

**PREVALENCE AND ANTIMICROBIAL RESISTANCE PATTERN OF PATHOGENIC
ESCHERICHIA COLI IN SMALL SCALE PIG FARMS**

BY

AUMA JILLIAN

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BSc. Educ (Hons), GULU UNIVERSITY

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APPROVAL

This dissertation has been submitted for examination with our approval as university supervisors.

Professor Emeritus Ipolto Okello Uma (RIP)

Department of Food Science and Post-Harvest Technology,

Faculty of Agriculture and Environment,

Gulu University

.....

.....

Signature

Date

Dr. Beyihayo Akiiki Geoffrey (PhD),

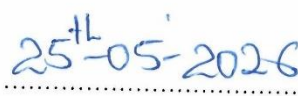
Department of Animal Production,

Faculty of Agriculture and Environment,

Gulu University



Signature



Date

DECLARATION

I, AUMA JILLIAN, hereby declare that this research dissertation is my original work and has not been presented for academic award in any University.



Signature



Date

DEDICATION

I dedicate this research to my beloved family, Mr. and Mrs. Cwa David, whose unwavering support, encouragement, and belief in me continually inspired my passion for academic pursuit and discovery. I also dedicate this work to my husband, Mr. Byamukama Didas, for his invaluable financial, spiritual, and emotional support throughout the course of this study. Special dedication goes to my children, Arinaitwe Jemimah and Amutuhire Maria Jasmine, whose love, patience, and emotional encouragement gave me strength and motivation during this research journey.

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LIST OF ABBREVIATIONS/ACRONYMNS

AMR	Antimicrobial Resistance
SDD	Single Disk Diffusion
PCR	Polymerase Chain Reaction
<i>E. coli</i>	<i>Escherichia coli</i>
MDR	Multidrug Resistance
WHO	World Health Organization
FAO	Food and Agricultural Organization
MAAIF	Ministry of Agriculture, Animal Industry and Fisheries
UNAS	Uganda National Academy of Science
US	United States
USD	United States Dollar
UBOS	Uganda National Bureau of Statistics
UTI	Urinary Tract Infection
MoH	Ministry of Health
Mcr-3	Colistin Resistant Gene
<i>aadA1</i>	Streptomycin Resistant Gene
<i>aac(6)-Ib-cr</i>	Ciprofloxacin Resistant Gene

<i>ereA</i>	Erythromycin Resistant Gene
<i>aac(3)-IV</i>	Gentamicin Resistant Gene
CDC	Center for Disease Control and Prevention
HUS	Hemolytic Uremic Syndrome
UNEP	United Nation Environment Program
LMIC	Low and Middle Income Countries
IPC	Infection Prevention and Control
ESBL- <i>Ec</i>	Extended-Spectrum Beta-Lactamase-producing <i>E. coli</i>
STEC	Shiga Toxin-Producing <i>E. Coli</i>
LAMP	Loop-Mediated Isothermal Amplification
PWD	Post-Weaning Diarrhea
ETEC	Entero Toxigenic <i>Escherichia coli</i>
MALDI-TOF MS Spectrum	Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass
DNA	Deoxyribonucleic Acid
UV	Ultra Violet Radiation
μL	Micro Liter
ml	Milli Liter
%	Percentage

nm	Nanometer
°C	Degree Celsius
NCCLS	Clinical and Laboratory Standards Institute guidelines
PPV	Positive Predictive Value
NPV	Negative Predictive Value
ROC	Receiver Operating Curve
AUC	Area Under Receiver Operating Curve
S	Second
rpm	Revolution per minute
kb	Kilo bytes
bp	Base pairs

ABSTRACT

The growing threat of antimicrobial resistance (AMR) is a critical global health issue largely driven by antibiotic misuse. Monitoring AMR levels, through bacteria like *Escherichia coli* (*E. coli*), is crucial. The research aimed at: 1) determining the prevalence of pathogenic *E. coli* in small scale pig farms in Gulu City, 2) assessing the antimicrobial resistance profile of *E. coli* O157 to six frequently administered antibiotics, and 3) determining antimicrobial resistance genes for six frequently administered antibiotics. Fecal samples from small-scale pig farms were analyzed for *E. coli* using culture and Polymerase Chain Reaction (PCR), targeting *E. coli* O157. Positive samples underwent resistance testing using Single Disk Diffusion (SDD), and finally PCR detecting resistance genes for specific antibiotics. The results indicated high prevalence of *E. coli* (87%) in pig feces, with a 55% prevalence of *E. coli* O157. *E. coli* O157 prevalence was associated with pig sex. Alarming antibiotic resistance rates were observed in *E. coli* O157, particularly to ampicillin (72.7%), erythromycin (65.5%), colistin (47.3%)%, gentamicin (34.5%), streptomycin (25.5%), and ciprofloxacin (7.27%), while 48% of isolates exhibited multidrug resistance (MDR). Colistin resistance was associated with pig age, and ampicillin resistance with geographic location. Genotypic analysis identified a high prevalence of resistance genes, including *mcr-3* for colistin (73%), *aac(6)-Ib-cr* ciprofloxacin (71%), *ereA* erythromycin (65%) , *aadA1* streptomycin (56%) , and *aac(3)-IV* gentamicin (53%). These findings highlight the need for more effective monitoring and management of AMR in animal husbandry.

Key words

Antimicrobial resistance, *Escherichia coli* O157, Pigs, Multidrug resistance, single disk diffusion, polymerase chain reaction.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Antimicrobial resistance (AMR) has become one of the most pressing global threats to public health, complicating the management of infections caused by bacteria, fungi, parasites, and viruses (WHO, 2023; FAO, 2017; MAIAF, 2023). This phenomenon occurs when microorganisms develop resistance to antimicrobial drugs that were once effective. Among these, *Escherichia coli* (*E. coli*), a common gut bacterium, has gained notoriety for its growing resistance to multiple antibiotics (WHO, 2023; FAO, 2017; MAIAF, 2023), including beta-lactams (e.g., ampicillin, amoxicillin), fluoroquinolones (e.g., ciprofloxacin), aminoglycosides, tetracyclines, and carbapenem (Mohsin et al., 2018; Paniagua-Contreras et al., 2018; UNAS et al., 2015).

Globally, the U.S. Centers for Disease Control and Prevention (CDC) reported approximately 2.8 million cases of antibiotic-resistant infections annually, with over 35,000 and 33,000 deaths in the U.S. and Europe, respectively CDC (2019). AMR is particularly prevalent in regions like Asia, Africa, and South America, where resistance in *E. coli* isolates exceeds levels observed in parts of Europe, North America, and Australia (Akrami et al., 2023; Munita & Arias, 2016; Farzan et al., 2010). Studies from sub-Saharan Africa reinforce these concerns. For instance, a research in Tanzania identified antimicrobial-resistant *E. coli* colonizing pigs (Mtemisika et al., 2022), while in South Africa, MDR- *E. coli* strains were identified in retail meat samples, including colistin-resistant isolates (Iweriebor et al., 2022). In Uganda, *E. coli* isolated from poultry and retail meats showed resistance to various antibiotics, raising concerns regarding the potential spread of these resistant strains within the food chain (Kakooza, 2021; Okubo et al., 2020).

The World Health Organization has warned that if AMR continues unchecked, it could result in over 10 million deaths annually by 2050 (Waswa et al., 2024). The rise in multidrug-resistant organisms severely limits treatment options for both human and animal infections, increasing the burden on public health systems and economies (Kimera et al., 2021). According to World Bank, antimicrobial resistance could reduce global GDP by up to 3.8% and push approximately 28 million people into extreme poverty by 2050 if interventions are not implemented (WB, 2024). The misuse and overuse of antibiotics, which create selection pressure for resistant strains (Oyebanji, 2018; Culyba et al., 2015), exacerbate the situation, as they develop mechanisms of resistance like antibiotic inactivation and target modification (Munita & Arias, 2016). Moreover, the zoonotic nature of *E. coli* means these resistant strains can easily spread from animals to humans through contaminated water, environments, or animal products (Samtiya et al., 2022).

E. coli O157:H7 is distinguished among pathogenic strains for its association with severe diseases such as hemolytic uremic syndrome (HUS), which can lead to renal failure, particularly in children and immunocompromised individuals (Palermo et al., 2009). This serotype remains a significant cause of morbidity and mortality worldwide (Llor & Bjerrum, 2014). Poor sanitation and inadequate animal health management, particularly in swine farms, may contribute to the spread of pathogenic and resistant strains like *E. coli* O157, underscoring the need for comprehensive surveillance of these bacteria (Coccia et al., 2021).

Despite growing concerns about AMR, research on the prevalence and resistance patterns of *E. coli* O157 in swine farms remains sparse, especially in low- and middle-income countries (LMICs). In Uganda, although a study by Ikwap et al. (2021) documented AMR in *E. coli* from smallholder pig farms, the specific resistance patterns of pathogenic *E. coli* O157 and prevalence were not explored. Furthermore, the study didn't explore the molecular resistance, since resistance

exist at both phenotypic and genotypic level. It is also necessary to determine genotypic resistance to design appropriate interventions to combat AMR.

1.2 PROBLEM STATEMENT

Antimicrobial resistance (AMR) is a growing threat to global public health. It has led to an increased incidence of infectious diseases in both animals and humans due to the failure of prescribed treatments (Kivumbi & Standley, 2021). A recent multi-hospital study conducted in Uganda estimated the total annual economic burden of AMR across nine regional referral hospitals at approximately US\$ 1.43 million, with an average cost of US\$4,983 per patient episode (Mayito et al., 2026) . This problem is partly driven by the misuse of antibiotics in livestock production, particularly in pig farming.

Evidence from a study by (Ikwap et al., 2021) reported the presence of AMR in *E. coli* isolates obtained from pig farms . Their findings further highlighted the use of antibiotics in healthy pigs as a preventive measure against African swine fever, a viral disease that does not respond to antibiotic treatment. This inappropriate practice is a major contributor to the development and spread of antimicrobial resistance and underscores the widespread presence of AMR in the region. If left unaddressed, this situation could lead to irreversible and devastating consequences for both animal and human health, as well as welfare and productivity (Dadgostar, 2019a).

However, there is scarce data on the prevalence of pathogenic *E. coli* O157:H7, its antimicrobial resistance patterns to commonly used agents, and antimicrobial resistance genes. This gap in knowledge hinders efforts to combat AMR and protect the public from these pathogens. Therefore, research is required to generate evidence and monitor trends to curb the emergence and spread of antimicrobial resistance.

1.3.0 General objective

To determine the occurrence, phenotypic antimicrobial resistance patterns, and genotypic resistance profiles of pathogenic *Escherichia coli* in small-scale pig farms.

1.3.1 Specific objectives

- i. To assess prevalence of pathogenic *E. coli* O157 in small- scale pig farms.
- ii. To assess the antimicrobial resistance pattern of pathogenic *E. coli* O157 to commonly used anti-microbial agents.
- iii. To evaluate antimicrobial resistance genes for the selected antibiotics.

1.3.2 Research Questions

- i. What is the prevalence of pathogenic *E. coli* O157 in small-scale pig farms?
- ii. What is the antimicrobial resistance pattern of pathogenic *E. coli* O157 to commonly used selected anti-microbial agents?
- iii. What are the frequencies of antimicrobial resistance genes associated with resistance to the selected antibiotics in pathogenic *E. coli* isolates?

1.4 Significance of the Study

This study provides important insights into the prevalence and AMR patterns of *E. coli* isolated from pigs. The findings are significant to several stakeholders, including farmers, veterinarians, and policymakers.

For farmers, the study provides information on the occurrence of antimicrobial resistant bacteria in pig production systems. This knowledge can increase awareness of the risks associated with the misuse or overuse of antibiotics in livestock production. Consequently, farmers may be encouraged to adopt improved husbandry practices, such as enhanced biosecurity, proper hygiene, and appropriate disease prevention strategies, which can reduce reliance on antibiotics and minimize economic losses associated with treatment failure and disease outbreak.

For veterinarians, the results of this study contribute to a better understanding of antimicrobial resistance patterns of *E. coli* circulating in pigs. Such information is essential for guiding evidence based treatment decisions and selecting appropriate antibiotics for managing bacterial infections in livestock. Furthermore, the findings support antimicrobial stewardship efforts aimed at promoting the prudent use of antibiotics in veterinary practice.

For policymakers and government authorities, the findings of this study provide scientific evidence that can inform the development and implementation of policies regulating antimicrobial use in animal production. This results may also contribute to strengthening antimicrobial resistance surveillance systems and support national and global efforts to combat AMR within the One Health framework promoted by the World Health Organization, Ultimately the study contributes to protecting both animal health and public health by addressing the growing challenge of AMR.

1.5 Scope of the Study

1.5.1 Content Scope

The research aimed to determine occurrence, phenotypic and genotypic resistance of pathogenic *E. coli* in pigs in order to inform public health control and antimicrobial stewardship strategies.

1.5.2 Geographical Scope

Samples for this study were collected from small scale farms in Gulu City, and laboratory analyses conducted at Gulu University's multifunctional laboratory.

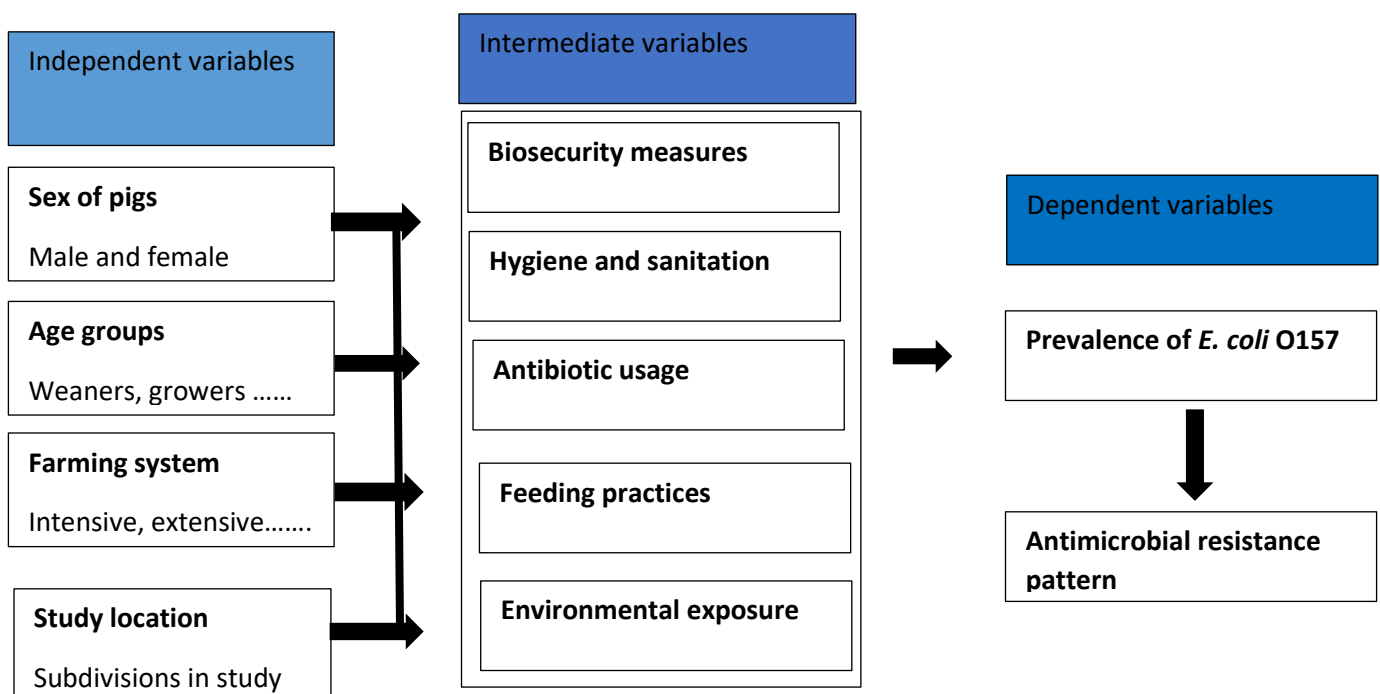
1.5.3 Time Scope

This research was conducted in one year, from December 2023 to March 2024.

1.6 Conceptual Framework

This study was guided by a conceptual framework in which host-related factors (sex and age group) and environmental/management factors (study location and farming system) are considered key independent variables influencing the occurrence and antimicrobial resistance of pathogenic *E. coli* O157. Sex and age are important biological determinants that may influence susceptibility to infection. For instance, younger animals often have immature immune systems, making them more vulnerable to colonization by enteric pathogens such as *E. coli* O157, while physiological differences associated with sex may also contribute to variation in infection dynamics (Chase-Topping et al., 2008; Kaper et al., 2004). Study location reflects geographical and environmental differences such as climate, sanitation, and microbial ecology, which can affect pathogen survival and transmission. Variations in agro-ecological zones have been shown to influence the prevalence

of zoonotic pathogens, including *E. coli* O157 (Rangel et al., 2005). Farming system (e.g., intensive, semi-intensive, or extensive) plays a critical role in determining animal exposure to pathogens. Intensive systems may increase transmission due to crowding, while extensive systems may expose animals to environmental contamination. Additionally, farming systems influence hygiene practices, biosecurity measures, and patterns of antimicrobial use, all of which are key drivers of pathogen prevalence and resistance (FAO, 2016; Marshall & Levy, 2011). These independent variables interact to shape intermediate processes, including exposure to contaminated environments, farm management practices, and antimicrobial usage. The use and misuse of antimicrobials in livestock production exert selective pressure on bacteria, promoting the emergence and spread of resistant strains (WHO, 2017). Ultimately, these factors influence the dependent variables, namely the prevalence of pathogenic *E. coli* O157 and its antimicrobial resistance (AMR) patterns. High prevalence indicates widespread exposure and transmission, while AMR patterns reflect the extent of resistance development due to selective pressures within the production system.



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Prevalence of Pathogenic *E. coli*

The issue of foodborne diseases attributable to the pathogenic *E. coli* O157:H7 serotype has received extensive attention, triggering scientific investigation about its prevalence across different spheres of the globe, including sub-Saharan Africa. To this effect, evidence from several studies such as Abdalla et al. (2021), Tanih et al. (2015), Ateba & Mbewe (2011), and Ateba et al. (2008) among others shows that the prevalence of pathogenic *E.coli* in the pork value chain in Sub-Saharan Africa is a significant concern. Precisely, a study by Abdalla et al. (2021) in South Africa's Mgungundlovu District found the prevalence of pathogenic *E.coli* in the pork value chain. Similarly, a study by Ateba et al. (2008) in South Africa found a higher prevalence of *E.coli* O157 in commercially farmed pigs. This was further supported by Tanih et al. (2015), who detected pathogenic *E.coli* isolates in cattle and pigs slaughtered in South African abattoirs. Ateba & Mbewe (2011) also documented an increased prevalence of *E. coli* O157 in the pork value chain. Similarly, despite a study by Karama et al. (2019) indicating that non-O157 serotypes such as STEC O26, O111, and O117 were predominantly associated with human diseases in South Africa, the occurrence of *E.coli* O157 was found to be 13%. In Cameroon, a study by Esemu (2015) detected *E. coli* O157 in 10.9% of cattle samples. Additionally, a review by Fletcher et al. (2011) of diarrheal pathogens in SSA found *E. coli* spp, with *E. coli* O157:H7 being a significant pathogen in the region. Furthermore, a study by Lupindu (2018) in Ethiopia reported *E. coli* O157 in 28.9% of isolates obtained from the feces of children with diarrhea.

In Uganda, several studies have indicated the prevalence of pathogenic *E. coli* (Nabatanzi et al., 2022; Obala et al., 2021; Walusansa et al., 2020). For example, research conducted by Walusansa et al. (2020) indicated 16% prevalence of Enterohemorrhagic *E. coli* (EHEC) among the pastoralist communities was approximately mainly from phylogroups B1 B2, and A with stx1, and stx2e virulence genes (Walusansa et al., 2020). Study conducted by Nabatanzi et al. (2022) also indicated a high prevalence of shigella toxin-producing *E.coli* among pastoral communities in northern Uganda. In the context of the pork value chain, a research conducted by (Obala et al, 2021) documented an increased prevalence of *E. coli* with enterotoxin gene markers among isolates from Eastern Uganda. A study by Kagambèga et al. (2011) found a 100% prevalence of *E. coli* in various types of raw meat in Burkina Faso, suggesting a similar prevalence in Uganda. Both studies conducted by Obala et al. (2021) and Ikwap et al. (2016) further identified *E. coli* as a key factor in piglet diarrhea, with the former reporting an elevated presence of virulence genes in *E. coli* isolates from piglets. A study by (Katushabe et al., 2022b) also indicated occurrence of *E. coli* in pigs and hence pork value chain from all the regions of Uganda was high. Findings by Selling (2020) equally indicated that a high presence of *E. coli* noted among weaning pigs in Lira.

However, even if these findings underscore the significance of *E. coli* pathogens throughout Uganda's health sector and pork value chain, most studies such as Nabatanzi et al. (2022), Obala et al. (2021), and Walusansa et al. (2020) that have been conducted in Uganda didn't specify the different serotypes. This implies that the evidence from these studies remains inadequate to be relied on for drawing conclusions and hence designing comprehensive strategies for addressing these pathogens and ensuring food safety and public health.

2.2 Antimicrobial Resistance among Pathogenic *E. coli*

Antimicrobial resistance is a phenomenon that describes the capacity of microorganisms to withstand the impact of antimicrobial agents. This occurs in a way that microorganisms develop resistance to the medications that were formerly potent in combating associated infection (Annunziato, 2019). Antimicrobial resistance is a global health nuisance because it reduces the effectiveness of commonly used and easily accessible essential drugs such as antibiotics, antivirals, and antifungals, critical for managing several diseases in humans, animals, and plants (Headley, 2021; Schulster & Chinn, 2003). As a result, AMR causes treatment failures, making control of infectious diseases more difficult and highly costly (Dadgostar, 2019b). This is largely attributed to the use of antibiotics in human, veterinary care, and various agricultural practices (S. Ibrahim et al., 2021). Additionally, AMR could also be driven by globalization which contributes to the spread and distribution of antibiotic-resistant strains (McEwen & Collignon, 2018; Mugerwa et al., 2021).

E. coli causes diseases such as dysentery sometimes known as hemorrhagic diarrhea and urinary tract infections (UTIs), among others (Fletcher et al., 2011; Nabatanzi et al., 2022). The resistance of these microbes to existing antibiotic agents leads to a reduction in treatment options and heightened risk of complications. Therefore, *E. coli* has been reported to exhibit resistance to various antimicrobial agents such as beta lactam-made drugs like ampicillin and amoxicillin; fluoroquinolones such as ciprofloxacin; aminoglycosides; tetracyclines; glycosamides, and carbapenems (Paniagua-Contreras et al., 2018b). Spread of AMR occurs through contaminated food, water, or direct exposure to infected individuals or animals. Muleme et al. (2023) reported that there exist a wider dissemination of ESBL- *E. coli* across different human, and animal

environments, an aspect which is indicative of poor management of infections. Due to AMR's negative implications, its presence among foodborne disease-causing pathogenic *E. coli* is of great interest to global health, environment, and agricultural actors. Its development in the pork value chain is particularly regarded as one of the greatest threats to global health, food security and development.

However, it is essential to recognize that even if antibiotic resistance is ubiquitous in nature, there are variations in its prevalence rates across geographical regions (Murray et al., 2022; Mugerwa et al., 2021; Headley, 2021). A study by Piu et al. (2019) indicates that regions such as Asia, Africa, and South America have higher prevalence and distribution rates of AMR, while North America, Europe, and Australia report lower rates, with the resistant strains of *E. coli* found in healthcare facilities, community settings, and agricultural environments (Campos et al., 2019; FAO, 2013; Huber et al., 2021; Miller et al., 2020). CDC (2019) also reported that each year in the US, there are approximately 2.8 million antibiotic-resistant infections, leading to over 35,000 fatalities. However, the level of resistance among bacterial serotypes varies from one serotype and geographical region to another, with some serotypes exhibiting resistance to similar antibiotics such as ampicillin and amoxicillin made with beta-lactam components (Bindelle et al., 2018; Bolton et al., 2012). Additionally, the susceptibility of *E. coli* to these antimicrobials varies depending on the source of the isolates and the specific antibiotics tested. For instance, a study conducted by Ninh et al. (2023) identified *E. coli* isolates from cancer patients exhibited high resistance rates to ampicillin, sulfamethoxazole/trimethoprim, and ceftazidime. A study by Abu-Aqil et al. (2022) indicated that resistance of *E. coli* from UTIs to different antibiotics ranged from 17% to 25%. Additionally, *E. coli* isolates from a bovine slaughterhouse demonstrated resistance to tetracycline, cephalothin, streptomycin, and ampicillin, with a significant percentage presenting

multidrug resistance (Jos et al., 2023). Limbachiya et al. (2022) further indicated that *E. coli* isolates from farms displayed high resistance to pefloxacin, moxifloxacin, and tetracycline while being highly susceptible to colistin, ceftriaxone, and spectinomycin.

In sub-Saharan Africa, research conducted by Hiko et al., (2008) showed the presence of *E. coli* O157, a major cause of diarrhea and other illnesses in Ethiopian retail raw meat products. Similarly, a study in Tanzania found that poultry and pigs in Mwanza harbored resistant *E. coli*, with different resistance patterns observed among the animal populations (Mtemisika et al., 2022). In South Africa, retail meat samples showed resistance to various antibiotics, with a troubling identification of colistin resistance among multidrug-resistant *E. coli* strains, highlighting public health implications (Iweriebor et al., 2022). Additionally, a study by Okubo et al. (2020) identified the existence of multidrug-resistant *E. coli*, encompassing *E. coli* O157, in meat samples from abattoirs and butcher shops in Uganda. Similarly, a study by Katushabe et al. (2022) conducted in Uganda's main pig abattoir revealed a high prevalence of multidrug-resistant *E. coli* in pigs, with resistance to erythromycin being common. A study by Muleme et al. (2023) on Uganda's main pig abattoirs revealed that there was a high incidence of multidrug-resistant *E. coli* in live pigs brought for slaughter. Additionally, research conducted by Muleme et al. (2022) and Katushabe et al. (2022) in central Uganda found widespread prevalence of extended-spectrum beta-lactamase-producing *E. coli* (ESBL-*E. coli*) in the environment, humans, and animals. A study by Kakooza (2021) further highlighted the issue of antimicrobial resistance in *E. coli* strains isolated from poultry, indicating a potential risk of transmission to the pork value chain.

Additionally, *E. coli* isolates, including ESBL- the producing group and post-weaning diarrhea (PWD) causing enterotoxigenic *E. coli* (ETEC) from the pork value chain in both non-intensive

and intensive production systems in Uganda, exhibited multidrug resistance to critical vital antibiotics for animal and human use and growth promoters (Kimera et al., 2021; Abdalla et al., 2021; Okello et al., 2015). Okubo et al. (2020) also found high levels of antimicrobial-resistant *E. coli* in retail meats, such as pork, sourced from roadside butcheries. These were mainly resistant to tetracycline, ampicillin, and sulfamethoxazole-trimethoprim, with the potential for simultaneous transfer of resistance to these antibiotics (Okubo et al., 2020). Similar findings were reported in dairy cattle farms in Uganda by Ball et al. (2019), who, in their study indicated that a considerable percentage of *E. coli* isolates including ESBLs showed resistance to various antibiotics.

Whereas these studies highlight the low sensitivity of *E. coli* to antibiotics, there's still scarcity of data on the susceptibility of *E. coli* in the pig farms to antibiotics in northern Uganda. This necessitates targeted studies to establish the extent to which these pathogens are susceptible or resistant to existing antibiotics. Such information will aid in regular monitoring of *E. coli* antibiotic resistance patterns to guide effective treatment strategies and interventions to improve public health.

2.3 Genotypic antimicrobial resistance in pigs

E. coli O157:H7 is a zoonotic foodborne pathogen of major public health concern, commonly associated with livestock, particularly pigs. The emergence of antimicrobial resistance (AMR) in this pathogen has been attributed to the acquisition of resistance genes through horizontal gene transfer, driven by extensive antimicrobial use in animal production systems. Genotypic resistance involves the detection of specific resistance genes or mutations responsible for antimicrobial resistance. In *E. coli* O157, these genes are often located on plasmids, integrons, and transposons, facilitating rapid dissemination within and between bacterial populations. Studies in pig

production systems have demonstrated a high prevalence of multidrug-resistant (MDR) *E. coli*, often harboring multiple resistance determinants simultaneously (Fair & Tor, 2014).

2.3.1 Colistin Resistance in Pig-Derived *E. coli* O157

Colistin is a last-resort antibiotic used to treat severe Gram-negative infections. However, resistance in *E. coli* O157 has increasingly been reported in livestock. The primary mechanism involves plasmid-mediated mobilized colistin resistance genes (*mcr-1* to *mcr-10*), which encode enzymes that modify lipid A, reducing colistin binding. The detection of *mcr-1* in pig isolates has raised global concern due to its ability to spread across bacterial species. In addition, chromosomal mutations in regulatory systems such as *pmrA/pmrB* contribute to resistance. Studies in intensive pig farming systems have linked colistin use as a growth promoter to increased prevalence of *mcr*-harboring *E. coli* strains (Liu et al., 2016).

2.3.2 Streptomycin Resistance

Resistance to streptomycin in *E. coli* O157 is widely reported in pig isolates and is mainly mediated by *aadA* genes encoding aminoglycoside adenylyltransferases. Mutations in ribosomal protein genes (*rpsL*). These genes are frequently located within **class 1 integrons**, which facilitate co-selection with other resistance genes. High prevalence of *aadA* genes has been documented in enteric bacteria from pigs due to long-term use of aminoglycosides in veterinary medicine (Sunde & Norstro, 2006).

2.3.3 Gentamicin Resistance

Gentamicin resistance in *E. coli* O157 is associated with aminoglycoside-modifying enzyme genes, including *aac (3)-IV*, *aph (3')* and *aadA*. These genes enzymatically modify gentamicin,

reducing its binding to bacterial ribosomes. Pig isolates often carry these genes on plasmids, contributing to multidrug resistance profiles. The co-occurrence of gentamicin resistance genes with other resistance determinants has been observed in *E. coli* from swine, suggesting linked selection pressures in antimicrobial usage (Aarestrup et al., 2008).

2.3.4 Ampicillin Resistance

Ampicillin resistance is one of the most commonly reported phenotypes in *E. coli* O157 from pigs. It is mainly mediated by β -lactamase genes, including *blaTEM*, *blaSHV*, *blaCTX-M* and *CITM*. These genes encode enzymes that hydrolyze the β -lactam ring of ampicillin. The widespread presence of *blaTEM* in pig isolates has been attributed to frequent use of β -lactams in veterinary practice. Additionally, extended-spectrum β -lactamases (ESBLs), particularly *blaCTX-M*, have been increasingly detected in livestock-associated *E. coli*, posing a serious public health risk due to reduced treatment options (Carattoli & Elena, 2009).

2.3.5 Erythromycin Resistance

Although *E. coli* is intrinsically less susceptible to macrolides, acquired resistance mechanisms have been reported in pig isolates. These include *erm(B)* genes encoding methylases that modify 23S rRNA, Efflux pump genes such as *mef(A)* and *ereA* gene. These genes reduce antibiotic binding or actively expel the drug from bacterial cells. The presence of macrolide resistance genes in *E. coli* from pigs is often linked to co-selection via other antimicrobials, rather than direct erythromycin use.

2.3.6 Ciprofloxacin Resistance

Ciprofloxacin resistance in *E. coli* O157 is primarily mediated by Mutations in *gyrA* and *parC* genes (target modification), Plasmid-mediated quinolone resistance genes such as *qnr* and Efflux

pumps (e.g., *acrAB*) Point mutations in the quinolone resistance-determining region (QRDR) reduce drug binding to DNA gyrase and topoisomerase IV. Studies in swine have shown increasing detection of *qnr* genes, indicating plasmid-mediated dissemination of fluoroquinolone resistance (Park et al., 2006).

Genotypic antimicrobial resistance in *E. coli* O157 from pigs is mediated by a wide range of transferable genes, including *mcr*, *bla*, *aad*, *erm*, and *qnr*. These genes confer resistance to critical antibiotics such as colistin, aminoglycosides, β -lactams, macrolides, and fluoroquinolones. The high prevalence of these genes in pig production systems underscores the role of livestock as reservoirs of antimicrobial resistance and emphasizes the need for continuous surveillance, antimicrobial stewardship, and integrated control strategies.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Design

A mixed study design, incorporating both cross-sectional and experimental approaches, was adopted for this study. A cross-sectional design was used to collect fecal samples while an experimental design was employed to gather information regarding the prevalence of pathogenic *E. coli* strain. Additionally, experimental design was used to determine phenotypic and genotypic resistance of pathogenic *E. coli* strains to commonly used antibiotics. The cross sectional design was chosen in order to save time since Msc. Research takes one year and the experimental design to obtain quantitative data to answer the research questions.

3.2 Study area and population

The research was conducted in Gulu City, located in northern Uganda, approximately 333 km from Kampala, the capital city, at coordinates 2°46'54.0"N 32°17'57.0"E and an altitude of about 1,100 m above sea level (UBOS, 2020; Gulu District Local Government, 2019). Gulu City comprises two divisions, 32 wards, and 120 cells (Gulu City Council, 2021). The area was selected due to its substantial pig population, estimated at 26,569 pigs (UBOS, 2020). The soils are predominantly ferruginous with sandy soils forming the major component (Bakamanume & Mutabuza, 1997). The climate is tropical with distinct dry and wet seasons, characterized by an average annual rainfall of approximately 1,500 mm, ranging from 1.4 mm in January to about 230 mm in August (Chen, 2024). Temperatures range between a minimum of 18°C and a maximum of about 30–32°C under normal conditions, with relative humidity being high during the wet season and low during the dry season (NEMA, 2019). The primary economic activity in the area is agriculture, which employs about 95% of the population (UBOS, 2020). The study population consisted of pig-

intensive and semi-intensive farms within Gulu City. Only small-scale farms i.e. farms containing 5-50 pigs.

3.3 Sample Size

Determination of sample size was done using Cochran's formula

$n = Z^2 Q^2 / E^2$; where n is the sample size, Z is 1.96 (standard score for a 95% confidence range), Q = 0.5 (estimated population standard deviation), and E is 0.009 (the desired margin of error).

Applying the formula using the stated values results in a sample size of 100 fecal samples.

3.4 Sampling Framework and Sampling Technique

A multistage sampling technique was used. There are two divisions i.e. Bardege-Layibi and Laroo - Pece. First, two sub-counties/divisions were selected from each division i.e. Laroo, Pece, Bardege, Layibi. Second, five farms were located using snowball sampling technique from each subdivision. Third, from each farm five pigs were simple sampled randomly from a population. To locate the farms snowball sampling technique was employed where the first farmer was identified by the help of the veterinary officer who then assisted in identifying others.

3.5 Data collection

3.5.1 Sample collection

During sample collection, sterile fecal containers were labeled and used to collect fresh fecal samples either directly from the pigs' rectum or from freshly passed feces. Samples were handled carefully to avoid contamination with soil, bedding, or other materials. Gloves and hand sanitizer were used to handle the samples aseptically. The samples obtained were then put in a cooler with ice packs to maintain a consistent temperature during the transportation process. All samples were

taken to the Gulu University Multifunctional Laboratories and analyzed immediately within 24 hours.

3.5.2 prevalence of *E. coli* O157:H7

Bacterial isolation

E. coli isolation was conducted using the method described by (Lim et al., 2007). Briefly, 1 gram of fecal sample was incubated in 9 milliliters of buffered peptone water (HiMedia, India) at a 1:10 ratio as a pre-enrichment medium in a 50ml falcon tube. Then, 0.5 milliliters of the buffered peptone water mixture were incubated in 5 milliliters of tryptone soya broth (HiMedia, India) in a 50ml falcon tube at 37°C for 24 hours as an enrichment medium and shaken the entire time. The tryptone soya mixture was then streaked onto MacConkey (HiMedia, India) agar selective medium using a sterile wire loop, incubated at 37°C for 24 hours. After 24 hours, the plates were examined for the presence of presumptive *E. coli* colonies (pink-colored colonies were considered *E. coli* positive). The suspected colonies were streaked again on MacConkey agar to obtain a pure culture of *E. coli* isolates. Pure cultures were confirmed using PCR.

3.5.3 DNA Extraction

DNA extraction was performed following the method outlined by (Dashti et al., 2009). Briefly, two colonies of overnight-grown bacteria were removed using a sterile pipette tip. The colonies were transferred into an Eppendorf tube containing 1 mL of distilled water, boiled for 10 minutes at 80°C in a water bath, and subsequently centrifuged for five minutes at 1000 rpm, 5 microliters of the supernatant were used for the PCR. The supernatant was taken because DNA is soluble so it settles as supernatant. The supernatant was stored at -20°C.

3.5.4 PCR detection of *E. coli* O157:H7

The normal conventional PCR, (SimpliAmp thermocycler Singapore, model EN61326) was used. The PCR reaction was carried out in the final volume of 25 µL, which consisted of 12.5 µL of 2x Master Mix (Macrogen, Europe), 0.5 µL of genomic DNA, 0.5 µL of each primer with concentration of 10 micro molar (Macrogen, Europe), and 11 µL of nuclease-free water (INQABA BIOTEC, South Africa). The PCR thermal cycling conditions included an initial denaturation at 94°C for 30 seconds, followed by 30 cycles consisting of denaturation at 94°C for 30 seconds, annealing at the optimal temperature for each primer set (table 1), extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. Amplified products were examined through electrophoresis on a 1 gram of agarose in 100ml buffer to form agarose gel. Ethidium bromide was employed for staining, gloves were used to prevent spill over and the PCR products were visualized using a UV transilluminator Chemidoc imaging system by BIO RAD laboratories, Singapore. A 1 kb DNA ladder served as a molecular weight marker.

Table 1: Primers for *E. coli*, *E. coli* O157:H7 and virulence factors stx1 and stx2

Target gene	Primer sequence (5'-3')	Approximate band size(bp)	Annealing temperature
16SRNAuidA	F: GACCTCGGTTTAGTTCACAGA R: CACACGCTGACGCTGACCA	585	55
<i>rfbO157</i>	F: AAGATTGCGCTGAAGCCTTTG R: CATTGGCATCGTGTGGACAG	497	66

Reference: (Sobur et al., 2019).

3.5.5 Anti-microbial resistance testing

Five colonies of similar morphological types were transferred into Falcon tubes and normal saline was added to reach a turbidity comparable to the 0.5 McFarland standard solution. The turbidity was assessed using a Spectrophotometer (7305 Jenway by Bobby scientific Ltd UK) at a wavelength of 600 nm, yielding an optical density of 0.085. Sterile swabs were employed to inoculate the bacterial suspension onto duplicate Mueller-Hinton agar (HiMedia, India) plates. Anti-microbial disks containing commonly used random selected antibiotics in the study area; Ampicillin 2µl, Gentamicin 10µl, Ciprofloxacin 5µl, Erythromycin 15µl, Streptomycin 10µl, and Colistin 10µl were then applied. The plates were incubated at 37°C for 16 to 18 hours. After incubation, each plate was assessed, and the diameters of the inhibition zones were measured in millimeters with a ruler. The results were recorded as sensitive, intermediately sensitive and resistant. The interpretation followed the guidelines established by the Clinical and Laboratory Standards Institute (Weinstein & Lewis, 2020), as detailed in the appendix 1. *E. coli* ATCC 25922 served as the control strain from Sigma Aldrich, Europe. The *E. coli* O157 found to be resistant to multiple antibiotics i.e. three or more antibiotics is said to be multidrug resistant. The antibiotics used in this study were selected based on their widespread use in both human and veterinary medicine. These antibiotics are also prioritized for antimicrobial resistance surveillance by the World Health Organization due to their public health importance (WHO, 2019).

3.5.6 PCR detection of Antibiotic resistance genes

The normal conventional PCR (SimpliAmp thermocycler, Model EN61326, Singapore) was used. The PCR reaction was carried out using a final volume of 25 µL, which consisted of 12.5 µL of

2x Master Mix (Macrogen, Europe), 0.5 µL of genomic DNA, 0.5 µL of each primer (Macrogen, Europe), and 11 µL of nuclease-free water (INQABA BIOTEC, South Africa). The PCR thermal cycling conditions included an initial denaturation at 94°C for 30 seconds, followed by 30 cycles consisting of denaturation at 94°C for 30 seconds, annealing at the optimal temperature for each primer set (table 1), extension at 72°C for 1 minute because the optimal working temperature for most Taq DNA polymerase enzymes is a round 72 °C, the usual rule is 1 minute per 1kb(1000bp) of DNA fragment, and a final extension at 72°C for 10 minutes to complete unfinished DNA strands, improve yields of full length amplicons and allow addition of A- overhangs when using Taq polymerase. Amplified products were examined through electrophoresis on 1gram of agarose in 100ml buffer to form agarose gel. Ethidium bromide was employed for staining, and the PCR products were visualized using a UV transilluminator. A 1 kb DNA ladder served as a molecular weight marker.

Table 2: Selected primers for resistance genes

Antibiotics	genes	primer sequence (5'-3')	base size (bp)	Annealing temp
Ampicillin	<i>CITM</i>	F: TGGCCAGAACTGACAGGCAAA R: TTTCTCCTGAACGTGGCTGGC	462	47
Ciprofloxacin	<i>aac(6)-Ib-cr</i>	F: TTGCGATGCTCTATGAGTGGCTA R: CTCGAATGCCTGGCGTGT	482	57
Colistin	<i>mcr-3</i>	F: TTGGCACTGTATTTGCATT R: TTAACGAAATTGGCTGGAACA	542	50
Erythromycin	<i>ereA</i>	F: GCCGGTGCTCATGAACTGAG R: CGACTCTATTCGATCAGAGGC	419	52
Gentamicin	<i>aac (3) - IV</i>	F: CTTCAAGATGGCAAGTTGGT R: TCATCTCGTTCTCCGCTCAT	286	55
Streptomycin	<i>aadA1</i>	F: TATCCAGCTAAGCGCGAACT R: ATTTGCCGACTACCTTGGTC	447	58

Reference: (Sobur et al., 2019).

3.6 Data analysis

R statistical package (version 4.4.1) was used to carry out all the data analysis. The following are the statistical packages used in the course of analysis; ggplot2, graphics, Base R, dplyr, janitor, gmodels.

To assess the prevalence of pathogenic *E. coli*, descriptive statistic such as frequencies and percentage were calculated, with prevalence reported as a percentage. To compare the prevalence of pathogenic *E. coli* across various farming systems, study location, sexes, and age groups, a Chi-square test for independence was conducted to evaluate the associations among these variables since it is a non-parametric test of association between categorical and nominal data.

To determine the antimicrobial resistance patterns of commonly administered antimicrobials, descriptive statistics such as frequencies, and percentages was obtained. To compare the percentage of resistance across different areas, sexes, age groups, and farming systems, a Chi-square test for independence was conducted to assess the associations.

To determine the occurrence of resistance genes descriptive statistics like frequency and percentage was obtained in order to provide the quantitative data.

3.7 Ethical Consideration

Approval of consent was done by the Gulu University Research Ethics Committee (GUREC) prior to the study (GUREC number 2024-916). Informed consent was sought from participants involved in the research process by having them sign the consent form.

CHAPTER FOUR: RESULTS

4.1.0 Prevalence of pathogenic *E. coli*

Out of the 100 fecal samples examined, 99 isolates produced characteristic pink colonies on MacConkey agar, indicating presumptive *E. coli*. Molecular confirmation using *E. coli*-specific primers identified 87 isolates as *E. coli*. Subsequent genotypic analysis using primers specific for *E. coli* O157 revealed that 55 out of the 87 confirmed *E. coli* isolates were positive for *E. coli* O157. This corresponds to a prevalence of 63.2% among confirmed *E. coli* and 55% overall prevalence in the sampled population.

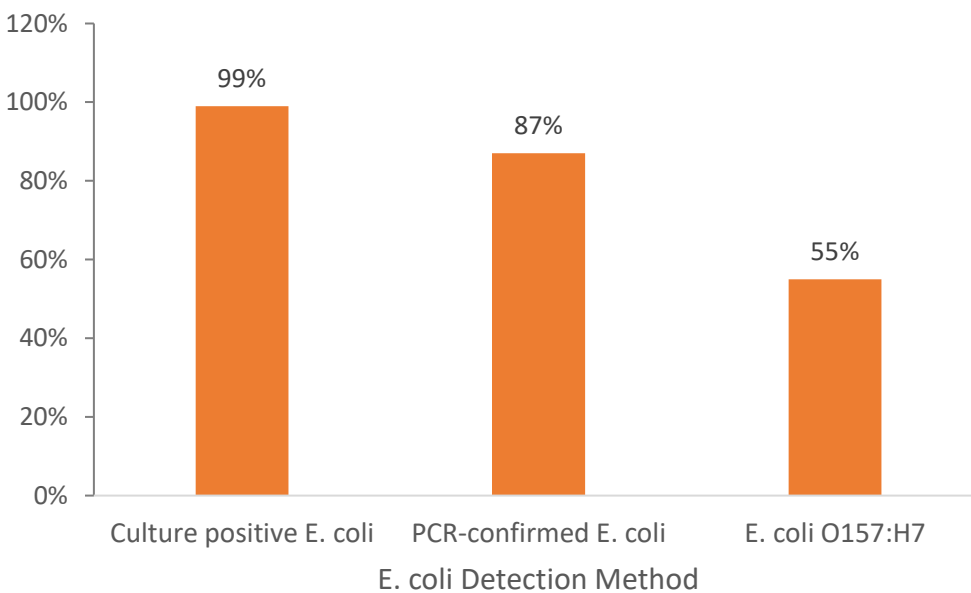


Figure 1: The prevalence of pathogenic *E. coli*

4.1.1 Comparison of the occurrence of pathogenic *E. coli* O157 with sex

The results demonstrated a statistically significant association between the prevalence of *E. coli* O157:H7 and the sex of pigs ($p < 0.05$), as shown in Table 3. Female pigs exhibited a higher percentage prevalence compared to male pigs.

Table 3: Comparison of prevalence of pathogenic *E. coli* with sex

Bacteria	Sex	percentage	P value
<i>E. coli</i>	Female	96.8	0.051
	Male	82.6	
<i>E. coli</i> O157:H7	Female	71.0	0.031
	Male	47.8	

4.1.2 Comparison of prevalence of pathogenic *E. coli* with division/ study area

There was no statistically significant association between the prevalence of *E. coli* and *E. coli* O157:H7 and the study area ($p > 0.05$), as shown in Table 4.

Table 4: Comparison of prevalence of pathogenic *E. coli* across the study area

DIVISION	<i>E. coli</i>	<i>E. coli</i> O157:H7
PECE	76.0	52.0
BARDEGE	87.5	54.2
LAYIBI	96.2	65.4
LAROO	88.0	55.0
P value	0.201	0.631

4.1.3 Comparison of prevalence of pathogenic *E. coli* with age categories

The Chi square test results in table 5 showed no significant difference between *E. coli*, *E. coli* O157 with age groups ($p > 0.05$)

Table 5: Comparison of prevalence of pathogenic *E. coli* and associated virulence with age categories

Bacteria		AGE			Overall N (%)	X ² (p-value)
		Weaners	Growers	Adults		
		n(%)	n(%)	n(%)		
E.coli O157H7	Negative	15 (41.7)	16 (37.2)	14 (66.7)	45 (45.0)	5.199 (0.074)
	Positive	21 (58.3)	27 (62.8)	7 (33.3)	55 (55.0)	
E.coli confirmed	Negative	4(11.1)	6(14.0)	3(14.3)	13(13.0)	0.179 (0.914)
	Positive	32(88.9)	37(86.0)	18(85.7)	87(87.0)	

4.1.4 Comparison of prevalence of pathogenic *E. coli* with farming system.

The chi-square test results presented in Table 6 indicated no statistically significant association between the prevalence of *E. coli* and *E. coli* O157 and the farming system ($p > 0.05$).

Table 6: Comparison of prevalence of pathogenic *E. coli* with the farming system

Bacteria	Semi intensive farming system	Intensive farming system	P values
<i>E. coli</i>	86.7	87.1	0.948
<i>E. coli</i> O157:H7	56.7	54.3	0.826

4.2.0 Antimicrobial resistance profile of pathogenic *E. coli* O157 to antibiotics.

The phenotypic antimicrobial resistance patterns of *E. coli*O157 isolates against six antibiotics are presented in Figure 3. Overall, a high level of resistance was observed across the tested antibiotics. The highest resistance was recorded against ampicillin, followed by erythromycin, colistin, gentamicin, streptomycin, and ciprofloxacin. In contrast, streptomycin exhibited comparatively higher susceptibility among the antibiotics tested. The antibiotic abbreviations used are as follows: AMP (ampicillin), CT (colistin), S (streptomycin), CIP (ciprofloxacin), and E (erythromycin).

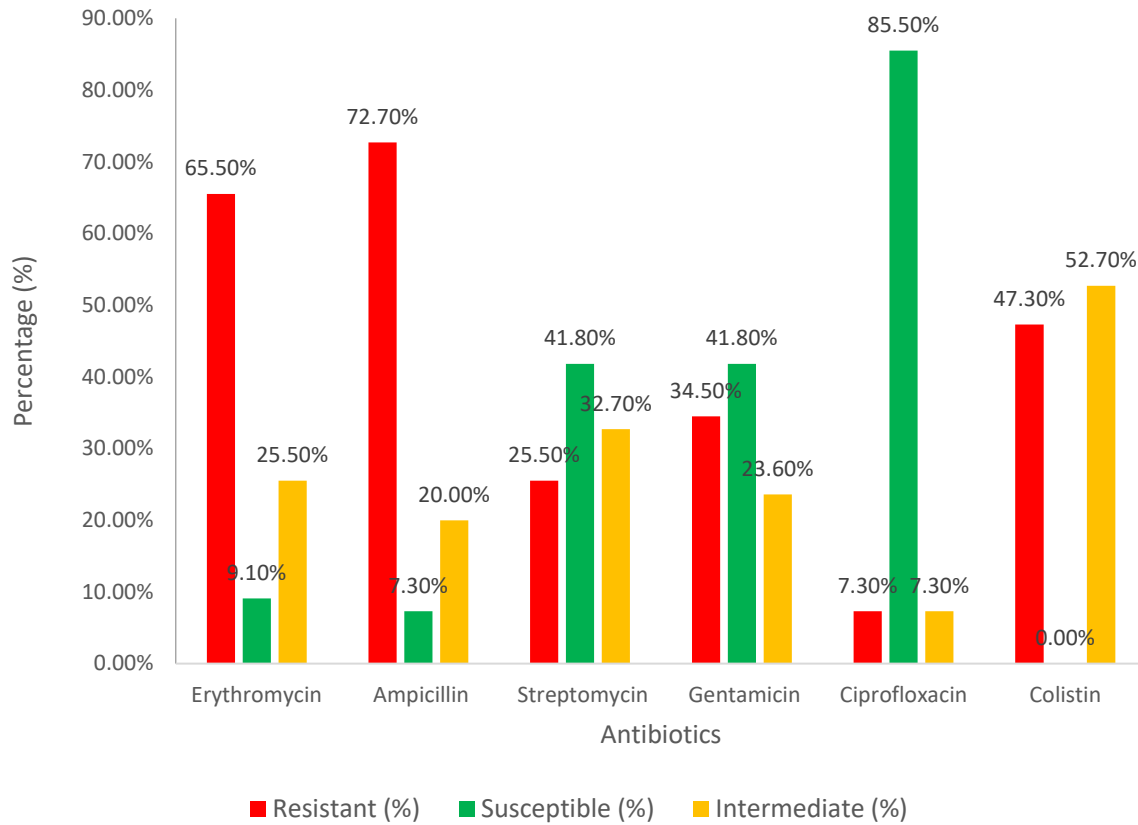


Figure 3 shows antimicrobial resistance pattern of six commonly used antibiotics

4.2.1 Comparison of percentage resistance of *E. coli* O157 with farming system

Chi-square analysis (Table 7) showed no significant association between farming system and the antimicrobial resistance of *E. coli* O157 to all six antibiotics tested ($p > 0.05$).

Table 7: Comparison of percentage resistance of *E. coli* O157 with farming system

Antimicrobial agent	Semi intensive	intensive farms	P value
Ampicillin (AMP)	68.8	74.4	0.834
Erythromycin (E)	68.8	69.4	0.889
Streptomycin(S)	25.6	25.0	0.290
Gentamycin(GN)	31.3	35.9	0.719
Ciprofloxacin (CIP)	0.0	10.3	0.147
Colistin (CT)	43,8	48.7	0.737

4.2.2 Comparison of percentage resistance of *E. coli* O157 with study area/ division

The results revealed a statistically significant difference in resistance of *E. coli* O157 to ampicillin across the study areas ($p < 0.05$), as shown in Table 8. Pece Division exhibited the highest level of resistance (92.3%) compared to the other divisions.

However, no statistically significant association was observed between study area and resistance to the other antibiotics tested ($p > 0.05$).

Table 8: Comparison of percentage resistance of *E. coli* O157 with the study area

Antimicrobial agents	Laroo	Pece	Layibi	Bardege	P- value
Ampicillin	66.7	92.3	58.8	76.9	0.026
Erythromycin	50.0	89.2	84.7	76.9	0.848
Streptomycin	25.0	30.8	5.9	46.2	0.109
Gentamycin	25.0	46.2	11.8	61.5	0.114
Ciprofloxacin	8.3	15.4	0.0	7.7	0.370
Colistin	58.3	46.2	35.3	53.8	0.615

4.2.3 Comparison of percentage resistance of *E. coli* O157:H7 with age categories

A statistically significant association was observed between age group and resistance to colistin ($p < 0.05$), with growers showing the lowest resistance (22.2%) compared to weaners and young adults (Table 9). No significant association was found between age group and resistance to the remaining antibiotics ($p > 0.05$).

Table 9: Comparison of percentage resistance of *E. coli* O157:H7 with age categories

Antimicrobial agent	Weaners	Growers	Young Adults	P value
Ampicillin	71.4(15/21)	77.8(21/27)	57.1(4/7)	0.543
Erythromycin	71.4(15/21)	55.5(15/27)	85.7(6/7)	0.250
Streptomycin	33.3(7/21)	18.5(5/27)	28.6(2/7)	0.495
Gentamycin	52.4(11/21)	25.9(7/27)	14.3(1/7)	0.078
Ciprofloxacin	0.0(0/21)	11.1(3/27)	14.3(1/7)	0.253
Colistin	71.4(15/21)	22.2(6/27)	71.4(5/7)	0.001

4.2.4 Comparison of percentage resistance of *E. coli* O157 with sex

The results presented in Table 10 indicate no statistically significant association between sex and the percentage resistance of *E. coli* O157 ($p > 0.05$).

Table 10 showing comparison of percentage resistance of *E. coli* O157 with sex

Antimicrobial agent	Male	Female	P value
Ampicillin	68.2	75.8	0.537
Erythromycin	59.1	69.7	0.418
Streptomycin	27.3	24.2	0.800
Gentamycin	22.7	42.4	0.132
Ciprofloxacin	4.5	9.1	0.525
Colistin	40.9	51.5	0.440

4.2.5 Multidrug resistance status

Out of the 55 samples, 24 (43.6%) were identified as multidrug-resistant (MDR).

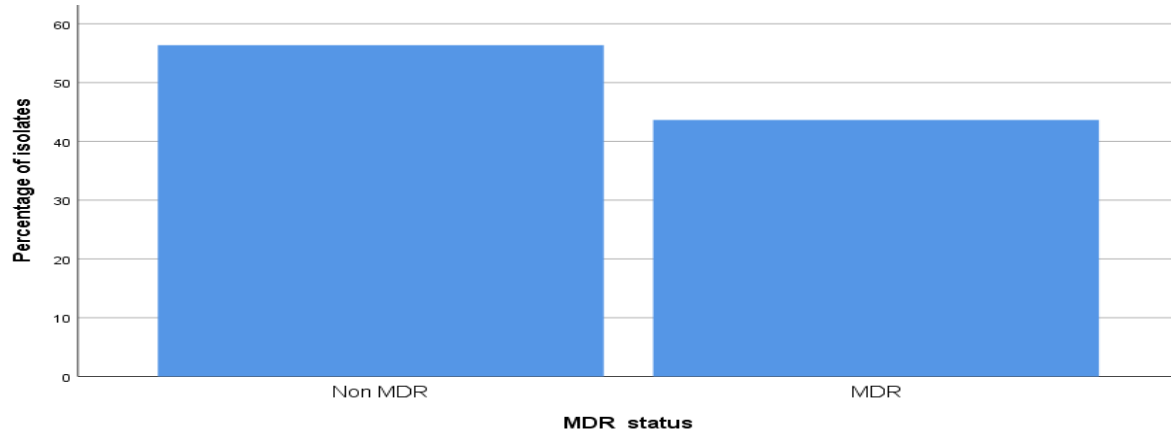
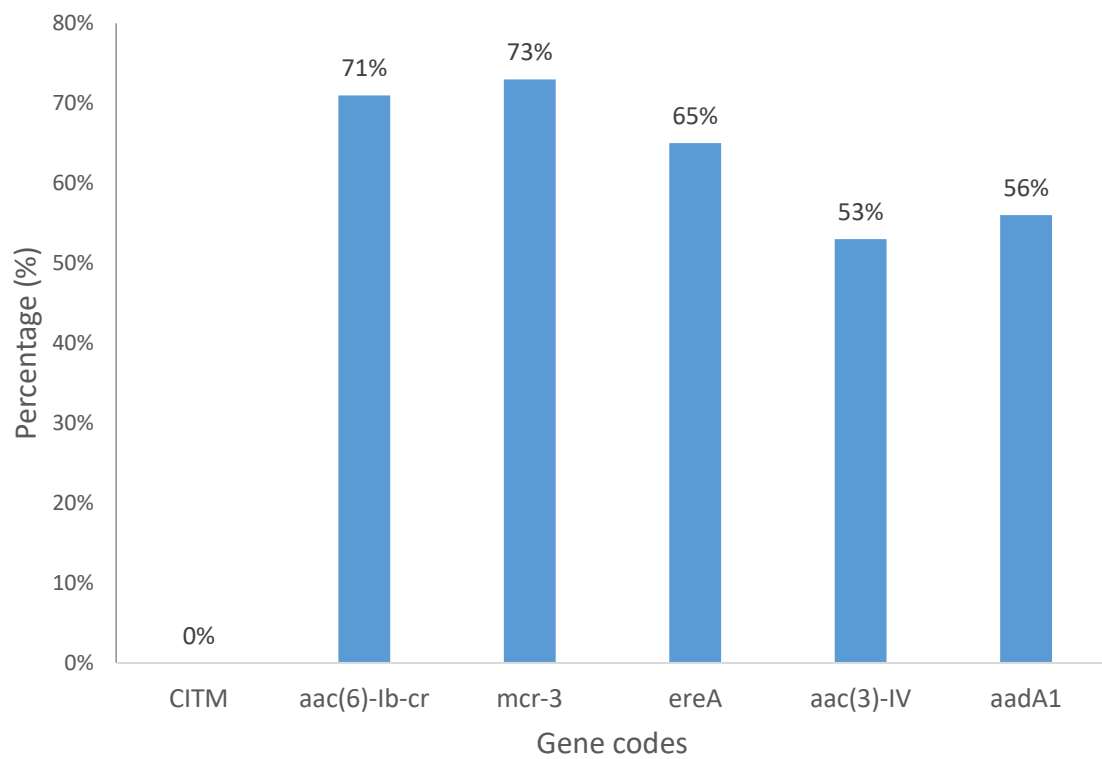


Figure 2: Multidrug resistance status for the six antibiotic

4.3 Resistance genes for antibiotics

The genotypic analysis revealed a high prevalence of antimicrobial resistance genes among the isolates. The most frequently detected gene was *mcr-3*, associated with colistin resistance, found in 73% of the isolates. This was followed by *aac(6')-Ib-cr* (71%), linked to ciprofloxacin resistance, and *ereA* (65%), associated with erythromycin resistance. Additionally, *aadA1* (56%) conferring streptomycin resistance and *aac(3)-IV* (53%) associated with gentamicin resistance were also detected.



CHAPTER FIVE: DISCUSSION

5.1 Prevalence of pathogenic *E. coli* O157

The current study found a high prevalence of *E. coli* at 87% in stool samples of pigs, with *E. coli* O157 present at 55%. The high prevalence of *E. coli* in fecal samples may reflect its role as a normal component of the pig intestinal microbiota (Nunes et al., 2023). Alternatively, this prevalence could be influenced by poor sanitary conditions on pig farms, including inadequate waste management, contaminated water, overcrowding, poor ventilation, and diet contamination from raw or improperly processed feeds (Selling, 2020). Additionally, existence of antibiotic-resistant strains that persist longer in the gut, as well as factors such as age, health status, and stress, may weaken pigs' immune defenses, making them more susceptible to *E. coli* infections.

A study in Jordan on broiler chickens found a relationship between multidrug-resistant *E. coli* and the use of groundwater as a drinking source (Ibrahim et al., 2019). Similarly, a study in India found an association between water source and the prevalence of resistant *E. coli* on pig farms (VinodhKumar et al., 2019), suggesting that contaminated water ingestion by farm animals may contribute to the high prevalence observed. In Lira, a study by Selling (2020) reported *E. coli* prevalence of 99% among weaning pigs, which could be explained by the natural decline in immunity from maternal antibodies during weaning. However, this contrasts with these findings, which showed no significant association between age categories and the prevalence of *E. coli* or *E. coli* O157. This discrepancy may be attributed not only to the relatively small sample size in the present study, which may have reduced statistical power, but also to possible differences in management practices, exposure levels, and environmental conditions across age groups. In

addition, variation in sampling design and herd structure could have influenced the distribution of infection.

Consistent with this findings, previous studies in Uganda have also reported high prevalence rates of *E. coli* as 85.1%, 88.3% and 89% (Obala et al, 2021; Katushabe et al., 2022b ; Tumusiime et al., 2024). In contrast, Ikwap et al. (2021) found a slightly higher prevalence of 98.1%. or farming system. Similarly, a study in South Africa reported an isolation rate of 44–50% for *E. coli* O157 in pig fecal samples, which is comparable to this finding of 55% (Ateba & Mbewe, 2011). The isolation of *E. coli* O157 in pig feces poses a significant risk of contamination to food and water sources, especially given the pathogen's low infectious dose and potential to cause severe illness and death (Kuan, 2012).

Conversely, some studies report lower prevalence rates of *E. coli*. For example, Begum et al. (2014) found a 59.33% prevalence in pigs in India, while Hanson et al. (2002) observed a prevalence of 47% in northern Thailand. Herrero-Fresno et al. (2015) reported a 67.2% prevalence in intensive nursery pig farms in Denmark. These differences may reflect geographic factors or the impact of better farm management, hygiene, and biosecurity measures in these countries compared to sub-Saharan Africa. In Uganda, for instance, pigs are often kept in housing with bare floors, which may create hygiene issues that promote bacterial survival and contamination of feeds, contributing to the high prevalence observed (Selling, 2020). .

The significant association observed between sex and the prevalence of *E. coli* O157 in pig fecal samples as indicated in table 3 suggests that biological and management-related factors may influence susceptibility and exposure to infection. Differences between male and female pigs in terms of physiology, hormonal influences, and immune response can contribute to variations in

colonization by pathogenic bacteria. For instance, sex hormones such as estrogen and testosterone are known to modulate immune function, potentially affecting the ability of animals to resist or harbor infections (Klein & Flanagan, 2016).

In addition to biological factors, management practices may also play a role. In many small-scale farming systems, male and female pigs are often managed differently for example, breeding sows may be kept longer and exposed to farm environments for extended periods compared to males, increasing their likelihood of acquiring and shedding pathogens. Similarly, differences in housing, feeding, and stress levels between sexes may influence bacterial carriage (Rossman et al., 2006). Studies have reported variable associations between sex and *E. coli* prevalence. Some studies suggest that females may have higher exposure due to prolonged retention on farms for reproduction, while others report no significant difference, indicating that the relationship may be context-specific and influenced by local management systems (Fairbrother & Nadeau, 2006). Therefore, the observed association in this study highlights the importance of considering sex as a potential risk factor in the epidemiology of *E. coli* O157. It also underscores the need for targeted interventions that account for management and biological differences to reduce the burden of pathogenic *E. coli* in pig production systems.

Numerous research studies, including Abdalla et al. (2021), Tanih et al. (2015), Ateba and Mbewe (2011), and Ateba et al. (2008), have isolated *E. coli* along the pig value chain, underscoring the public health threat posed by pathogenic microorganisms in the food chain. The presence of such pathogens has serious implications for public health and agriculture, with potential impacts on food.

security (Elbehiry et al., 2023; Mainardi & Bidoia, 2024). Pathogens such as bacteria, fungi, parasites, and Viruses can be transmitted to humans via contact with infected animals or their waste, fecal contamination of soil or water, and consumption of contaminated foods or animal products (Saleh et al., 2009). Therefore, raising awareness of good farm management practices, hygiene standards, and biosecurity measures is essential to mitigate the risk of transmission of this pathogen to humans.

5.2 Antimicrobial resistance profile of pathogenic *E. coli* O157

Antimicrobial resistance poses a major concern for global health. The development of resistance of pathogenic *E. coli* O157 to antimicrobials poses significant challenges for both veterinary and human medicine. The study revealed a high level of resistance among pathogenic *E. coli* O157 isolates. These results indicate a critical resistance pattern that aligns with the findings of previous studies. For instance, a study by Adzitey et al. (2020) in Ghana observed 71.6% resistance to ampicillin, which is comparable to this finding. A similar study carried out in Uganda found 41.8% resistance to ampicillin in piglets (Obala et al., 2021).

The observed AMR development is likely influenced by both rational and irrational antibiotic use for various purposes, such as prophylaxis, therapy, and growth promotion in livestock (Ikwap et al., 2021). The selection pressure exerted by inappropriate antimicrobial use, including over-the-counter purchase of antibiotics by farmers, exacerbates the AMR burden (Selling, 2020). The presence of multidrug resistance (MDR) in 48% of the isolates aligns closely with previous studies, such as Ikwap et al. (2021), who reported 47% MDR in *E. coli* isolates from livestock in Uganda. Similarly, a study conducted at Wambizi, Uganda's main abattoir, found an even higher prevalence of MDR at 85.1% (Katushabe et al., 2022b). This alarmingly high prevalence underscores the

increasing threat posed by MDR strains, which complicate treatment options in both veterinary and human healthcare. As MDR pathogens become more difficult to manage, their persistence represents a significant challenge to public health and animal welfare.

More concerning is the 47.3% resistance to colistin observed in this study. Colistin is often considered the last line of defense against multidrug-resistant bacteria (Wenjuan et al., 2017), and resistance at such high levels suggests potential therapeutic failures in both veterinary and clinical settings. Comparable findings have been documented in South Africa, where isolates of *E. coli* from retail meat exhibited colistin resistance (Iweriebor et al., 2022). In Uganda studies carried out on cattle in high acaricide resistant area showed 100% resistance to colistin and 97.8% in low acaricide resistant area (Byaruhanga et al., 2022). This underscores the urgent need for alternative treatment strategies, such as vaccines, to mitigate the spread of colistin-resistant bacteria.

A significant association was observed between colistin resistance and age categories, with higher resistance levels detected in weaners and adult pigs compared to growers. This variation in resistance may be attributed to differences in environmental exposures and immune status at distinct life stages. Weaners, for instance, often experience stress, dietary changes, and a decline in immunity due to the loss of maternal antibodies, as noted by Obala et al. (2021). These factors frequently lead to increased antimicrobial usage, thereby promoting the development of antimicrobial resistance (AMR). Similarly, adult pigs may accumulate resistance over time due to prolonged or repeated exposure to antimicrobials throughout their lifespan, whether for prophylactic or therapeutic purposes. In contrast, growers, being in an intermediate stage of development, may have comparatively lower exposure to antimicrobials. This could be because their immune system is more stable than that of weaners and they have not yet been exposed to the same levels of antimicrobial treatments. These findings suggest that interventions aimed at

reducing colistin use, particularly in weaners and adult pigs, could be crucial in lowering the observed resistance levels.

An association was also identified between ampicillin resistance and geographic study areas. This variation could be linked to differences in the frequency and dosage of ampicillin usage across regions. In areas where ampicillin is used more frequently or in higher doses, there may be a greater selective pressure, leading to the development of AMR. Additionally, differences in management practices, such as hygiene standards, biosecurity measures, and stocking density, may influence the transmission of resistant pathogens and contribute to the observed patterns of resistance.

No statistical difference was found between antibiotic resistance and factors such as sex or farming system. Likewise, no association was detected between antibiotic resistance and study area or age categories for antibiotics other than ampicillin and colistin. This absence of significant findings could be attributed to small sample size, which could have limited the analytical power. Future research with larger sample sizes is recommended to determine whether any associations might emerge with a more robust dataset.

The findings of this study highlight the critical need for enhanced AMR surveillance, stricter regulations on antimicrobial use, and alternative strategies like vaccination to mitigate the growing AMR threat. Given the global importance of maintaining effective antibiotics for both human and veterinary medicine, coordinated efforts are essential in Uganda to address this issue.

5.3 The genotypic resistance

The genotypic analysis in this study revealed high prevalence of antimicrobial resistance genes among *E. coli* isolates. These findings indicate widespread dissemination of resistance

determinants, particularly against critically important antibiotics, posing a serious public health concern. The high prevalence of the *mcr-3* gene (73%) is particularly alarming, given that colistin is considered a last-resort antibiotic for treating infections caused by multidrug-resistant Gram-negative bacteria. This finding was higher than those reported in other regions. For example, a study by Iweriebor et al. (2022) in South Africa detected colistin resistance genes in *E. coli* isolates from retail meat, and similarly Mtemisika et al. (2022) in Tanzania reported the presence of colistin-resistant *E. coli* in pigs, but at relatively lower levels (10-25%). The higher prevalence in this study may be attributed to unregulated antibiotic use in livestock, particularly in small-scale farming systems.

The high prevalence of ciprofloxacin resistance genes *aac(6')-Ib-cr*; (71%) is consistent with findings from other studies in developing countries. For instance, Mohsin et al. (2018) reported widespread fluoroquinolone resistance genes in *E. coli* isolates from food animals, attributing this to the frequent use of these antibiotics in veterinary practice. Similarly, a study found high levels of plasmid-mediated quinolone resistance genes, indicating their rapid dissemination across bacterial populations (Paniagua-Contreras et al., 2018b).

Resistance to aminoglycosides and macrolides, as demonstrated by the presence of *aac(3)-IV* (53%), *aadA1* (56%), and *ereA* (65%), is also consistent with previous studies. M. Munita and C. A. Arias (2016) highlighted that genes such as *aadA1* and *aac* variants are commonly associated with mobile genetic elements, facilitating their spread among bacterial populations. In Uganda, F. Okubo et al. (2020) reported high levels of antimicrobial-resistant *E. coli* in retail meats, including resistance to aminoglycosides and other commonly used antibiotics, supporting the findings of the present study.

The overall high prevalence of these resistance genes suggests the role of horizontal gene transfer, particularly via plasmids, in the dissemination of resistance within pig production systems. This is further exacerbated by selective pressure from antibiotic misuse and overuse, as noted by Munita and Arias (2016), who emphasized that antimicrobial use in animals drives the emergence and spread of resistance genes.

CHAPTER SIX: RECOMMENDATION AND CONCLUSION

6.1 Prevalence of pathogenic *E. coli* O157

Conclusion

This study highlighted a high prevalence of *E. coli* O157, highlighting a significant public health concern in the studied population. Importantly, association was observed between *E. coli* O157 prevalence and sex of pigs, suggesting that certain sex-related factors may influence susceptibility to infection. However, no statistical difference observed between *E. coli* O157 prevalence and other demographic factors, such as age, study area, and farming system indicating that these factors alone may not be a determining factor in the risk of infection within the pig population.

Recommendation

These findings underscore the necessity for targeted public health initiatives to mitigate the prevalence of *E. coli* O157. Such interventions could include promoting better farm hygiene practices, food safety measures, and public education on preventing infection.

Strengthen surveillance and monitoring programs to track the occurrence of *E. coli* O157 across various populations and environments. This would provide more comprehensive data over time and help in identifying trends of *E. coli* O157 related to sex, age, or other demographics of pigs.

Future studies should aim to include larger sample sizes to adequately assess potential associations between *E. coli* O157 prevalence and demographic factors such as age, farming system and study area. The small sample size in this study may have limited the ability to detect such associations, and larger cohorts are necessary to draw more robust conclusions. Additional, further research to explore other potential risk factors (e.g., dietary habits, occupation, etc.) associated with *E. coli*

O157 infection, beyond sex categories, age, farming system and study location is also needed. Lastly, further research could also compare the occurrence of *E. coli* O157 in rural and urban settings.

6.2 Antimicrobial resistance pattern of pathogenic *E. coli* O157

Conclusion

This study found a significant level of antimicrobial resistance (AMR) in *E. coli*O157 isolated from pig fecal samples, particularly highlighting a concerning resistance to colistin and widespread multidrug resistance. The findings revealed a significant association between the study area and resistance to ampicillin, suggesting geographic or management-related factors may influence the development of resistance. Additionally, there was a statistical difference among age group and resistance to colistin, with weaners and adult pigs exhibiting higher resistance compared to growers. The elevated resistance to colistin, a last-resort antibiotic for human health, underscores the critical need for enhanced stewardship in veterinary antimicrobial use to prevent further escalation of resistance. The presence of multidrug-resistant *E. coli* strains further emphasizes the growing threat of AMR in agricultural settings, which may have serious implications for both animal and human health.

Recommendation

These results suggest that targeted interventions like antimicrobial resistance stewardship program, vaccination should be implemented, focusing on reducing antimicrobial use.

Additionally, future research should explore the underlying causes in these areas and age-related differences in resistance patterns to develop more effective strategies for controlling AMR in livestock populations.

6.3 Genotypic resistance of pathogenic *E. coli* O17:H7

Conclusion

The genotypic analysis demonstrated a high prevalence of antimicrobial resistance (AMR) genes among *E. coli* isolates from pigs, indicating widespread dissemination of resistance determinants within the study area. Notably, the high occurrence of the *mcr-3* gene (73%) highlights a serious public health threat, as it confers resistance to colistin, a last-resort antibiotic used in the treatment of multidrug-resistant Gram-negative infections. The prevalence observed in this study exceeds reports from other regions such as South Africa and Tanzania, suggesting a more intense selection pressure, likely driven by unregulated antimicrobial use in livestock production systems. Similarly, the high prevalence of fluoroquinolone resistance genes (*aac(6')-Ib-cr*; 71%) and genes conferring resistance to aminoglycosides and macrolides *aac(3)-IV* (53%), *aadA1* (56%), and *ereA* (65%) indicates multidrug resistance at the genetic level. The presence of these genes, many of which are associated with mobile genetic elements, suggests that horizontal gene transfer plays a significant role in the spread of resistance within pig populations. Overall, these findings confirm that pig production systems serve as important reservoirs of antimicrobial resistance genes, with potential transmission to humans through direct contact, the food chain, and the environment. This poses a substantial risk to both animal and public health, particularly in settings with limited antimicrobial stewardship.

Recommendations

1. Strengthen regulation of antimicrobial use

There is an urgent need for stricter enforcement of policies regulating the use of antibiotics in

livestock, particularly critically important antibiotics such as colistin and fluoroquinolones. Veterinary oversight should be mandatory to minimize misuse and overuse.

2. Promote antimicrobial stewardship programs

Implementation of antimicrobial stewardship programs in animal production systems should be prioritized. This includes educating farmers on prudent antibiotic use, appropriate dosages, and alternatives such as vaccination, improved hygiene, and biosecurity measures.

3. Enhance surveillance of antimicrobial resistance

Continuous monitoring of both phenotypic and genotypic resistance patterns in livestock is essential. National surveillance systems should be strengthened to track emerging resistance genes and guide policy decisions.

4. Improve farm management and biosecurity

Farmers should be encouraged to adopt improved husbandry practices, including proper sanitation, controlled animal movement, and adequate housing, to reduce disease occurrence and the need for antibiotic use.

5. Promote One Health approaches

A coordinated One Health approach involving human, animal, and environmental health sectors is necessary to address the spread of AMR. Collaboration between veterinary and public health authorities should be strengthened.

6. Further research

Additional studies should be conducted to:

- Investigate the transmission pathways of resistance genes between animals, humans, and the environment
- Characterize plasmids and other mobile genetic elements involved in resistance gene transfer
- Assess the impact of interventions aimed at reducing antimicrobial use in livestock

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APPENDICES

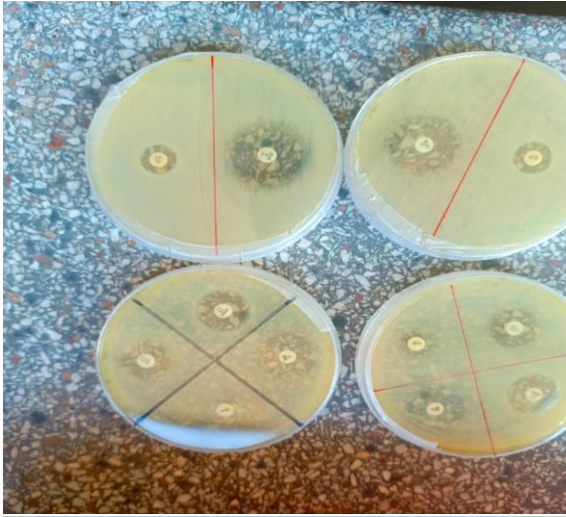
Appendix I: Interpretation of AST Results

Interpretive category	MIC, µg/mL	Zone diameter, mm
Susceptible	≤4	≥20
Intermediate	8-16	15-19
Resistant	≥32	≤14

Appendix II: Work Plan

s/n	activity	Time frame
1	Training of research assistants and the community	Jan 2024
2	Sample collection	Jan 2024
3	Laboratory analysis	Feb 2024
4	Data analysis and dissertation writing	August to November 2024
5	Submission of dissertation and marking	November 2024

APPENDIX III: PHOTOS

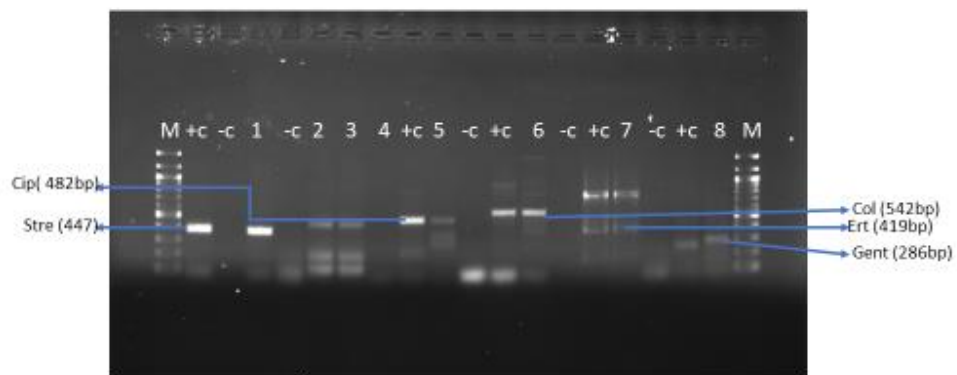


Meuller Hinton plates with antibiotics

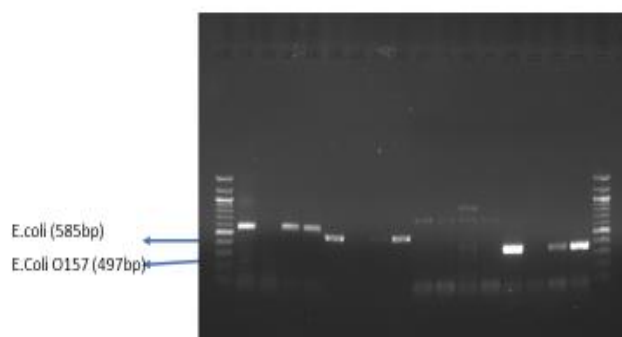


Pink E.coli colonies on MacConkey agar

APPENDIX IV: GEL IMAGES



Amplicons of the antibiotic resistance genes observed



Amplicons of *E. coli* and *E. coli* O157 observed

APPENDIX V: ZONE OF INHIBITION (mm)

Sample id	E	AMP	S	GN	CIP	CT
F1B	I(17.0)	R(00.0)	R(10.0)	R(13.5)	S(34.0)	R(09.0)
F1C	R(14.0)	R(13.0)	S(28.5)	S(22.0)	S(29.5)	I(17.5)
F1D	R(11.0)	R(10.0)	S(26.0)	S(20.0)	S(32.5)	I(17.5)
F1E	I(15.0)	R(11.0)	S(29.5)	S(21.5)	S(43.0)	I(17.5)
F2B	R(12.0)	I(18.0)	S(30.0)	I(19.0)	S(32.0)	R(14.0)
F2C	R(00.0)	I(17.0)	S(32.0)	I(16.0)	S(20.0)	R(13.0)
F2D	R(09.0)	R(11.0)	I(16.0)	I(18.0)	S(30.0)	I(18.0)
F2E	R(11.5)	R(00.0)	S(28.5)	S(21.5)	S(33.0)	I(17.0)
F3A	R(12.0)	R(00.0)	S(29.0)	S(32.0)	S(23.0)	R(08.0)
F3C	I(17.0)	R(10.0)	I(16.0)	R(12.0)	S(24.0)	R(10.0)
F4A	R(11.0)	R(00.0)	I(15.0)	I(17.0)	I(16.0)	I(17.0)
F4B	R(00.0)	R(00.0)	I(16.0)	S(20.0)	S(27.0)	I(17.0)
F4D	R(10.0)	R(08.0)	R(07.0)	R(13.0)	S(21.0)	R(10.0)
F5B	I(17.0)	I(18.0)	S(33.0)	I(15.0)	S(24.0)	I(18.0)
F5C	I(18.0)	R(32.0)	I(17.0)	S(23.0)	S(32.0)	I(16.0)
F5D	R(12.0)	R(10.0)	S(22.0)	S(22.0)	S(40.0)	I(17.0)
F5E	R(07.0)	I(16.0)	S(32.0)	I(17.0)	I(18.5)	R(12.5)
F6B	R(12.0)	R(08.0)	R(12.0)	R(13.0)	S(32.0)	R(14.0)
F6C	R(13.0)	I(15.0)	R(09.0)	R(08.0)	S(31.0)	R(12.0)
F7C	R(12.0)	R(10.0)	I(17.0)	I(16.0)	S(32.0)	I(16.0)
F8E	R(12.0)	R(09.0)	I(19.0)	I(18.0)	I(16.5)	R(00.0)
F9B	S(00.0)	S(30.0)	S(26.0)	S(19.0)	S(29.0)	R(13.5)
F9D	R(00.0)	R(00.0)	R(10.0)	S(21.0)	S(24.0)	R(12.0)
F10A	I(17.0)	R(08.0)	R(07.0)	S(26.5)	S(28.0)	I(17.0)
F10B	I(16.0)	I(18.0)	I(16.0)	S(29.0)	S(30.0)	R(10.0)
F10C	R(10.0)	R(13.0)	S(31.0)	I(17.0)	S(32.0)	I(17.0)
F10E	I(10.0)	S(26.0)	S(32.0)	I(16.0)	S(25.0)	I(16.0)
F11B	R(10.0)	R(13.0)	I(17.0)	I(16.0)	R(11.0)	R(10.0)
F11C	R(11.0)	R(09.0)	I(16.0)	S(30.0)	S(30.0)	I(16.0)
F11D	R(14.0)	R(07.0)	R(10.0)	R(10.0)	S(26.0)	R(13.0)
F11E	R(12.0)	R(12.0)	I(16.0)	I(16.0)	S(26.5)	R(10.0)
F12B	R(11.0)	R(14.0)	I(17.0)	R(10.0)	S(28.0)	I(16.0)
F12C	S(27.0)	I(18.0)	I(16.0)	S(27.0)	S(30.0)	I(17.0)
F12E	I(16.0)	R(10.0)	I(16.0)	R(11.0)	R(14.0)	I(17.0)
F13C	S(31.0)	S(27.0)	S(29.0)	S(24.0)	S(20.0)	I(16.0)
F14B	I(17.0)	I(16.0)	S(31.0)	S(28.0)	S(29.0)	I(17.0)
F14C	I(17.0)	I(16.0)	S(25.0)	S(30.0)	S(24.0)	I(16.0)
F14D	S(31.0)	I(17.0)	S(24.0)	S(28.0)	S(31.0)	I(16.0)
F14E	R(12.0)	R(08.0)	S(26.0)	S(31.0)	S(26.5)	I(15.0)

F15A	S(30.0)	S(31.0)	S(28.0)	S(32.0)	S(28.0)	I(17.0)
F15C	R(12.0)	R(10.0)	S(34.0)	S(32.0)	S(30.0)	I(17.0)
F15D	I(16.0)	I(17.0)	S(31.0)	S(26.0)	S(32.0)	I(16.0)
F15E	I(16.0)	R(07.0)	I(17.0)	S(32.0)	S(31.0)	I(17.0)
F17A	R(00.0)	R(09.0)	R(00.0)	R(11.5)	S(21.5)	R(00.0)
F17E	R(09.0)	R(00.0)	R(00.0)	R(06.0)	S(20.0)	R(00.0)
F18A	R(09.0)	R(00.0)	R(00.0)	R(09.0)	R (00.0)	R(00.0)
F18B	R(00.0)	R(00.0)	I(19.0)	R(11.0)	I(15.0)	R(00.0)
F18C	I(15.0)	R(00.0)	S(24.0)	R(11.5)	S(24.5)	R(09.5)
F19A	R(00.0)	R(00.0)	I(15.0)	R(10.0)	S(21.0)	I(16.0)
F19C	R(00.0)	R(00.0)	R(11.5)	R(08.0)	R(00.0)	R(00.0)
F19D	R(00.0)	R(00.0)	R(00.0)	R(13.5)	S(21.0)	R(10.0)
F19E	R(07.0)	R(00.0)	R(14.0)	R(11.0)	S(21.5)	R(00.0)
F20A	R(08.5)	R(00.0)	I(15.0)	R(09.0)	S(21.0)	R(00.0)
F20E	R(06.0)	R(00.0)	R(00.0)	R(10.0)	S(20.5)	R(00.0)
F10D	R(05.0)	R(06.0)	S(27.0)	I(16.0)	S(27.0)	I(17.0)