC), *Enterobacter cloacae* (*bla*DHA, *bla*ACT), and *K. pneumoniae* (*bla*CMY). Additional clinically relevant AmpC variants include *bla*ACC, *bla*EBC, *bla*FOX, *bla*MOX, and *bla*CIT (Richter et al., 2020).

It is against this broader phenotypic backdrop, narrow-spectrum penicillinases, through IRT and CMT variants, to plasmid-mediated AmpC enzymes, that true ESBLs represent the clinically most consequential category: enzymes that have acquired, through further point mutation or horizontal acquisition, the ability to hydrolyse extended-spectrum

(third-generation) cephalosporins while generally remaining inhibitable by clavulanic acid, which is precisely the property exploited by phenotypic ESBL confirmatory testing. This study's exclusive focus on ESBL genes specifically, rather than the full beta-lactamase spectrum outlined above, reflects both this category's disproportionate clinical significance in the region, as the following review of ESBL gene epidemiology demonstrates, and the practical scope constraints of a single MSc thesis; carbapenemase and AmpC gene characterisation were addressed separately within the wider KONSERVE-AB One Health project of which this study forms a part.

2.8.2 Genes Mediating ESBL Resistance

Resistance genes are often carried on mobile genetic elements (MGEs), enabling both vertical transmission across bacterial generations and horizontal transfer between species. Multiple genes are associated with ESBL production. In an animal study, Ndukui et al. (2022) demonstrated that ESBL production in poultry farm isolates was mediated by common ESBL genes, including *bla*TEM, *bla*CTX-M, *bla*OXA, and *bla*SHV, all detectable using conventional PCR; *bla*OXA was the most frequently detected gene (n = 20, 26%), followed by *bla*TEM (n = 16, 21%), predominantly in *E. coli* and *Salmonella* spp., while *Klebsiella* spp. and *Shigella* spp. isolates lacked these genotypes.

Human clinical studies confirm a similar genetic basis for AMR in Enterobacteriaceae isolates causing gastrointestinal infection. Mutua et al. (2023) identified TEM-type (83%), CTX-M-type (59%), and SHV-type (100%) beta-lactamase enzymes as key resistance determinants. Correspondingly, Muraya et al. (2022) reported high carriage rates of *bla*CTX-M (95.6%) and *bla*TEM (95.6%) in *E. coli* isolates, alongside lower detection of *bla*SHV (21.7%). A regional meta-analysis by Onduru et al. (2021) also found CTX-M predominance across most studies (81.5%), with CTX-M-15 the most widely disseminated subtype, a finding echoed by Peirano and Pitout (2019) in their global review of CTX-M epidemiology, which identified CTX-M-15 as the single most geographically

widespread ESBL variant worldwide, underscoring that the gene-level pattern observed regionally in East Africa mirrors a genuinely global epidemiological trend rather than a locally idiosyncratic one. Collectively, the literature identifies *bla*CTX-M, *bla*TEM, *bla*SHV, and *bla*PER among the most reported ESBL-associated resistance genes across human and animal studies (Ghanavati et al., 2017; Mohamed et al., 2020; Ndukui et al., 2022; Mutua et al., 2023).

Molecular epidemiology studies in Kenya consistently highlight CTX-M as the most pervasive ESBL genotype among clinical Enterobacteriaceae isolates, particularly the CTX-M-15 subtype (Sonda et al., 2016; Saravanan et al., 2018; Onduru et al., 2021). Other ESBL genes, such as TEM, SHV, and OXA, are also reported but less widespread (Ghanavati et al., 2017; Mohamed et al., 2020; Ndukui et al., 2022; Mutua et al., 2023). Given the shared resistance profiles across human and animal isolates, and the rapid dissemination facilitated by MGEs, sustained molecular surveillance and evidence-based intervention remain critical. Reflecting this epidemiological priority, the present study focuses on five central ESBL resistance genes: *bla*TEM, *bla*SHV, and three *bla*CTX-M variants (*bla*CTX-M-8, *bla*CTX-M-9, and *bla*CTX-M-15), based on their documented clinical significance and regional predominance.

2.9 Research Gap

Taken together, the literature reviewed above establishes three consistent findings. First, gastrointestinal infections caused by Enterobacteriaceae carry a substantial and likely underestimated burden globally, regionally, and within Kenya specifically, with locally documented prevalence in Kenyan children reaching as high as 36.4% for diarrhoeagenic *E. coli* alone (Webale et al., 2020). Second, a significant and growing proportion of these infections are complicated by antimicrobial resistance, particularly ESBL-mediated resistance, with Kenyan hospital-based prevalence estimates reaching 40.8% (Mutua et al., 2023) and regional pooled estimates for ESBL-producing Enterobacterales reaching as high as 67% (Mutua et al., 2023; Maina et al., 2017). Third,

the molecular determinants of this resistance, principally *bla*CTX-M, *bla*TEM, and *bla*SHV, have been reasonably well characterised at both regional and national levels, including in poultry and clinical isolates elsewhere in Kenya (Ndukui et al., 2021, 2022; Mutua et al., 2023; Muraya et al., 2022).

Despite this body of evidence, a specific and consequential gap remains: none of the studies reviewed above, whether addressing GIT infection prevalence, AMR burden, or ESBL molecular epidemiology, drew specimens from the southwestern region of Kenya, and specifically not from Kisii County or KTRH. This is not a trivial omission. KTRH is the principal referral facility for over five million people across five counties, with a documented burden of gastrointestinal infection in its own facility records, yet clinicians there currently have no local evidence base, of the kind generated for Nairobi (Kimathi et al., 2022), Kilifi (Kagia et al., 2019), or other Kenyan sites reviewed above, on which to base empirical antibiotic selection. Given the well-established regional and national variability in both ESBL prevalence (ranging from 18% pooled regionally to over 67% in specific Kenyan cohorts) and dominant gene profile, extrapolating findings from Nairobi- or Kilifi-based studies to guide treatment decisions at KTRH is neither statistically nor clinically justified. This study addresses that specific, geographically and institutionally defined gap: no prior study has characterized the prevalence, antimicrobial susceptibility patterns, or molecular epidemiology of ESBL-producing Enterobacteriaceae among patients presenting with gastrointestinal infection at KTRH, or within Kisii County more broadly.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter outlines the specific details of how the study was accomplished. Descriptions include the study area, population of interest, sampling technique, and eligibility criteria for selecting patients. Further, the chapter also details specimen collection, transport, and handling procedures. The technical procedures followed during the laboratory experiments for isolation, identification, antibiotic susceptibility testing, and the methods for determining AMR genes are also outlined in this chapter. The chapter concludes by detailing the methodology for data analysis and the ethical considerations adhered to in the study.

3.2 Study Area

This study was conducted at Kisii Teaching and Referral Hospital (KTRH), located in Kisii Town in southwestern Kenya (0°40'S, 34°46'E), from October 2023 to April 2024 among patients presenting with gastrointestinal infections (Figure 3.1). KTRH is a 650-bed referral hospital that provides full inpatient and outpatient services, recording an average bed occupancy rate of approximately 150% and around 200 new outpatient registrations daily, with total outpatient visits, including follow-ups, often exceeding 400. The hospital serves as the principal referral facility for over five million people across Kisii, Nyamira, Homa Bay, Migori, and Bomet counties. The study catchment area is densely populated, comprising approximately 60% rural and 40% semi-urban settlements, with agriculture and small-scale trade as the predominant sources of household income.

KTRH was selected as the study site given the size and diversity of the population it serves, the documented burden of gastrointestinal infections in facility records, the near-total absence of prior AMR research within its catchment population, and its frequent

reliance on empirical antibiotic prescribing in the absence of local susceptibility data. As a regional referral center drawing patients across five counties, KTRH is also epidemiologically well-positioned to capture a diverse range of circulating resistant *Enterobacteriaceae* strains, and represents a site where antimicrobial-resistant infections are likely to cluster, evolve, and be detected. Collectively, these factors position KTRH as a critical surveillance point for understanding the molecular drivers of AMR among gastrointestinal pathogens in western Kenya, with direct relevance to local and national antibiotic stewardship priorities.



Figure 3.1: Map of Kenya Showing Kisii County and Location of KTRH

3.3 Study Design

The proposed study was conducted by following a laboratory-based cross-sectional design. This was nested within the Kenyan (Kisii) One Health Research in Environment, Veterinary and Human Antibiotic (KONSERVE-AB) study. Herein, patient data and samples were collected only once and subjected to laboratory analysis (Wang & Cheng, 2020). A cross-sectional study gives a snapshot view of the scenario of interest at a moment in time and therefore fails to account for long-term changes in its numbers over

time. The cross-sectional approach was beneficial in this research as it provided a snapshot of AMR patterns at a single point in time, enabling the identification of prevalent resistant strains. However, it was limited by its inability to capture temporal changes in resistance, which could have offered insights into trends over time. Nonetheless, it was the most suitable method for assessing the current state of AMR and informing immediate interventions.

3.4 Study Population

Patients attending the KTRH outpatient department with clinical suspicion of bacterial gastrointestinal infections were the target population of this study. This clinical suspicion was judged by a clinical care provider, and its definition typically included high fever (38°C) or temperature below 36°C , vomiting, bloody or mucoid stool, and diarrhoea. Patients of all ages presenting to the hospital during the proposed study period (1 June 2024 to 30 August 2024) were eligible for inclusion in the study. Since all outpatients report to the hospital's outpatient department (OPD) regardless of age, patients were sampled from the OPD.

3.4.1 Inclusion Criteria

- i. All patients presenting at KTRH with infections that meet the clinical criteria for gastrointestinal bacterial infections, including high fever above 38°C or temperature below 36°C , vomiting, bloody or mucoid stool, and diarrhoea.
- ii. Patients of all ages, including adults and children.
- iii. Patients who provided consent to participate in the study.
- iv. Patients who were permanent residents of Kisii County, having resided within the county for a minimum period of one month prior to enrollment.

3.4.2 Exclusion Criteria

- i. Patients who had used any antibiotic within the previous 2 weeks before presenting at the hospital.
- ii. Patients unable or unwilling to provide a stool specimen for analysis.
- iii. Patients presenting with a repeat episode of gastrointestinal infection who had already been enrolled and sampled earlier in the study period.
- iv. Patients who were critically ill and required immediate emergency intervention, precluding specimen collection prior to urgent clinical management.

3.5 Sample Size

The Fischer formula for calculating sample sizes for cross-sectional studies was used to determine the sample size for this study (Amatya et al., 2013). The calculation is as follows:

$$n = \frac{Z^2 p (1-p)}{e^2}$$

In the formula, Z corresponds to the 95% confidence level (1.96), while E is the margin of error, 5% (0.05). P is the prevalence of the phenomenon of interest as previously determined. The study by Edwards et al. (2023) determined the prevalence of ESBL-producing *Enterobacteriaceae* at 87% among neonates and was used as a basis for the calculation of the sample in this study. Substituting in the formula, the sample size was determined as follows:

$$n = \frac{1.96^2 \cdot 0.87 (1-0.87)}{0.05^2}$$

Therefore, based on this formula, the minimum sample size determined was 174 patients. This sample size was adjusted upwards by 10% to account for non-response or attrition. The primary factor contributing to attrition was the lack of bacterial growth upon culturing the samples. In the end, this study included 200 patients.

3.6 Sampling Method

A consecutive sampling approach was adopted to target patients presenting at KTRH with symptoms of GIT infections, including high fever above 38⁰C or temperature below 36⁰C, vomiting, bloody or mucoid stool, and diarrhoea. In the consecutive sampling approach, every next patient meeting the inclusion criteria and consenting to the study is included until the sample size is attained or exceeded. Thus, for this study, a stool specimen was collected from every patient presenting to the hospital with the symptoms of interest after they consented in writing.

3.7 Recruitment Procedures

Patients who qualified for inclusion in the study were approached by the researcher and asked to consent to the study. This encounter involved the researcher explaining the details about the study, answering any questions they had, providing necessary clarifications, and administering consent documents. For children under the age of 12 years, their parents or guardians were approached to provide parental consent before specimen collection. For those between 13 and 17 years, written assent was first obtained before seeking legal consent from their parents. After consenting, a questionnaire was administered by the researcher to gain demographic data. Following completion of the questionnaire, the researcher issued the adult patients or guardians with instructions on how to collect a stool specimen.

3.8 Data Collection

3.8.1 Demographic and Location Data

Following confirmation of eligibility, written informed consent was obtained from all participants. Subsequently, an interviewer-administered questionnaire (Appendix 1) was used to collect demographic data systematically. The instrument captured core participant characteristics, including age, sex, ethnicity, marital status, educational attainment, employment status, and household income level. To ensure comprehension and inclusivity across the study population, the questionnaire was professionally translated into Swahili, which is the primary local language spoken by a majority in the area. The data obtained were used for participant characterization and subsequent subgroup analysis where applicable. Location geopoints of the villages from which the participants came were abstracted from participant datasets maintained under the mother KONSERVE-AB Project.

Since the questionnaire was limited to demographic variables only, psychometric reliability testing was not done. This is because such variables are considered low-inference measures based on recent methodological guidance studies that emphasize that reliability statistics and extensive piloting are most critical when instruments assess subjective attitudes or latent constructs (Lenzner et al., 2024). For such variables, item interpretation may not vary, and measurement error is not likely. For factual demographic questions, cognitive pretesting or expert review is generally sufficient to ensure clarity and comprehensibility, and the risk of systematic bias is minimal (Duero & Namoco, 2024; Chhetri & Khanal, 2024).

Participant confidentiality was maintained through the assignment of unique, sequentially generated anonymous identifiers (such as K001–A1) to each participant and their corresponding specimen at the point of enrolment. This identifier linked the participant's

questionnaire, clinical data, and laboratory results without recording any personally identifiable information on the data collection or laboratory forms themselves.

3.8.2 Specimen Collection and Transport

A stool sample was obtained from all consenting patients on the day of reporting to KTRH. Patients were issued with clear instructions and asked to self-collect the specimen using aseptic techniques as described by Leber (2016) and deliver them to the specimen collection point. A similar approach was described by Leber (2016). Specifically, patients were instructed to scoop about a peanut-sized amount of faecal matter into a sterile container. For children and patients who could not self-collect stool specimens, their guardians received the same instructions and were asked to assist with specimen collection. These samples were transported to the KTRH Microbiology Laboratory and cultured within an hour of collection; hence, there was no need for transport media.

3.9 Specimen Processing

3.9.1 Isolation and Identification

The specimen was cultured on MacConkey agar, which inhibits Gram-positive bacteria and yeasts and blocks the swarming of *Proteus spp.* The plates were incubated at 37°C aerobically and observed for growth after 24 hours, as Lewis (2023) described. Similarly, a portion of the stool specimen was inoculated in Selenite F broth for approximately 8 hours for enrichment and subcultured on *Salmonella-Shigella* Agar to increase the chances of isolation of *Salmonella spp.* and *Shigella spp.* Isolates were then described using cultural morphology, Gram reaction, and biochemical tests. Specific tests done included the Indole test, Methyl Red (MR) test, Voges–Proskauer (VP) test, and Triple Sugar Iron (TSI) agar test. Identified isolates were then inoculated into stocking media (Tryptone Soy Broth with 20% glycerol) and stored at -20°C, awaiting molecular analysis. Table 3.1 below is a summary of the identification scheme followed. The overall laboratory

workflow followed in this study, from specimen collection through to molecular confirmation of ESBL genes, is summarized in Figure 3.2.

Table 3.1: Isolate Identification Scheme Based on Morphology and Biochemical Tests

Test		Isolate reaction Pattern				<i>E. coli</i> (ATCC 25922)	<i>K. pneumoniae</i> (ATCC 700603)
MacConkey Agar		LF	NLF	NLF	LF+ mucoid	LF	LF+ mucoid
SS Agar		Pink	Colorless + black	Colorless	Pink	Pink	Pink
Gram Staining		GNR	GNR	GNR	GNR	GNR	GNR
TSI Agar	Slant	A	K	K	A	A	A
	Butt	A	A	A	A	A	A
	H₂S	-	+	-	-	-	-
Indole		+	-	-	-	+	-
Citrate		-	+	-	+	-	+
Urease		-	-	-	+	-	+
Motility		+	+	-	-	+	-
Probable Pathogen		<i>Escherichia coli</i>	<i>Salmonella spp.</i>	<i>Shigella spp.</i>	<i>Klebsiella spp.</i>	—	—

Note: LF: Lactose Fermenter; NLF: Non-Lactose Fermenter; GNR: Gram-Negative Rods; A: Acidic (Yellow); K: Alkaline (Red); H₂S: Hydrogen Sulfide Production; +: Positive; -: Negative.

3.9.2 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was conducted using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar, following Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines (CLSI, 2024). Isolates were tested against a panel of antibiotics, including: amoxicillin-clavulanate (AMC, 20/10 µg), ampicillin (AMP, 10 µg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg), imipenem (IPM, 10 µg), cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), amikacin (AK, 30 µg), ceftriaxone (CRO, 30 µg), piperacillin-tazobactam (TZP, 100/10 µg), kanamycin (KAN, 30 µg), cefepime (FEP, 30 µg), chloramphenicol (CHL, 30 µg), and ciprofloxacin (CIP, 5 µg) (Oxoid, USA). Bacterial suspensions were prepared by suspending 3-5 isolated colonies from a fresh culture into 4-5 mL of normal saline and adjusting the turbidity to a 0.5

McFarland standard. For inoculation, a sterile swab was dipped into the suspension, excess liquid was removed by pressing the swab against the side of the tube, and the swab was used to evenly spread the suspension onto Mueller-Hinton agar plates using the lawn culture technique. Plates were incubated at 35°C for 16-18 hours, and inhibition zone diameters were measured. Inhibition zone diameters were measured, and isolates were classified as susceptible, intermediate, or resistant according to CLSI 2024 breakpoints (CLSI, 2024). Table 3.2 below summarises the classifications and mechanisms of action of each included drug.

Table 3.2: Antibiotics Tested, Mechanisms of Action, and Pharmacological Classification

Broad Class	Subclass	Mechanism of Action	Drug (Abbreviation)
Beta-lactams	Aminopenicillins	Bind penicillin-binding proteins (PBPs), inhibiting peptidoglycan cross-linking and causing cell lysis (Saikia & Chetia, 2024)	Ampicillin (AMP)
	Aminopenicillin + β -lactamase inhibitor	As above; clavulanic acid additionally inhibits bacterial β -lactamase enzymes, protecting the partner drug from hydrolysis.	Amoxicillin-clavulanate (AMC)
	Ureidopenicillin + β -lactamase inhibitor	As above, with an extended spectrum against Gram-negative and <i>Pseudomonas</i> species; tazobactam inhibits β -lactamase activity.	Piperacillin-tazobactam (TZP)
	3rd-generation cephalosporins	Bind PBPs with enhanced stability against many β -lactamases relative to earlier-generation agents (Saikia & Chetia, 2024)	Cefotaxime (CTX), Ceftazidime (CAZ), Ceftriaxone (CRO)
	4th-generation cephalosporin	As above, with broader Gram-negative coverage and improved penetration through the outer membrane	Cefepime (FEP)
	Carbapenems	Bind PBPs with a structurally distinct side chain that confers resistance to hydrolysis by most β -lactamases, including many ESBLs (Saikia & Chetia, 2024)	Imipenem (IPM)
Aminoglycosides	—	Bind the 30S ribosomal subunit, causing misreading of the mRNA codon sequence and inhibiting	Amikacin (AK), Kanamycin (KAN)

Broad Class	Subclass	Mechanism of Action	Drug (Abbreviation)
Fluoroquinolones	—	protein synthesis (Saikia & Chetia, 2024) Inhibit bacterial DNA gyrase and topoisomerase IV, blocking DNA supercoiling and replication (Saikia & Chetia, 2024)	Ciprofloxacin (CIP)
Folate pathway inhibitors	Sulfonamide-trimethoprim combination	Sequentially block two enzymes in the bacterial folate synthesis pathway, producing a synergistic bacteriostatic effect (Saikia & Chetia, 2024)	Trimethoprim-sulfamethoxazole (SXT)
Amphenicols	—	Bind the 50S ribosomal subunit, inhibiting peptidyl transferase activity and blocking protein synthesis (Saikia & Chetia, 2024)	Chloramphenicol (CHL)

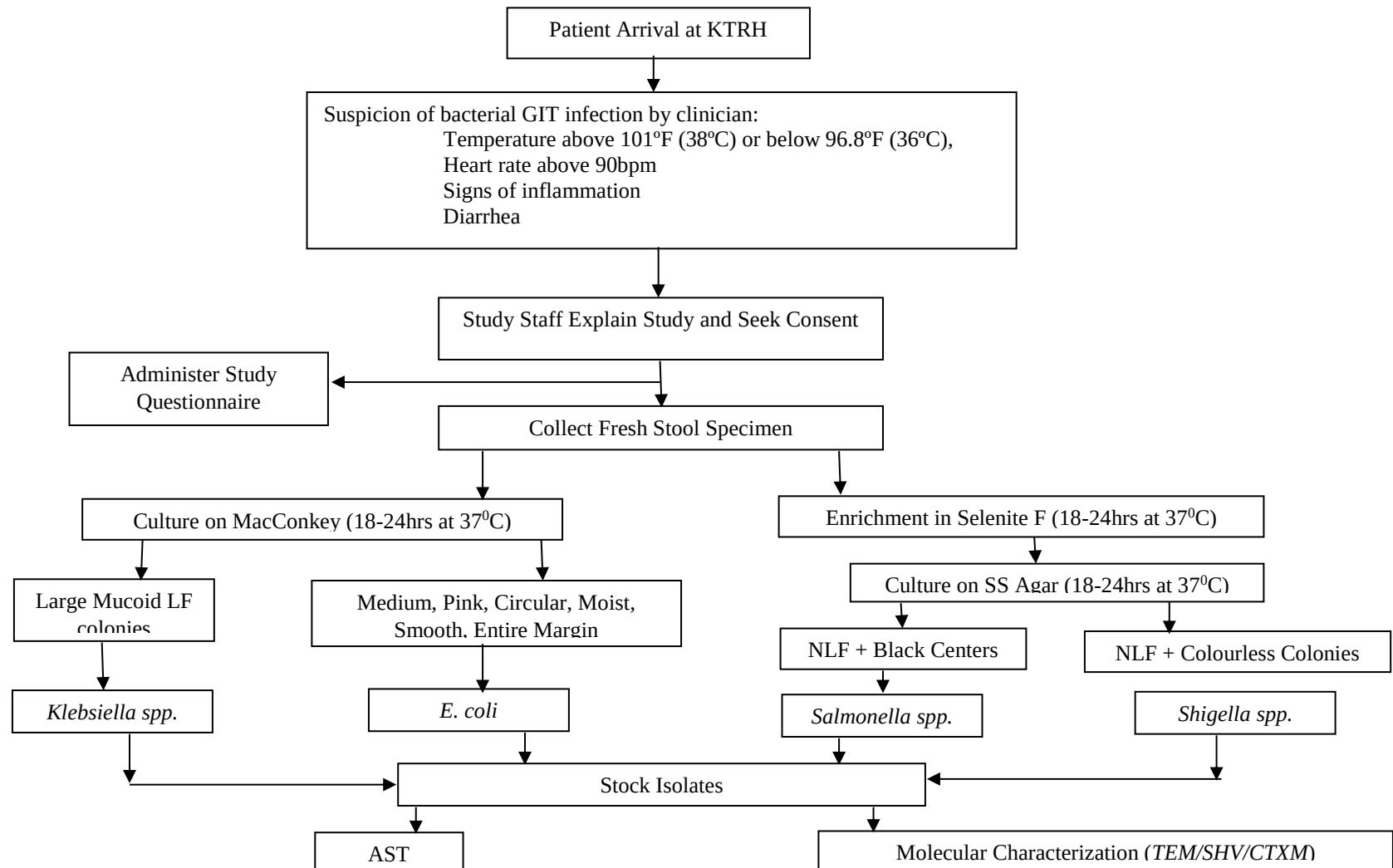


Figure 3.2: Flow Chart of the Study Methodology

3.9.3 Identification of ESBLs Using the Modified Double-Disk Synergy Test

Phenotypic detection of ESBL production, a key test underpinning this study's specific objectives, was performed using the Modified Double-Disk Synergy Test (MDDST). This method exploits the ability of clavulanic acid, a beta-lactamase inhibitor, to restore susceptibility in ESBL-producing isolates that would otherwise resist third-generation cephalosporins. Cefotaxime (CTX; 30 µg) and ceftazidime (CAZ; 30 µg) disks were placed on Mueller-Hinton agar at a fixed distance from a central disk containing amoxicillin-clavulanate (AMC; 30/10 µg), following the protocol previously described by Silago et al. (2021). Following incubation, a clear enhancement or distortion of the inhibition zone around either cephalosporin disk, oriented toward the amoxicillin-clavulanate disk, was considered indicative of ESBL production if the increase was at least 5mm. Isolates demonstrating this synergistic zone enhancement were classified as phenotypic ESBL producers and were subsequently subjected to molecular characterization, as described in Section 3.8.4.

3.9.4 Molecular Detection and Characterization of ESBL-Encoding Genes

3.9.4.1 Genomic DNA Extraction Using the PureLink™ Kit

Bacterial DNA was extracted using the Invitrogen™ PureLink™ Genomic DNA Mini Kit. The process involved cell lysis with Proteinase K, followed by the addition of RNase A to remove RNA. The lysate was mixed with ethanol and applied to a spin column for DNA binding. After washing the column, the DNA was eluted using the PureLink™ Genomic Elution Buffer. The purified DNA was stored at -20°C until further analysis.

3.9.4.2 Detection of ESBL Genes by Uniplex PCR

Uniplex PCR was performed using Thermo Scientific™ DreamTaq PCR Master Mix (2X), with a total reaction volume of 25 µL. The reaction mixture contained 12.5 µL of the master mix, 1 µL each of forward and reverse primers in Table 3.3, 2 µL of DNA

template, and 8.5 µL of nuclease-free water. The amplification was carried out using an initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 95°C, primer annealing temperature determined based on manufacturer details, primer BLAST results, and optimized experimental conditions, and extension at 72°C. A final extension was performed at 72°C for 10 minutes.

Primer specificity and reaction optimization were first validated using no-template controls (nuclease-free water in place of DNA) to rule out reagent or primer-dimer contamination prior to sample analysis. For all subsequent diagnostic PCR runs, a previously characterized isolate confirmed positive for each respective gene served as the positive control, and a confirmed-negative isolate verified to lack all target genes served as the negative control to validate amplification specificity in each run.

Table 3.3: Primers for the Genes of Interest

Gene name	Primer sequence	Annealing Temp (°C)	Fragment Size(bp)	Reference
<i>blaTEM</i>	F: 5'-AAACGCTGGTGAAAGTA-3'	46	822	(Mohamed et al., 2020)
	R: 5'-AGCGATCTGTCTAT-3'			
<i>blaSHV</i>	F: 5'-ATGCGTTATATTCGCCTGTG-3'	53	753	
	R: 5'-TGCTTTGTTATTCGGGCCAA-3'			
<i>blaCTX-M - 9</i>	F: 5'-ATG GTGACA AAGAGAGTGCA-3'	53	850	
	R: 5'-CCC TTC GGC GAT TCT C-3'			
<i>blaCTX-M - 8</i>	F: 5'-TCGCGTTAAGCGGATGATGC-3'	57	666	
	R: 5'-AACCCACGATGTGGGTAG-3'			
<i>blaCTX-M-15</i>	F: 5'-GTGATACCACTTCACCTC -3'	53	255	(Khalifa et al., 2021)
	R: 5'-AGTAAGTGACCAGAATCAG-3'			

3.9.3.3 Gel Electrophoresis and Interpretation of PCR Products

Amplified PCR products were separated and visualized by electrophoresis using a 1% agarose Tris-acetate-EDTA gel containing 0.5 µg of SYBR™ Safe DNA Gel Stain. A 100–1,000 bp GeneRuler DNA Ladder was used to determine the molecular weight of the PCR products. The gel was run at the appropriate voltage, and the bands were visualized under UV light to confirm the presence of the targeted genes. A band appearing at the expected fragment size for a given gene, as indicated in Table 3.3, was scored as positive for that gene; the absence of a band at the expected size was scored as negative. Figure 3.3 below presents a collage of sample gel electrophoresis images taken and their interpretation.

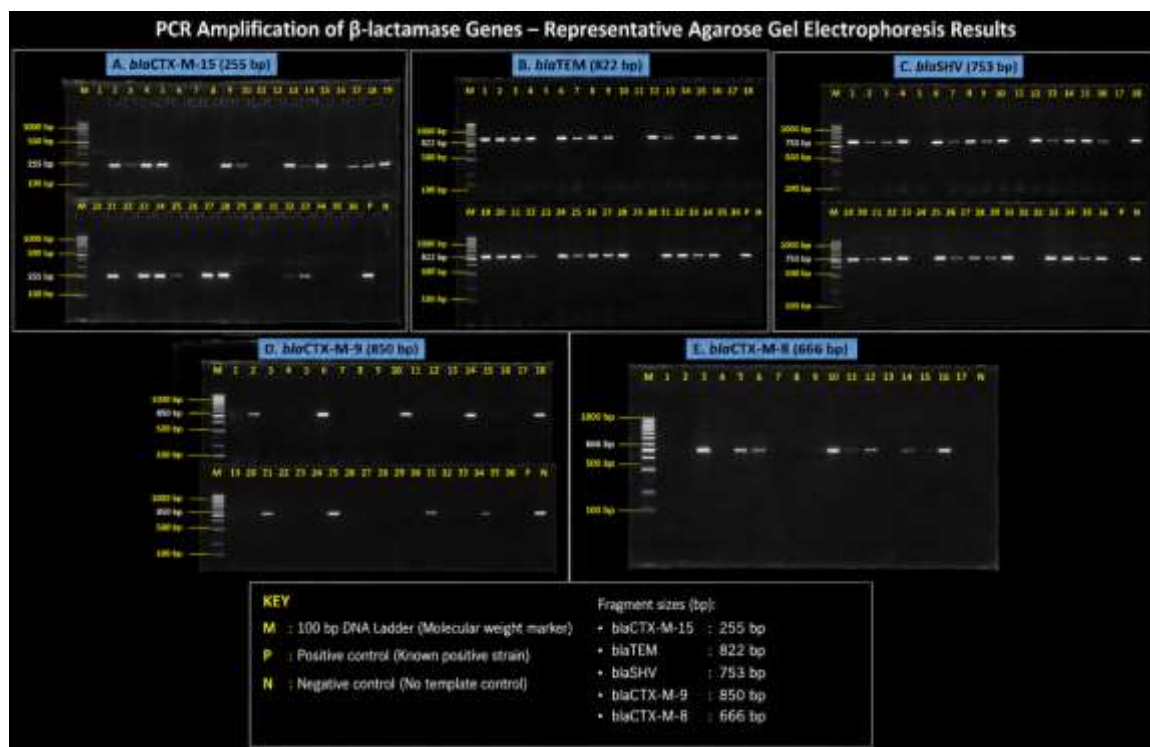


Figure 3.3: Representative Agarose Gel Electrophoresis Results Confirming PCR Amplification of the Five ESBL Genes Screened in this Study

Note: (A) *bla*CTX-M-15 (255 bp), (B) *bla*TEM (822 bp), (C) *bla*SHV (753 bp), (D) *bla*CTX-M-9 (850 bp), and (E) *bla*CTX-M-8 (666 bp). Lane M denotes the 100 bp DNA ladder used to estimate fragment size; lanes are otherwise numbered according to isolate identity. Lane P represents the positive control, and lane N represents the negative control. Amplicon sizes correspond to the expected fragment sizes listed in Table 3.3.

3.10 Data Analysis

Data were entered into a Microsoft Excel Spreadsheet (Microsoft Corporation, USA) and cleaned before analysis and visualisation using Stata 15 (StataCorp, USA). Descriptive statistics were used to summarise the demographic information of the participants. The prevalence of *Enterobacteriaceae* pathogens was described as a percentage of the total number of specimens collected. Percentages were calculated for each of the species of *Enterobacteriaceae* identified. Similarly, the antimicrobial susceptibility patterns of *Enterobacteriaceae* were analysed as percentages out of all isolates screened. Bacteria isolates showing resistance to more than three antibiotics in different classes were described as multidrug-resistant, as per the work of Ndukui et al. (2022). Since all bacterial isolates were subjected to an ESBL screening, the proportion of phenotypically screened ESBL-producing *Enterobacteriaceae* was analysed as a percentage of the total number of isolates screened. Finally, the rates of detection of common ESBL genes in *Enterobacteriaceae* pathogens isolated from stool specimens of patients presenting with symptoms of gastrointestinal infection at KTRH were analysed as a percentage of all the isolates that had screened positive for ESBL production using the double-disk diffusion method.

To assess associations between ESBL phenotype and participant demographic, clinical, and exposure characteristics, as well as associations between specific ESBL gene pairs, the Pearson Chi-square test of association was applied for 2×2 and larger contingency tables where all expected cell counts were ≥ 5 . Fisher's exact test was used in place of Chi-square wherever any expected cell count fell below 5, as the Chi-square approximation

becomes unreliable under these conditions. For pairwise gene associations, odds ratios with 95% confidence intervals were additionally calculated to quantify the strength and direction of each association, using Woolf's log-method. Statistical significance was set at $p < 0.05$ throughout. All visualisations were designed using Python (SciPy). As all reported percentages represent complete counts within the isolates tested rather than estimates drawn from a further sub-sample, error bars were not applied to descriptive figures; uncertainty is instead reported via confidence intervals and p-values specifically where inferential tests were conducted.

3.11 Quality Assurance

Validity and reproducibility were confirmed by doing all laboratory experiments in duplicate. For standardisation and adherence to international guidelines, quality control strains were used in all runs of laboratory experiments. Known control strains, including *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603, were used to verify the accuracy of biochemical tests and antimicrobial susceptibility results (Ndukui et al., 2021). Purity plating was done before downstream processing for any mixed growths obtained. Strict adherence to a pre-developed and tested standard operating procedure was also observed.

3.12 Biosafety Issues

All specimen handling, culture, and isolate manipulation were conducted under Biosafety Level 2 (BSL-2) practices at the KTRH Microbiology Laboratory, with personal protective equipment (gloves, laboratory coats, and eye protection) worn throughout, and all bacterial manipulation performed within a Class II biosafety cabinet to minimize aerosol exposure. Contaminated materials, including used culture plates, swabs, and disposable labware, were autoclaved prior to disposal in accordance with the hospital's infectious waste management protocol. Confirmed isolates of interest, including ESBL-

producing strains, were preserved in Tryptone Soy Broth with 20% glycerol and stored at -80°C to maintain viability for any subsequent confirmatory or molecular analyses.

3.13 Risks and Potential Benefits for the Study Participants

Participation in this study posed minimal risk, as specimen collection was limited to a single, non-invasive stool sample requiring no clinical procedure beyond routine self-collection. Confidentiality was maintained throughout via anonymized participant identifiers, minimizing any risk of personal information disclosure. While participants received no direct financial compensation, they benefited from free bacteriological culture, antimicrobial susceptibility testing, and, where clinically relevant, communication of actionable results to their attending clinicians to inform treatment. More broadly, participants contributed to generating the first localized antimicrobial resistance data for KTRH, with potential downstream benefit to the wider patient population through improved, evidence-based treatment protocols.

3.14 Study Limitations and Delimitations

This cross-sectional design captured a single snapshot of *Enterobacteriaceae* prevalence and resistance at KTRH between October 2023 and April 2024, and cannot show how these patterns shift over time. Gastrointestinal infections and their causative organisms are known to vary seasonally (Zama et al., 2022), so a seven-month window may understate the true range of prevalence and resistance the facility experiences across a full year. This temporal boundary was, however, a deliberate delimitation rather than an oversight, set by the practical constraints of a single MSc research timeline, and it does not undermine the validity of the patterns observed within that period; it simply means those patterns should be read as representative of this specific season rather than assumed stable across all months.

The findings are also confined to a single referral facility. KTRH serves a large, multi-county catchment population, yet what was observed reflects patients who specifically sought care there, and may not generalise cleanly to primary care centres or community settings within the same counties, where healthcare-seeking behaviour and prior antibiotic exposure likely differ (van Boeckel et al., 2019). This single-site scope was itself a deliberate delimitation, chosen precisely because KTRH's referral status and patient volume made it the most epidemiologically informative site available for this study's objectives, rather than an attempt at countywide representativeness.

Recruitment relied on consecutive sampling of symptomatic outpatients rather than random sampling from the wider community, meaning the sample may not proportionately reflect the full spectrum of gastrointestinal infection severity across the catchment population, since individuals who did not seek hospital care were never captured. This, too, was a considered delimitation appropriate to a hospital-based prevalence study of this kind, though it does mean the 44.9% ESBL figure reported here should be understood as a facility-based estimate, not a community-wide one.

Bacterial identification and susceptibility testing depended on culture-based methods, which remain the established gold standard for this type of investigation (Kagia et al., 2019), but which are limited to organisms capable of growing under the specific conditions used. Thirteen of the 200 stool samples yielded no growth at all, and it is plausible that fastidious organisms, or those present at low bacterial load, escaped detection entirely rather than simply being classified as negative.

Molecular characterisation was intentionally delimited to five ESBL genes, *bla*TEM, *bla*SHV, and three *bla*CTX-M variants, selected for their documented regional relevance (Onduru et al., 2021; Ndukui et al., 2022), while carbapenemase and plasmid-mediated AmpC genes were addressed separately within the wider KONSERVE-AB One Health project this thesis sits within (Tamma et al., 2019). Within that chosen panel, targeted PCR can only detect the exact sequences its primers were built against, so any novel or highly

divergent gene variant would have gone unidentified, even in isolates whose resistance phenotype strongly suggested an ESBL mechanism was genuinely present.

Finally, while the sample size proved adequate for the study's primary prevalence and susceptibility objectives, it constrained the statistical power available to detect smaller sociodemographic associations with ESBL status. Several associations examined under Objective Three, notably occupation and water source, approached but did not reach conventional significance, and a larger, more highly powered sample may ultimately have resolved whether these represent genuine, modest effects or simply chance variation within this particular cohort.

3.15 Ethical Considerations

Written informed consent was obtained from all adult participants above 18 years before enrolment into the study using the consent form in Appendix 2. For minors between 12-17 years, an assent form with simplified language explaining the study details was administered and signed, as in Appendix 3. However, their participation had to be approved by obtaining written consent from their parents or guardians. For children below 12 years, only parental/guardian consent was required, which was obtained using the tool in Appendix 4. All consent forms contained details about the study background, procedures, risks/benefits, compensation, and confidentiality as mandated by ethical guidelines. They also explicitly stated that participation was fully voluntary, allowing withdrawal at any time without consequences. To enhance comprehension across literacy levels, the forms were translated into Swahili and Kisii and then back-translated to ensure accuracy. In cases where participants or their guardians were illiterate, they affirmed participation by using a thumbprint, which one neutral witness countersigned.

Participant confidentiality was ensured by assigning unique study codes that delinked personal identifiers from laboratory/questionnaire data. All hard copy records were stored securely in locked cabinets accessible only to authorised study personnel. The electronic

database was password-protected and only contained coded data. During dissemination, no individually identifiable information was shared. The proposed research protocol and informed consent documents were subjected to ethical review at the KEMRI Scientific Ethics Review Unit (Approval Number SERU 4970) and Moi Teaching and Referral Hospital and Moi University Institutional Scientific and Research Ethics Committee (MTRH-MU ISERC) (Approval number 0004012) for approval before commencement (Appendix 5). The review examined if adequate provisions were in place to safeguard participants' dignity, rights, safety, and well-being. The research team committed fully to additional oversight and auditing by the KEMRI-SERU during implementation. Further to ethical review, final approvals were sought from the National Commission for Science, Technology, and Innovation (NACOSTI) (License No: NACOSTI/P/24/41335) and the County Government of Kisii before the commencement of the study. Whereas there was no payment or compensation for participation in the study, patients' laboratory results were sent to their attending clinician as a written report to help in treatment.

3.16 Dissemination of Study Findings

The findings from this study were widely disseminated through various channels. First, this thesis dissertation adhered to the institutional guidelines of Jomo Kenyatta University of Agriculture and Technology (JKUAT) and was prepared as the primary scholarly output. Second, the results were presented at relevant conferences focused on antimicrobial resistance, including the KEMRI Annual Scientific and Health Conference 2025. These avenues facilitated sharing the data with the wider scientific community and fostering collaborative discussions. Finally, the findings of this study were published as a peer-reviewed paper in the Kavulavu et al. (2025) article (Appendix 6). Open-access publishing was prioritised to enable widespread accessibility. At the study site, a presentation was administered to KTRH Antimicrobial Stewardship Committee members, in addition to depositing a concise policy brief highlighting key findings and recommendations at the hospital's resource centre.

CHAPTER FOUR

RESULTS

4.1 Introduction

This chapter presents the findings of the study based on the specific objectives outlined in Chapter One. The results are organized according to the study objectives, with detailed analysis and visualizations provided for each. First, the geographical distribution of samples collected is presented before the demographic and clinical profiles of the study participants. Subsequently, the objectives are addressed in sequence, including the prevalence of *Enterobacteriaceae*, antimicrobial susceptibility patterns, ESBL production, and the molecular characterization of ESBL genes.

4.2 Distribution of Samples

In this study, samples were collected from patients reporting for care at the OPD of KTRH as long as they met the inclusion criteria. The distribution of the 200 samples collected was mapped to show their spatial distribution, which is also reflective of patient trends. Among the locations, Suneka (15%, 30/200) had the highest number of samples, followed by Nyanchwa (13%, 26/200) and Mwembe (11.5%, 23/200). On the other hand, low sample locations included Bobasi, Bomachoge, Isecha, Kerongo, among others. Each of these sites had only one sample. The distribution of these samples was mapped (Figure 4.1) using GIS tools as shown below.

48.5%). Participants represented a range of educational backgrounds and occupations, with students/pupils (57/200, 28.5%) and farmers (49/200, 24.5%) forming the largest occupational groups. All patients presented with diarrhea, alongside varying proportions of fever, vomiting, and mucoid or bloody stool, consistent with the clinical criteria for enrolment. Participants also reported diverse water sources, meat sources, and levels of animal contact, reflecting the semi-rural and peri-urban character of the study catchment area. The full socio-demographic, clinical, and exposure profile of the study population is presented in Table 4.2 and discussed further under Section 4.5.

4.4 Prevalence of *Enterobacteriaceae* in Stool Specimens

The prevalence of *Enterobacteriaceae* and other bacterial isolates from stool specimens at KTRH is presented in Figure 1. The most prevalent pathogen was *Escherichia coli* (n=139/200 [69.5%]), followed by *Salmonella* spp. (n=23/200 [11.5%]), *Klebsiella* spp. (n=16/200 [8%]), and *Shigella* spp. (n=9/200 [4.5%]). Notably, 6.5% of stool samples (13/200) showed no bacterial growth. A total of 187 isolates were identified from the samples as shown in Figure 4.2.

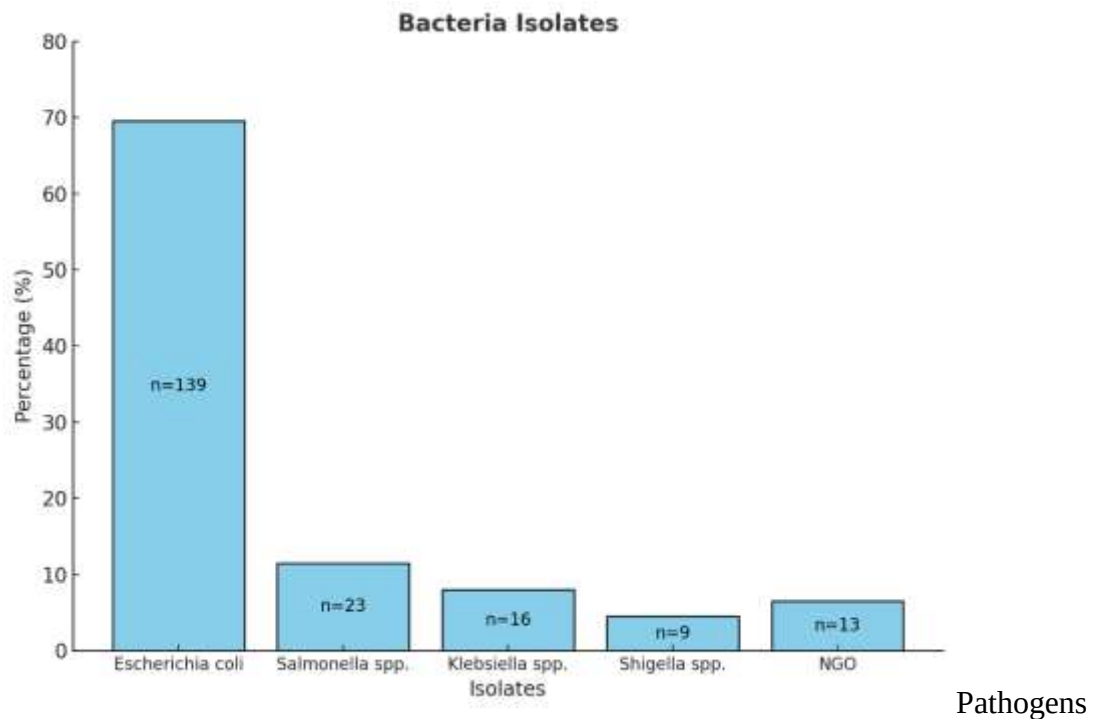


Figure 4.2: Prevalence of Enterobacteriaceae Species among 187 Bacterial Isolates Recovered from 200 Human Stool Samples

Note: Percentages represent the proportion of each species among total isolates recovered; 13 samples (6.5%) yielded no bacterial growth and are excluded from the species breakdown henceforth.

4.5 Antimicrobial Susceptibility Patterns of *Enterobacteriaceae*

The antimicrobial resistance profiles of the isolates demonstrated variable susceptibility across the antibiotics tested. *Salmonella* spp. exhibited the highest resistance to amoxicillin–clavulanate (17/23, 73.9%), followed by *Escherichia coli* (78/139, 56.1%) and *Klebsiella* spp. (5/16, 31.3%). Resistance to ampicillin was most pronounced in *E. coli* (109/139, 78.4%) and *Shigella* spp. (7/9, 77.8%), and *Klebsiella* spp. (12/16, 75.0%). Trimethoprim–sulfamethoxazole resistance was also common, particularly among *E. coli* (82/139, 59.0%) and *Shigella* spp. (7/9, 77.8%). Susceptibility to imipenem was observed

in 46.0% (64/139) of *E. coli* isolates and 56.3% (9/16) of *Klebsiella* spp. isolates. Cefotaxime demonstrated good activity against *E. coli* (92/139, 66.2%) and *Salmonella* spp. (15/23, 65.2%), and *Klebsiella* spp. (10/16, 62.5%), while ceftazidime showed comparable efficacy, with susceptibility rates of 75.5% (105/139) in *E. coli* and 78.3% (18/23) in *Salmonella* spp.

Amikacin demonstrated good activity across the major species, with susceptibility observed in *E. coli* (90/139, 64.8%) and *Salmonella* spp. (16/23, 69.6%), and *Klebsiella* spp. (11/16, 68.8%). Ceftriaxone also exhibited appreciable activity, with susceptibility rates of 48.9% (68/139) in *E. coli* and 37.5% (6/16) in *Klebsiella* spp. High levels of resistance to piperacillin–tazobactam were recorded in *E. coli* (100/139, 71.9%) and *Klebsiella* spp. (13/16, 81.3%). Kanamycin resistance was identified in 27.3% (38/139) of *E. coli* isolates and 37.5% (6/16) of *Klebsiella* spp. isolates. Resistance to cefepime and chloramphenicol was also notable, occurring in *E. coli* at rates of 30.9% (43/139) and 31.6% (44/139), respectively, and in *Klebsiella* spp. at rates of 43.8% (7/16) and 56.3% (9/16), respectively. Ciprofloxacin resistance was observed in *E. coli* (47/139, 33.8%) and *Salmonella* spp. (8/23, 34.8%). The complete susceptibility profile for each species is presented in Table 4.1

Table 4.1: Antimicrobial Resistance Profiles of Bacterial Isolates per Species

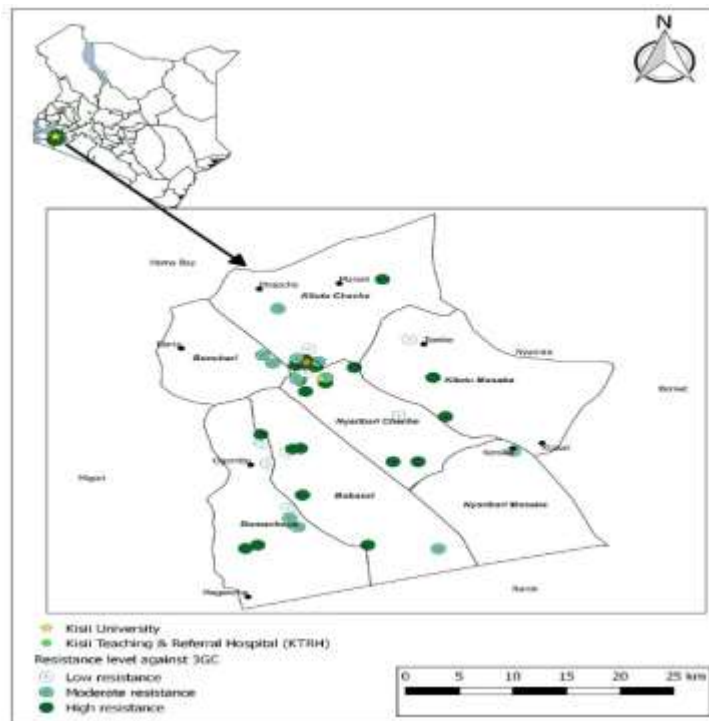
Organism	Susceptibility	Antibiotics [n (%)]												
		AMC	AMP	SXT	IPM	CTX	CAZ	AK	CRO	TZP	KAN	FEP	CHL	CIP
<i>Escherichia coli</i> (n=139)	S	43 (30.9)	23 (16.6)	49 (35.3)	64 (46.0)	92 (66.2)	105 (75.5)	90 (64.8)	68 (48.9)	22 (15.83)	38 (27.3)	88 (63.31)	92 (66.2)	80 (57.6)
	I	17 (12.2)	7 (5.0)	8 (5.8)	46 (33.1)	7 (5.0)	6 (4.3)	15 (10.8)	36 (25.9)	17 (12.23)	63 (45.3)	8 (5.76)	3 (2.2)	12 (8.6)
	R	78 (56.1)	109 (78.4)	82 (59.0)	29 (20.8)	40 (28.8)	28 (20.1)	34 (24.5)	35 (25.3)	100 (71.94)	38 (27.3)	43 (30.94)	44 (31.6)	47 (33.8)
<i>Salmonella</i> <i>spp.</i> (n=23)	S	3 (13.0)	10 (43.5)	5 (21.7)	8 (34.9)	15 (65.2)	18 (78.3)	16 (69.6)	10 (43.5)	4 (17.39)	9 (39.1)	14 (60.87)	11 (47.8)	12 (52.2)
	I	2 (8.7)	--	3 (13.0)	4 (17.4)	2 (8.7)	2 (8.7)	3 (13.0)	5 (21.7)	2 (8.70)	10 (43.5)	3 (13.04)	2 (8.7)	3 (13.0)
	R	17 (73.9)	12 (52.2)	15 (65.2)	11 (47.8)	6 (26.1)	3 (13.0)	4 (17.4)	8 (34.8)	17 (73.91)	4 (17.4)	6 (26.09)	10 (43.5)	8 (34.8)
<i>Klebsiella</i> <i>spp.</i> (n=16)	S	7 (43.8)	3 (18.8)	3 (18.8)	9 (56.3)	10 (62.5)	11 (68.8)	8 (50.0)	6 (37.5)	2 (12.50)	4 (25.0)	8 (50.00)	7 (43.8)	8 (50.00)
	I	3 (18.8)	--	1 (6.3)	2 (12.5)	1 (6.25)	--	2 (12.5)	4 (25.0)	1 (6.25)	6 (37.5)	1 (6.25)	--	1 (6.3)
	R	5 (31.3)	12 (75.0)	11 (68.8)	5 (31.3)	5 (31.3)	5 (31.3)	6 (37.5)	6 (37.5)	13 (81.25)	6 (37.5)	7 (43.75)	9 (56.3)	7 (43.8)
<i>Shigella</i> <i>spp.</i> (n=9)	S	5 (55.6)	2 (22.2)	2 (22.2)	4 (44.4)	5 (55.6)	7 (77.8)	6 (66.7)	4 (44.4)	1 (11.11)	3 (33.3)	5 (55.56)	5 (55.6)	5 (55.6)
	I	2 (22.2)	--	--	2 (22.2)	--	1 (11.1)	1 (11.1)	2 (22.2)	1 (11.11)	4 (44.4)	2 (22.22)	1 (11.1)	1 (11.1)
	R	2 (22.2)	7 (77.8)	7 (77.8)	3 (33.3)	4 (44.4)	1 (11.1)	2 (22.2)	3 (33.3)	7 (77.78)	2 (22.2)	2 (22.22)	3 (33.3)	3 (33.3)

Note S: Sensitive; I: Intermediate, R: Resistant; AMC: Amoxicillin-Clavulanate; AMP: Ampicillin; SXT: Trimethoprim-Sulfamethoxazole; IPM: Imipenem; CTX: Cefotaxime; CAZ: Ceftazidime; AK: Amikacin; CRO: Ceftriaxone; TZP: Piperacillin-Tazobactam; KAN: Kanamycin; FEP: Cefepime; CHL: Chloramphenicol; CIP: Ciprofloxacin

--: No isolate, or 0% fell in this category.

Special focus was directed towards third-generation cephalosporins (3GC) and imipenem (IPM) because these antibiotics are essential for treating infections caused by members of the family *Enterobacteriaceae*, including GIT infections marked by severe diarrhea in adults and children. Imipenem is a representative carbapenem drug. Resistance to these classes of antibiotics may mean limited options for treating similar illnesses. The distribution of resistance levels to these categories is shown in the maps below, showing areas with varying rates of resistance to 3GC (a) and IPM (b) (Figure 4.3). It was noted that samples from some areas had high resistance, while others showed moderate resistance or low resistance levels. These resistance levels reflect the differing prevalence of resistance to the classes of drugs across Kisii County. However, it is important to note that some locations had very few samples, which means the resistance rates calculated for these areas might be unreliable due to the small sample sizes.

(a)



(b)

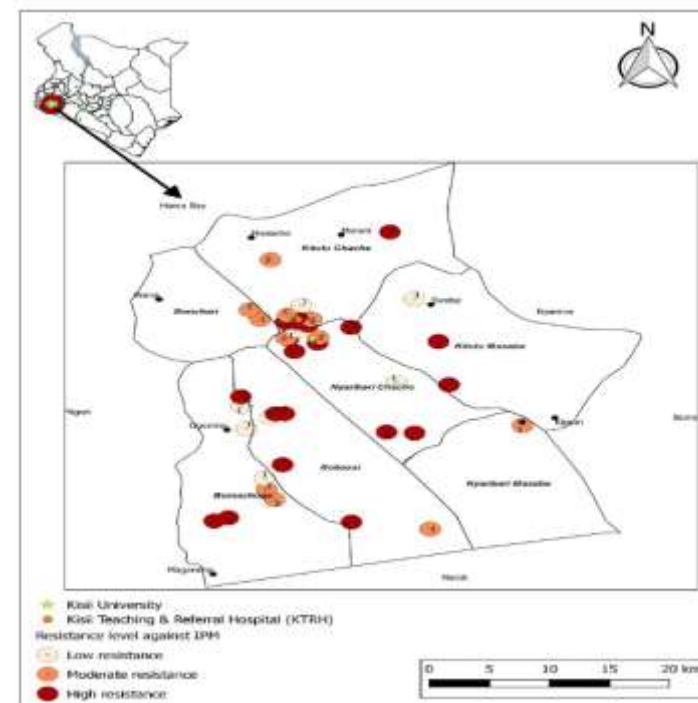


Figure 4.2: Distribution of Resistance to Third-Generation Cephalosporins (a) and Imipenem (b) in Human Samples across the Different Locations in Kisii County

Note: Shading intensity reflects the proportion of isolates from each location exhibiting resistance to the respective antibiotic class; locations with very few samples should be interpreted with caution given limited statistical reliability at low sample sizes.

The data on resistance across various organisms and antibiotics revealed notable trends. *Escherichia coli* exhibited the highest resistance to Ampicillin (78.4%) and Piperacillin-Tazobactam (71.9%), while resistance to Imipenem and Ceftazidime was relatively lower (20.9% and 20.1%, respectively). *Salmonella spp.* showed high resistance to Amoxicillin-Clavulanate (73.9%) and Piperacillin-Tazobactam (73.9%), with lower resistance to Ceftazidime (13.0%). *Klebsiella spp.* demonstrated significant resistance to Piperacillin-Tazobactam (81.2%) and Ampicillin (75%), while other antibiotics like Cefotaxime and Ceftazidime had resistance rates around 31.2%. *Shigella spp.* showed the highest resistance to Ampicillin and Trimethoprim-Sulfamethoxazole (77.8% each), with lower resistance to Ceftazidime (11.1%) and other drugs like Imipenem and Ceftriaxone (33.3%). Overall, the data highlighted variable resistance levels across different antibiotics and organisms, with some antibiotics showing higher resistance rates, particularly in common pathogens such as *E. coli* and *Salmonella spp.* (Figure 4.4).

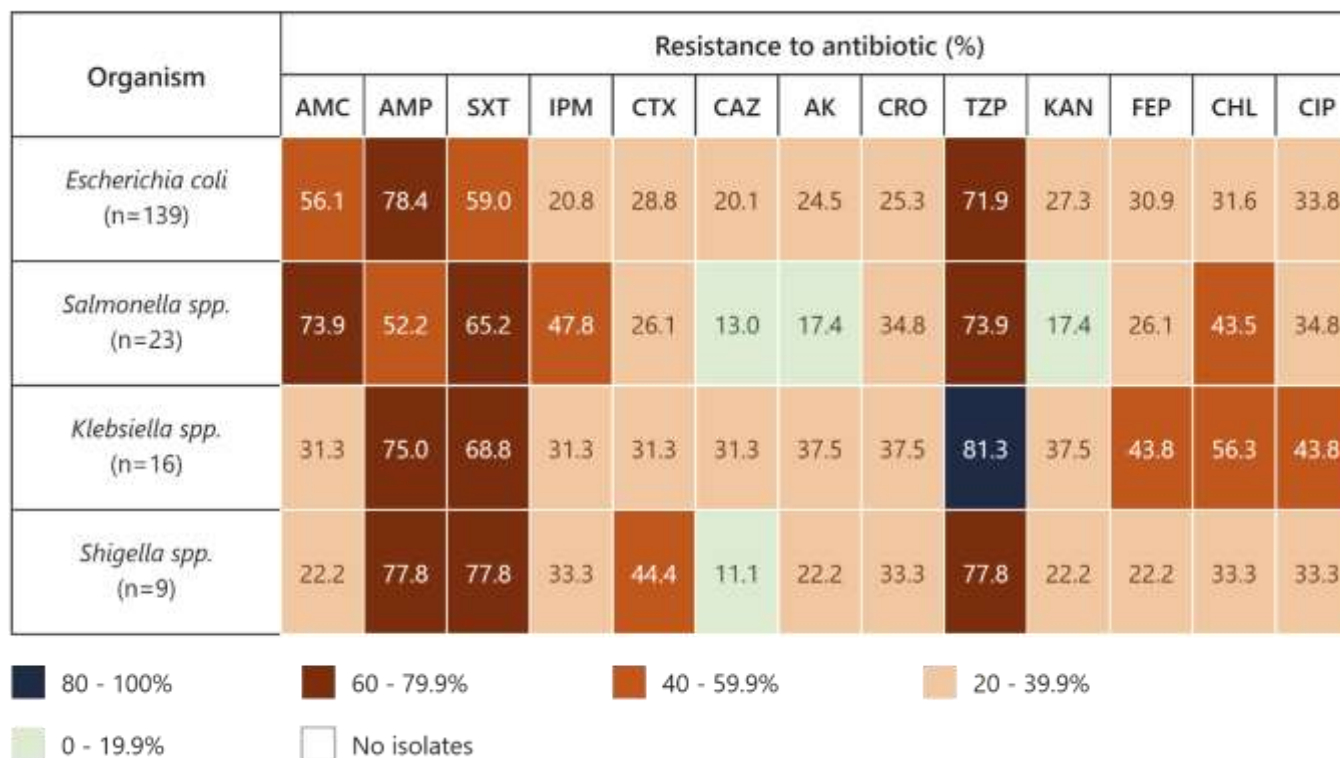


Figure 4.3: Antibigram Patterns - Resistance Trends among *Enterobacteriaceae* Isolates from Human Stool Samples

The AWARe classification of antibiotics by the WHO was developed to guide the usage of antibiotics in a judicious manner. Thus, the resistance trends in the data collected in this study were also visualized in line with this classification, as in Figure 4.5 below. Resistance rates were generally higher in the Access group antibiotics such as Amoxicillin-Clavulanate (54.5%) and Ampicillin (74.9%). In the Watch group, represented by antibiotics such as Piperacillin-Tazobactam (73.3%) and Ciprofloxacin (34.8%), resistance was also notable, including for Kanamycin (26.7%), an aminoglycoside classified under Watch rather than Reserve. Resistance in the Reserve group was comparatively lower, as shown by Imipenem (25.7%), though this remains concerning since carbapenems are considered last-resort agents for multidrug-resistant infections.

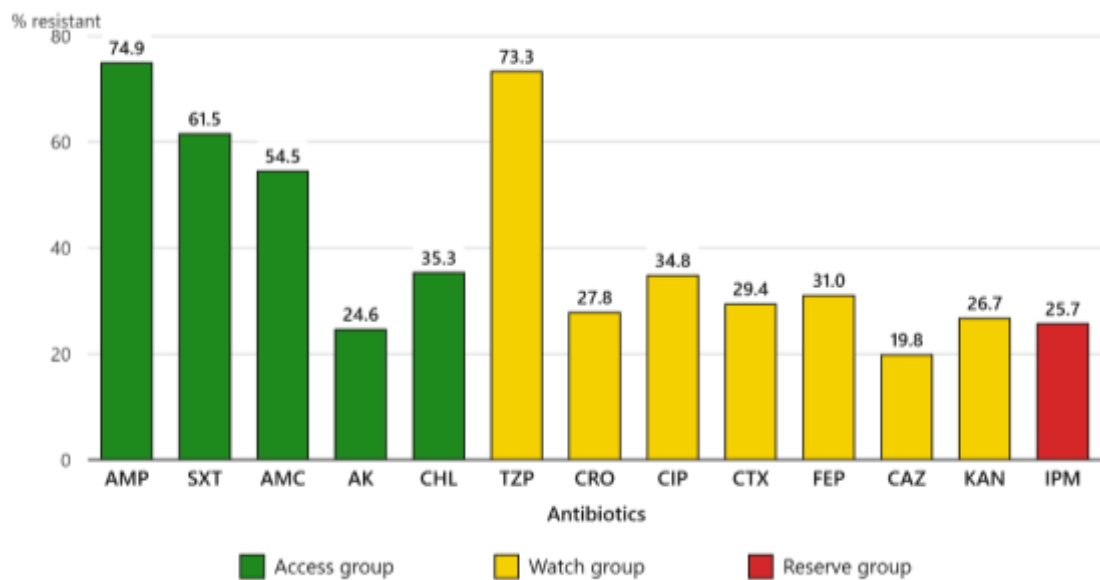


Figure 4.4: Resistance Rates as per the WHO AWARe Classification of Antibiotics

Note: Resistance rates among Enterobacteriaceae isolates, classified according to the WHO Access, Watch, and Reserve (AWARe) antibiotic framework. Bars represent the percentage of isolates resistant to each antibiotic, grouped by AWARe category; percentages are calculated from complete isolate counts.

4.6 Proportion of ESBL-Producing *Enterobacteriaceae* and Association with Participant Characteristics

Determining ESBL production in clinical isolates is vital as it may help with better antibiotic selection to prevent treatment failure. The prevalence of ESBL-producing and non-ESBL-producing isolates was determined using the Modified Double-Disk Synergy Test (MDDST). Among the 187 isolates, 103 (55.1%) were non-ESBL producers, while 84 (44.9%) were identified as ESBL producers. The pie chart clearly depicts the proportion of these isolates, with non-ESBL producers comprising the majority at 103/187 (55.1%) and ESBL producers making up 84/187 (44.9%) (Figure 4.6). It is important to note that isolates classified as non-ESBL producers were screened specifically for ESBL phenotype via MDDST and were not further characterized for other beta-lactamase mechanisms, such as AmpC production; it is therefore possible that some non-ESBL isolates harbor alternative resistance mechanisms not captured by this study's design.

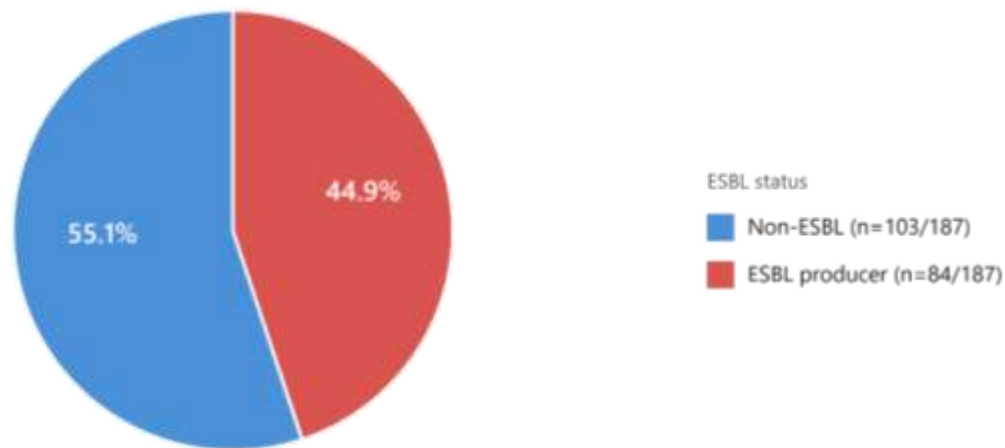


Figure 4.5: Proportion of ESBL-Producing *Enterobacteriaceae* among the Samples

Beyond establishing the overall proportion of ESBL producers, this study examined whether ESBL phenotype was associated with the demographic, clinical, and exposure

characteristics presented in Table 4.2. Chi-square and Fisher's exact tests were conducted for each characteristic against confirmed ESBL status.

No statistically significant associations were found (all $p > 0.05$). The strongest, though still non-significant, association was with occupation ($p = 0.083$), where ESBL-positivity was highest among the "other" occupational category (77.8%) and healthcare workers (64.3%), compared to business (29.0%) and farming (35.6%). Age group also approached significance ($p = 0.421$), with the 5–14 years group showing the highest ESBL-positivity (64.3%) against the lowest among those aged 60 years and above (25.0%). Primary water source showed a similar pattern ($p = 0.273$ – 0.984 across sources), with patients relying on rain, spring, or river water showing higher ESBL-positivity (48.1–59.5%) than those using treated piped water (40.4%). Sex ($p = 0.549$), animal exposure ($p = 0.323$), and antibiotic use in animals ($p = 0.172$) showed no notable pattern.

These findings suggest ESBL carriage was broadly distributed across demographic and exposure groups in this sample, rather than concentrated within an identifiable high-risk subgroup.

Table 4.2: Socio-Demographic and Clinical Characteristics of Study Participants and Association with ESBL Phenotype (N = 200)

Characteristic	Category	n (% ^b)	Non-ESBL (%) ^c	n ESBL+ (%) ^c	n p-value
Sex	Female	116 (58.0)	62 (57.4)	46 (42.6)	0.549*
	Male	84 (42.0)	41 (51.9)	38 (48.1)	
Age Group^s	Below 5 years	6 (3.0)	5 (62.5)	3 (37.5)	0.421#
	5–14 years	16 (8.0)	5 (35.7)	9 (64.3)	
	15–24 years	37 (18.5)	28 (52.8)	25 (47.2)	
	25–44 years	97 (48.5)	40 (54.8)	33 (45.2)	
	45–59 years	26 (13.0)	12 (54.5)	10 (45.5)	
	≥ 60 years	18 (9.0)	12 (75.0)	4 (25.0)	
Education	Primary	26 (13.0)	14 (63.6)	8 (36.4)	0.573#
	Secondary	83 (41.5)	39 (49.4)	40 (50.6)	
	Tertiary ^d	81 (40.5)	45 (57.7)	33 (42.3)	
	None	10 (5.0)	5 (62.5)	3 (37.5)	
Occupation	Students/Pupils	57 (28.5)	27 (51.9)	25 (48.1)	0.083#
	Farming	49 (24.5)	29 (64.4)	16 (35.6)	
	Business	35 (17.5)	22 (71.0)	9 (29.0)	

Characteristic	Category	n (% ^b)	Non-ESBL (%) ^c	n	ESBL+ (%) ^c	n	p-value
Presenting Symptoms^a	Teachers	28 (14.0)	13 (48.1)	14 (51.9)			
	Healthcare	14 (7.0)	5 (35.7)	9 (64.3)			
	Unemployed	6 (3.0)	2 (40.0)	3 (60.0)			
	Manual Laborers	3 (1.5)	2 (66.7)	1 (33.3)			
	Other	6 (3.0)	2 (22.2)	7 (77.8)			
	Diarrhea	200 (100.0)	—	—			— ^c
Meat Sources^a	Fever	182 (91.0)	97 (56.7)	74 (43.3)			0.224*
	Vomiting	106 (53.0)	50 (52.1)	46 (47.9)			0.485*
	Mucoid/Bloody Stool	105 (52.5)	52 (54.7)	43 (45.3)			1.000*
	Market	198 (99.0)	89 (57.1)	67 (42.9)			0.309*
	Own Animal	35 (17.5)	14 (45.2)	17 (54.8)			
	Not Applicable	2 (1.0)	—	—			—
Water Sources^a	Treated Piped Water	101 (50.5)	56 (59.6)	38 (40.4)			0.273*
	Underground Well	73 (36.5)	40 (56.3)	31 (43.7)			0.905*
	Rain Water	25 (12.5)	14 (51.9)	13 (48.1)			0.877*
	Springs	37 (18.5)	16 (50.0)	16 (50.0)			0.660*
	River	48 (24.0)	27 (56.2)	21 (43.8)			0.984*
	Other (water vendors)	1 (0.5)	—	—			—
Animal and Antibiotic Exposure							
<i>Prior unprescribed antibiotic use</i>	Yes	18 (9.0)	10 (55.6)	8 (44.4)			1.000*
<i>Animal exposure</i>	Yes	68 (34.0)	23 (47.9)	25 (52.1)			0.323*
<i>Pets in homestead</i>	Yes	40 (20.0)	20 (51.3)	19 (48.7)			0.723*
<i>Keeps domestic animals</i>	Yes	64 (32.0)	30 (46.9)	34 (53.1)			0.141*
<i>Works with animals</i>	Yes	65 (32.5)	31 (47.7)	34 (52.3)			0.184*
<i>Type of animal contact^a</i>	Feeding and Cleaning	55 (27.5)	25 (47.2)	28 (52.8)			0.228*
	Farm						
	Processing Meat	13 (6.5)	6 (60.0)	4 (40.0)			1.000#
<i>Antibiotic usage in animals</i>	Yes	23 (11.5)	15 (71.4)	6 (28.6)			0.172*
	No	177 (88.5)	88 (52.7)	78 (47.3)			

Note.^a Multiple responses were permitted; percentages are based on the total sample (N = 200) and may therefore exceed 100%.

^b Percentages are rounded to one decimal place.

^c Row percentages are calculated within category (Non-ESBL % + ESBL+ % = 100%), based on n = 187 isolates with a confirmed ESBL phenotype; the 13 no-growth samples among the 200 enrolled patients could not be assigned an ESBL status and are excluded from these columns only.

^d Tertiary education category includes participants reporting any levels of education beyond secondary.

^e Not tested — Diarrhea was a universal presenting symptom (100%) among enrolled patients and therefore shows no variation to test against ESBL phenotype.

* Pearson Chi-square test. # Fisher's exact test. $p < 0.05$ considered statistically significant. No association reached statistical significance in this sample.

[§] Participants were categorized into six age groups (below 5, 5–14, 15–24, 25–44, 45–59, and 60 years and above), consistent with standard World Health Organization age-group classification conventions (WellFr, 2025a, 2025b).

4.7 Molecular Characterization of Common ESBL Genes

The 84 Enterobacteriaceae isolates confirmed as ESBL producers by the phenotypic Modified Double-Disk Synergy Test were subjected to PCR to detect the main genes they harboured. The results of the PCR are summarized in Figure 4.7 below. The gene *bla*TEM had the highest prevalence, found in 63.1% (53/84) of the isolates, followed by *bla*CTX-M-15 at 48.8% (41/84). The *bla*SHV gene was detected in 38.1% (32/84) of the isolates, while *bla*CTX-M-8 showed a prevalence of 28.6% (24/84). The *bla*CTX-M-9 gene had the lowest prevalence, found in 9.5% (8/84) of the isolates.

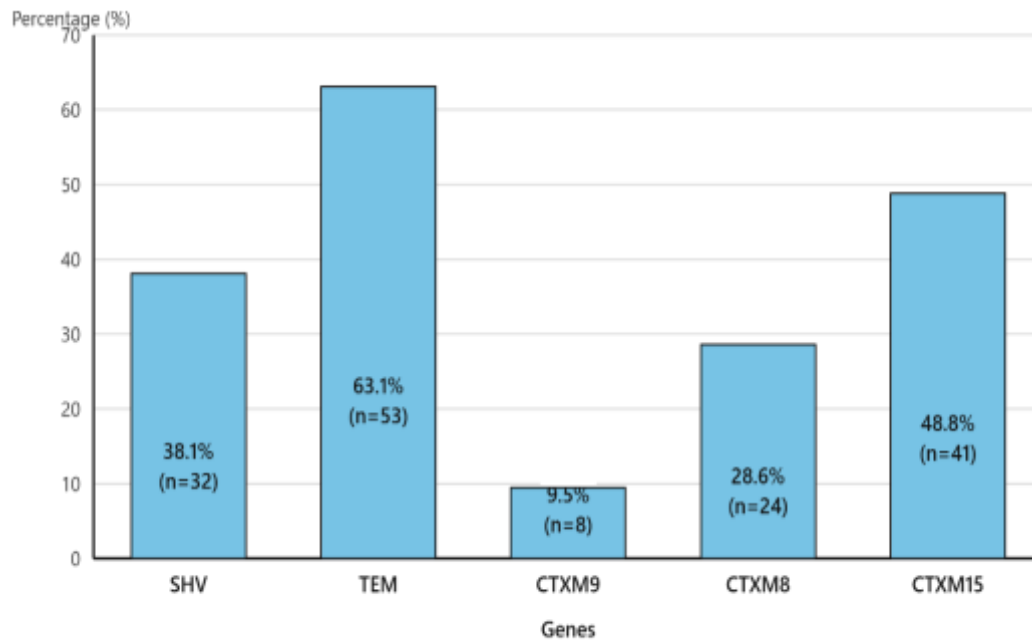


Figure 4.7: Prevalence of ESBL Resistance Genes among Phenotypically Confirmed ESBL-producing Isolates (n=84)

Note: *bla*TEM was the most frequently detected gene

Analysis of the co-carriage patterns among the 84 phenotypically confirmed ESBL-producing *Enterobacteriaceae* isolates subjected to PCR revealed substantial diversity in gene combinations, with eighteen unique co-carriage patterns observed. The most frequent multi-gene pattern was *bla*TEM + *bla*SHV + *bla*CTX-M-15, present in 11 isolates (13.1%). Other notable patterns included *bla*TEM alone in 9 isolates (10.7%) and *bla*TEM + *bla*CTX-M-8 + *bla*CTX-M-15 in 8 isolates (9.5%).

Pairwise co-carriage analysis identified *bla*TEM + *bla*CTX-M-15 as the most common combination, detected in 35 isolates (41.7%), followed by *bla*TEM + *bla*SHV in 25 isolates (29.8%) and *bla*TEM + *bla*CTX-M-8 in 19 isolates (22.6%). Less frequent pairwise combinations included *bla*CTX-M-9 + *bla*CTX-M-15 (7.1%) and *bla*SHV +

*bla*CTX-M-9 (4.8%). Testing each pair against the null hypothesis of independent gene assortment showed that *bla*TEM and *bla*CTX-M-15 co-occurred significantly more often than chance alone would predict (OR = 8.10, 95% CI: 2.82–23.31, $p < .001$), as did *bla*TEM and *bla*SHV (OR = 3.06, 95% CI: 1.13–8.32, $p = .025$), while the remaining pairs did not reach statistical significance, suggesting that certain gene combinations, particularly *bla*TEM with *bla*CTX-M-15, may be preferentially co-located on shared mobile genetic elements rather than accumulating independently.

Distribution by number of genes positive indicated that 26.2% of isolates carried three genes, 26.2% carried a single gene, 17.9% carried two genes, and 11.9% harboured four genes, while 17.9% carried none of the five genes tested. This distribution differed significantly from what independent gene assortment would predict ($\chi^2(4) = 28.96$, $p < .001$), with isolates clustering disproportionately at the extremes, more carrying no genes and more carrying three or four genes than expected by chance, and fewer falling in the one-to-two gene middle range. Collectively, these patterns point to considerable heterogeneity in ESBL carriage, consistent with the presence of multi-gene resistance clusters and possible horizontal gene transfer events, rather than genes accumulating one at a time through unrelated acquisition. The co-carriage and pairwise data presented in Table 4.3, alongside the co-occurrence matrix in Figure 4.9, offer insight of direct relevance to molecular surveillance, antibiotic stewardship, and targeted infection control planning at KTRH.

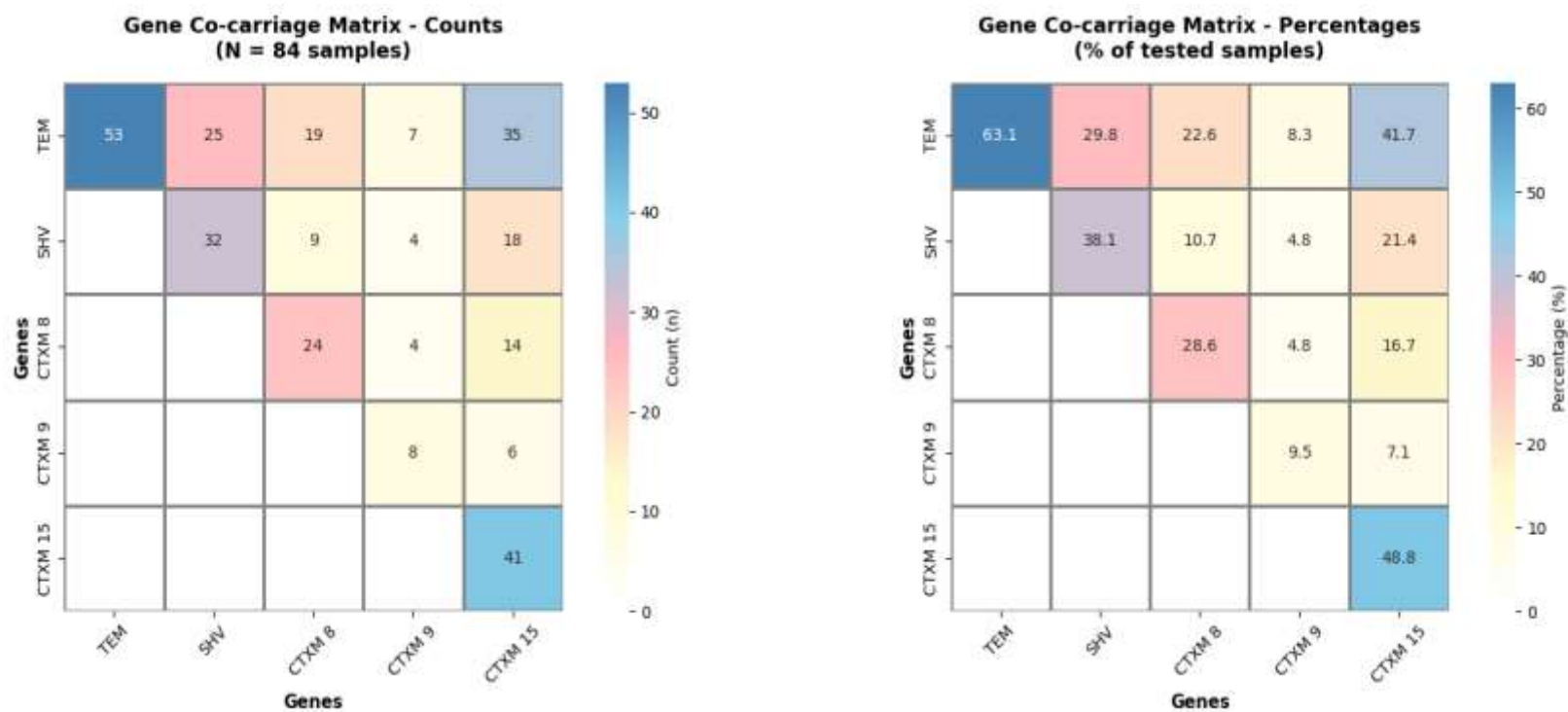


Figure 4.8: Co-occurrence Matrix of ESBL Genes among Phenotypically Confirmed ESBL-Producing *Enterobacteriaceae* (n = 84)

Table 4.3: Co-carriage Patterns, Pairwise Gene Associations, and Distribution of ESBL Genes among ESBL-Positive Isolates (N = 84)

Pattern / Gene Pair / Number of Genes	n	%	Expected ^c	OR [95% CI]	p
Co-carriage patterns					
No genes	15	17.9	—	—	—
TEM + SHV + CTXM 15	11	13.1	—	—	—
TEM	9	10.7	—	—	—
TEM + CTXM 8 + CTXM 15	8	9.5	—	—	—
TEM + CTXM 15	7	8.3	—	—	—
CTXM 15	5	6.0	—	—	—
SHV	4	4.8	—	—	—
TEM + SHV	4	4.8	—	—	—
TEM + CTXM 8 + CTXM 9 + CTXM 15	3	3.6	—	—	—
TEM + SHV + CTXM 8 + CTXM 15	3	3.6	—	—	—
TEM + SHV + CTXM 8	3	3.6	—	—	—
CTXM 8	3	3.6	—	—	—
TEM + SHV + CTXM 9 + CTXM 15	3	3.6	—	—	—
SHV + CTXM 8	2	2.4	—	—	—
CTXM 9	1	1.2	—	—	—
TEM + CTXM 8	1	1.2	—	—	—
TEM + SHV + CTXM 8 + CTXM 9	1	1.2	—	—	—
SHV + CTXM 15	1	1.2	—	—	—
Pairwise co-carriage^d					
TEM + SHV	25	29.8	20.2	3.06 [1.13, 8.32]	.025 ^{a*}
TEM + CTXM 8	19	22.6	15.1	2.91 [0.96, 8.81]	.054 ^a
TEM + CTXM 9	7	8.3	5.0	4.57 [0.53, 39.00]	.248 ^b
TEM + CTXM 15	35	41.7	25.9	8.10 [2.82, 23.31]	<.001 ^{a****}
SHV + CTXM 8	9	10.7	9.1	0.97 [0.36, 2.56]	.943 ^a
SHV + CTXM 9	4	4.8	3.0	1.71 [0.40, 7.40]	.473 ^b
SHV + CTXM 15	18	21.4	15.6	1.62 [0.67, 3.94]	.285 ^a
CTXM 8 + CTXM 9	4	4.8	2.3	2.80 [0.64, 12.26]	.217 ^b
CTXM 8 + CTXM 15	14	16.7	11.7	1.71 [0.66, 4.46]	.269 ^a
CTXM 9 + CTXM 15	6	7.1	3.9	3.51 [0.67, 18.53]	.151 ^b
Distribution by number of genes positive					
0 genes	15	17.9	6.4	—	
1 gene	22	26.2	24.0	—	
2 genes	15	17.9	31.5	—	
3 genes	22	26.2	17.7	—	
4 genes	10	11.9	4.4	—	
Overall goodness-of-fit				$\chi^2(4) = 28.96, p < .001^{a****}$	

Note: OR = odds ratio, calculated as $(a \times d) / (b \times c)$ from the 2×2 contingency table for each gene pair, where the joint co-occurrence count and each gene's individual marginal total (blaTEM 53/84, blaSHV 32/84, blaCTX-M-8 24/84, blaCTX-M-9 8/84, blaCTX-M-15 41/84) together fully determine all four cells of the table. OR quantifies the strength and direction of association (OR > 1 indicates the genes co-occur more often than chance; OR < 1, less often). 95% CIs for OR were calculated using Woolf's log-method. Expected = expected joint count/frequency under the null hypothesis of independent gene assortment. Co-carriage patterns are presented descriptively only since sparse cell counts across several individual patterns ($n \leq 3$) preclude reliable statistical testing at this level of granularity.

^aPearson chi-square test of independence, applied where all four cells of the 2×2 table had expected counts ≥ 5 .

^bFisher's exact test, applied where any expected cell count was <5; no odds ratio confidence interval could be computed reliably for TEM+CTXM9 given its small expected cell count, and this estimate should be interpreted with caution.

^cExpected count/frequency under the null hypothesis that genes are distributed independently of one another, given each gene's marginal prevalence.

^dEach row represents an independent 2×2 test for that specific gene pair; no correction for multiple comparisons was applied given the exploratory nature of this analysis.

CHAPTER FIVE

DISCUSSION OF FINDINGS, CONCLUSIONS, AND RECOMMENDATIONS

5.1 Introduction

This final chapter presents a detailed discussion of the study's findings and draws conclusions and recommendations from them. It begins with an interpretation of the results for each objective, moves through the clinical and epidemiological meaning of what was found, and closes with recommendations grounded firmly in the data. Rather than simply restating results, this chapter asks what each finding actually means for AMR management, infection control, and surveillance at KTRH, and why the patterns observed took the shape they did. Where a result diverges from what regional literature would predict, an explanation is offered rather than left unexamined. This chapter integrates the study's results with broader regional and global trends and offers insight critical to addressing *Enterobacteriaceae* infections and antimicrobial resistance in the local and wider healthcare context.

5.2 Prevalence of *Enterobacteriaceae*

Escherichia coli emerged as the most prevalent pathogen among the analysed human stool samples, and this finding sits comfortably alongside its established role as a primary gastrointestinal pathogen across sub-Saharan Africa, where the organism is frequently implicated in both community-acquired and healthcare-associated infection (Ndukui et al., 2021; Rashid et al., 2019; Webale et al., 2020). *E. coli* is commonly reported among patients in the region for urinary tract infections and bloodstream infections too, particularly among vulnerable populations such as children and the immunocompromised (Rashid et al., 2019). What this study cannot say, however, is which pathotype of *E. coli* was actually responsible for each infection, since pathotyping was not undertaken. Different pathotypes vary considerably in virulence and clinical presentation, and

increasingly, metagenomic and whole-genome sequencing approaches are proving far more reliable than culture alone for teasing this apart (Medugu et al., 2025; Robins-Browne et al., 2016). This is acknowledged here as an interpretive boundary on the *E. coli* findings rather than a flaw in the culture-based methodology itself, which remains entirely appropriate for answering the prevalence question this study set out to answer.

The isolation of *Salmonella* and *Shigella* species, though less frequent, is consistent with documented disease burdens across the region. *Salmonella enterica* serovar Typhi and invasive non-typhoidal *Salmonella* remain significant contributors to morbidity and mortality in sub-Saharan Africa (Balasubramanian et al., 2018). *Shigella* spp. similarly rank among the leading causes of diarrhoeal disease in children under five, and a recent meta-analysis placed the pooled African prevalence at 5.9%, rising slightly to 6.2% within East Africa specifically (Nyarkoh et al., 2024). The prevalence rates observed here track closely with those figures, which suggests this study's sample, though drawn from a single facility, reflects a genuinely regional pattern of enteric infection rather than a local anomaly.

The comparatively lower prevalence of *Klebsiella* and *Shigella* species relative to *E. coli* deserves closer thought, because it is not simply a smaller number; it is a different biological story. *Klebsiella pneumoniae* is fundamentally an opportunistic pathogen, thriving especially in healthcare settings among neonates and immunocompromised patients (Abbas et al., 2024), and its lower isolation rate here may partly reflect the fact that this study's population was largely community-presenting outpatients rather than a hospitalised, more vulnerable cohort. *Shigella* spp., meanwhile, are recognised causes of diarrhoeal disease throughout the region, yet their lower prevalence in this study could plausibly be shaped by improvements in sanitation, differences in vaccination coverage, or simply variation in diagnostic sensitivity between laboratories (Ongadi et al., 2025). None of these explanations can be confirmed definitively from this dataset alone, but together they offer a more textured account than transmission dynamics could provide on its own. Taken as a whole, these findings reinforce the need for continued surveillance

and targeted intervention to address the enteric pathogen burden across the region, with particular attention to how local patient populations may differ from hospital-based cohorts elsewhere.

5.3 Antimicrobial Susceptibility Patterns

The resistance rates recorded for *Escherichia coli* and *Klebsiella* spp. against ampicillin and piperacillin-tazobactam were, frankly, alarming. Ampicillin resistance stood at 78.4% for *E. coli* and 75.0% for *Klebsiella* spp., while resistance to piperacillin-tazobactam reached 71.9% and 81.3% respectively. These figures sit within the range reported elsewhere in the region, where high resistance to penicillins and cephalosporins has become something of a grim constant (Kimathi et al., 2022; Misau et al., 2023). In Ethiopia, for instance, resistance to piperacillin reached 88% in *E. coli* and 97% in *K. pneumoniae* (Kitaba et al., 2024), and within Kenya itself, comparably high resistance to ampicillin and other penicillins has been documented in both clinical and meat isolates from Nairobi (Chepkemei et al., 2022; Kariuki et al., 2021). Such widespread resistance compromises the reliability of first-line treatment and complicates clinical decision-making at the point of care.

The piperacillin-tazobactam finding, though, merits particular attention, because on its face it should not have happened this way. Tazobactam is a beta-lactamase inhibitor, chemically related to clavulanic acid, and its role in the combination is precisely to neutralise the very enzymes that would otherwise destroy piperacillin. That resistance nonetheless reached 71.9% and 81.3% in this study's isolates suggests something other than a simple ESBL story is at play. Several mechanistic explanations are worth considering here. One possibility, well established in the beta-lactamase literature reviewed in Chapter Two, is that some isolates may carry Complex Mutant TEM (CMT) variants, enzymes that combine the extended substrate range of an ESBL with mutations conferring reduced susceptibility to clavulanate-class inhibitors including tazobactam, which would produce exactly this pattern of resistance despite an inhibitor being present

(Matlock et al., 2025). A second explanation lies in what microbiologists call the inoculum effect, where a sufficiently high bacterial load or high level of enzyme production can simply overwhelm the fixed concentration of inhibitor included in the combination drug, allowing enough unprotected piperacillin hydrolysis to occur that the isolate still tests resistant. A third, non-enzymatic possibility involves porin loss or efflux pump upregulation, mechanisms entirely independent of beta-lactamase activity that reduce intracellular drug concentration regardless of how effectively the inhibitor performs its job. Any of these, or some combination of them, would explain why tazobactam's presence did not translate into the expected clinical benefit here, and this ambiguity itself argues strongly for the molecular surveillance this study went on to undertake.

Interestingly, ceftazidime susceptibility among *E. coli* isolates was relatively high, at 75.5%. This stands in contrast to findings from Ethiopia, where *E. coli* showed 65% resistance to extended-spectrum cephalosporins (Kitaba et al., 2024), and the gap invites explanation rather than simple acknowledgement. One plausible driver is cost and access. Third- and fourth-generation cephalosporins such as ceftazidime and cefotaxime remain comparatively expensive and less freely available over the counter in many parts of sub-Saharan Africa, and this restricted access likely translates into lower selection pressure at the population level, since a drug that patients cannot easily obtain cannot easily drive resistance through repeated, unsupervised use. Local prescribing habits at KTRH, along with whatever infection control practices are already in place there, may compound this effect further, though this study's design does not allow that contribution to be quantified precisely. Whatever the exact balance of causes, the presence of resistance to piperacillin-tazobactam and ampicillin alongside preserved ceftazidime susceptibility hints at extended-spectrum beta-lactamase or AmpC-type mechanisms operating selectively rather than uniformly across the antibiotic panel, which again underscores why molecular characterisation, and not phenotypic testing alone, was necessary to properly interpret these isolates. Kenyan surveillance data more broadly echo rising resistance to extended-spectrum cephalosporins such as ceftriaxone and ceftazidime in clinical isolates (Muraya

et al., 2022; Rwigy et al., 2024), so the relatively favourable ceftazidime picture at KTRH should be read as a local, possibly transient advantage rather than a national trend.

Resistance to imipenem, a reserve antibiotic by any classification, was also observed, and this is concerning regardless of how modest the absolute rate appears. The emergence of resistance among last-resort agents threatens the very safety net that reserve antibiotics are meant to provide (Haines et al., 2022; Karlowsky et al., 2023). Ethiopia again offers a cautionary parallel, where carbapenem resistance among *K. pneumoniae* isolates rose markedly between 2017 and 2021 (Kitaba et al., 2024), and carbapenem-resistant Enterobacteriaceae infections carry substantial morbidity and mortality wherever they take hold. Drivers of this trend commonly include antibiotic overuse, weak infection control, and the spread of resistant strains through the hospital environment itself (Karlowsky et al., 2023), and in Kenya specifically, carbapenemase genes have already been identified co-existing with ESBL genes in *K. pneumoniae* isolates, a combination that should worry anyone responsible for antimicrobial stewardship (Muraya et al., 2022). Viewed through the lens of the WHO's Access, Watch, and Reserve classification, these resistance patterns make a certain uncomfortable sense. Access group antibiotics, being the most commonly used first resort, would be expected to show the highest resistance, and that expectation held true here. But resistance was also detectable within the Watch and Reserve categories, and that should not be true if stewardship were working as intended (World Health Organization, 2023). Regional data from Western Kenya show a similar pattern of widespread multidrug-resistant *Enterobacteriaceae* (Rwigy et al., 2024), which suggests these are not isolated local quirks but part of a broader national trajectory. Addressing this will require stringent stewardship programmes, stronger infection prevention practices, and surveillance systems robust enough to catch these shifts before they become entrenched.

5.4 Proportion of ESBL-Producing *Enterobacteriaceae*

Nearly half, specifically 44.9%, of *Enterobacteriaceae* isolates in this study were phenotypically confirmed as ESBL producers, and this figure alone speaks to how far antimicrobial resistance has progressed in this setting. It sits considerably above the 18% pooled estimate reported in Ethiopia (Kiros et al., 2021), though comparisons across studies always carry some caveat given differences in population and methodology. In Kenya, a Kilifi County Hospital study observed a 10% ESBL carriage rate at neonatal admission that rose sharply to 55% during hospitalisation (Kagia et al., 2019), a trajectory that illustrates just how dynamic ESBL prevalence can be within a single institution, let alone across a country. Rwanda reported 23.4% among clinical isolates (Kayinamura et al., 2024), while a Nigerian review found prevalence ranging from as low as 7.5% to as high as 82.3% depending on setting (Tanko et al., 2020), and a systematic review across East, Central, and Southern Africa placed the regional figure at 38% (Onduru et al., 2021). Against this backdrop of wide variability, the findings of this study corroborate patterns seen elsewhere in sub-Saharan Africa (Edwards et al., 2023; Richter et al., 2023), and the sheer spread of reported figures across the continent is itself an argument for localised, facility-specific surveillance rather than reliance on national or regional averages.

It is worth pausing here to consider who, specifically, carried these ESBL-producing isolates, since Objective Three of this study was concerned not only with how common ESBL production was but with understanding it within the population sampled. Analysis of participant sociodemographic characteristics against ESBL status did not reveal statistically significant associations with age, sex, education, or occupation in this cohort, though certain patterns edged toward significance and are worth noting rather than dismissing outright. Occupational category showed the closest approach to significance, with higher ESBL positivity observed among healthcare workers and those in loosely defined "other" occupations than among farmers or those in business, a pattern that would fit plausibly with differential healthcare exposure even though it did not reach conventional statistical thresholds here. Water source told a similar, suggestive story, with

patients relying on rain, spring, or river water showing somewhat higher ESBL positivity than those on treated piped water, consistent with the household sanitation and water access pathways to enteric resistance already discussed in Chapter Two (Berendes et al., 2023). None of this establishes causation, and the absence of significance should be stated plainly rather than glossed over, but the direction of these associations is at least internally coherent with the transmission pathways the wider literature would predict.

The clinical implications of such high ESBL prevalence are considerable regardless of which sociodemographic group carries the greatest burden. These pathogens resist commonly used penicillins and cephalosporins, narrowing therapeutic options meaningfully. In Rwanda, ESBL-producing isolates were entirely resistant, 100%, to amoxicillin-clavulanic acid and third-generation cephalosporins, with only amikacin, meropenem, and polymyxin B retaining reasonable activity (Kayinamura et al., 2024). This kind of pattern forces clinicians toward alternative agents that are frequently less accessible or considerably more expensive in resource-limited settings, and the growing emergence of carbapenem resistance specifically threatens to close off even those alternatives (Peirano & Pitout, 2019). Treating ESBL-producing infections is therefore rarely straightforward. Limited first-line efficacy tends to prolong hospital stays and drive up costs, and in Rwanda, mortality among patients with ESBL-producing infections reached 10.3%, more than double the general hospital mortality rate recorded over the same period (Kayinamura et al., 2024). The rapid in-hospital acquisition documented among neonates in Kilifi (Kagia et al., 2019) only compounds the urgency. What emerges from all of this is a clear case for targeted intervention: stronger infection prevention and control, routine surveillance, rational antibiotic policy, and molecular characterisation of resistance mechanisms to properly understand what is actually driving the numbers (Onduru et al., 2021).

5.5 Molecular Characterization of ESBL Genes

Molecular characterisation of the ESBL-positive isolates revealed genuine diversity in the genetic mechanisms underlying resistance. *bla*TEM proved the most prevalent gene, detected in 63.1% (n=53/84) of confirmed ESBL producers, followed closely by *bla*CTX-M-15 at 48.8% (n=41/84), with *bla*SHV at 38.1% (n=32/84) and *bla*CTX-M-8 at 28.6% (n=24/84). These findings align closely with previous regional work identifying *bla*TEM and *bla*CTX-M as the primary contributors to beta-lactam resistance across East Africa (Mutua et al., 2023; Onduru et al., 2021). The prevalence of *bla*TEM is worth dwelling on, because this gene's association with resistance to multiple penicillins and cephalosporins is long established, and its persistence in clinical settings, particularly where antibiotic use is high, is neither surprising nor reassuring. It is also worth recalling, from Chapter Two, that even a well characterised gene like *bla*TEM does not translate uniformly into a single phenotype; Matlock et al. (2025) demonstrated that the resistance phenotype conferred by *bla*TEM-1 varies considerably depending on the surrounding bacterial genetic background, and this genotype-phenotype complexity is a useful caution against reading gene prevalence figures as a direct, one-to-one predictor of clinical resistance.

*bla*CTX-M-15 was similarly well represented, and its detection here reflects the well-documented global spread of CTX-M genes generally, and CTX-M-15 specifically. This variant has been described as the most prevalent ESBL gene worldwide, particularly among Gram-negative pathogens such as *E. coli*, which cause everything from gastrointestinal to urinary tract infections (Richter et al., 2020). These findings sit comfortably alongside earlier regional surveys identifying CTX-M as a key driver of resistance across sub-Saharan Africa (Ghanavati et al., 2017; Sonda et al., 2016), and the spread of CTX-M genes has repeatedly been linked to horizontal transfer via mobile genetic elements, which helps explain how a single successful gene variant can disseminate so rapidly across bacterial species and, indeed, across continents.

The detection of *blaSHV* and *blaCTX-M-8*, at 38.1% and 28.6% respectively, adds further texture to this genetic picture. *blaSHV*, while somewhat less prevalent than *blaTEM* or *blaCTX-M-15*, remains a significant contributor to beta-lactam resistance, particularly within *Klebsiella pneumoniae* and *Enterobacter* spp. (Tamma et al., 2019), and its continued presence here reinforces the need for ongoing surveillance of this gene across both human and animal populations. *blaCTX-M-8* is typically associated with outbreaks of multidrug-resistant infection in hospital settings (Etemadi et al., 2020), so its presence in nearly three in ten ESBL-positive isolates is not a trivial finding. The comparatively lower prevalence of *blaCTX-M-9*, at just 9.5% (n=8/84), fits expectations set by the wider literature, where this gene has consistently been reported less frequently than other CTX-M variants globally. It is worth noting, as Cantón et al. (2012) described, that *blaCTX-M-8* and *blaCTX-M-9* belong to genetically distinct phylogenetic groups despite sharing the CTX-M family name, with *blaCTX-M-8* falling within the CTX-M-1 group and *blaCTX-M-9* forming its own separate lineage, each having followed a distinct evolutionary path within the broader CTX-M radiation.

The spread of these genes owes much to mobile genetic elements, which allow resistance determinants to move between bacterial species via plasmids and integrons (Grover et al., 2013). Work by Ndukui et al. (2022) and Mutua et al. (2023) has shown that the same genes detected here, *blaTEM*, *blaCTX-M*, and *blaSHV*, mediate resistance not only in human isolates but in animal populations too, particularly poultry, and this cross-species pattern speaks directly to the complex, interconnected dynamics of AMR that a purely clinical lens cannot fully capture. The co-existence of these genes across environmental and clinical isolates complicates containment efforts considerably, and argues for integrating molecular epidemiology with targeted stewardship rather than treating them as separate concerns.

Taken together, this molecular characterisation offers real insight into the genetic diversity and spread of resistance within this setting. The predominance of *blaTEM* and *blaCTX-M-15*, alongside meaningful detection of *blaSHV* and *blaCTX-M-8*, reflects patterns

consistent with regional and global literature and contributes to a fuller understanding of the genetic factors driving AMR across sub-Saharan Africa. These results point toward an urgent need for continued monitoring, rational antibiotic use, improved infection control, and, where feasible, the development of alternative therapeutic strategies. A coordinated response spanning both human and animal health sectors will be necessary if the transmission of these resistance genes across populations is to be meaningfully curbed.

The co-carriage of multiple ESBL genes observed here reflects a pattern increasingly documented across Kenya, where *Enterobacteriaceae* isolates frequently harbour more than one resistance determinant simultaneously. Recent molecular surveillance has shown *E. coli* and *K. pneumoniae* from both clinical and community sources carrying combinations of *bla*TEM, *bla*SHV, and *bla*CTX-M variants, with *bla*CTX-M-15 consistently emerging as the dominant allele in these combinations (Maveke et al., 2024; Waithaka et al., 2024). This kind of multi-gene carriage raises the probability of true multidrug resistance and narrows the treatment options available to clinicians considerably. Studies from Nairobi and Kiambu have reported similar co-existence of ESBLs alongside plasmid-mediated quinolone resistance genes, underscoring just how readily horizontal transfer can occur across bacterial populations once the right mobile elements are in play (Ndukui et al., 2022). The predominance in this study of isolates carrying three or more ESBL genes parallels findings from Western Kenya, where hospitalised children were found to harbour *Klebsiella* spp. with multiple beta-lactamase genes simultaneously (Rwigi et al., 2024), and genomic epidemiology elsewhere in Kenya has shown ESBL genes combining with carbapenemase genes too, raising legitimate concern over how much longer last-resort antibiotics will remain effective (Muraya et al., 2022). The additive effect of these co-carried genes broadens resistance spectra and appears to accelerate the spread of resistant clones through both healthcare and community settings alike, which is precisely why strengthening surveillance and embedding molecular diagnostics into routine clinical practice matters so much going forward.

5.6 Implications for Antimicrobial Resistance Management at KTRH

The findings of this study offer critical, actionable evidence that should directly shape AMR management at KTRH. The documented burden of ESBL-producing *Enterobacteriaceae*, together with the resistance profiles described above, argues for a deliberate shift away from intuition-based empirical prescribing toward a structured, evidence-driven stewardship programme.

First, high resistance to ampicillin and amoxicillin-clavulanate, together with substantial resistance to piperacillin-tazobactam, means these agents can no longer be relied upon for empirical treatment of suspected Gram-negative infection at KTRH. This pattern aligns with regional surveillance showing escalating resistance to penicillins and cephalosporins more broadly (Kariuki et al., 2021; Onduru et al., 2021), and it follows that the hospital's essential medicines list and treatment protocols require urgent revision. Ceftazidime and amikacin, both of which showed higher susceptibility here, should be prioritised in empirical protocols for severe infection, consistent with the WHO's AWaRe framework, which explicitly encourages preserving Watch and Reserve group antibiotics for situations where they are genuinely needed (World Health Organization, 2023).

Second, embedding routine, facility-level antimicrobial susceptibility testing into everyday clinical workflow is no longer optional. Reliance on outdated or national-level data risks compromising patient outcomes, precisely because this study has shown that resistance patterns unique to KTRH exist and matter. Regular testing, paired with a properly disseminated, facility-specific antibiogram, would give clinicians the tools to make informed, timely therapeutic decisions, which is really the cornerstone of any functioning stewardship programme (Gandra et al., 2020).

Third, the molecular identification of prevalent genes, particularly *bla*TEM and *bla*CTX-M-15, adds a layer of surveillance that phenotypic testing alone simply cannot provide. Because these genotypes are known to travel on mobile genetic elements, they can spread

rapidly within and between bacterial species (Mutua et al., 2023; Ndukui et al., 2022), and building the capacity for periodic molecular surveillance at KTRH would allow early detection of high-risk resistance clones and a clearer picture of how resistance genes actually move through the hospital environment over time. This kind of proactive surveillance is essential if outbreaks are to be contained rather than merely documented after the fact.

Finally, these findings underscore the ongoing need for provider education on local AMR trends and prudent prescribing. Empirical therapy must be anchored in local evidence, and the selection pressure driving resistance forward will only ease if broad-spectrum antibiotic use is genuinely curtailed where it is not clinically necessary (Schuts et al., 2016; Sulis et al., 2023). In sum, AMR management at KTRH needs to evolve to incorporate robust local surveillance, guidelines built from local evidence rather than assumption, molecular epidemiological insight, and sustained professional education, all working together to protect the antibiotics still working and to improve outcomes for the patients who depend on them.

5.7 Conclusions

1. **Prevalence of *Enterobacteriaceae*:** *Enterobacteriaceae* were highly prevalent among patients with gastrointestinal symptoms at Kisii Teaching and Referral Hospital, with *Escherichia coli* (69.5%, n = 139/200) and *Klebsiella* spp. (8.0%, n = 16/200) among the most commonly isolated species. These findings confirm that *Enterobacteriaceae* are key causative agents of gastrointestinal infection in this setting and align closely with regional and global epidemiological trends.
2. **Antimicrobial Susceptibility Patterns:** High resistance was observed among *Enterobacteriaceae* isolates to Ampicillin (74.9%, n = 140/187), Trimethoprim Sulfamethoxazole (61.5%, n = 115/187), and Piperacillin Tazobactam (73.3%, n = 137/187), with multidrug resistance common across isolates. Resistance to Imipenem (25.7%, n = 48/187) and Amikacin (24.6%, n = 46/187) was

comparatively lower, though notably still present given their status as reserve and later-line agents. Together, these patterns point to real, present therapeutic challenges for managing gastrointestinal infections at KTRH, and the unexpectedly high resistance to piperacillin-tazobactam in particular suggests resistance mechanisms beyond simple ESBL production may be contributing.

3. **Proportion of ESBL Producers:** Phenotypic testing revealed that 44.9% (n = 84/187) of *Enterobacteriaceae* isolates were ESBL producers, a substantial burden that constrains the usefulness of frontline beta-lactam antibiotics at this facility. Sociodemographic and exposure characteristics, including age, sex, education, occupation, water source, and animal contact, showed no statistically significant association with ESBL status in this population, though occupation and water source approached significance, suggesting ESBL carriage here was broadly distributed rather than concentrated in one identifiable group.
4. **Most Common ESBL Genes:** Molecular analysis identified *bla*TEM (63.1%, n = 53/84) and *bla*CTX-M-15 (48.8%, n = 41/84) as the most prevalent ESBL genes, followed by *bla*SHV (38.1%, n = 32/84) and *bla*CTX-M-8 (28.6%, n = 24/84), with a significant proportion of isolates carrying three or more genes simultaneously. This genetic diversity, and the frequency of multi-gene carriage specifically, underscores the complexity now driving ESBL-mediated resistance in *Enterobacteriaceae* at KTRH.

5.8 Recommendations

1. **Prevalence of *Enterobacteriaceae* in Stool Specimens.** Given the confirmed high burden of *Enterobacteriaceae* among gastrointestinal presentations at KTRH, there is a need for continuous surveillance of these pathogens rather than reliance on periodic or one-off assessment. It is important for the hospital laboratory to strengthen capacity for accurate species identification, of the kind used in this study, as this would improve diagnostic precision and support timely patient

management, while regular data collection would allow shifts in pathogen prevalence to be caught early rather than discovered retrospectively.

2. **Antimicrobial Susceptibility Patterns.** Because ampicillin and piperacillin-tazobactam showed such high resistance in this study, there is a case for KTRH to revisit its empirical treatment protocols with these local findings in mind, rather than continuing to prescribe on the basis of national or assumed patterns. Antimicrobial stewardship efforts, supported by ongoing provider training and treatment guidelines updated as local susceptibility data change, would be worth reinforcing specifically around these two agents. Given the comparatively higher susceptibility observed for ceftazidime and amikacin here, these agents merit consideration where clinically appropriate, in line with the WHO AWaRe framework's emphasis on preserving Watch and Reserve category antibiotics for situations where they are genuinely needed.
3. **Proportion of ESBL Producers.** Since nearly half of isolates in this study were ESBL producers, there is value in integrating routine phenotypic ESBL screening into standard diagnostic protocols at KTRH, so that resistant infections can be identified promptly rather than assumed. Given that this study found no strong sociodemographic predictor of ESBL carriage, such screening would be best applied broadly across presenting patients rather than targeted narrowly at any single risk group, as the evidence here does not support such targeting.
4. **Common ESBL Genes Detected.** With *bla*TEM and *bla*CTX-M-15 confirmed as the dominant genes at this facility, it would be worthwhile for laboratory capacity to be developed specifically toward molecular diagnostics capable of detecting these genes, rather than a broader, less targeted panel. Periodic, facility-based molecular surveillance is worth considering to track whether this gene profile shifts over time, and collaboration with regional and national reference laboratories could support this without requiring KTRH to build full sequencing capacity independently.

5. **Pathotyping of *E. coli* Isolates.** Because pathotyping fell outside this study's scope yet would meaningfully extend its findings, future studies at this facility stand to benefit from incorporating molecular pathotyping of *E. coli* isolates to clarify the virulence mechanisms behind the resistant strains already characterised here, building directly on rather than duplicating this study's gene-level data.
6. **Further Molecular Characterisation.** There is merit in future research applying whole genome sequencing to the isolates and gene combinations identified in this study specifically, to resolve the transmission dynamics and mobile genetic element context behind the high rate of multi-gene carriage observed here, particularly the *bla*TEM and *bla*CTX-M-15 co-occurrence this study's own data flagged as significant.

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APPENDICES

Appendix I: Demographic Data Questionnaire

English Version

Participant's Study Number	
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1. Basic details:

a	Age	
b	Gender	
c	Highest Education Level	
d	Occupation	

2. Symptoms *(Please note down all symptoms like wound infection and other acute conditions as well):*

	Symptoms	Tick if present
a	Fever	
b	Diarrhea	
c	Vomiting	
d	Mucus or bloody stool	
e	Others (Please describe them)	

3. Have you used any antibiotics before this moment?

No ☐ Yes ☐

If Yes, please indicate the name of the drug (indicate the name on the box/packaging)_____

4. If Yes in 4 above, was the antibiotic prescribed by a medical professional or you self-medicated?

Prescribed by a professional ☐₀

Self-medicated ☐

5. **Do you usually ensure you finish antibiotic doses?** No ☐₀ Yes ☐₁
6. **Do you sometimes share antibiotics with family and friends, such as leftover doses from last time?** No ☐₀ Yes ☐₁

Swahili Translation

Nambari ya Utafiti wa Mshiriki	
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1. Maelezo ya msingi:

a	Umri	
b	Jinsia	
c	Kiwango cha Elimu ya Juu	
d	Kazi	

2. Dalili (Tafadhali kumbuka dalili zote kama vile maambukizi ya jeraha na hali nyingine kali pia):

	Symptoms	Tick if present
a	Hali ya joto jingi mwilini	
b	Kuharisha	
c	Kutapika	
d	Kamasi au kinyesi chenye damu	
e	Eleza dalili zingine zozote	

3. Je, umetumia dawa zozote za kuua viuavijasumu kabla ya wakati huu?

- Hapana
- Ndiyo

Ikiwa Ndiyo, tafadhali onyesha jina la dawa (onyesha jina kwenye kisanduku/kifungashio)_____

4. Ikiwa Ndiyo katika 3 hapo juu, je, dawa ya kukinga dawa ilitolewa na mtaalamu wa matibabu au wewe ulijitibu mwenyewe?

- Imetolewa na mtaalamu
- Kujitibu

5. Je, huwa unahakikisha umemaliza dozi za antibiotiki?

- **Hapana**
- **Ndiyo**

6. Je, wakati mwingine unashiriki dawa za kuua viuavijasumu na familia na marafiki, kama vile dozi zilizobaki mara ya mwisho?

- **Hapana**
- **Ndiyo**

Appendix II: Informed Consent Forms

Informed Consent Form (English Version)

Study Title: Molecular Characterization of ESBL-Producing *Enterobacteriaceae* from Patients with Gastrointestinal Infections at Kisii Teaching and Referral Hospital

Name of Principal Investigator(s):

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Name of Organization: KEMRI Graduate School, Kisii University

This Informed Consent Form has two parts:

- Part I: Information Sheet [to share information about the study with you]
- Part II: Certificate of Consent [for signatures if you choose to participate]

Summary

The purpose of this study is to determine the rate of resistant enteric bacteria, and the genes that cause these bacteria to be non-responsive to antibiotic treatment. It is planned to include 174 patients with symptoms of infection such as fever and diarrhoea. This is a cross-sectional study hence no follow-up or experimental treatment will be involved. Participants will be asked to complete a questionnaire. Stool samples will be collected at the hospital. The total duration of participation is three months. Participation in this study is entirely voluntary.

Part I: Patient Information Sheet

Dear Sir/Madam,

You have been invited to participate in this research study. Before you decide to participate, please read this information sheet carefully.

1. Who is organizing and financing this study?

This study is organized by the PRINCIPAL INVESTIGATOR, Kavulavu Briton, who is a Master of Science (Medical Microbiology) student at KEMRI Graduate School. The study is primarily financed by the Universities of Hohenheim and Tübingen in Germany.

2. What is the purpose of this study?

We all carry a large number of non-harmful bacteria that live in our gut, also known as gut flora. When taking antibiotics, these bacteria can develop resistance to antibiotics called Extended-spectrum beta-lactamase-producing *Enterobacteriaceae*. In simple terms, the bacteria produce substances to break down antibiotics and therefore cannot be killed by these antibiotics. The main reservoir for patients colonized by these ESBL-PE bacteria is the digestive tract. The purpose of this study is to assess the genes that cause such resistance.

3. Why have I been chosen?

You were chosen for one of two reasons: You are in the hospital with fever and/or diarrhoea and you are suspected to have a gastrointestinal bacterial infection in your gut by the clinician.

4. Who cannot participate?

You cannot participate if you have taken antibiotics before coming to the hospital. In addition, those who refuse to give consent or have been subjected to antibiotic usage within the last week will be excluded.

5. Can I be excluded from the study after I give my consent?

Yes! Participation in this study is purely voluntary and you have freedom to withdraw at any time.

6. Do I have to take part in this study and what are my rights?

No. It is not a must. Your participation in this study is completely voluntary. Not taking part in the study will not affect your future medical care. The same principle applies if you revoke your initial consent after starting the study. You can therefore withdraw your participation at any time without having to justify your decision.

7. What should I do if I choose to participate in this study?

If you agree to take part in this study, the study investigator will ask you to sign a consent form to confirm that you understand the study and agree to participate. You will be asked questions about your illness, medical history, contact with animals and dietary preferences. You will be asked to provide a stool sample.

8. What are my obligations in the study?

Aside from giving your specimen, you have no other obligations.

9. What microbiological samples will be taken? Will these tests cause any discomfort?

To search for the presence of resistant bacteria, a stool sample is necessary. This sampling does not cause any physical discomfort.

10. What happens to the samples once they are collected?

The specimen will be subjected to microbiological analysis to determine any bacteria of interest present. The bacteria will be checked for sensitivity to common drugs before molecular methods are used to check for the genes harboured by the bacteria.

11. What are the benefits and drawbacks of the study?

Participation in the study does not create any direct benefit for you. However, if pathogenic bacteria that may aid in your treatment are noted, the result will be communicated to you to aid in your treatment. The main benefit is that you will help improve the understanding of antimicrobial resistance, its transmission and persistence. The results of this research may help to improve antibiotic treatment of infections with resistant bacteria in the future. The only disadvantage of participation is the time required for sample collection and the questionnaire.

12. What will these results be used for?

The results of this study will be shared with other researchers and physicians in Kenya and Europe. They will be used to improve antibiotic treatment and minimize the risk of antibiotic resistance.

13. Will I be informed of new findings from the study?

Once the study is completed and analyzed, you will also be informed of the results in writing.

14. Is my participation confidential?

All of your personal and medical data collected during the study will be treated as confidential. Only the investigators of this study are authorized to access the data. Published findings will be de-identified.

Contact Information for questions about the study

If you have any more questions, please feel free to contact the principal investigators of the study:

Kavulavu, Briton Matata

Phone: 0702086822

Email: kavulavubright@gmail.com

For questions about your rights as a participant, you may contact the KEMRI Scientific Ethics Review Unit, P.O Box 54840-00200, Nairobi; telephone 0717719477, 0776399979; email address: seru@kemri.go.ke; Kemriseru18@gmail.com

Part II: Informed Consent Form

I declare that I have been informed orally and in writing by the signing health worker about the above-mentioned study and agree to participate.

By signing this form,

- ☐ I agree to my stool being tested for resistance to antibiotics and any other tests relevant to the mentioned study
- ☐ I agree to my specimen and/or bacteria isolated therein being stored for future analyses.

Place, date		Signature of the patient
Place, date		Name and signature of the Principal Investigator / Co-investigator

If illiterate

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Illiterate participants should include their thumbprints as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

*Print name of witness*_____

AND

Thumb print of

Signature of witness _____

Date _____

Informed Consent Form (Swahili Version)

Study Title: Molecular Characterization of ESBL-Producing *Enterobacteriaceae* from Patients with Gastrointestinal Infections at Kisii Teaching and Referral Hospital

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Jina la Shirika: Chuo Kikuu cha Kisii, Chuo Kikuu cha KEMRI

Fomu hii ya idhini iliyo na habari ina sehemu mbili:

Sehemu ya 1: Habari kwa washiriki

Sehemu ya II: Hati ya idhini

Muhtasari

Madhumuni ya utafiti huu ni kuamua kiwango cha bakteria sugu ya enteric, na jeni zinazosababisha bakteria hawa kuwa wasio na majibu kwa matibabu ya antibiotiki. Inatarajiwa kujumuisha wagonjwa 174 wenye dalili za maambukizi kama vile homa na kuhara. Hii ni utafiti wa sehemu ya msalaba kwa hivyo hakuna ufuatiliaji au matibabu ya majaribio yatahusika. Washiriki wataulizwa kukamilisha dodoso. Sampuli za Stool zitakusanywa katika hospitali. Muda wote wa kushiriki ni miezi minne. Kushiriki katika utafiti huu ni kwa hiari kabisa.

Sehemu I: Habari Kwa Washiriki

Umealikwa kushiriki katika utafiti huu. Kabla ya kuamua kushiriki, tafadhali soma karatasi hii ya habari kwa umakini.

1) Ni nani wanaoendesha na kufadhili utafiti huu?

Utafiti huu umeandaliwa na shule ya Tiba ya Chuo Kikuu cha Kisii na shule ya Chuo Kikuu cha Kemri na imeidhinishwa na kamati ya maadili ya Utafiti ya chou kikuu cha Moi.

2) Kusudi la utafiti huu ni nini?

Sisi sote hubeba idadi kubwa ya bakteria wasio na madhara ambao hukaa ndani ya utumbo wetu, pia hujulikana kama mimea ya utumbo. Wakati tunakunywa dawa ya antibiotic, mimea asili ya tumbo na pia bakteria inayosababisha magonjwa hutoa madini yanayoharibu antibiotic ili ziwe sugu. Madini haya yanaitwa ESBL na yakiadhiri utumbo wale bakteria wanayoyatoa huitwa ESBL-PE. Kuenea kwa bakteria sugu ya dawa ni moja wapo ya shida muhimu katika upinzani wa antimicrobial. Kuenea kwa bakteria sugu ya dawa ni moja wapo ya shida muhimu katika upinzani wa antimicrobial. Ni tishio ulimwenguni kwa afya ya umma, sio tu katika vituo vya huduma za afya lakini pia katika jamii. Madhumuni ya utafiti wa KONSERVE-AB ni kutathmini ikiwa upinzani wa bakteria kwa watu binafsi hupitishwa kutoka au kwa wanyama au mazingira katika kaya zao. Inalenga kuamua kiwango cha maambukizo haya na sababu zinazoweka watu kwa hatari ya maambukizi haya. Imepangwa kujumuisha wagonjwa 191 huko Kisii kuanzia Mei hadi Septemba 2021. Kwa wagonjwa sugu tutakusanya data kutoka kwa mazingira ya nyumba zao pamoja na kinyesi cha wanyama, udongo na maji. Huu ni utafiti wa uchunguzi, na hakuna matibabu ya majaribio yanayotekelezwa.

3) Kwa nini nimechaguliwa?

Ulichaguliwa kwa sababu moja wapo: Uko hospitalini na homa na kuhara na unashukiwa kuwa na maambukizo ya bakteria ya *Enterobacteriaceae* ndani ya utumbo wako.

4) Nani hawezi kushiriki?

Huwezi kushiriki ikiwa umechukua antibiotics kabla ya kuja hospitali. Zaidi ya hayo, wale wanaokataa kutoa kibali au wamepewa matumizi ya viuvijasumu ndani ya wiki iliyopita hawatajumuishwa.

5) Je! Ninaweza kutengwa kwenye utafiti baada ya kutoa idhini yangu?

Ndiyo! Kushiriki katika utafiti huu ni kwa hiari tu na una uhuru wa kujiondoa wakati wowote.

6) Je! Lazima nishiriki katika utafiti huu na haki zangu ni zipi?

Hapana. Sio lazima. Kushiriki kwako katika utafiti huu ni kwa hiari kabisa. Kutoshiriki katika utafiti hakutaathiri huduma yako ya matibabu ya baadaye. Kanuni hiyo hiyo inatumika ikiwa utabatilisha idhini yako ya awali baada ya kuanza utafiti. Kwa hivyo unaweza kuondoa ushiriki wako wakati wowote bila kuhitaji kuhalalisha uamuzi wako.

7) Nifanye nini ikiwa nitashiriki katika utafiti huu?

Ikiwa unakubali kushiriki katika utafiti huu, mchunguzi wa utafiti atakuuliza utie sahihi fomu ya idhini ili kuthibitisha kwamba unaelewa utafiti na kukubali kushiriki. Utaulizwa maswali kuhusu ugonjwa wako, historia ya matibabu, kuwasiliana na wanyama na mapendekezo ya chakula. Utaulizwa kutoa sampuli ya kinyesi.

8) Majukumu yangu katika utafiti ni nini?

Kando na kutoa sampuli yako ya kinyesi, huna majukumu mengine.

9) Ni sampuli gani za microbiolojia zitakazochukuliwa? Je! Vipimo hivi vitasababisha usumbufu wowote?

Ili kutafuta uwepo wa bakteria sugu, sampuli ya kinyesi inahitajika. Sampuli hii haisababishi usumbufu wowote wa mwili.

10) Ni nini hufanyika kwa sampuli mara zinapokusanywa?

Sampuli hiyo itafanyiwa uchanganuzi wa kibiolojia ili kubaini bakteria yoyote ya kuvutia iliyopo. Bakteria hao watachunguzwa ili kubaini unyeti kwa dawa za kawaida kabla ya mbinu za molekuli kutumika kuangalia jeni zilizowekwa na bakteria.

11) Je! Kuna faida gani na mapungufu gani katika utafiti huu?

Kushiriki katika utafiti hakuleti manufaa yoyote ya moja kwa moja kwako. Walakini, ikiwa bakteria ya pathogenic ambayo inaweza kusaidia katika matibabu yako itatambuliwa, matokeo yatawasilishwa kwako ili kusaidia katika matibabu ya yoru. Faida kuu ni kwamba utasaidia kuboresha uelewa wa upinzani wa antimicrobial, maambukizi yake na kuendelea. Matokeo ya utafiti huu yanaweza

kusaidia kuboresha matibabu ya antibiotiki ya maambukizo na bakteria sugu katika siku zijazo. Hasara pekee ya ushiriki ni muda unaohitajika kwa ajili ya kukusanya sampuli, na dodoso.

12) Matokeo haya yatatumika kwa njia gani?

Matokeo ya utafiti huu yatashirikiwa na watafiti na madaktari wengine nchini Kenya na Ulaya. Zitatumika kuboresha matibabu ya antibiotic na kupunguza hatari ya upinzani wa antibiotics.

13) Je! Nitaarifiwa juu ya matokeo mapya kutoka kwa utafiti?

Baada ya utafiti kukamilika na kuchambuliwa, utafahamishwa pia matokeo kwa maandishi.

14) Je! Ushiriki wangu ni wa siri?

Data yako yote ya kibinafsi na ya matibabu iliyokusanywa wakati wa utafiti itachukuliwa kuwa siri. Wachunguzi wa utafiti huu pekee ndio wameidhinishwa kufikia data. Matokeo yaliyochapishwa yatatambuliwa.

Maelezo ya Mawasiliano

Ikiwa una maswali yoyote zaidi, tafadhali jisikie huru kuwasiliana na wachunguzi wakuu wa utafiti:

Kavulavu, Briton Matata

Phone: 0702086822

Email: kavulavubright@gmail.com

Kwa maswali kuhusu haki zako kama mshiriki, unaweza kuwasiliana na Kamati ya Maadili na Utafiti ya Kitaasisi ya KEMRI, P.O Box 54840-00200, Nairobi; kwa simu 0717719477, 0776399979; au barua pepe: seru@kemri.go.ke; Kemriseru18@gmail.com

Hati ya Ithini

Ninatangaza kwamba nimearifiwa kwa mdomo na kwa maandishi na mfanyakazi wa afya aliyetia saini kuhusu utafiti uliotajwa hapo juu na kukubali kushiriki.

Kwa kusaini fomu hii,

- Ninakubali kinyesi changu kupimwa ukinzani dhidi ya viuavijasumu na vipimo vingine vyovyote vinavyohusiana na utafiti uliotajwa.
- Ninakubali kielelezo changu na/au bakteria zilizowekwa humo kuhifadhiwa kwa uchanganuzi wa siku zijazo.

Mahali, Tarehe	Sahihi ya mgonjwa
Mahali, Tarehe	Jina na sahihi ya Mtafiti mkuu/ Mtafiti mwenza

Ikiwa hajui kusoma na kuandika

Shahidi anayesoma lazima asaini (ikiwezekana, mtu huyu anapaswa kuchaguliwa na mshiriki na haipaswi kuwa na uhusiano na timu ya utafiti). Washiriki ambao hawajui kusoma na kuandika wanapaswa kujumuisha alama zao za vidole pia

Nimeshuhudia usomaji sahihi wa fomu ya idhini kwa mshiriki anayeweza kushiriki, na mtu huyo amepata nafasi ya kuuliza maswali. Ninathibitisha kuwa mtu huyo ametoa idhini kwa uhuru.

Chapisha Jina la shahidi _____ NA Uchapishaji wa kidole

gumba cha mshiriki

Sahihi ya shahidi _____

Tarehe _____

Informed Consent Form (Kisii Version)

Efomu Y'ogwancherana

Omotwe bw'erisomo: Okomanyekana gwa ekemorekiura kwa chibakteria chitari koigwa amariogo(ESBL-PE) ase abarwaire baigwete enda bobbe ase enyagitari ya Kisii Teaching and Referral.

Omochungusi omonene:

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MSc Student

Abakonyi baye:

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Rieta ria esukuru: KEMRI Graduate School, Kisii University

Efomu eye y’ogwancherana ebwate chinsemo ibere:

- Ensemo entang’ani: Ensemo y’obomanyi [gokomanyia igoro y’obochungusi obo]
- Ensemo ya kabere: Esatibiketi y’ogwancherana [obeke esiginecha onye kogwancherana]

Obweng’e

Esababu y’ogotwara obochungusi obo n’okomanya ekerengo kia ogoitaigwera amariogo kia chibakteria chia amatumbo (ESBL-PE), na kobwekania na amasingira korigia gose okwambokera nkoroo ase abarwaire batokire na bakteria echi. Ebangire korigereria abarwaire mia mbili babwate riberera nainde bagosaa. Obo n’obochungusi borenetege egekundi; tokobwatereria totari korwari.

Baria bagoshiriki bagosabwa baichori amachibu. Echoo enene n’eganeiri nyagitari. Engaki y’ogoshiriki n’emetienyi ene. Ogoshiriki n’ogwancha kwago.

Ensemo entang’ani: Obomanyia gochia ase omorwaire

Aye,

Gwachikirwe goshiriki ase obochungusi obo. Nyuma otarachora goshiriki, soma ensemo eye.

1. Ning’o orosirie na gosiira obochungusi obo?

Risomo eri erirosiri na OMOCHUNGUSI OMONENE , Kavulavu Briton, ogosomera Masters y’ebinyinyi ase esukuru ya KEMRI Graduate. Chibesa chio’gosira risomo eri chikorwegwa ne chiyunivasiti chia Hohenheim na Tübingen chia Bocherumani.

2. Ninki ekerengo kia obochungusi obo?

Intwe twensi nigo tobwate chibakteria chiri chitari koreta oborwaire gochakera ase omonwe baka ase amatumbo. Kere tokonywa amariogo, chibakteria echi nabo chitaigwere amariogo gete, echikorokwa(Extended-spectrum beta-lactamase-producing *Enterobacteriaceae*). Chibakteria echi nigo chikorusi ebinto bikorwani na goita amariogo. Ase igo, chibakteria echi nchikonyara goitwa n’amariogo aya. Chibakteria echi mono echimenyete ase amatumbo. Ekerengo ki’erisomo eri n’okorigia chichini chikoreta ogoitaigwera amariogo oko.

3. 'Nasekie nachorirwe?

Nigo gwachorirwe ase yeching'encho ibere: 'Gwachire nyagitari neriberera gose ogosaa nainde okageire gotwara chibakteria chia amatumbo n'omonyagitari.

4. Barabie batanyare goshiriki?

Tokonyara goshiriki onye komenyete goetania chikilomita emerongo ebere na isano korwa Bosongo. Komenta naigo, barie bagokana goancherana gose baria batimiire amariogo ase ewiki eyemo yaetire.

5. Ninyare korusigwa ase risomo eri nonya konyora naancheranire?

Eeeh! Ogoshiriki ase risomo eri n'ogwancha kwago, ase igo nabo okwerusia chinsa chinde chionsi oeigwete.

6. Nerasima n'shiriki naende okobua kwane nkoroki?

Yaya. Tori korasimiswa. Ogoshiriki n'ogwancha kwago. Okwanga kwago goshiriki nkobwati boiri bonde bwensi n'okorwarigwa kwago mosuko. Nabo ere igo noerusia kere gwa'ncheranire. Nabo okoerusia otari goetetea.

7. Ninki mbwenerete gokora kere naacheranire?

Kere gw'ancheranire omochungusi nagocike obeke esiginecha yago inse egere amanye gose kwamanyire obochungusi obo naende ing'a gw'anchiranirie. N'oborigwe amaswari igoro y'oborwaire bwago, gose kwanya korwara rinde, gose nkwomerana ore n'etugo na endagera yago. Nochikwe orete echo enene.

8. Ninki naende imbwenerete gokora?

Gotatiga na koreta echo enene, tobwate egasi yende.

9. N'esampo ki eganeirie? Chitest echi nchindetere obokong'u bonde bwonsi?

Echoo enene nero eganeirie egere tonyare korigia chibakteria chitari koigu amariogo. Korusia echo enene ngokonyara gokoretera obokong'u bonde bwonsi.

10. Ninki chisampo chogokorerwa kere chiarusigwe?

Ebinyiyi bia bakteria ebikorigigwa naende bipimu gose nkoigwera bire amariogo akawaida arafu chichi chikoreta ogotaigwera amariogo nachironywe chirigigwe onye bakteria echio nchiri koigwera amariogo.

11. Naki ‘ngokonyeka gose ‘ntari gokonyeka korwa ase risomo eri?

Ogoshiriki ase risomo eri ‘nkori n’obokonyi bwa enchera eyemo ase eyemo gocha asore. Rakini, onye chibakteria chikoreta oborwaire nchitoke nabo okomanyigwa egere orwarigwe. Obokonyi obonene ‘mbori ing’a orakonye komenta okomanya igoro ya ogoitaigwa amariogo n’okwambokera kwa ebinyinyi. Amatokeo y’obochungusi obo ngokonye korigia amariogo aria aranyare goita chibakteria echi chitari koigwa amariogo. Ne’chinsa nkeigo orasirie koreta echo na goichoria risakara eri.

12. Amatokeo ninki agotumeka gokora?

Amatokeo y’obochungusi obo nigo yagosangwa na abachungusi bande amo na abanyagitari korwa ense ya Kenya na korwa mache ng’umbu. Nigo agotumekwa komenta oborwari bwe ebinyinyi birie bitari koigwa amariogo na gopungusa ekerengo kia ebinyinyi gotaigwera amariogo.

13. Inee, nimanyigwe obomanyi obonyia boratoke korwa ase obochungusi obo?

Kere obochungusi bwaerire, nomanyigwe obomanyi oboyia obwe boratoke goetera enchera y’amariko.

14. Ogoshiriki kwane ‘mbobisi kore?

Ebimanyerie biago bionsi pi nigo tokobigacha bobisi. Abachungusi boka nabwo baancheire korora. Amachibu y’omoerio tagotwara ebimanyerio biago.

Chinamba ase oraborie amaswari igoro y’obochungusi obo

Onye kobwate amaswari ande, moborie omochungusi omonene ase enamba ekobwatia:

Briton Kavulavu

0702086822

kavulavubright@gmail.com

Ase amaswari y’okobua kwago buna omoshiriki, nabo okorigia ekomitii y’obochungusi KEMRI Scientific Ethics Review Unit, P.O Box 54840-00200, Nairobi; ase enamba 0717719477, 0776399979; gose seru@kemri.go.ke; Kemriseru18@gmail.com

Ensemu ya kabere : Efomu y’ogwancherana

Natianire ing'a namanyigwe igoro y'obochungusi obo nainde naanchire goshiriki.

Ase ogosaini efomu eye,

- ☐ Naanchire echoo yane epimwe gose ebinyi birie bitari koigwera amariogo mbiroo ne chitesti chinde chia manna ase obochungusi obo.
- ☐ Naanchire echoo yaane gose ebinyinyi ebio biratoke bigachwe bitumeke mosuko.

Ase, chinsa	Esiginecha y'omorwaire
Ase, chinsa	Rieta n'esiginecha y'omochungusi omonene/ omokonyi oye

Onye tomanyeti gosoma na korika

Omonto ekabuna oyo baka akonywe gosaini na barie bamanyete gosoma na korika(Onye gekonyarekana, omoshiriki tika aechorere oyo oramosaineirie nainde tatwara boiri bonde bwensi n'abachungusi). Abashiriki buna aba nabarabwo baka babeke ekiara.

Narorire omoshiriki kagosoma buya efomu eye y'ogwancherana, naende omoshiriki nanyora ribaga ri'okoboria amaswari. Naanchire ing'a nere bweka oerwera.

Rieta ria oy'orora _____ NA Ekiara kia omoshiriki

Esiginecha ya oy'orora _____

Chitariki _____

Rituko/omotienyi/omwaka

Appendix III: Assent Forms for Minors

Assent Forms for Minors

Study Title: Molecular Characterization of ESBL-Producing *Enterobacteriaceae* from Patients with Gastrointestinal Infections at Kisii Teaching and Referral Hospital

Name of Principal Investigator(s):

Kavulavu, Briton Matata

Phone: 0702086822

Email: kavulavubright@gmail.com

MSc Student

Co-investigators:

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Phone: 0722689073

knyerere@jkuat.ac.ke

Name of Organization: KEMRI Graduate School, Kisii University

This Informed Consent Form has two parts:

- Part I: Information Sheet [to share information about the study with you]
- Part II: Certificate of Consent [for signatures if you choose to participate]

Summary

We're studying bacteria in people's bodies that don't respond to antibiotics. We're asking 174 people with symptoms like fever and diarrhoea to help. They'll answer questions and give a small sample of their poop. The study lasts three months. It's up to them if they want to join. We won't give them any special treatment, and we won't follow up on them afterwards. It's just about understanding these bacteria better so we can help people get better when they're sick. They don't have to do it if they don't want to. It's all their choice.

Part I: Patient Information Sheet

Dear,

You have been invited to participate in this research study. Before you decide to participate, please read this information sheet carefully.

1. Who is organizing and financing this study?

A student named Kavulavu Briton and universities in Germany are organizing and funding this study.

2. What is the purpose of this study?

We want to know why some bacteria in our stomachs can resist antibiotics. We're studying these bacteria and the genes that make them resistant.

3. Why have I been chosen?

You're chosen because you're in the hospital with a fever and/or diarrhoea, and the doctor thinks you might have a gut infection.

4. Who cannot participate?

You can't participate if you've already taken antibiotics before coming to the hospital, or if you refuse to agree to participate.

5. Can I be excluded from the study after I give my consent?

Yes! You can leave the study anytime you want. It's totally up to you.

6. Do I have to take part in this study and what are my rights?

No, it's your choice. Not joining won't affect your treatment. You can leave anytime without having to explain.

7. What should I do if I choose to participate in this study?

If you agree to join, you'll sign a paper to show you understand and agree. You'll answer some questions and give a stool sample.

8. What are my obligations in the study?

You just need to give a stool sample. There's nothing else you have to do.

9. What microbiological samples will be taken? Will these tests cause any discomfort?

We'll take a stool sample to check for resistant bacteria. It won't hurt at all.

10. What happens to the samples once they are collected?

We'll test them to see if there are any resistant bacteria and what genes they have.

11. What are the benefits and drawbacks of the study?

You won't get any direct benefits, but if we find bacteria that can help your treatment, we'll let you know. The main benefit is that you will help improve the understanding of antibiotic resistance. The only downside is the time it takes for the tests and questions.

12. What will these results be used for?

The results will be shared with other scientists and doctors to improve antibiotic treatment and prevent antibiotic resistance.

13. Will I be informed of new findings from the study?

Yes, we'll tell you what we find once we're done with the study.

14. Is my participation confidential?

Yes, we'll keep all your info private. Only the people running the study can see it. When we share our findings, we won't include any personal details.

Contact Information for questions about the study

If you have any more questions, please feel free to contact the principal investigators of the study:

Kavulavu, Briton Matata

Phone: 0702086822

Email: kavulavubright@gmail.com

For questions about your rights as a participant, you may contact the KEMRI Scientific Ethics Review Unit, P.O. Box 54840-00200, Nairobi; telephone 0717719477, 0776399979; email address: seru@kemri.go.ke; Kemriseru18@gmail.com

Part II: Informed Consent Form

I declare that I have been informed orally and in writing by the signing health worker about the above-mentioned study and agree to participate.

By signing this form,

- ☐ I agree to my stool being tested for resistance to antibiotics and any other tests relevant to the mentioned study
- ☐ I agree to my specimen and/or bacteria isolated therein being stored for future analyses.

Place, date		Signature of the patient
Place, date		Name and signature of the Principal Investigator / Co-investigator

If illiterate

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Illiterate participants should include their thumbprint as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness _____
participant

AND

Thumb print of

Signature of witness _____

Date _____

--

Appendix IV: Assent Forms for Minors (Swahili Version)

Kichwa cha Utafiti: Tabia ya Masi ya ESBL-Kuzalisha *Enterobacteriaceae* kutoka kwa Wagonjwa walio na Maambukizi ya Tumbo katika Hospitali ya Kufundisha na Rufaa ya Kisii

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MSc Student

Watafiti Wenza:

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Jina la Shirika: KEMRI Graduate School, Kisii University

Fomu hii ya idhini ya taarifa ina sehemu mbili:

- Sehemu ya I: Karatasi ya Habari [kushiriki habari kuhusu utafiti na wewe]
- Sehemu ya II: Cheti cha idhini [kwa saina ikiwa utachagua kushiriki]

Muhtasari

Tunajifunza bakteria katika miili ya watu ambayo haijibu antibiotics. Tunaomba watu 174 wenye dalili kama vile homa na kuhara wasaidie. Watajibu maswali na kutoa sampuli ndogo ya poop yao. Utafiti huu huchukua miezi mitatu. Ni juu yao kama wanataka kujiunga. Hatutawapa matibabu yoyote maalum, na hatutawafuata baadaye. Ni juu ya kuelewa bakteria hawa vizuri ili tuweze kuwasaidia watu kupata nafuu wakati wanapokuwa wagonjwa. Hawana haja ya kufanya hivyo ikiwa hawataki. Ni uamuzi wao wote.

Sehemu ya I: Karatasi ya Habari

Wapendwa

Umealikwa kushiriki katika utafiti huu. Kabla ya kuamua kushiriki, tafadhali soma karatasi hii ya habari kwa uangalifu.

1. Ni nani anayeandaa na kufadhili utafiti huu?

Mwanafunzi anayeitwa Kavulavu Briton na vyuo vikuu nchini Ujerumani wanaandaa na kufadhili utafiti huu.

2. Kusudi la utafiti huu ni nini?

Tunataka kujua kwa nini baadhi ya bakteria katika tumbo zetu wanaweza kupinga antibiotics. Tunajifunza bakteria hawa na jeni zinazowafanya kuwa sugu.

3. Kwa nini nimechaguliwa?

Unachaguliwa kwa sababu uko hospitalini na homa na / au kuhara, na daktari anadhani unaweza kuwa na maambukizi ya utumbo.

4. Ni nani asiyeweza kushiriki?

Huwezi kushiriki ikiwa tayari umechukua antibiotics kabla ya kuja hospitali, au ikiwa unakataa kukubali kushiriki.

5. Je, ninaweza kutengwa na utafiti baada ya kutoa idhini yangu?

Ndiyo! Unaweza kuacha utafiti wakati wowote unataka. Ni juu yako kabisa.

6. Je, ni lazima nishiriki katika utafiti huu na haki zangu ni zipi?

Hapana, ni uamuzi wako. Kutojiunga hakutaathiri matibabu yako. Unaweza kuondoka wakati wowote bila ya kueleza.

7. Nifanye nini ikiwa nitachagua kushiriki katika utafiti huu?

Ikiwa unakubali kujiunga, utasaini karatasi ili kukuonyesha kuelewa na kukubaliana. Utajibu maswali kadhaa na kutoa sampuli ya kinyesi.

8. Ni nini wajibu wangu katika utafiti?

Unahitaji tu kutoa sampuli ya kinyesi. Hakuna kitu kingine unapaswa kufanya.

9. Ni sampuli gani za microbiological zitachukuliwa? Je, majaribio haya yatasababisha usumbufu wowote?

Tutachukua sampuli ya kinyesi ili kuangalia bakteria sugu. Haitakuwa na madhara hata kidogo.

10. Ni nini hufanyika kwa sampuli mara tu zinapokusanywa?

Tutawajaribu kuona ikiwa kuna bakteria yoyote sugu na ni jeni gani wanazo.

11. Ni faida na hasara gani za utafiti?

Hutapata faida yoyote ya moja kwa moja, lakini ikiwa tunapata bakteria ambayo inaweza kusaidia matibabu yako, tutakujulisha. Faida kuu ni kwamba utasaidia kuboresha uelewa wa upinzani wa antibiotiki. Ubaya pekee ni wakati inachukua kwa majaribio na maswali

12. Matokeo haya yatatumika kwa ajili ya nini?

Matokeo yatashirikiwa na wanasayansi wengine na madaktari ili kuboresha matibabu ya antibiotiki na kuzuia upinzani wa antibiotiki.

13. Je, nitajulishwa kuhusu matokeo mapya kutoka kwa utafiti?

Ndio, tutakuambia kile tunachopata mara tu tutakapomaliza na utafiti.

14. Je, ushiriki wangu ni wa siri?

Ndio, tutaweka maelezo yako yote faragha. Ni watu tu wanaoendesha utafiti wanaweza kuona. Tunaposhiriki matokeo yetu, hatutajumuisha maelezo yoyote ya kibinafsi.

Maelezo ya Mawasiliano

Ikiwa una maswali yoyote zaidi, tafadhali jisikie huru kuwasiliana na wachunguzi wakuu wa utafiti:

Kavulavu, Briton Matata

Phone: 0702086822

Email: kavulavubright@gmail.com

Kwa maswali kuhusu haki zako kama mshiriki, unaweza kuwasiliana na Kamati ya Maadili na Utafiti ya Kitaasisi ya KEMRI, P.O Box 54840-00200, Nairobi; kwa simu 0717719477, 0776399979; au barua pepe: seru@kemri.go.ke; Kemriseru18@gmail.com

Hati ya Ithini

Ninatangaza kwamba nimearifiwa kwa mdomo na kwa maandishi na mfanyakazi wa afya aliyetia saina kuhusu utafiti uliotajwa hapo juu na kukubali kushiriki.

Kwa kusaini fomu hii,

- Ninakubali kinyesi changu kupimwa ukinzani dhidi ya viuavijasumu na vipimo vingine vyovyote vinavyohusiana na utafiti uliotajwa.
- Ninakubali kielelezo changu na/au bakteria zilizowekwa humo kuhifadhiwa kwa uchanganuzi wa siku zijazo.

Mahali, Tarehe	Sahihi ya mgonjwa
Mahali, Tarehe	Jina na sahihi ya Mtafiti mkuu/ Mtafiti mwenza

Ikiwa hajui kusoma na kuandika

Shahidi anayesoma lazima asaini (ikiwezekana, mtu huyu anapaswa kuchaguliwa na mshiriki na haipaswi kuwa na uhusiano na timu ya utafiti). Washiriki ambao hawajui kusoma na kuandika wanapaswa kujumuisha alama zao za vidole pia

Nimeshuhudia usomaji sahihi wa fomu ya idhini kwa mshiriki anayeweza kushiriki, na mtu huyo amepata nafasi ya kuuliza maswali. Ninathibitisha kuwa mtu huyo ametoa idhini kwa uhuru.

Chapisha Jina la shahidi _____

NA

Uchapishaji wa

Sahihi ya shahidi _____

Tarehe _____

gumba cha mshiriki

--

Appendix V: Parental Consent Forms

Parental Permission Consent form (English Version)

Study Title: Molecular Characterization of ESBL-Producing *Enterobacteriaceae* from Patients with Gastrointestinal Infections at Kisii Teaching and Referral Hospital

Name of Principal Investigator(s):

Kavulavu, Briton Matata

Phone: 0702086822

Email: kavulavubright@gmail.com

Co-investigators:

Dr Eric Omori Omwenga

Department of Medical Microbiology & Parasitology

School of Health Sciences

Kisii University

Phone: 0725806875

Email: omorieric@gmail.com

Dr Elizabeth Matey

Senior Research Scientist

Centre for Microbiology Research

Kenya Medical Research Institute

Phone: 0721604849

ematey@kemri.go.ke

Dr Oscar Nyangiri

Department of Medical Microbiology & Parasitology

School of Health Sciences

Kisii University

Phone: 0720709392

oasanya@gmail.com

Dr Andrew Nyerere

Department of Medical Microbiology

School of Biomedical Sciences

Jomo Kenyatta University of Agriculture and Technology

Phone: 0722689073

knyerere@jkuat.ac.ke

Name of Organization: KEMRI Graduate School, Kisii University

This Informed Consent Form has two parts:

- Part I: Information Sheet [to share information about the study with you]
- Part II: Certificate of Consent [for signatures if you choose to participate]

Summary

This study aims to assess the prevalence of antibiotic-resistant enteric bacteria and identify associated genetic markers. It will involve the enrollment of 174 patients presenting symptoms indicative of infection, such as fever and diarrhoea, in a cross-sectional study design. No follow-up or experimental treatments are planned. Participation entails the completion of a questionnaire and the provision of stool samples at the hospital. The duration of involvement is three months. Participation of your child is voluntary.

Part I: Patient Information Sheet

Dear Sir/Madam,

Your child has been invited to participate in this research study. Before you decide to participate permit the participation of your child, please read this information sheet carefully.

15. Who is organizing and financing this study?

This study is organized by the PRINCIPAL INVESTIGATOR, Kavulavu Briton, who is a Master of Science (Medical Microbiology) student at KEMRI Graduate School. The study is primarily financed by the Universities of Hohenheim and Tübingen in Germany.

16. What is the purpose of this study?

We all carry a large number of non-harmful bacteria that live in our gut, also known as gut flora. When taking antibiotics, these bacteria can develop resistance to antibiotics called Extended-spectrum beta-lactamase-producing *Enterobacteriaceae*. In simple terms, the bacteria produce substances to break down antibiotics and therefore cannot be killed by these antibiotics. The main reservoir for patients colonized by these bacteria is the digestive tract. The purpose of this study is to assess the genes that cause such resistance.

17. Why has my child been chosen?

Your child was chosen for one of two reasons: They are in the hospital with fever and/or diarrhoea and are suspected to have a gastrointestinal bacterial infection in your gut by the clinician.

18. Who cannot participate?

Your child cannot participate if you have taken antibiotics before coming to the hospital. In addition, those who refuse to give consent or have been subjected to antibiotic usage within the last week will be excluded.

19. Can my child be excluded from the study after I give my consent?

Yes! Participation in this study is purely voluntary and you have the freedom to withdraw your child at any time.

20. Do I have to take part in this study and what are my rights?

No. It is not a must. Your participation in this study is completely voluntary. Not taking part in the study will not affect your future medical care. The same principle applies if you revoke your initial consent after starting the study. You can therefore withdraw your participation at any time without having to justify your decision.

21. What should I do if I choose to participate in this study?

If you agree to take part in this study, the study investigator will ask you to sign a consent form to confirm that you understand the study and agree to participate. You will be asked questions about your illness, medical history, contact with animals and dietary preferences. You will be asked to provide a stool sample.

22. What are my obligations in the study?

Aside from giving your specimen, you have no other obligations.

23. What microbiological samples will be taken? Will these tests cause any discomfort?

To search for the presence of resistant bacteria, a stool sample is necessary. This sampling does not cause any physical discomfort.

24. What happens to the samples once they are collected?

The specimen will be subjected to microbiological analysis to determine any bacteria of interest present. The bacteria will be checked for sensitivity to common drugs before molecular methods are used to check for the genes harboured by the bacteria.

25. What are the benefits and drawbacks of the study?

Participation in the study does not create any direct benefit for you. However, if pathogenic bacteria that may aid in your treatment are noted, the result will be communicated to you to aid in your treatment. The main benefit is that you will help improve the understanding of antimicrobial resistance, its transmission and persistence. The results of this research may help to improve antibiotic treatment of infections with resistant bacteria in the future. The only disadvantage of participation is the time required for sample collection and the questionnaire.

26. What will these results be used for?

The results of this study will be shared with other researchers and physicians in Kenya and Europe. They will be used to improve antibiotic treatment and minimize the risk of antibiotic resistance.

27. Will I be informed of new findings from the study?

Once the study is completed and analyzed, you will also be informed of the results in writing.

28. Is my participation confidential?

All of your personal and medical data collected during the study will be treated as confidential. Only the investigators of this study are authorized to access the data. Published findings will be de-identified.

Contact Information for questions about the study

If you have any more questions, please feel free to contact the principal investigators of the study:

Kavulavu, Briton Matata

Phone: 0702086822

Email: kavulavubright@gmail.com

For questions about your rights as a participant, you may contact the KEMRI Scientific Ethics Review Unit, P.O Box 54840-00200, Nairobi; telephone 0717719477, 0776399979; email address: seru@kemri.go.ke; Kemriseru18@gmail.com

Part II: Informed Consent Form

I declare that I have been informed orally and in writing by the signing health worker about the above-mentioned study and agree to my child participating in the study.

By signing this form,

- ☐ I agree to my child's stool being tested for resistance to antibiotics and any other tests relevant to the mentioned study
- ☐ I agree to my child's stool specimen and/or bacteria isolated therein being stored for future analyses.

Place, date		Signature of the parent
Place, date		Name and signature of the Principal Investigator / Co-investigator

If illiterate

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Illiterate participants should include their thumbprints as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely for their child to participate in the study.

Print name of witness _____

AND

Thumb print of parent

Signature of witness _____

Date _____

Parental Permission Consent Form (Swahili Version)

Study Title: Molecular Characterization of ESBL-Producing *Enterobacteriaceae* from Patients with Gastrointestinal Infections at Kisii Teaching and Referral Hospital

Jina la Mchunguzi Mkuu:

Kavulavu, Briton Matata

Phone: 0702086822

Email: kavulavubright@gmail.com

Wachunguzi-wenza:

Dr Eric Omori Omwenga

Department of Medical Microbiology & Parasitology

School of Health Sciences

Kisii University

Phone: 0725806875

Email: omorieric@gmail.com

Dr Elizabeth Matey

Senior Research Scientist

Centre for Microbiology Research

Kenya Medical Research Institute

Phone: 0721604849

ematey@kemri.go.ke

Dr Oscar Nyangiri

Department of Medical Microbiology & Parasitology

School of Health Sciences

Kisii University

Phone: 0720709392

oasanya@gmail.com

Dr Andrew Nyerere

Department of Medical Microbiology

School of Biomedical Sciences

Jomo Kenyatta University of Agriculture and Technology

Phone: 0722689073

knyerere@jkuat.ac.ke

Jina la Shirika: Chuo Kikuu cha Kisii, Chuo Kikuu cha KEMRI

Fomu hii ya idhini iliyo na habari ina sehemu mbili:

Sehemu ya 1: Habari kwa washiriki

Sehemu ya II: Hati ya idhini

Muhtasari

Madhumuni ya utafiti huu ni kuamua kiwango cha bakteria sugu ya enteric, na jeni zinazosababisha bakteria hawa kuwa wasio na majibu kwa matibabu ya antibiotiki. Inatarajiwa kujumuisha wagonjwa 174 wenye dalili za maambukizi kama vile homa na kuhara. Hii ni utafiti wa sehemu ya msalaba kwa hivyo hakuna ufuatiliaji au matibabu ya majaribio yatahusika. Washiriki wataulizwa kukamilisha dodoso. Sampuli za Stool zitakusanywa katika hospitali. Muda wote wa kushiriki ni miezi minne. Kushiriki katika utafiti huu ni kwa hiari kabisa. Fomu hii ina maelezo yatakayo kuelekeza kupeana ruhusa ya mtoto wako kushirikishwa katika utafiti huu.

Sehemu I: Habari Kwa Washiriki

Mtoto wako amelikwa kushiriki katika utafiti huu. Kabla ya kuamua kushiriki, tafadhali soma karatasi hii ya habari kwa umakini.

15) Ni nani wanaoendesha na kufadhili utafiti huu?

Utafiti huu umeandaliwa na shule ya Tiba ya Chuo Kikuu cha Kisii na shule ya Chuo Kikuu cha Kemri na imeidhinishwa na kamati ya maadili ya Utafiti ya chou kikuu cha Moi.

16) Kusudi la utafiti huu ni nini?

Sisi sote hubeba idadi kubwa ya bakteria wasio na madhara ambao hukaa ndani ya utumbo wetu, pia hujulikana kama mimea ya utumbo. Wakati tunakunywa dawa ya antibiotic, mimea asili ya tumbo na pia bakteria inayosababisha magonjwa hutoa madini yanayoharibu antibiotic ili ziwe sugu. Madini haya yanaitwa ESBL na yakiadhiri utumbo wale bakteria wanayoyatoa huitwa ESBL-PE. Kuenea kwa bakteria sugu ya dawa ni moja wapo ya shida muhimu katika upinzani wa antimicrobial. Kuenea kwa bakteria sugu ya dawa ni moja wapo ya shida muhimu katika upinzani wa antimicrobial. Ni tishio ulimwenguni kwa afya ya umma, sio tu katika vituo vya huduma za afya lakini pia katika jamii. Madhumuni ya utafiti wa KONSERVE-AB ni kutathmini ikiwa upinzani wa bakteria kwa watu binafsi hupitishwa kutoka au kwa wanyama au mazingira katika kaya zao. Inalenga kuamua kiwango cha maambukizo haya na sababu zinazoweka watu kwa hatari ya maambukizi haya. Imepangwa kujumuisha wagonjwa 191 huko Kisii kuanzia Mei hadi Septemba 2021. Kwa wagonjwa sugu tutakusanya data kutoka kwa mazingira ya nyumba zao pamoja na kinyesi cha wanyama, udongo na maji. Huu ni utafiti wa uchunguzi, na hakuna matibabu ya majaribio yanayotekelezwa.

17) Kwa nini nimechaguliwa?

Mtoto wako alichaguliwa kwa sababu moja wapo: Uko hospitalini na homa na kuhara na unashukiwa kuwa na maambukizo ya bakteria ya *Enterobacteriaceae* ndani ya utumbo wako.

18) Nani hawezi kushiriki?

Huwezi kushiriki ikiwa umechukua antibiotics kabla ya kuja hospitali. Zaidi ya hayo, wale wanaokataa kutoa kibali au wamepewa matumizi ya viuavijasumu ndani ya wiki iliyopita hawatajumuishwa.

19) Je! Ninaweza kutengwa kwenye utafiti baada ya kutoa idhini yangu?

Ndiyo! Kushiriki katika utafiti huu ni kwa hiari tu na una uhuru wa kujiondoa wakati wowote.

20) Je! Lazima nishiriki katika utafiti huu na haki zangu ni zipi?

Hapana. Sio lazima. Kushiriki kwako katika utafiti huu ni kwa hiari kabisa. Kutoshiriki katika utafiti hakutaathiri huduma yako ya matibabu ya baadaye. Kanuni hiyo hiyo inatumika ikiwa utabatilisha idhini yako ya awali baada ya kuanza utafiti. Kwa hivyo unaweza kuondoa ushiriki wako wakati wowote bila kuhitaji kuhalalisha uamuzi wako.

21) Nifanye nini ikiwa nitashiriki katika utafiti huu?

Ikiwa unakubali kushiriki katika utafiti huu, mchunguzi wa utafiti atakuuliza utie sahihi fomu ya idhini ili kuthibitisha kwamba unaelewa utafiti na kukubali kushiriki. Utaulizwa maswali kuhusu ugonjwa wako, historia ya matibabu, kuwasiliana na wanyama na mapendekezo ya chakula. Utaulizwa kutoa sampuli ya kinyesi.

22) Majukumu yangu katika utafiti ni nini?

Kando na kutoa sampuli yako ya kinyesi, huna majukumu mengine.

23) Ni sampuli gani za microbiolojia zitakazochukuliwa? Je! Vipimo hivi vitasababisha usumbufu wowote?

Ili kutafuta uwepo wa bakteria sugu, sampuli ya kinyesi inahitajika. Sampuli hii haisababishi usumbufu wowote wa mwili.

24) Ni nini hufanyika kwa sampuli mara zinapokusanywa?

Sampuli hiyo itafanyiwa uchanganuzi wa kibiolojia ili kubaini bakteria yoyote ya kuvutia iliyopo. Bakteria hao watachunguzwa ili kubaini unyeti kwa dawa za kawaida kabla ya mbinu za molekuli kutumika kuangalia jeni zilizowekwa na bakteria.

25) Je! Kuna faida gani na mapungufu gani katika utafiti huu?

Kushiriki katika utafiti hakuleti manufaa yoyote ya moja kwa moja kwako. Walakini, ikiwa bakteria ya pathogenic ambayo inaweza kusaidia katika matibabu yako itatambuliwa, matokeo yatawasilishwa kwako ili kusaidia katika matibabu

ya yoru. Faida kuu ni kwamba utasaidia kuboresha uelewa wa upinzani wa antimicrobial, maambukizi yake na kuendelea. Matokeo ya utafiti huu yanaweza kusaidia kuboresha matibabu ya antibiotiki ya maambukizo na bakteria sugu katika siku zijazo. Hasara pekee ya ushiriki ni muda unaohitajika kwa ajili ya kukusanya sampuli, na dodoso.

26) Matokeo haya yatatumika kwa njia gani?

Matokeo ya utafiti huu yatashirikiwa na watafiti na madaktari wengine nchini Kenya na Ulaya. Zitatumika kuboresha matibabu ya antibiotic na kupunguza hatari ya upinzani wa antibiotics.

27) Je! Nitaarifiwa juu ya matokeo mapya kutoka kwa utafiti?

Baada ya utafiti kukamilika na kuchambuliwa, utafahamishwa pia matokeo kwa maandishi.

28) Je! Ushiriki wangu ni wa siri?

Data yako yote ya kibinafsi na ya matibabu iliyokusanywa wakati wa utafiti itachukuliwa kuwa siri. Wachunguzi wa utafiti huu pekee ndio wameidhinishwa kufikia data. Matokeo yaliyochapishwa yatatambuliwa.

Maelezo ya Mawasiliano

Ikiwa una maswali yoyote zaidi, tafadhali jisikie huru kuwasiliana na wachunguzi wakuu wa utafiti:

Kavulavu, Briton Matata

Phone: 0702086822

Email: kavulavubright@gmail.com

Kwa maswali kuhusu haki zako kama mshiriki, unaweza kuwasiliana na Kamati ya Maadili na Utafiti ya Kitaasisi ya KEMRI, P.O Box 54840-00200, Nairobi; kwa simu 0717719477, 0776399979; au barua pepe: seru@kemri.go.ke; Kemriseru18@gmail.com.

Hati ya Ithini

Ninatangaza kwamba nimearifiwa kwa mdomo na kwa maandishi na mfanyakazi wa afya aliyetia saini kuhusu utafiti uliotajwa hapo juu na kukubali kushirikisha mtoto wangu.

Kwa kusaini fomu hii,

- Ninakubali kinyesi cha mtoto wangu kupimwa ukinzani dhidi ya viuavijasumu na vipimo vingine vyovyote vinavyohusiana na utafiti uliotajwa.
- Ninakubali kielelezo cha mtoto wangu na/au bakteria zilizowekwa humo kuhifadhiwa kwa uchanganuzi wa siku zijazo.

Mahali, Tarehe	Sahihi ya mzazi
Mahali, Tarehe	Jina na sahihi ya Mtafiti mkuu/ Mtafiti mwenza

Ikiwa hajui kusoma na kuandika

Shahidi anayesoma lazima asaini (ikiwezekana, mtu huyu anapaswa kuchaguliwa na mshiriki na haipaswi kuwa na uhusiano na timu ya utafiti). Washiriki ambao hawajui kusoma na kuandika wanapaswa kujumuisha alama zao za vidole pia

Nimeshuhudia usomaji sahihi wa fomu ya idhini kwa mshiriki anayeweza kushiriki, na mtu huyo amepata nafasi ya kuuliza maswali. Ninathibitisha kuwa mtu huyo ametoa idhini kwa uhuru.

Chapisha jina la shahidi _____
kidole

NA

Uchapishaji wa

gumba cha mshiriki

Sahihi ya shahidi _____

Tarehe _____

Parental Permission Consent Form (Kisii Version)

Efomu Y'ogwancherana

Omotwe bw'erisomo: Okomanyekana gwa ekemorekiura kwa chibakteria chitari koigwa amariogo(ESBL-PE) ase abarwaire baigwete enda bobbe ase enyagitari ya Kisii Teaching and Referral.

Omochungusi omonene:

Kavulavu, Briton Matata

Phone: 0702086822

Email: kavulavubright@gmail.com

MSc Student

Abakonyi baye:

Dr Eric Omori Omwenga

Department of Medical Microbiology & Parasitology

School of Health Sciences

Kisii University

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Email: omorieric@gmail.com

Dr Elizabeth Matey

Senior Research Scientist

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Dr Andrew Nyerere

Department of Medical Microbiology

School of Biomedical Sciences

Jomo Kenyatta University of Agriculture and Technology

Phone: 0722689073

knyerere@jkuat.ac.ke

Rieta ria esukuru: KEMRI Graduate School, Kisii University

Efomu eye y’ogwancherana ebwate chinsemo ibere:

- Ensemo entang’ani: Ensemo y’obomanyi [gokomanyia igoro y’obochungusi obo]
- Ensemo ya kabere: Esatibiketi y’ogwancherana [obeke esiginecha onye kogwancherana]

Obweng’e

Esababu y’ogotwara obochungusi obo n’okomanya ekerengo kia ogoitaigwera amariogo kia chibakteria chia amatumbo (ESBL-PE), na kobwekania na amasingira korigia gose okwambokera nkoroo ase abarwaire batokire na bakteria echi. Ebangire korigereria abarwaire mia mbili babwate riberera nainde bagosaa. Obo n’obochungusi borenete egekundi; tokobwatereria totari korwari.

Baria bagoshiriki bagosabwa baichori amachibu. Echoo enene n’eganeiri nyagitari. Engaki y’ogoshiriki n’emetienyi ene. Ogoshiriki n’ogwancha kwago.

Ensemo entang’ani: Obomanyia gochia ase omorwaire

Aye,

Gwachikirwe goshiriki ase obochungusi obo. Nyuma otarachora goshiriki, soma ensemo eye.

15. Ning’o orosirie na gosiira obochungusi obo?

Risomo eri erirosiri na OMOCHUNGUSI OMONENE , Kavulavu Briton, ogosomera Masters y’ebinyinyi ase esukuru ya KEMRI Graduate. Chibesa chio’gosira risomo eri chikorwegwa ne chiyunivasiti chia Hohenheim na Tübingen chia Bocherumani.

16. Ninki ekerengo kia obochungusi obo?

Intwe twensi nigo tobwate chibakteria chiri chitari koreta oborwaire gochakera ase omonwe baka ase amatumbo. Kere tokonywa amariogo, chibakteria echi nabo chitaigwere amariogo gete, echikorokwa(Extended-spectrum beta-lactamase-producing *Enterobacteriaceae*). Chibakteria echi nigo chikorusi ebinto bikorwani na goita amariogo. Ase igo, chibakteria echi nchikonyara goitwa n’amariogo aya. Chibakteria echi mono echimenyete ase amatumbo. Ekerengo ki’erisomo eri n’okorigia chichini chikoreta ogotaigwera amariogo oko.

17. ’Nasekie nachorirwe?

Nigo gwachorirwe ase yeching’encho ibere: ’Gwachire nyagitari neriberera gose ogosaa nainde okageire gotwara chibakteria chia amatumbo n’omonyagitari.

18. Barabie batanyare goshiriki?

Tokonyara goshiriki onye komenyete goetania chikilomita emerongo ebere na isano korwa Bosongo. Komenta naigo, barie bagokana goancherana gose baria batimiire amariogo ase ewiki eyemo yaetire.

19. Ninyare korusigwa ase risomo eri nonya konyora naancheranire?

Eeeh! Ogoshiriki ase risomo eri n’ogwancha kwago, ase igo nabo okwerusia chinsa chinde chionsi oeigwete.

20. Nerasima n’shiriki naende okobua kwane nkoroki?

Yaya. Tori korasimiswa. Ogoshiriki n’ogwancha kwago. Okwanga kwago goshiriki nkobwati boiri bonde bwensi n’okorwarigwa kwago mosuko. Nabo ere igo noerusia kere gwa’ncheranire. Nabo okoerusia otari goetetea.

21. Ninki mbwenerete gokora kere naancheranire?

Kere gw’ancheranire omochungusi nagocike obeke esiginecha yago inse egere amanye gose kwamanyire obochungusi obo naende ing’a gw’anchiranirie.

N’oborigwe amaswari igoro y’oborwaire bwago, gose kwanya korwara rinde, gose nkwoomerana ore n’etugo na endagera yago. Nochikwe orete echo enene.

22. Ninki naende imbwenerete gokora?

Gotatiga na koreta echo enene, tobwate egasi yende.

23. N’esampo ki eganeirie? Chitest echi nchindetere obokong’u bonde bwonsi?

Echoo enene nero eganeirie egere tonyare korigia chibakteria chitari koigu amariogo. Korusia echo enene ngokonyara gokoretera obokong’u bonde bwonsi.

24. Ninki chisampo chogokorerwa kere chiarusigwe?

Ebinyiyi bia bakteria ebikorigigwa naende bipimu gose nkoigwera bire amariogo akawaida arafu chichi chikoreta ogotaigwera amariogo nachironywe chirigigwe onye bakteria echio nchiri koigwera amariogo.

25. Naki ‘ngokonyeka gose ‘ntari gokonyeka korwa ase risomo eri?

Ogoshiriki ase risomo eri ‘nkori n’obokonyi bwa enchera eyemo ase eyemo gocha asore. Rakini, onye chibakteria chikoreta oborwaire nchitoke nabo okomanyigwa egere orwarigwe. Obokonyi obonene ‘mbori ing’a orakonye komenta okomanya igoro ya ogotaigwa amariogo n’okwambokera kwa ebinyinyi. Amatokeo y’obochungusi obo ngokonye korigia amariogo aria aranyare goita chibakteria echi chitari koigwa amariogo. Ne’chinsa nkeigo orasirie koreta echo na goichoria risakara eri.

26. Amatokeo ninki agotumeka gokora?

Amatokeo y’obochungusi obo nigo yagosangwa na abachungusi bande amo na abanyagitari korwa ense ya Kenya na korwa mache ng’umbu. Nigo agotumekwa komenta oborwari bwe ebinyinyi birie bitari koigwa amariogo na gopungusa ekerengo kia ebinyinyi gotaigwera amariogo.

27. Inee, nimanyigwe obomanyi obonyia boratoke korwa ase obochungusi obo?

Kere obochungusi bwaerire, nomanyigwe obomanyi oboyia obwe boratoke goetera enchera y’amariko.

28. Ogoshiriki kwane ‘mbobisi kore?

Ebimanyerie biago bionsi pi nigo tokobigacha bobisi. Abachungusi boka nabwo baancheire korora. Amachibu y'omoerio tagotwara ebimanyerio biago.

Chinamba ase oraborie amaswari igoro y'obochungusi obo

Onye kobwate amaswari ande, moborie omochungusi omonene ase enamba ekobwatia:

Kavulavu, Briton Matata

Phone: 0702086822

Email: kavulavubright@gmail.com

Ase amaswari y'okobua kwago buna omoshiriki, nabo okorigia ekomitii y'obochungusi KEMRI Scientific Ethics Review Unit, P.O Box 54840-00200, Nairobi; ase enamba 0717719477, 0776399979; gose seru@kemri.go.ke; Kemriseru18@gmail.com

Ensemu ya kabere : Efomu y'ogwancherana

Natianire ing'a namanyigwe igoro y'obochungusi obo nainde naanchire goshiriki.

Ase ogosaini efomu eye,

- ☐ Naanchire echoo yane epimwe gose ebinyi birie bitari koigwera amariogo mbiroo ne chitesti chinde chia manna ase obochungusi obo.
- ☐ Naanchire echoo yaane gose ebinyinyi ebio biratoke bigachwe bitumeke mosuko.

Ase, chinsa	Esiginecha y'omorwaire
Ase, chinsa	Rieta n'esiginecha y'omochungusi omonene/ omokonyi oye

Onye tomanyeti gosoma na korika

Omonto ekabuna oyo baka akonywe gosaini na barie bamanyete gosoma na korika(Onye gekonyarekana, omoshiriki tiga aechorere oyo oramosaineirie nainde tatwara boiri bonde bwensi n'abachungusi). Abashiriki buna aba nabarabwo baka babeke ekiara.

Narorire omoshiriki kagosoma buya efomu eye y'ogwancherana, naende omoshiriki nanyora ribaga ri'okoboria amaswari. Naanchire ing'a nere bweka oerwera.

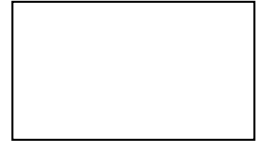
Rieta ria oy'orora _____ *NA*

Ekiara kia omoshiriki

Esiginecha ya oy'orora _____

Chitariki _____

Rituko/omotienyi/omwaka



Appendix VI: Ethical Approvals



In Search of Better Health

KENYA MEDICAL RESEARCH INSTITUTE

OFFICE OF THE DIRECTOR RESEARCH & DEVELOPMENT

Tell: +254 020 2722541, 2713349,
0722 205 901, 0733 400 003

P.O. Box 54840-00200, Nairobi
Email: ddrt@kemri.go.ke
Website: www.kemri.go.ke

KEMRI/RD/22

July 9, 2024

TO: KAVULAVU BRITON MATATA,
PRINCIPAL INVESTIGATOR,

THROUGH: THE DEPUTY DIRECTOR, CMR
NAIROBI,

Dear Madam,

RE: SERU 4970 (RESUBMISSION OF INITIAL SUBMISSION): MOLECULAR
CHARACTERIZATION OF EXPANDED-SPECTRUM BETA-LACTAMASE-
PRODUCING *ENTEROBACTERIACEAE* FROM STOOL OF PATIENTS WITH
GASTROINTESTINAL INFECTIONS AT KISII TEACHING AND REFERRAL
HOSPITAL.

Reference is made to your letter dated June 13, 2024. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the following revised study documents on June 15, 2024:

1. Cover Letter dated 13th June 2024.
2. Investigators Documents-CITI and CVs
3. Study Tools
4. Clean and tracked versions of the protocol

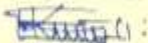
This is to inform you that the Committee notes that the issues raised during the 347th Committee A meeting of the KEMRI Scientific Ethics Review Unit (SERU) held on **May 14, 2024**, have been adequately addressed.

Consequently, the study is **granted approval** for implementation effective this day, **July 07, 2024**, for a period of **one (1) year**. Please note that authorization to conduct this study will automatically expire on **July 06, 2025**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **May 25, 2025**.

Please note that only approved documents including (informed consents, study instruments, Material Transfer Agreement) will be used. You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) <https://oris.nacosti.go.ke> and also obtain other clearances needed.

Yours faithfully,


ENOCK KEBENEI,
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT

In Search of Better Health



MTRH/MU-INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)
MDI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471020
Reference: IREC/2021/040
Approval Number: 0004012



MOI UNIVERSITY
COLLEGE OF HEALTH SCIENCES
P.O. BOX 4606
ELDORET
3rd November, 2024

Dr. Oscar Nyangiri & Team,
Kisii University,
P.O Box 408-40200,
KISII-KENYA

Dear Dr. Nyangiri & Team,

RE: CONTINUING APPROVAL

The Institutional Research and Ethics Committee has reviewed your request for continuing approval for your study titled:-

"Investigating Antibiotics Resistance and Transmission in Patients Presenting with Symptoms of Gastrointestinal Tract Infection in Kisii County: A Pilot Study"

Your request has been granted Approval with effect from 3rd November, 2024. You are therefore permitted to continue with your study.

Note that this approval is for 1 year; it will thus expire on 2nd November, 2025. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE



cc:	CEO	-	MTRH	Dean	-	SPH	Dean	-	SOM
	Principal	-	CHS	Dean	-	SOD	Dean	-	SON

Appendix VI: Publication



RESEARCH ARTICLE

One health approach unravels worrying antimicrobial resistance patterns: A cross-sectional study in Kisii, Kenya

Briton M. Kavulavu^{1,2,3*}, Eric O. Omwenga^{2,4*}, Oscar Asanya Nyangiri^{2,5}, Andrew K. Nyerere¹, Rael J. Too³, Elizabeth J. Matey³, Siri Göpel^{4,6}, Wycliffe Mogoa⁶, Thorben Schilling⁷, Ludwig E. Hoelzle⁷, Beryl Primrose Gladstone^{4,8}

1 Department of Medical Microbiology, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya, **2** Department of Medical Microbiology & Parasitology, School of Health Sciences, Kisii University, Kisii, Kenya, **3** Center for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya, **4** German Center for Infection Research (DZIF), partner site Tübingen, Germany, **5** Department of Internal Medicine 1, University Hospital of Tübingen, Tübingen, Germany, **6** Department of Child Health and Pediatrics, School of Health Sciences, Kisii University, Kisii, Kenya, **7** Livestock Infectiology and Environmental Hygiene, University of Hohenheim, Stuttgart, Germany

* These authors contributed equally to this work.

† LEH and BPG also contributed equally to this work.

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OPEN ACCESS

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Editor: Mabel Kamweli Aworh, Fayetteville State University, UNITED STATES OF AMERICA

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Data availability statement: All relevant data are within the manuscript and its [Supporting Information Files](#).

Funding: This research was supported by initial funding (seed grant) for joint projects on Africa by the Universities of Hohenheim and

Abstract

Background

Antimicrobial resistance (AMR) is a major public health challenge, particularly in Sub-Saharan Africa, where diagnostic and surveillance capacities are limited. *Enterobacteriales* significantly contribute to AMR, with environmental reservoirs facilitating transmission between humans, animals, and the environment.

Methods

This study investigated the prevalence and antimicrobial susceptibility of selected *Enterobacteriales* in human, water, animal feces, and soil samples in Kenya. A cross-sectional study including 200 patients with gastrointestinal symptoms was conducted at Kisii Teaching and Referral Hospital and surrounding areas. AMR testing was performed using the disk diffusion method.

Results

A total of 365 samples were collected: 200 human and 55 each of water, animal feces, and soil specimens from the homesteads of patients with resistant isolates. 343 isolates were obtained (*Escherichia coli*: 280/343 [81.6%], *Salmonella* spp.: 28/343 [8.2%], *Klebsiella* spp.: 25/343 [7.3%], *Shigella* spp.: 10/343 [2.9%]). A significant proportion of isolates exhibited AMR, particularly to piperacillin-tazobactam (up to 87%) and ampicillin (up to 79%). Resistance to piperacillin-tazobactam in *E. coli*