MAKERERE UNIVERSITY

ANTIMICROBIAL RESISTANCE PROFILES OF EXTENDED-SPECTRUM BETA-LACTAMASE (ESBL)-PRODUCING *Escherichia Coli* ISOLATED FROM WASTEWATER, POULTRY, AND SWINE SAMPLES AT THE ANIMAL–ENVIRONMENT INTERFACE IN KAMPALA, UGANDA

BY

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DECLARATION AND APPROVAL

I, **Ahabwe Sixtus** affirm that this research project report is original and has not been submitted for any other degree award to any other university before.

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DEDICATION

To my dear beloved parents, my one and only beloved wife and the drivers of public health safety in Uganda and at the global stage.

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I wish to express my profound gratitude to God Almighty for provision for health and financial sustenance throughout my studies. I also very grateful to all who dedicated their time to support me throughout my academic journey, in particular, my dear esteemed lecturers, fellow students and personnel working under the Walter-reed project at COVAB. Your assistance has been instrumental in the completion of this research endeavor and my undergraduate program.

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LIST OF ABBREVIATIONS AND ACRONYMS

AMC:	Amoxicillin-clavulanate
AMR:	Antimicrobial Resistance
ARB:	Antimicrobial-Resistant Bacteria
ARGs:	Antimicrobial Resistant Genes
C:	Chloramphenicol
CAZ:	Ceftazidime
CFM:	Cefixime
CIP:	Ciprofloxacin
CLSI	Clinical Laboratory Standards Institute
CN:	Gentamicin
CNS	Central Nervous System
COVAB	College of Veterinary medicine, Animal resources and Biosecurity
CRO:	Cefuroxime
CTX	Cefotaxime
E. coli	*Escherichia coli*
ESBL	Extended Spectrum Beta lactamase
FEP	Cefepime
GRASS	Global antimicrobial resistance surveillance system
HGT	Horizontal Gene Transfer
LMICs	Low to Middle-Income Countries
MDR	Multi-Drug Resistance
STP	Sewage Treatment Plant
SXT:	Trimethoprim-sulfamethoxazole
TE:	Tetracycline
TOB:	Tobramycin
WHO	World Health Organization.
WWTP	Waste water treatment plant

ABSTRACT

Antimicrobial resistance (AMR) is a growing global public health challenge that threatens the effectiveness of antimicrobial agents in both human and veterinary medicine. Extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* are among the priority antimicrobial-resistant pathogens. This study aimed to determine the antimicrobial resistance profiles of ESBL-producing *E. coli* at the animal-environment interface in Kampala, Uganda.

A cross-sectional study was conducted using 120 samples comprising wastewater collected from Bugolobi Sewage Treatment Plant and Kampala City Abattoir, poultry cloacal swabs collected from Kalerwe, Kasubi, Nakawa, and Nakasero live bird markets, and swine rectal swabs collected from Nansana Abattoir. Isolation and identification of *E. coli* were performed using standard microbiological and biochemical methods. Phenotypic confirmation of ESBL production and antimicrobial susceptibility testing were conducted using the Kirby–Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) 2026 guidelines.

Overall, *E. coli* was isolated from 79 of the 120 samples, giving an overall proportion of 65.8%. Recovery rates were highest in wastewater (78%), followed by poultry (65.0%) and swine (55.0%). Among the *E. coli* isolates, 32% were confirmed as ESBL producers. Poultry had the highest proportion of ESBL-producing *E. coli* (50.0%), followed by swine (32%) and wastewater (16.1%), with ($p = 0.023$). Antimicrobial resistance was common, with 79% of isolates resistant to at least one antimicrobial agent, while 34% exhibited multidrug resistance. Multidrug resistance was highest among poultry (46%) and wastewater (42%) isolates compared with swine (9%) ($p = 0.01$).

The findings demonstrate that wastewater, poultry, and swine constitute important reservoirs of ESBL-producing *E. coli* within Kampala's animal-environment interface. Strengthening antimicrobial stewardship, improving wastewater treatment and abattoir waste management, and implementing integrated One Health surveillance are essential to limit the spread of antimicrobial resistance in Uganda.

CHAPTER ONE

INTRODUCTION

1.1 Background

Antimicrobial resistance (AMR) has become a global public health emergency threatening the effectiveness of life-saving antibiotics (Abbas et al., 2024) AMR is recognized by the World Health Organization (WHO) as one of the top ten global public health threats(Walsh et al., 2023), AMR poses a significant global health challenge, affecting humans, animals, and the environment alike (Aslam et al., 2024). Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* are among the most clinically and epidemiologically significant resistant pathogens (Ribeiro et al., 2024).

Bacteria that produce extended-spectrum β-lactamases (ESBLs) are considered one of the critical priority pathogens by the World Health Organization (Ramatla et al., 2023). ESBLs are a type of β-lactamase that can hydrolyze penicillin, first, second, and third-generation cephalosporins, and aztreonam but are unable to break down cephamycin or carbapenems (Husna et al., 2023). The rising problem of extended spectrum beta lactamase (ESBL) producing bacteria has become great importance during the last decades (Coque et al., 2008). Since the first report of an ESBL producing organism in the 1980s, they have received increasing attention due to their wide spread and constant evolution, becoming increasingly resistant to most of the commonly used antibiotics. The most worrisome amongst antibiotic-resistant bacteria are those with resistance to several antibiotics, also known as superbugs (Chandrasekhar et al., 2024). The improper and uncontrolled uses of antibiotics against pathogenic bacteria have resulted in the occurrence of multi drug resistance (MDR) strains. MDR bacteria is defined as nonsusceptibility to one or more antimicrobials on three or more antimicrobial classes, at the same time the strains that are non-susceptible to all antimicrobials, (Sweeney et al., 2018). MDR are classified as extreme drug-resistant strains infections due to MDR pathogens that increase both morbidity and mortality.

In low- and middle-income countries like Uganda, urban interfaces create ideal conditions for the emergence and spread of ESBL-*E. coli* (Kavulavu et al., 2026). The Bugolobi Sewage Treatment Plant receives mixed inputs from households, healthcare facilities, and industries(McConville et al., 2019), while the Kampala City Abattoir generates high-organic-load effluent rich in animal feces, blood, and antimicrobial residues (Gufe et al., 2025). Animal sources also amplify the problem. Live bird markets in Kalerwe, Nakasero,

Kasubi, and Nakawa are hubs for intensive poultry trading where overcrowding and frequent antibiotic use for prophylaxis and growth promotion select for resistant strains (Ssajjakambwe et al., 2023). Similarly, the Nansana Swine Abattoir handles large numbers of pigs, with runoff contributing to environmental contamination (Nigussie et al., 2025). The Kampala City Abattoir and the city's sewage treatment plant are critical but poorly monitored hotspots for microbial contamination and AMR dissemination (Katumba et al., 2024).

These compartments form a dynamic animal-environment interface in Kampala, where ESBL-producing *E. coli* can cycle between wastewater and livestock. Studies have already detected multidrug-resistant Gram-negative bacteria in urban drainage channels, but few have systematically focused specifically on the phenotypic resistance profiles of the ESBL-producing bacteria, especially in Uganda, that are both clinically relevant and environmentally persistent. Recent scientific advances such as metagenomic sequencing for resistance surveillance, innovative wastewater treatment technologies such as ozonation, Ultra-violet radiation and membrane filtration, and the development of vaccines and probiotics as alternatives to antibiotics in livestock and are helping to mitigate resistance. Generating such data would be essential for Uganda's National Action Plan on AMR and integrates animal and environmental dimensions of AMR control. The purpose of this research study therefore, will be to ascertain the phenotypic resistance profiles of the ESBL-producing E coli in waste-water, poultry and swine slaughtered in the selected area through strategic sampling of the waste water effluents, cloacal swabs and rectal swabs and laboratory test at the Microbiology laboratory at CoVAB, Makerere University.

1.2 Statement of the Problem

Although AMR is recognized as a national priority in Uganda, surveillance remains heavily biased toward clinical human isolates (Mwanja et al., 2025). There is a significant knowledge gap regarding the environmental and livestock dimensions, particularly the distribution, and detailed antimicrobial resistance profiles of ESBL-producing *E. coli* in Kampala's key hotspots. Inadequately treated effluent from Bugolobi STP and Kampala City Abattoir, combined with high carriage rates in poultry and swine, creates continuous release of resistant bacteria into receiving water bodies and the food chain.

Without site-specific data on incidence and resistance patterns including multi-drug resistance, Uganda cannot fully quantify the animal and environmental contribution to the AMR burden or design targeted mitigation measures such as enhanced wastewater

treatment, improved abattoir waste management, or market-level hygiene protocols. This gap threatens the effectiveness of the National Action Plan on AMR.

1.3 Research Objectives

1.3.1 General Objective

To determine the antimicrobial resistance profiles of ESBL-producing *Escherichia coli* isolated from waste water from Bugolobi STP and Kampala City Abattoir, poultry from Kalerwe, Nakasero, Kasubi, and Nakawa live bird markets and swine from Nansana Abattoir.

1.3.2 Specific Objectives

i. To determine the proportion of *E. coli* in wastewater from Bugolobi STP and Kampala City Abattoir, poultry from Kalerwe, Nakasero, Kasubi, and Nakawa live bird markets and swine from Nansana Abattoir.
ii. To determine the proportion of ESBL-producing *E. coli* in wastewater from Bugolobi STP and Kampala City Abattoir, poultry from Kalerwe, Nakasero, Kasubi, and Nakawa live bird markets, and swine from Nansana Abattoir.
iii. To determine the phenotypic antimicrobial resistance patterns (including multi-drug resistance) exhibited by the isolated *E. coli*?

1.4 Research questions

1. What is the proportion of *E. coli* in wastewater from Bugolobi STP and Kampala City Abattoir, poultry from Kalerwe, Nakasero, Kasubi, and Nakawa live bird markets, and swine from Nansana Abattoir?
2. What is the proportion of ESBL-producing *E. coli* in wastewater from Bugolobi STP and Kampala City Abattoir, poultry from Kalerwe, Nakasero, Kasubi, and Nakawa live bird markets and swine from Nansana Abattoir?
3. What are the phenotypic antimicrobial resistance patterns (including multi-drug resistance) exhibited by the isolated *E. coli*?

1.5 Justification and Significance

This study addresses a critical knowledge gap in environmental and livestock AMR surveillance in Uganda by integrating wastewater and animal sources, it provides actionable data to support policy on wastewater treatment, antibiotic stewardship in poultry and swine production, and market hygiene. The findings will provide actionable, locally

generated data to strengthen Uganda's National Action Plan on AMR, particularly the environmental, inform improvements in wastewater management policies and infrastructure at municipal level such as KCCA and National Water and Sewerage Corporation, Identify environmental hotspots for resistance dissemination and guide targeted interventions to reduce the release of resistant bacteria into ecosystems, Support antimicrobial stewardship initiatives across animal, and environmental sectors, Serve as a replicable model for similar studies in other Ugandan or African urban centers facing similar challenges.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter reviews the existing body of knowledge relevant to the antimicrobial resistance profiles of extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* at the animal environment interface. It begins with a global overview of antimicrobial resistance (AMR) and the clinical and epidemiological significance of ESBL-producing *E. coli*. Subsequent sections examine the biology of ESBL enzymes, the role of *E. coli* as a sentinel organism for One Health surveillance, reported prevalences in wastewater, poultry, and swine, and the specific pathways through which wastewater and livestock systems interact to sustain and disseminate resistant bacteria.

2.2 Global Overview of AMR and ESBL-Producing *E. coli*

Antimicrobial resistance (AMR) is the ability of microorganisms to resist the effects of medicines that were previously used to treat such diseases (Ferraz, 2024). Globally, it has become an increasing health concern (Salam et al., 2023). In 2019, AMR was linked to approximately 5 million deaths, with 1.27 million of these deaths directly attributable to drug-resistant bacterial infections (Qin et al., 2026). Including blood stream infections, pneumonia, tuberculosis, and intra-abdominal infections. These deaths disproportionately affect low- and middle-income countries (LMICs), where inadequate surveillance, limited access to new antimicrobials, and insufficient healthcare infrastructure exacerbate the problem (Sulis et al., 2022).

Resistance to commonly used antibiotics, such as fluoroquinolones and beta-lactams, accounted for more than 70% of these deaths, underscoring the urgent need for coordinated action to combat AMR (Gajic et al., 2025). If current trends continue, AMR could lead to up to 10 million deaths annually by 2050, with profound consequences for public health and the global economy. (Tang et al., 2023). While developing countries are battling an accelerated spread of AMR, developed countries are also experiencing the same trend (Samtiya et al., 2022). AMR has increased as a result of the use of broad-spectrum antibiotics to treat invasive infections, such as bloodstream infections and central nervous system (CNS) infections (Namubiru et al., 2024). As a result, treating these infections is becoming more challenging, which increases treatment failures and death. Over-the-counter medication access in developing countries such as Uganda is one of the drivers of

AMR in such nations. Indiscriminate usage of antimicrobials exerts increased selection pressure on the bacterial population, resulting in accelerated emergence of AMR. Antibiotic usage has led to the rise of widely multidrug-resistant germs, rendering even the most potent medications useless (Oliveira et al., 2024). AMR arises when certain bacteria harbor antimicrobial resistance genes (ARGs) that confer resistance that can be horizontally transferred to other bacteria, accelerating resistance spread in the environment.

Escherichia coli producing extended-spectrum beta lactamases (ESBLs) are categorized as critical priority pathogens by the World Health Organization (WHO) (Lincopan et al., 2023). Surveillance data from the Global Antimicrobial Resistance and Use Surveillance System (GLASS) indicated that median resistance rates to third-generation cephalosporins in *E. coli* had reached 42% across 76 reporting nations. For standard community infections like urinary tract infections (UTIs), approximately 1 in 5 *E. coli* cases exhibited reduced susceptibility to frontline treatments such as ampicillin, co-trimoxazole, and fluoroquinolones. This forces reliance on last-resort agents like carbapenems, against which resistance is already climbing. Projections indicate a potential twofold increase in resistance to these last-resort antibiotics by 2035 (Dagli et al., 2024).

2.3 Overview of ESBL-Producing *Escherichia coli*

Extended-Spectrum Beta-Lactamase (ESBL)-producing *Escherichia coli* are Gram-negative bacteria that produce enzymes capable of hydrolyzing and inactivating a wide range of beta-lactam antibiotics (Akpaka et al., 2021). These include penicillin, first-, second-, and third-generation cephalosporins, and monobactams (e.g., aztreonam), but not cephamycin's or carbapenems. ESBLs are inhibited by beta-lactamase inhibitors such as clavulanic acid, which forms the basis for phenotypic confirmation tests (Drieux et al., 2008). The most common ESBL families are CTX-M (especially CTX-M-15 and CTX-M-1), TEM, and SHV(Castanheira et al., 2021). *E. coli* is the most frequent carrier due to its ubiquity in the gut of humans and animals, environmental persistence, and genetic plasticity (Beaumont et al., 2026). Pathogenic and commensal strains (phylogroups A, B1, B2, D) can acquire ESBL genes, ESBL producers are often multidrug-resistant (MDR), showing resistance to fluoroquinolones, aminoglycosides, tetracyclines, and trimethoprim-sulfamethoxazole due to co-located resistance genes (ELTarabili et al., 2026).

Escherichia coli is a Gram-negative, rod-shaped, non-spore-forming, facultative anaerobic bacterium usually motile by peritrichous flagella, belonging to the family Enterobacteriaceae (Bhattacharya, 2025). Enterobacteriaceae is a heterogeneous group of

Gamma-proteobacteria that are facultative anaerobes, oxidase-negative, catalase-positive, nitrate-to-nitrite reducers, glucose-fermenters producing various final products, and having simple nutritional requirements. On average, they measure 2-4 μm in length by 0.4-0.6 μm in width, with rounded ends, and an in vitro generation time between 20- and 30-minutes. *Escherichia coli* was initially believed to mainly inhabit the lower intestinal tract of warm-blooded animals, including humans, and be discharged to the environment through feces and wastewater treatment plants (Hassan et al., 2021).

2.4 ESBL-Producing *E. coli* as an Indicator of Antimicrobial Resistance (AMR) in Wastewater, Poultry, and Swine

Extended-Spectrum Beta-Lactamase (ESBL)-producing *Escherichia coli* (ESBL-*E. coli*) is widely recognized as an excellent sentinel/indicator organism for monitoring AMR across the One Health spectrum, particularly in environmental (wastewater), animal (poultry and swine), and food production systems (Di Marcantonio et al., 2025). It is recommended by the WHO through the Tricycle protocol (part of GLASS) as a standardized indicator for integrated surveillance. Ubiquitous and Easy to Detect: Present in the gut of humans and animals; survives well in water, soil, and manure. Selective culture and phenotypic confirmation (CLSI double-disk synergy test). ESBL genes (*bla*CTX-M, *bla*TEM, *bla*SHV) are often on mobile plasmids that co-carry other resistance determinants such as fluoroquinolones, aminoglycosides, tetracyclines (Wojnarowski et al., 2025). High ESBL prevalence signals widespread selective pressure and potential for multidrug-resistant (MDR) strains, reflects transmission across humans ,animals and the environment (Habib, 2023). ESBL-*E. coli* causes difficult-to-treat infections such as UTIs, and bacteremia and indicates risk of carbapenemase emergence (Husna et al., 2023).

Municipal sewage systems collect waste from households, healthcare facilities, and industries, making them a critical nexus in the transmission of AMR across human, environmental, and animal health domains (Al-Khalaifah et al., 2025). Human excreta contain unmetabolized antibiotics, ARB, and ARGs, especially in communities with high antibiotic consumption or poor pharmaceutical stewardship (Al-Khalaifah et al., 2025). Effluents discharged into rivers, lakes, or used for irrigation can disseminate resistance genes into natural ecosystems and agricultural soils, where environmental bacteria or crop-associated microbiota may take them up (Al-Khalaifah et al., 2025). These may ultimately re-enter the human population via drinking water, food chains, or direct environmental contact, completing the One-Health cycle. (Al-Khalaifah et al., 2025).

Poultry is a high-risk compartment: High antibiotic use for prophylaxis and growth promotion selects for ESBL strains (Gonçalves et al., 2025). Cloacal sampling from markets captures cross-contamination and food-chain risks. Correlates with MDR and zoonotic potential via meat and direct contact, Sensitive to on-farm practices and market hygiene. Serves as an early warning for animal and human health risks (Elbehiry & Marzouk, 2025).

Swine represents another key ESBL livestock reservoir (Di Marcantonio et al., 2025). Pigs receive significant veterinary antibiotics. Some of the initial phases where pigs can be colonized by ESBL-producing *E. coli* is at trading places, livestock transport vehicles, through introduction of new animals into herds, or at lairage in the slaughterhouse (Bergšpica et al., 2020). As drinking water, surface water and wastewater, all can be contaminated with ESBL-producing *E. coli*, a possible transmission of ESBLs from the environment to pigs can occur (Bergšpica et al., 2020). Holding pens in stables and at lairage in abattoirs are recognized as major hotspots for the transmission of ESBL producing Enterobacteriaceae along the pig production chain (Bergšpica et al., 2020). At slaughterhouses, a risk of cross-contamination of meat exists, especially during evisceration, where carcasses can be contaminated by AMR bacteria from the feacal content of the same or different pigs (Saenkankam et al., 2026). Food processing environments are considered to be important intermediate reservoirs and vectors of AMR bacteria, and also food handlers pose a risk of transmission of ESBL producing bacteria (Ribeiro et al., 2024).

2.5 Prevalence of ESBL *E. coli* in Waste Water, Poultry, and Swine

Under a One Health framework, ESBL *E. coli* constantly cycles through environmental reservoirs and animal value chains.

wastewater provide favorable conditions for the growth of a diverse bacterial community, which constitutes a basis for the further selection and spread of antibiotic resistance (La Rosa et al., 2025). Wastewater treatment plants (WTPs)are important reservoirs of human and animal micro-organisms that can enter into the environment again through the plant outlet, such as with water and food, and are likely to infect human and animals (Li et al., 2019) .

The poultry industry is affected by the emergence of ESBL-producing bacteria. Africa including Nigeria Egypt and Tanzania has reported ESBL-*E. coli* presence in chickens The highest record of ESBL (65.3%) was found in 2021 in Tanzania. Asia has numerous reports

of ESBL-*E. coli* isolated from poultry with high prevalence rates in India, Pakistan, Malaysia, and Indonesia, averaging 60.6%. In the Americas, countries such as Chile the United States and Cuba have noted ESBL-*E. coli* in broiler chickens, with an average prevalence of 14.3%. Europe observed its highest ESBL-*E. coli* prevalence in chickens in 2021, with countries such as Hungary, Belgium, Denmark, France, Germany, Bosnia, Spain, Poland, Italy, and the United Kingdom showing an average prevalence of 31.4%. The variation in the prevalences of ESBL-*E. coli* isolated from farm animals worldwide could be attributed to factors such as climate, customs, traditions, animal management, and antibiotic use (Mandujano-Hernández et al., 2024).

Pig production is widespread throughout the world, especially in industrialized countries, where these animals are raised on farms that provide controlled conditions. In a six-year retrospective, Africa reported the highest prevalence (65.3%) of ESBL-*E. coli* in pigs in 2021 in Tanzania. in Asia, the highest figure (98.0%) was reported in 2019 in Thailand in the Americas, few studies focusing on ESBL-*E. coli* in pigs have been conducted, especially in the northern region, where countries such as the United States and Mexico reported a similar prevalence of approximately 7.0%. In Oceania, specifically in the Australasian subregion, few reports of ESBL-*E. coli* in farm animals, especially pigs, are expected. However, Abraham et al. Reported a 35.9% prevalence rate for Australia. In Europe in 2021, papers focused on pigs were observed in Greece, France, Germany, Spain, the United Kingdom, Italy, and Hungary, with an average prevalence of 18.8% for ESBL-*E. coli* isolated from swine fecal samples (Mandujano-Hernández et al., 2024).

2.6 Antibiotic Resistance Mechanisms of ESBL producing *E. coli*

Antibiotic resistance is a growing global problem in which antibiotics are no longer effective in the treatment of infectious diseases for which they were specifically designed (Chinemerem Nwobodo et al., 2022). The predominant mechanism of antibiotic resistance is the production of beta lactamase enzymes capable of binding and hydrolyzing the beta lactam ring (Kaderabkova et al., 2022). ESBL enzymes break down penicillin, first-, second-, and third-generation cephalosporins (e.g., cefotaxime, ceftazidime), and monobactams (aztreonam) and unable to hydrolyze cephamycin or carbapenems and remain susceptible to beta lactamase inhibitors like clavulanic acid (Alfei & Schito, 2022). The genes encoding these enzymes blaCTX-M, blaTEM, blaSHV families are heavily blooded on mobile genetic elements such as plasmids for example IncF, IncI, IncK), transposons, and insertion sequences. (Gelalcha).

This plasmid-mediated localization allows rapid horizontal gene transfer (HGT) between pathogenic and commensal bacterial populations is the production of ESBL which is responsible for the resistance to the third-generation cephalosporins (López et al., 2026). The most problematic mechanisms in *E. coli* correspond to the acquisition of genes coding for extended-spectrum β-lactamases conferring resistance to broad-spectrum Cephalosporins. Since the last two decades, ESBL producing multidrug resistance *E. coli* and K. pneumoniae have emerged as a major problem in many settings (Padmini et al., 2017). Multi drug resistance can take place as a consequence of various biochemical mechanisms. It is important to realize that resistance to a particular drug can be achieved by more than one mechanism. In the juncture, particularly in the case of changes in either drug transportation or drug detoxication, protection against more than one chemical is in variably observed. This can be marked as cross resistance to structurally related compounds or structurally unrelated chemicals.

2.7 AMR in Wastewater and Its Impact on Veterinary Health in Relation to Poultry and Swine

Wastewater from municipal, hospital, and industrial effluents, including abattoirs serves as a critical reservoir and mixing vessel for antimicrobial-resistant bacteria and antibiotic resistance genes (ARGs). In urban settings like Kampala, facilities such as Bugolobi Sewage Treatment Plant and abattoirs receive mixed inputs from humans, healthcare, and livestock, creating ideal conditions for persistence, selection, and horizontal gene transfer (HGT) of resistance.

Untreated or inadequately treated wastewater affects poultry and swine production through several interconnected routes: Direct Contamination of Water Sources: Livestock drink from contaminated rivers, ponds, or runoff. ESBL-*E. coli* and other resistant bacteria colonize the gut, establishing resistant flora. Irrigation and Crop Contamination: Wastewater reuse for forage or feed crops introduces ARGs into the food chain. Poultry and swine ingest contaminated feed. Manure and Sludge Application: Abattoir and STP sludge applied as fertilizer spreads resistant bacteria into soil. Runoff during rains carries them back into water bodies used by farms. Abattoir and Market Interfaces: Kampala City Abattoir generate high-organic waste. Poor management leads to environmental contamination that cycles back to live bird markets and farms.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted in the Kampala Metropolitan Area, central Uganda. Kampala lies between latitudes 0°15′N and 0°25′N and longitudes 32°30′E and 32°40′E, at an average altitude of 1,190 meters above sea level and is experiencing rapid urbanization, leading to increased wastewater generation and intensive livestock activities.

Bugolobi Sewage Treatment Plant (STP) is the largest wastewater treatment facility serving Kampala city. It receives influent from domestic households, healthcare facilities, and industrial establishments. it represents a major environmental reservoir where ESBL-producing *E. coli* from human sources can persist and be discharged into the environment, even after treatment.

Kampala City Abattoir is the principal livestock slaughter facilities in the city, handling large numbers of cattle, goats, and sheep daily with high volume of organic waste such as blood, fecal material, and rumen contents that create a conducive environment for the proliferation of antimicrobial-resistant bacteria. Inadequate waste management at this site makes it a critical hotspot for environmental contamination.

Poultry Markets (Kalerwe, Nakasero, Kasubi, and Nakawa): These are the major live bird markets in Kampala, characterized by high bird density, frequent trading, slaughtering, and poor hygiene practices. these facilitate cross-contamination among birds and serve as important points of human exposure to ESBL-producing *E. coli* through direct contact and contaminated meat.

Nansana Swine Abattoir: A key peri-urban facility handling large numbers of pigs from various production units. swine production is a growing sector in Uganda and represents an important animal reservoir for ESBL genes that can be transferred to the environment through runoff and waste discharge.

These sites were deliberately chosen as they represent critical nodes at the animal-environment interface where antimicrobial-resistant bacteria can persist, multiply, and disseminate. Together, they provide a comprehensive framework for assessing the incidence and resistance patterns of ESBL-producing *E. coli* in Kampala.

3.2 Study Design

This study employs a cross-sectional design to assess the resistance profiles of ESBL-producing *Escherichia coli* from multiple compartments.

3.3 Study Population

Wastewater samples, Cloacal swabs and Rectal swabs from swine

Inclusion criteria

- Fresh wastewater samples collected during peak flow periods.
- Freshly slaughtered poultry and swine from the selected sites.

Exclusion criteria

- Highly decomposed or contaminated samples unsuitable for microbiological analysis.
- Samples collected outside the designated sites or during non-operational hours.

3.4 Sample Size determination

A total of 120 samples were collected as follows:

- Wastewater: 40 samples (20 from Bugolobi Sewage Treatment Plant and 20 from Kampala City Abattoir)
- Poultry: 40 cloacal swabs (10 from each of the four live bird markets: Kalerwe, Nakasero, Kasubi, and Nakawa)
- Swine: 40 rectal swabs from Nansana Abattoir

This sample size provided adequate power for overall proportion estimation and comparative analysis of ESBL and multidrug-resistance rates across the three compartments.

3.5 Sampling Strategy and Collection Procedures

Wastewater: Grab and 24-hour composite samples (500ml) were collected during peak flow hours using pre-sterilized polypropylene bottles. Sampling for waste water occurred for over four months.

Poultry: Aseptic cloacal swabs were collected from randomly selected birds. Swabs were immediately placed in 10 mL of sterile buffered peptone water (0.1%) for pre-enrichment and transportation.

Swine: Fresh rectal swabs were collected at lairage or immediate post-slaughter points. Samples were placed in sterile containers containing 10 mL of buffered peptone water (0.1%) to maintain moisture and support bacterial viability during transport.

Samples were immediately placed in cool boxes maintained at 4°C and transported to the Microbiology Laboratory at the College of Veterinary Medicine, Animal Resources and Biosecurity (CoVAB), Makerere University, for processing within 6 hours of collection. This timeframe minimizes changes in microbial viability and resistance profiles.

3.6 Isolation, Screening of ESBL-Producing *E. coli*

MacConkey agar supplemented with 2 µg/mL cefotaxime was used for selective isolation. Plates were incubated aerobically at 37°C for 18–24 hours. Pink colonies obtained from primary culture were considered presumptive *E. coli* and sub-cultured for purity.

Presumptive isolates underwent standard biochemical test tests including Indole, Methyl Red, Citrate, Triple Sugar Iron agar, urease tests were performed from the subculture according to the guidelines of Bergey's manual of determinative bacteriology and *E. coli*. API 20E strips were used for confirmation. Quality control strains included *E. coli* ATCC 25922 (ESBL-negative control). Only isolates that met the standard biochemical characteristics of *E. coli* were subjected to antimicrobial susceptibility testing to determine their resistance patterns.

3.7 Antimicrobial Susceptibility Testing (AST)

The antimicrobial resistance profiles of *E. coli* isolates were determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar following Clinical and Laboratory Standards Institute (CLSI) 2026 guidelines. A standardized bacterial suspension was made by emulsifying a 24-hour colony in 1 mL of sterilized normal saline. The turbidity of the formed bacterial suspension was matched a 0.5M MacFarland standard, thus forming a standardized bacterial suspension of approximately 1.5×108 cfu/ml. A sterile swab was immersed in the standardized bacterial suspension, and excess suspension was removed by pressing the swab on the walls of the tube using a firm pressure. The bacterial suspension on the swab was introduced onto the freshly prepared sterile MHA and a bacterial lawn made by surface spreading of the inoculum on MHA plates until the whole media surface was covered. The antibiotic disks were independently placed approximately 20mm apart onto inoculated agar plates.

A panel of clinically and veterinary-relevant antibiotics was tested, including amoxicillin-clavulanic acid(AMC),ceftazidime(CAZ), cefuroxime(CRO), tobramycin(TOB), cefepime (FEP),cefixime(CFM),gentamicin(CN),ciprofloxacin(CIP),tetracyclines(TE),trimethopri m-sulfamethoxazole(SXT) and chloramphenicol(C) and incubated at 37°C for 24 hours, after which zone diameters of inhibition were measured using a foot ruler with the plate held at an angle of 45 degrees against a dark background to enhance contrast and visibility of zone edges and zone diameters recorded in the workbooks against respective samples. Zone diameters were interpreted as susceptible, intermediate, or resistant based on CLSI 2026 breakpoints.

Phenotypic confirmation of ESBL production was done using Disk diffusion method where the antibiotic disks were independently placed approximately 20mm apart onto inoculated agar plates from amoxicillin-clavulanic acid (AMC) for cefepime (FEP) and aztreonam (ATM) in opposite direction, and then ceftazidime (CAZ), cefotaxime (CTX) placed approximately 15mm apart onto inoculated agar plates. The inhibition zone difference of ≥ 5 mm in the amoxicillin clavulanic acid versus the inhibition zone in the third-generation cephalosporin (cefotaxime (CTX)) tested alone was interpreted as positive for ESBL production.

3.8 Data Analysis

Data obtained from the microbiological analyses were systematically recorded in Microsoft Excel spreadsheets. Each sample entry included detailed information such as sample identification code, sampling location, and laboratory results, including the presence or absence of *Escherichia coli*, as well as the outcomes of the antibiotic susceptibility tests.

Following data entry and validation in Excel, the cleaned dataset was imported into STATA version 15 for statistical analysis. The analysis proceeded chronologically, beginning with descriptive statistics to determine the overall proportion of *E. coli* in all the sample sources sampled followed by determining proportion of antimicrobial-resistant ESBL producing *E. coli* in the wastewater samples, poultry and swine samples. antimicrobial resistance profiles for *E. coli* isolates were determined and that exhibited resistance to one or more antibiotics, The results were presented using frequency tables and graphs to provide a clear visual representation of the proportion data.

To determine the antibiotic resistance profiles of the isolated *E. coli* strains, results from the Kirby-Bauer disk diffusion method were interpreted according to the Clinical and

Laboratory Standards Institute (CLSI) guidelines. Each isolate's response to the tested antibiotics was categorized as susceptible, intermediate, or resistant. The data were then used to calculate the proportion of resistant isolates for each antibiotic.

Further analysis was conducted to compare resistance patterns and multi drug resistance between the different Sample sources. The fisher exact test was applied to determine whether there were statistically significant in the resistance patterns and multi drug resistance observed across sample sources.

3.9 Quality Control and Assurance

To ensure the reliability and integrity of results, rigorous quality control and assurance measures were implemented throughout the study. During sampling, sterile techniques were meticulously followed to prevent contamination. All sampling equipment, including 500 ml polypropylene bottles, sterile swabs was pre-sterilized, and field blanks were included as negative controls to monitor potential contamination during transportation and sample handling. Personal protective equipment (PPE), such as gloves, was changed between each sampling point to minimize cross-contamination risks.

Culture media were quality-checked for sterility before use, and only freshly prepared or properly stored media were employed. Positive and negative control strains were included during isolation and biochemical identification to validate the accuracy and performance of the procedures.

3.10 Ethical consideration

Permissions were also secured from the Kampala Capital City Authority (KCCA), National Water and Sewerage Corporation (NWSC) for Bugolobi STP, and management of Kampala City Abattoir and Wakiso district local government. The study aligned with Uganda’s National Action Plan on AMR (2024-2026). Biosafety and biosecurity protocols were strictly followed to protect laboratory personnel and the environment.

CHAPTER FOUR

RESULTS

4.1 Sample sources and sampling sites

From the study a total of 120 samples were collected from three different sources: wastewater, poultry, and swine. Each source contributed equally (40 samples), representing 33.3% of the total study samples.

Table 1: Samples and sample sources for the study

Sample source	Site	Number of samples, n (%)
Waste water	Bugolobi	20 (16.7)
	City abattoir	20 (16.7)
Poultry	Nakasero	10 (8.3)
	Nakawa	10 (8.3)
	Kasubi	10 (8.3)
	Kalerwe	10 (8.3)
Swine	Nansana	40 (33.3)

4.2 Proportion of *E. coli* in the sampled sources (waste water, poultry and swine)

From the study, *E. coli* was isolated from 79 samples, giving an overall proportion of 65.8%.

Table 2: Proportion of *E. coli* in the sampled subjects

Source	Number of samples	Proportion of *E. coli*, n
Waste water	40	31 (77.5)
Poultry	40	26 (65.0)
Swine	40	22 (55.0)
Overall	120	79 (65.8)

4.3 Proportion of ESBL *E. coli* in sampled subjects (waste water, poultry and swine)

4.3.1 Proportion of ESBL *E. coli* in samples from waste water

From the study, 31 *E. coli* isolates recovered from wastewater, 5 isolates (16.1%) were confirmed as ESBL-producing *E. coli*

Table 3: Occurrence of ESBL *E. coli* isolates from waste water

Site	Number of *E. coli* isolates	Proportion of ESBL *E. coli* (%)	*p*-value
Bugolobi	16	4 (25.0)	0.689
City abattoir	15	1 (6.7)	
Overall	31	5 (16.1)	

4.3.2 Proportion of ESBL *E. coli* in samples from poultry

From the study, 26 *E. coli* isolates obtained from poultry, 13 isolates (50.0%) were ESBL producers.

Table 4: Proportion of ESBL *E. coli* isolates from poultry

Site	Number of *E. coli* isolates	Proportion of ESBL *E. coli* n (%)	*p* – value
Nakasero	7	1 (14.3)	< 0.001
Nakawa	9	9 (100.0)	
Kasubi	6	0 (0.0)	
Kalerwe	4	3 (75.0)	
Overall	26	13 (50.0)	

4.3.3 Proportion of ESBL *E. coli* in samples from swine from Nansana

From the study, the 22 *E. coli* isolates recovered from swine samples.

Table 5: Proportion of ESBL *E. coli* isolates from swine

ESBL result	Frequency (n)	Percent (%)
Positive	7	31.8
Negative	15	68.2

4.3.4 Comparison of ESBL *E. coli* occurrence in the sampled subjects

From the study of the three sample sources showed that poultry had the highest proportion of ESBL-producing *E. coli* followed by swine and wastewater

Table 6: ESBL *E. coli* occurrence in the sampled subjects

Source	Number of *E. coli* isolates	Proportion of ESBL *E. coli* n (%)	*p* – value
Waste water	31	5 (16.1)	0.023
Poultry	26	13 (50.0)	
Swine	22	7 (31.8)	
Overall	79	25 (31.7)	

4.4 Antimicrobial resistance in *E. coli* isolates from waste water, poultry and swine

From the study of the 79 *E. coli* isolates, 62 isolates (78.5%) were resistant to at least one antimicrobial agent.

Regarding multidrug resistance (MDR**),** 27 isolates (34.2%) were resistant to three or more classes of antimicrobials.

Table 7: Occurrence of resistance and MDR in the sampled subjects

Source	Number of *E. coli* isolates	*Proportion of Resistant *E. coli* n (%)	#Proportion of MDR *E. coli* n (%)
Waste water	31	27 (87.1)	13 (41.9)
Poultry	26	23 (88.5)	12 (46.2)
Swine	22	12 (54.6)	2 (9.1)
Overall	79	62 (78.5)	27 (34.2)

*p-value for resistance = 0.08 and #p-value for mdr = 0.01, mdr – multidrug resistance

4.5 Antimicrobial susceptibility patterns of *E. coli* isolates from waste water

From the study of waste water isolates**,** antimicrobial susceptibility patterns were as below.

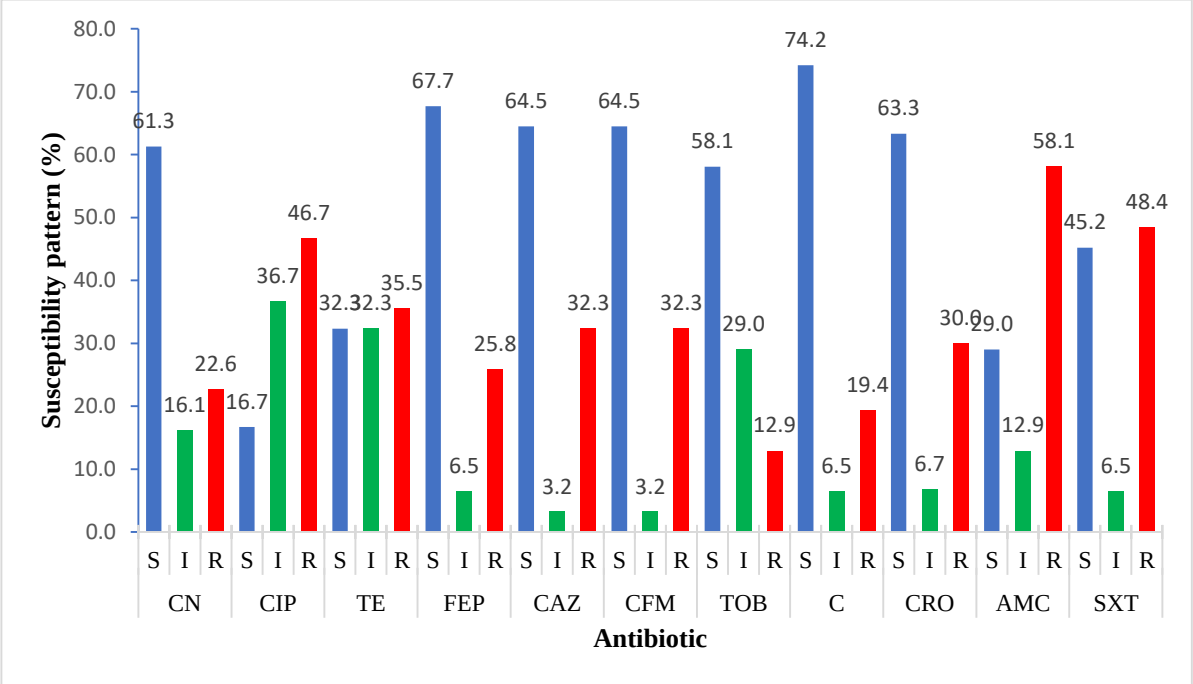


Figure 1: AMR susceptibility of *E. coli* from waste water

4.6 Antimicrobial susceptibility patterns of *E. coli* isolates from poultry

From the study, poultry isolates antimicrobial susceptibility patterns were as below

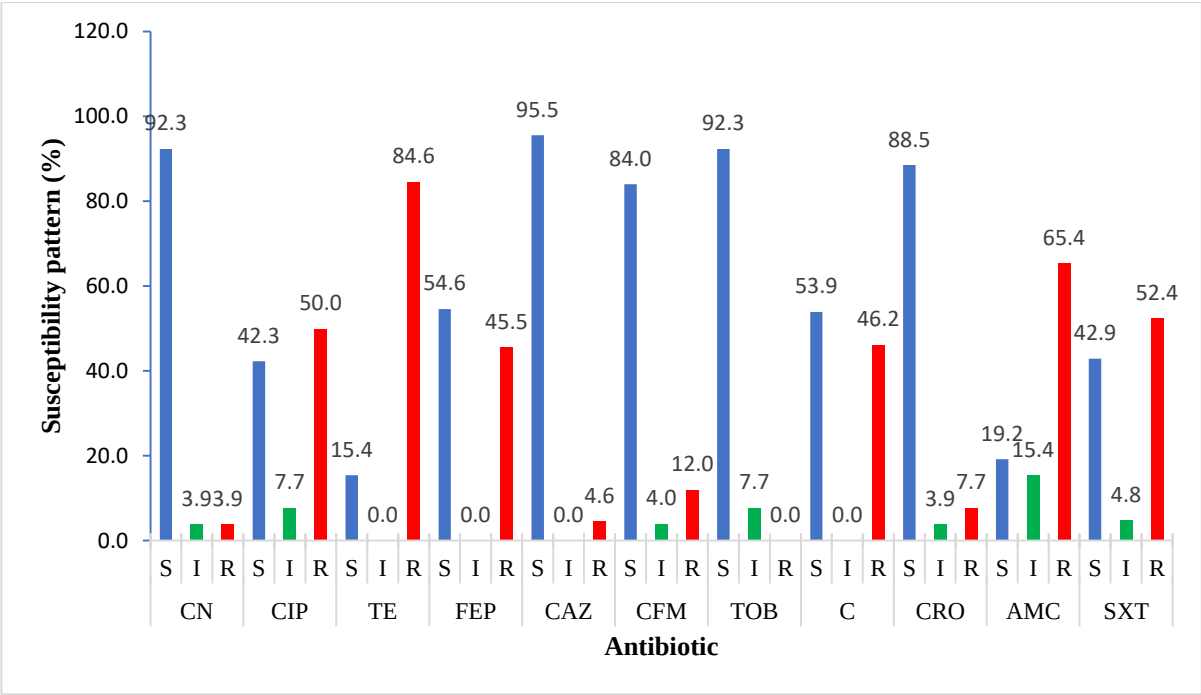


Figure 2: AMR susceptibility of *E. coli* from poultry

4.7 Antimicrobial susceptibility patterns of *E. coli* isolates from swine

From the study, the antimicrobial susceptibility pattern for swine

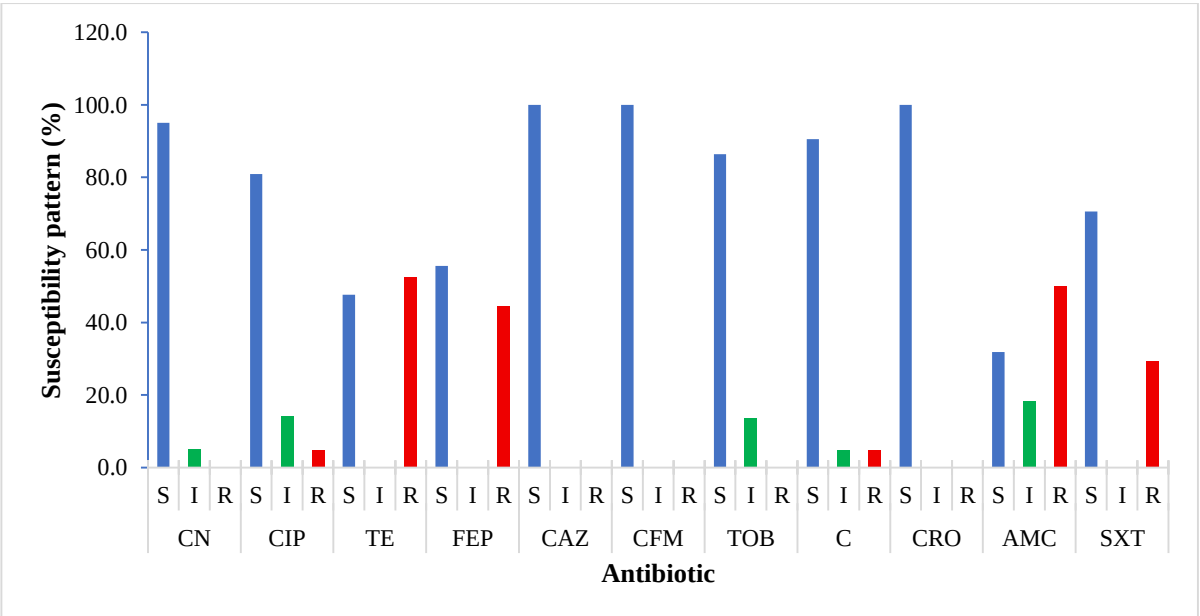


Figure 3: AMR susceptibility of *E. coli* from swine

CHAPTER FIVE

DISCUSSION

5.1 Introduction

This study investigated the antimicrobial resistance profiles of Extended-Spectrum Beta-Lactamase (ESBL)-producing *Escherichia coli* isolated from wastewater, poultry, and swine sources within the Kampala Metropolitan Area, Uganda to examine the animal–environment interface. The findings demonstrate that *E. coli* is widely distributed across the sampled compartments, that ESBL-producing strains are established within these reservoirs, and that multidrug resistance is common, particularly among isolates recovered from poultry and wastewater.

5.2 Proportion of *Escherichia coli* across Sample Sources

The overall recovery of *E. coli* was 65.8% (79/120 samples). Recovery rates varied by source: wastewater yielded the highest proportion (77.5%, 31/40), followed by poultry (65.0%, 26/40) and swine (55.0%, 22/40). These findings confirm that enteric bacteria are abundant at the animal environment interface in Kampala and that wastewater systems function as major sinks for fecal contamination (Muleme et al., 2023).

The high recovery from wastewater is consistent with the nature of the sampling sites. Bugolobi Sewage Treatment Plant receives mixed domestic, healthcare, and industrial influent, while Kampala City Abattoir generates high organic loads containing animal faeces, blood, and rumen contents. Such environments provide nutrients and moisture that support the persistence of enteric organisms. Previous studies have similarly identified wastewater treatment plants and abattoir effluents as important environmental reservoirs of *E. coli* and other Enterobacteriaceae in both high- and low-resource settings.(Tufa & Birhanu, 2025).

The proportion observed in poultry (65.0%) aligns with the ecology of intensive and semi-intensive poultry production. High stocking densities, continuous environmental contamination of housing and market environments, and frequent handling at live bird markets favor colonization and cross-contamination. The recovery rate from swine (55.0%), although lower than the other two sources, still indicates that more than half of the animals sampled carried *E. coli* detectable by the methods employed. This finding underscores the role of pigs as reservoirs capable of introducing resistant bacteria into abattoir environments and, through runoff and waste, into the surrounding ecosystem.

The recovery rates demonstrate that wastewater, poultry, and swine compartments in Kampala routinely harbor *E. coli* and therefore constitute legitimate targets for antimicrobial resistance surveillance.

5.3 Proportion of ESBL-Producing *Escherichia coli*

Among the 79 *E. coli* isolates recovered, 25 (31.7%) were phenotypically confirmed as ESBL producers. This overall proportion indicates that ESBL-producing strains are well established at Kampala's animal environment interface. The distribution was markedly uneven across sources: poultry recorded the highest proportion (50.0%, 13/26), followed by swine (31.8%, 7/22) and wastewater (16.1%, 5/31). The difference among sources was statistically significant ($p = 0.023$).

Poultry has the highest proportion of 50% as the Dominant Reservoir of ESBL Live bird markets in Kalerwe, Nakasero, Kasubi, and Nakawa are characterized by high bird density, frequent trading, on-site slaughter, and limited hygiene infrastructure. These conditions facilitate both the selection and the amplification of resistant strains. In addition, the widespread use of antibiotics for prophylaxis and growth promotion in poultry production systems in Uganda and other low- and middle-income countries exerts strong selective pressure favoring ESBL-producing organisms. (Mandujano-Hernández et al., 2024). The present findings therefore situate Kampala's live bird markets within a broader regional pattern in which poultry production constitutes a major reservoir of ESBL-producing Enterobacteriaceae.

Swine isolates were one-third (31.8%) of ESBL producers. This proportion is lower than that observed in poultry but remains epidemiologically significant. Pigs can acquire ESBL-producing *E. coli* through multiple routes, including contaminated drinking water, feed, introduction of new animals, transport vehicles, and holding pens at abattoirs (Bergšpica et al., 2020).

Wastewater had the lowest with proportion of (16.1%) among the three sources, the absolute number of resistant isolates and the continuous nature of wastewater discharge confer substantial public health importance (Mutuku et al., 2022). The higher ESBL recovery from Bugolobi STP (25.0%) compared with Kampala City Abattoir (6.7%) reflect differences in the composition of influent (domestic and healthcare contributions versus primarily animal-origin waste

5.4 Antimicrobial Resistance Patterns

Overall, 78.5% (62/79) of *E. coli* isolates were resistant to at least one antimicrobial agent. Resistance was highest among poultry isolates (88.5%) and wastewater isolates (87.1%), while swine isolates showed comparatively lower resistance (54.6%). These figures indicate widespread exposure of bacterial populations to antimicrobial selective pressure within the studied compartments. The high resistance rates to tetracycline, ciprofloxacin, and trimethoprim-sulfamethoxazole among poultry and wastewater isolates mirror patterns documented in national and regional surveillance reports. This is due to these agents are frequently used for treatment, disease prevention and sometimes growth promotion in livestock production. Inappropriate antimicrobial use creates selective pressure favoring resistant bacterial populations. These agents, or closely related compounds, have been widely used in both human medicine and livestock production in Uganda, generating sustained selective pressure. The comparatively lower resistance burden among swine isolates may indicate less intensive antimicrobial use or different management practices in the swine production systems supplying Nansana Abattoir; however, the presence of any ESBL-producing and resistant strains in this compartment remains a concern.

5.5 Multidrug Resistance

Multidrug resistance (MDR), defined as non-susceptibility to agents from three or more antimicrobial classes, was detected in 34.2% (27/79) of isolates. The distribution was significantly uneven ($p = 0.01$): poultry isolates exhibited the highest MDR proportion (46.2%), followed closely by wastewater isolates (41.9%), while swine isolates showed a markedly lower rate (9.1%).

The high MDR rates in poultry and wastewater are particularly alarming. In poultry production, repeated exposure to multiple antimicrobial classes creates an environment in which only highly resistant strains survive and proliferate. Live bird markets further amplify this problem by concentrating birds from diverse sources under conditions that favor cross-contamination. Wastewater systems, in turn, receive resistant bacteria from hospitals, households, pharmaceutical residues, and livestock operations. The high bacterial density, nutrient availability, and residual antimicrobial concentrations typical of wastewater promote conjugation. The structural similarity in MDR proportion between poultry (46.2%) and wastewater (41.9%) therefore supports that veterinary selective pressures and municipal waste streams jointly drive the emergence and persistence of multidrug-resistant phenotypes.

The low MDR proportion among swine isolates (9.1%) is encouraging relative to the other sources, yet it does not eliminate risk. Even a minority of multidrug-resistant strains can expand under favorable conditions and contaminate the food chain or the environment. Continued surveillance of swine production remains necessary.

5.6 Study Limitations

The study relied on phenotypic methods for identifying ESBL-producing *E. coli*. Molecular techniques such as polymerase chain reaction (PCR) or whole genome sequencing were not performed to identify specific resistance genes including *blaCTX-M, blaTEM* and *blaSHV*. In addition, the cross-sectional design provides information at one point in time and does not capture seasonal variations in antimicrobial resistance. Despite these limitations, the study provides valuable baseline data on ESBL-producing *E. coli* within Kampala Metropolitan Area and contributes to understanding antimicrobial resistance.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study successfully evaluated the phenotypic antimicrobial resistance (AMR) profiles of *Escherichia coli* and extended-spectrum beta-lactamase (ESBL)-producing *E. coli* at the animal environment interface in the Kampala Metropolitan Area. Based on the experimental findings and statistical analyses, the following conclusions are drawn

Escherichia coli is highly present across all sampled compartments (65.8% overall recovery), with environmental wastewater systems serving as the largest bacterial sink (77.5%), followed closely by poultry value chains (65.0%) and swine networks (55.0%).

Phenotypic ESBL production is firmly established at Kampala's urban interface, affecting 31.7% of all recovered *E. coli* isolates. The distribution of these resistant pathogens is significantly compartment-dependent ($p = 0.023$).

Commercial poultry networks represent the most volatile reservoir of ESBL-producing strains, exhibiting a 50.0% proportion rate followed by Swine (31.8% ESBL) and wastewaters (16.0% ESBL represent dangerous secondary reservoirs that actively maintain and release resistant phenotypes into the local food supply and environmental waterways.

Multidrug resistance is dangerously common at the urban interface, affecting 34.2% of all isolates, with statistically significant divergence across sources ($p = 0.01$). The alarming structural convergence of MDR levels between poultry (46.2%) and wastewater (41.9%) confirms that high veterinary antibiotic selection pressures and municipal waste stream mixing are driving co-selection for pan-resistant traits.

Ultimately, these findings substantiate the One Health premise that AMR in Kampala cannot be addressed in sectoral isolation, as resistant pathogens cycle dynamically between livestock populations, urban market environments, and wastewater infrastructures.

6.2 Recommendations

To mitigate the dissemination of ESBL-producing and multidrug-resistant *E. coli* within Kampala, a coordinated, multi-sectoral framework must be operationalized. The following specific actions are recommended:

The National Water and Sewerage Corporation (NWSC) should enhance tertiary treatment capabilities at the Bugolobi Sewage Treatment Plant. Transitioning from basic secondary

processes or standard chlorination to more robust advanced oxidation processes (AOPs), such as UV-C combinations, is necessary to successfully degrade residual beta-lactam compounds and eliminate persistent ESBL-producing pathogens before environmental discharge.

The Kampala Capital City Authority (KCCA) must strictly enforce environmental compliance guidelines requiring the Kampala City Abattoir to install functional on-site solid-liquid separation and sedimentation tanks. Effluents containing high organic loads, animal feces, and blood must not be discharged untreated into municipal drainage systems.

The Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) should restrict the over-the-counter access and indiscriminate use of medically important antibiotics especially tetracyclines and fluoroquinolones for flock prophylaxis and growth promotion. Farm-level veterinary oversight must be reinforced to curb the extreme selective pressures identified in poultry supply chains.

Technical outreach programs should be rolled out to swine and poultry producers to improve structural biosecurity, waste management, and hygiene. Proper composting of swine manure and poultry litter should be mandated to heat-inactivate enteric pathogens before agricultural land application, breaking the environmental recycling loop.

Uganda's National Action Plan on AMR must structurally expand its baseline monitoring beyond human clinical isolates to include routine, programmatic testing of municipal wastewater effluents, live bird markets, and livestock abattoirs.

Future research initiatives should build upon this phenotypic baseline by utilizing whole-genome sequencing (WGS) or polymerase chain reaction (PCR) assays. This will allow investigators to map the specific plasmid tracking networks (such as blaCTX-M-15 lineages) and precisely quantify horizontal gene transfer rates across the human-animal-environment continuum.

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APPENDICES

Appendix I: Research pictorial evidence



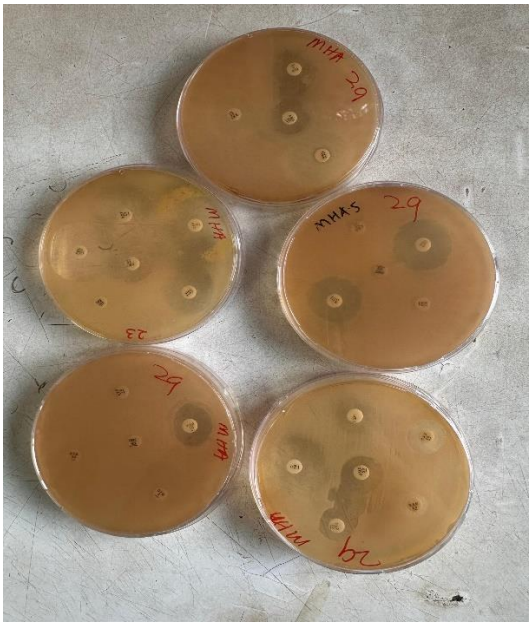
Waste water sample collection



AST determination using different drugs



AST prepared plates waiting incubation.



Zones of inhibition.