

**RISK FACTORS, PREVALENCE AND CHARACTERISATION OF  
PATHOGENIC DRUG RESISTANT *Escherichia coli* ISOLATED FROM BEEF  
AND CHICKEN MEAT IN EASTERN KENYA**

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**A Thesis Submitted to the Graduate School in Partial Fulfilment of the  
Requirements for the Award of the Degree of Doctor of Philosophy in Food  
Science of Chuka University**

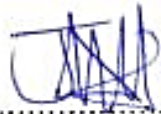
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## DECLARATIONS AND RECOMMENDATION


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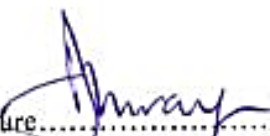
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## **DEDICATION**

To my beloved children, Marie-Becka Gudda, Jakes-Israel Gudda, and Mayaan-Bracha Gudda. Remember, when life calls you to face hard things, you already have the strength and ability to overcome.

## **ACKNOWLEDGEMENT**

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## ABSTRACT

Globally, *Escherichia coli* (*E. coli*) accounts for over 25% of food borne disease (FBD) outbreaks. In Kenya, diarrhoea is the leading cause of death after malaria and pneumonia in Kenya with 100 deaths reported daily. Of all foodborne diseases, food of animal origin account for over 70% of all cases. Eastern Kenya is leading in all diarrhoea cases at 20.8% in the country. The main objective of this study was to determine hygiene handling practices and characterise pathogenic drug resistance genes of *E. coli* isolated from raw and ready-to-eat beef and chicken meat. This study was carried out in Meru, Embu and Tharaka-Nithi counties of Eastern Kenya. Personnel from a total of 216 outlets of RTE and Butcheries were assessed for knowledge, attitudes and practices using a structured questionnaire and samples bought for isolation and characterization of *E. coli* taken. Total coliform counts, *E. coli* and antibiotic resistant (AR) *E. coli* were analysed using standard methods. Antibiotic resistant *E. coli* isolates were exposed to Antibiotic sensitivity test using the Kirby Bauer antibiotic sensitivity discs. Antibiotic sensitivity against sixteen antibiotics from eight were assessed for antibiotic sensitivity including; penicillins, cephalosporins, tetracyclines, aminoglycosides, polymixins, sulphonamides, lincosamides, chloramphenicol and macrolide. Resistant isolates were taken through DNA extraction and the isolates were characterised for presence of the following five virulence genes against the markers; *VirF* (Enteroinvasive *E. coli*), *eae* (Enteropathogenic *E. coli*), *daaE* (Diffusively adherent *E. coli*), *aafII* (Entero-aggressive *E. coli*), *stx1* (Enterohaemorrhagic *E. coli*) and *stII* (Enterotoxigenic *E. coli*). Using specific primers, the presence of the following seven antibiotic resistance genes (ARGs) were also sought; *Bla<sub>TEM</sub>*, *Bla<sub>SHV</sub>*, *Bla<sub>OXA</sub>*, *Bla<sub>CTX-M-15</sub>*, *Mcr-1*, *Sul1*, *tet A*. Data analysis for survey, microbial counts and virulence genes was done using R, SPSS, SAS and STATA. Significant means were separated using the Least Significant Difference (LSD) at  $\alpha = 0.05$ . The results showed that 94% (102) male were the majority in butchereries while females were the majority in RTE establishments (53.3%, 59). In butchereries meat was sold after approximately 3 days (71.3%, 77) while only 25% (27) had refrigeration facilities. All participants had medical certificates but only 46% (50) had it renewed in the last three months. The KAP analysis reported that as food safety hygiene knowledge and attitude was satisfactory but food safety practices were unsatisfactory. The microbial quality of raw beef and chicken meat fell below the regulatory standards set by KEBS of 2 CFU/g for *E. coli* in all raw meat samples. Resistance against Methicillin was the highest for *E. coli* isolated from RTE beef ((24/60) 40%). The multiple antibiotic resistance (MAR) index ranged between 0.2 to 0.67. The most prevalent *E. coli* pathotype was EHEC at ((30/60) 51%) while the least was EIEC with 23.3% (14/60). The *stx1* gene was prevalent in 90% (9/10) of raw beef from Meru county. The resistance gene *Bla<sub>Oxa</sub>* was most prevalent of all at 26% (16/60), followed by *tet A* (40%, (24/60)). The presence of pathogenic *E. coli* in RTE meat is a public health and trade concern. There is need for strict regulation, education and training on Food safety handling methods and their consequences not only to the involved stakeholders but to the general public as well.

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## LIST OF ABBREVIATIONS AND ACRONYMS

<b>AAC</b>	Aminoglycoside Acetyl Transferase
<b>AMR</b>	Antimicrobial Resistance
<b>ANOVA</b>	Analysis of variance
<b>ARG</b>	Antimicrobial Resistance Genes
<b>AST</b>	Antimicrobial Sensitivity Testing
<b>CA</b>	Codex Alimentarius
<b>CBD</b>	Central Business District
<b>CDC</b>	Centre for Disease Control and Prevention
<b>CFU</b>	Colony Forming Units
<b>CLSI</b>	Clinical and laboratory Standard Institute
<b>CTX-M</b>	Cefotaximase enzyme encoding gene
<b>DAE</b>	Diarrheagenic <i>Escherichia coli</i>
<b>DAEC</b>	Diffusively adherent <i>Escherichia coli</i>
<b>DHFR</b>	Dihydrofolate reductase
<b>DHPS</b>	Dihydropteroate Synthase
<b>DNA</b>	Deoxyribonucleic Acid
<b><i>E. coli</i></b>	<i>Escherichia coli</i>
<b>EAEC</b>	Enteraggresive <i>Escherichia coli</i>
<b>EFSA</b>	European Food Safety Authority
<b>EHEC</b>	Enterohaemorrhagic <i>Escherichia coli</i>
<b>EIEC</b>	Enteroinvasive <i>Escherichia coli</i>
<b>EPEC</b>	Enteropathogenic <i>Escherichia coli</i>
<b>ESBL</b>	Extended Spectrum Beta-lactamase
<b>ESVAC</b>	European Surveillance of Veterinary Antimicrobial Consumption
<b>ETEC</b>	Enterotoxigenic <i>Escherichia coli</i>
<b>EUCAST</b>	European Committee on Antimicrobial Susceptibility Testing
<b>FAO</b>	Food and Agriculture Organisation of the United Nations
<b>FAOSTAT</b>	Statistics by Food and Agriculture organisation of the United Nations
<b>FBD</b>	Food Borne Diseases
<b>GI</b>	Gastrointestinal
<b>GIS</b>	Geographic Information System
<b>GMP</b>	Good Manufacturing Practices

<b>HACCP</b>	Hazard Analysis Critical Control Points
<b>HPCI</b>	Highest Priority Clinically Important
<b>KAP</b>	Knowledge Attitude and Practices
<b>KDHS</b>	Kenya Demographic Health Survey
<b>KEBS</b>	Kenya Bureau of Standards
<b>KNBS</b>	Kenya National Bureau of Statistics
<b>LLC</b>	Limited Liability Company
<b>LMICs</b>	Low- and Middle-Income Countries
<b>LSD</b>	Least Significant Difference
<b>MAR</b>	Multiple Antibiotic Resistance
<b>MDR</b>	Multi Drug Resistance
<b>MIC</b>	Minimum Inhibitory Concentration
<b>MoH</b>	Ministry of Health
<b>NDM</b>	New Delhi metallo- $\beta$ -lactamase
<b>NDM -1</b>	New Delhi Mettalo- $\beta$ -lactamase
<b>OECD</b>	Organisation for Economic Co-operation and Development
<b>PBC</b>	Perceived Behavioural Control
<b>PCR</b>	Polymerase Chain Reaction
<b>PMQR</b>	Plasmid Mediated Quinolone Resistance
<b>QNR</b>	Quinolone Resistance
<b>RNA</b>	Ribonucleic Acid
<b>RTE</b>	Ready-to-Eat
<b>SHV</b>	Sulfhydryl-Variable enzymes of beta-lactamase
<b>STEC</b>	Shinga-toxin Producing <i>Escherichia coli</i>
<b>TBX</b>	Tryptone Bile Agar
<b>TCC</b>	Total Coliform Count
<b>TEM</b>	Gene encoding the TEM-1 beta-lactamase enzyme,
<b>TNC</b>	Tharaka – Nithi County
<b>UK</b>	United Kingdom
<b>USDA</b>	United States Department of Agriculture
<b>VRBA</b>	Violet Red Bile Agar
<b>WHO</b>	World Health Organisation

# CHAPTER ONE

## INTRODUCTION

### 1.1 Background Information to the Study

Meat is a major component of human diet as it provides, protein, energy, minerals and vitamins (McAfee *et al.*, 2010). Food-producing animals are the most preferred sources of proteins due to their high biological value (Mallhi *et al.*, 2019). The demand for meat is rising due to increased population, urbanisation and higher purchasing power especially in low and middle-income countries (LMiCs). The global meat consumption is projected to reach 382 Metric tonnes by 2032 a 16% increase from 2022 according to the agricultural outlook by Organisation for Economic Co-operation and Development (OECD) and the Food and Agricultural Organization (FAO) of the United Nations (OECD-FAO, 2022). In Kenya the meat value chain is an important economic activity. It provides nutrition and employment to the agricultural sector by contributing about 40-50% to the agricultural Gross Domestic Product (FAOSTAT, 2022). Due to the increasing urban population, Kenya has witnessed an increased demand in meat in most towns at a rate of 2.2 tons annually from 2017 with beef and chicken as the most consumed meats (Kunyanga *et al.*, 2021).

Unfortunately, foods from animal sources have been incriminated as the leading contributors to food borne diseases by 70% (WHO, 2023a). Foodborne diseases (FBD) remain a major global public health challenge, with significant socio-economic and health implications, particularly in low- and middle-income countries (LMiCs). These include; morbidity, loss of productivity and strain in health care facilities. Centre for disease control and prevention has reported that approximately 600 million illnesses occur worldwide due to food borne diseases with children under the age of five being the most affected (CDC, 2019). The burden of FBD is made up of mostly LMICs economies which are disproportionately struggling with poor access to health facilities, non-structured food value chains and minimal regulations (Kunyanga *et al.*, 2021; Onyeaka *et al.*, 2023). The prevalence of Diarrheagenic diseases in Kenya by 2024 was reported to be highest in Eastern Kenya at 20.8% followed by Nairobi at 16% with North Eastern Kenya recording least prevalence of 3.4% (Ogumbo *et al.*, 2024). According to the Kenya Demographic Health Survey (KDHS), the overall prevalence of diarrheagenic cases in Kenya is at 15% (KDHS, 2022). Top epidemiological caused

of diarrhoea was lack of hygiene practices like hand washing and consumption of retail meats where contamination with causative agents like *E. coli* is rampant.

Ready-to-eat (RTE) food refers to food which can be consumed immediately or after taking a few steps such as heating before consuming (Thienhirun & Chung, 2018). Since customers rarely reprocess these foods before consuming, food poisoning cases on account of RTE meat occasionally happen. Many sanitary surveys have revealed that RTE meat is a kind of instant food easily contaminated by microbes (Baloch *et al.*, 2017; Yang *et al.*, 2016) and would be a major source of foodborne diseases. Meat and meat products are considered to be excellent sources of support the growth of pathogenic microorganisms (El-Shenawy, *et al.*, 2016). As a result, foodborne diseases from food animals are also increasing. As the urban population rises so does the demand for RTE meat and meat products, on the international market due to a changing urban lifestyle as people prefer food with little or no preparation time before consumption (Sofos, 2008; Havelaar *et al.*, 2010).

In Kenya, the meat retail sector, encompassing both raw and RTE meat vendors, playing a pivotal role in the food supply chain. However, this sector is fraught with hygiene and sanitation challenges that heighten the risk of transmission of FBD agents (CDC, 2019). These dynamics, coupled with weak enforcement of food safety regulations and limited awareness of proper hygiene practices, make urban meat retail outlets potential hotspots for foodborne disease outbreaks. In Kenya meat value chain actors have been documented to fail to adhere to good manufacturing practices (GMP) in handling raw and RTE meat and meat products (Aduah *et al.*, 2021; Mwove *et al.*, 2021; Siluma *et al.*, 2023).

Top epidemiological cause of diarrhoea is lack of hygiene practices like hand washing and consumption of retail meats where contamination with causative agents (like *E. coli*) is rampant (CDC, 2019). The Kenyan meat value chain involves movement of meat through slaughtering, transportation and retail where each step poses a risk of microbial contamination. Although there exists meat hygiene and handling requirements for butcheries in Kenya (GOK, 2023), adherence to this requirement by most butcheries and RTE retailers is low and not adequately documented (Lamuka *et al.*, 2015; Wambui *et al.*, 2020; Mwove, 2025). Risks factors associated with meat

contamination with pathogenic microorganisms have been categorized into three aspects; personnel hygiene, food storage and design of premises, according to the Essential Food Safety Requirement by WHO (WHO, 2018). These aspects are usually dependent on value chain actors' knowledge attitudes and practices (KAP). Therefore, the KAP analysis of food handlers in these establishment would directly influence the likelihood of foodborne disease outbreak. Unfortunately, food handlers' practices such as lack of hand washing, lack of refrigeration, handling meat and money simultaneously have been reported to contribute over 50% of microbial contamination to foods (Soon *et al.*, 2020; Gichunge *et al.*, 2023; Koech *et al.*, 2024).

Microbial quality assessments of meat products provide insights into the extent of contamination and potential health risks. Research indicates that both raw and RTE meat products in Kenyan urban centres are contaminated with high levels of microorganisms including *Escherichia coli* (*E. coli*) (Odwar *et al.*, 2014; Ronald *et al.*, 2023; Koech *et al.*, 2024). *Escherichia coli* as the most prevalent microorganism in food borne diseases (CDC, 2019,) and is responsible for 25% of all food borne diseases globally (Mir & Kudva, 2019; Kassaye *et al.*, 2021). It is a significant contaminant during animal slaughter and an indicator microorganism for faecal contamination (FAOSTAT, 2022).

Among the pathogenic *E. coli*, diarrheagenic *E. coli* (DEC) is the main cause of global epidemic and endemic diarrhoea (CDC, 2019). Diarrheagenic *E. coli* strains are grouped into six pathotypes which include enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), entero-invasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC) and diffusive adherent *E. coli* (DAEC) which have also been reported in meat (Muinde *et al.*, 2023; Okumu *et al.*, 2025; Wang *et al.*, 2025). However, it is not clear to what extent the Eastern Kenya population is exposed to the risk of pathogenic *E. coli* in RTE meats due to the minimal information since majority of the studies are concentrated in Nairobi and its environs (Chilanga *et al.*, 2025; Okumu *et al.*, 2025). These studies were concentrated around Central and Rift Valley regions based on RTE meats. (Chilanga *et al.*, 2025; Okumu *et al.*, 2025). Since these strains are associated with diarrhoea in most cases, Eastern Kenya region has been reported as the leading region in prevalence of diarrheagenic diseases in Kenya (Ogumbo *et al.*, 2024). Literature has indicated that diarrheagenic *E. coli* (DEC) is the

leading cause of endemic diarrhoea in most diagnosed cases globally and has been isolated from the RTE meats (Okumu *et al.*, 2025. Wang *et al.*, 2025) it is postulated that it might be involved in the prevalent diarrhoea cases in Eastern Region of Kenya.

When sickness occur, antimicrobials are among the first line of defence. However, antimicrobial resistance (AMR) is becoming a major problem to treatment of illnesses (CDC, 2019). Antimicrobial resistance occurs when microorganisms are no longer susceptible to antibiotics previously used to eliminate them at minimum inhibitory concentrations (MIC) (Mahtab *et al.*, 2021). It can be facilitated by human practices such as poor hygiene, self-medication, inappropriate drugs prescription (Martínez *et al.*, 2015; Holmes *et al.*, 2016), overuse of chemicals and antibiotics, inaccurate diagnosis and extensive agricultural application (Munita & Arias, 2016; MacGowan & MacNaughton, 2017). Animal production uses a lot of antibiotics, according to European surveillance of veterinary antimicrobial consumption participating countries (ESVAC). Of 1.5 tonnes of active microbials, 98% of these are used in animal production with over 84% used as feed additives (ESVAC, 2019). Therefore, beef and chicken meat were selected as representatives for this study. In Kenya the most dispensed antibiotics for animal production have been reported to be tetracyclines, beta-lactams, sulphonamides and macrolides while in human health the most dispensed antibiotics are reported to be beta-lactams, fluoroquinolones and metronidazole (Muloi *et al.*, 2019; Kemp *et al.*, 2021). Majority of antibiotics used in animal production are also used in human medicine (CDC, 2019).

*Escherichia. coli* isolated from raw and RTE beef and poultry products have reported resistance against some of the major broad-spectrum antibiotics (Muloi *et al.*, 2019, Kemp *et al.*, 2021, Ronald *et al.*, 2023, Koech *et al.*, 2024). However, minimal studies present the effect of food chain combined with species in reporting antimicrobial resistance patterns. There is also an alteration in the frequency and pattern of antimicrobial resistance within species (Abass *et al.*, 2020; Liu *et al.*, 2020), antibiotics class (Kemp, 2021) and geographical location (CDC, 2019; GLASS - WHO, 2021). The main threat of AMR is the presence of untreatable infections due to reduced number of drug options available for treatments, which presents a challenge to the global healthcare system (OIE, 2020; Plackett, 2020). Death may occur or patients may stay longer in hospitals hence incurring more medical bills (GLASS - WHO, 2021). In 2021,

antimicrobial resistance was responsible for approximately 700,000 deaths and projected a rise in this figure to 10 million by 2050 (O'Neill, 2016; WHO, 2023a).

AMR pattern of a microorganism is controlled by its antimicrobial resistance genes (ARGs) (Mahtab *et al.*, 2021). The gradual increase of ARG found in food animals and products makes them an important source and environmental repository of drug-resistant bacteria (Muloi *et al.*, 2019; Kemp, 2021; Ronald *et al.*, 2023). These genes can be transmitted between microorganism and the risk of their presence in one variant of *E. coli* is extrapolated within other pathogenic types. The ARGs may enter into humans by food chain, resulting in deleterious consequences to human health (Huang *et al.*, 2019). Majority of studies on ARG are concentrated in hospitals and farms where administration of antibiotics takes place with little information on food chain contribution as a point of transfer of ARG to human population (Moor *et al.*, 2020; Chilanga *et al.*, 2025; Brito-Junior *et al.*, 2025).

Diarrheagenic *E. coli* strains are mainly treated with the  $\beta$ -lactam group of antibiotics (CDC, 2019). They are the leading microorganisms in the production of the beta lactamase enzymes which hydrolyse the beta lactam ring in beta lactam antibiotics. This further complicates treatment by inducing resistance. Furthermore, *E. coli* strains have been reported to transfer resistance genes from one strain to another. Studies have shown that some DEC strains have resistance genes such as *bla<sub>TEM</sub>*, *bla<sub>CTX-M</sub>*, *bla<sub>SHV</sub>*, and *bla<sub>OXA</sub>* and have been reported in Raw and RTE beef and other meat products (Kemp *et al.*, 2019, Ronald *et al.*, 2023, Koech *et al.*, 2024).

To combat AMR, animal food value chains characterised with chances of high bacterial loads like meat need to be studied and antibacterial resistance genes characterised. Pathogenic *E. coli* is a clinically important pathogen whose incubation and spread to the environment should be under utmost restraint (GLASS - WHO, 2021; Kemp, 2021; Liu *et al.*, 2021). The meat value chain is a possible driver to *E. coli* pathogenesis and could be used to stop the spread of food borne illness and unsure food safety by improved hygiene practices. Currently it is not clear to which extent beef and chicken meat value chain is responsible for culmination and discharge of pathogenic *E. coli* and resistance genes to the environment in Meru, Embu and Tharaka Nithi counties in Eastern Kenya. The incidences of pathogens vary with bacterial species, antimicrobial groups and most of all geographical locations in the world hence the one in Eastern

Kenya could be different from those around the country (Mahtab *et al.*, 2021). Therefore, there is need to assess the patterns of pathogenic *E. coli* antimicrobial resistance and characterise antimicrobial resistance genes under Eastern Kenya environmental conditions. By combining environmental assessments, hygiene practices, microbiological testing, antimicrobial resistance profiling, molecular characterization, and practical interventions, this study adopts a holistic, One Health approach to addressing the threat of foodborne diseases in Kenya. The outcomes of this research will not only contribute to academic knowledge but will also inform national food safety policy, consumer protection strategies, and public health interventions aimed at safeguarding meat quality and preventing possible foodborne disease outbreaks.

### **1.2 Statement of the Problem**

Food of animal sources are the most implicated sources in the disease outbreaks. Environmental contamination with pathogenic strains from the animals during slaughter, retail in butcheries, storage and personnel handling during processing are major drivers of contamination and illnesses. Reports on prevalence of *E. coli* in raw and RTE meat is reported in many studies in the country, but there is little information on antimicrobial resistance or virulence factors of diarrheagenic *E. coli* strains reported from raw and RTE meats in Eastern regions of Kenya. The information that exists is mostly from central and Rift Valley regions of Kenya. The regions may be having different ecological diversities and practices that may influence the occurrence of the diarrheagenic *E. coli* types that may be associated with high prevalence of diarrheagenic diseases in Eastern region of Kenya. Hence; the assessment of the risk factors, the prevalence and nature of pathogenic drug resistant *E. coli* that could be responsible for the high prevalence of diarrhoea in Eastern Kenya region. The Kenyan government has meat hygiene and handling requirements for butcheries in Kenya, however, compliance to these regulations has been reported to be minimal. Antimicrobial resistance is projected to cause 10 million deaths by 2050. It is not clear to which extent beef and chicken meat are responsible for the discharge of drug-resistant pathogenic *E. coli* in Eastern Kenya which led in diarrheagenic diseases prevalence nationally in 2024. Most studies on ARG associated with *E. coli* are also within farms with few focusing on butcheries and/or RTE meats.

### **1.3 Objectives of the Study**

#### **1.3.1 General Objective**

To assess the risk factors contributing to contamination of beef and chicken meat with pathogenic *Escherichia coli* and to characterise its virulence and antibiotic resistance genes in Eastern Kenya.

#### **1.3.2 Specific Objectives**

- i. To determine the risk factors associated with handling and processing of raw and ready-to-eat beef and chicken meat that pre-disposing them to contamination with pathogenic *Escherichia coli* in Meru, Embu and Tharaka-Nithi counties
- ii. To determine the prevalence of pathogenic and antimicrobial resistant *E. coli* isolated from raw and ready-to-eat beef and chicken meat in Meru, Embu and Tharaka-Nithi counties.
- iii. To determine the antibiotic susceptibility profiles of pathogenic drug-resistant *E. coli* isolated from raw and ready to eat beef and chicken meat in Meru, Embu and Tharaka-Nithi counties
- iv. To characterise antimicrobial resistance and virulence genes of pathogenic drug-resistant *E. coli* isolated from raw and ready-to-eat beef and chicken meat in Meru, Embu and Tharaka-Nithi Counties

### **1.4 Hypotheses**

H0<sub>1</sub>: There is no association between handling and processing risk factors and contamination of raw and ready-to-eat beef and chicken meat with pathogenic *Escherichia coli* in Meru, Embu and Tharaka-Nithi counties

H0<sub>2</sub>: There is no significant difference in the prevalence of pathogenic drug resistant *Escherichia coli* isolated from raw and ready-to-eat beef and chicken meat in Meru, Embu and Tharaka- Nithi counties

H0<sub>3</sub>: There is no significant difference in the antibiotic susceptibility profiles of pathogenic drug-resistant *E. coli* isolated from raw and ready to eat beef and chicken meat in Meru, Embu and Tharaka-Nithi counties

H0<sub>4</sub>: There is no significant difference in antimicrobial resistance and virulence genes of pathogenic drug-resistant *E. coli* isolated from raw and ready-to-eat beef and chicken meat in Meru, Embu and Tharaka-Nithi Counties

### **1.5 Justification of the Study**

As meat moves along the value chain, poor handling practices and hygiene contribute to the multiplication of pathogenic *E. coli* and by extension resistance genes. Poor hygiene of personnel and handling equipment is the most common source of microorganisms in meat value chains (Wang *et al.*, 2020). Hospital environment and animal farms have received a lot of attention when it comes to studies on antimicrobial resistance of pathogenic organisms leaving a gap in market samples and RTE meats in Kenya (Kemp, 2021; Muloi *et al.*, 2019; WHO, 2022). Therefore, there is need to explore practices and antimicrobial resistance of *E. coli* to facilitate targeted interventions. Improved hygiene will enhance environmentally friendly practices around the meat value chain especially in butchereries and RTE establishments and improve trade. This will also further the identification of reservoirs of resistance genes in beef and chicken meat by effecting policy decision with evidence-based information. By uncovering the connection between beef and chicken meat handling in the value chain, and contamination with *E. coli* resistance and virulence genes in this study, there could be more effective mitigation strategies to enhance the ability to protect the Eastern Kenya population from diarrheagenic diseases caused by *E. coli*, reduce transmission of AMR bacteria and contribute to the global effort towards food safety.

## CHAPTER TWO

### LITERATURE REVIEW

#### **2.1 Risk Factors to Meat Contamination with Pathogenic *Escherichia coli***

##### **2.1.1 Risk Factors Pre-disposing Raw Meat to Contamination with *Escherichia coli* in Butcherries**

Meat is a major component of human diet because it provides, protein, energy, minerals and vitamins (McAfee *et al.*, 2010). On the other hand, animal products like meat are the most preferred sources of proteins due to their high biological value (McAfee *et al.*, 2010; Mallhi *et al.*, 2019). Meat is rich in nutrients and highly susceptible to microbial contamination that can causes foodborne illness to consumers and meat spoilage (Mallhi *et al.*, 2019). This can result in quality deterioration hence quality losses, economic losses and public health concerns (Erick, 2012). The types and extent of microbial contamination depends on sanitation procedures and hygienic practices during meat handling, storage, distribution and processing (Lamuka *et al.*, 2015; Kunyanga *et al.*, 202; Mwove *et al.*, 2024).

The spread of pathogens can be facilitated by some human activities one of them being movement along the food value chains. Poor hygiene, poor sanitation and poor infection control are three interconnected key factors contributing to the spread of pathogenic bacteria in communities (Mahtab *et al.*, 2021). Poor hygiene has been cited as the leading cause of contamination of foods and disease outbreaks (WHO, 2023) In another study aimed at assessing post-harvest handling practices of meat in eastern Kenya, it was reported that hygiene practices were poor since over 50% of post-harvest meat handlers did not have any formal training on hygiene practices (Kunyanga *et al.*, 2021).

Practices such as frequent hand washing has been reported to be little among meat handlers (Ronald *et al.*, 2023). Handling money while serving food without washing hands between the intervals has been reported in Kenyan food serving establishments (Mwove *et al.*, 2021). Some studies have reported that some food handlers acknowledge the risks of handing money while selling food (79.4%) while a relatively small amount (35%) do not involve in this practice (Ma *et al.*, 2019; Araceli *et al.*, 2023) this is a risk to food consumers.

Studies have shown that food handlers have good knowledge on the significance of hand washing before work (77%) (Ma *et al.*, 2019) but a very small percentage of street food vendors are actually equipped with hand washing stations (26.7%) (Sabbithi *et al.*, 2023). The combination of washing hands with soap and also using sanitizers on surfaces is a recommended practice for food handlers (FAO, 2022). According to Azanaw and others, 81% of ready to eat meat retailers rinse cutting boards with sanitizers after washing with soap and detergents (Azanaw *et al.*, 2022).

Certification ensures that trained personnel on hygiene are found at food processing and handling establishments (FAO, 2022). Personnel need to understand the importance of wearing protective clothing such as hair nets and aprons (Sabbithi *et al.*, 2023). Sick individuals should also not be allowed to handle food since they could spread the sickness through foods (CDC, 2019). Several studies have reported that food handling agents are not fully knowledgeable on the impact of personal hygiene to food hygiene (Stephen & Wakhungu, 2019; Azanaw *et al.*, 2022).

### **2.1.2 Risk Factors in Knowledge Attitude and Practices of Ready-to-Eat Retailers of Beef and Chicken Meat**

Among the factors contributing to outbreak of foodborne diseases, food handling is the leading contributor to pathogenic foodborne agents in meats (Tegegne & Phyto, 2017). While hotels are the major sellers of RTE foods in urban centres (Rashmore & Bagley, 2014), the KAP analysis of meat handlers in these establishment would provide a better analysis of the risks that the urban population is exposed to. Risks factors associated with meat contamination with pathogenic microorganisms have been categorized into three aspects; personnel hygiene, food storage and design of premises, according to the Essential Food Safety Requirement (WHO, 2018). These aspects are usually dependent on value chain actors' knowledge attitudes and practices (KAP). Moreover, the KAP of RTE meat retailers significantly influence food safety outcomes. Food handlers have been reported to contribute approximately 50% of microbial contamination (Ehuwa *et al.*, 2021, Teferi *et al.*, 2022), these values could even be higher in LMICs like Kenya where regulations in the informal market is minimal (Mulat *et al.*, 2024). This is because a food handlers' knowledge on food safety, handling, storage and processing will have a positive effect on hygiene practices which have a cumulative effect on microbial contamination and proliferation in food. Understanding these factors and

their implications can be used to develop mitigation measures such as training targeting the knowledge and attitude gaps (Segbedzi *et al.*, 2024).

Assessment of KAP is anchored on the theory of planned behavior (Nickell & Hinsz, 2023). This theory speculates that the best predictor of an individual's behavior is the intention to perform the behavior (Divianjella *et al.*, 2020). This is grounded on three antecedents: subjective norms, attitudes, and perceived behavioral control (PBC) (Tao-Ing, 2022). Attitude, on the other hand, determines the level at which individuals have favorable or unfavorable assessment of a behaviour in question (Vamvaka *et al.*, 2020). This is influenced by beliefs individuals hold regarding such behavior and expectations from subjective norms.

To facilitate the performance of safe food behaviours, there is a need to equip food service employees with knowledge, skills, equipment, and opportunities to build their confidence. Attitudes towards behaviour comprise individual's appraisal of how positive or negative performing a behaviour would affect the outcome (Sezbedzi *et al.*, 2024). Attitudes are based on beliefs, and individual's intentions serve as the mediation factor for behaviour to be performed. For instance, if food handlers believe that frequent hand washing is undesirable, they are less likely to engage in it. This attitude reflects a critical factor that determines food handlers' intentions as well as the successive likelihood to execute the behaviour (Nickell & Hinz, 2023).

It is therefore imperative for food handlers to have a thorough understanding of food safety protocols and training on safety with elaborate consequences. Different factors affecting food hygiene practices among Food handlers have been identified in different settings. Factors such as good knowledge on food safety, having a formal education and over five years of experience have been reported to have a positive influence on improved hygiene practices (Zenbaba *et al.*, 2022, Chekol *et al.*, 2019). In African LMICs, RTE meat handlers often work in informal environments where food safety knowledge and infrastructure are minimal. In Nigeria, Ilesanmi *et al.* (2020) reported low knowledge scores among meat vendors, with only 15% understanding proper meat storage temperatures. Yet, 80% of respondents expressed positive attitudes toward improving hygiene if provided with training. In Ghana, Aikins *et al.* (2021) revealed that while many RTE meat handlers were aware of the risks associated with poor

hygiene, their practices were constrained by lack of clean water, refrigeration, and waste disposal facilities. In Sudan, Osman *et al.* (2020) identified a disparity between attitude and practice, whereby vendors acknowledged the importance of cleanliness but used unsafe meat handling techniques due to lack of infrastructure and supervision. In Rwanda, Uwiringiyimana *et al.* (2021) highlighted that meat handlers in Kigali markets rarely used protective clothing and had poor handwashing habits, largely due to low enforcement of food safety regulations. Across these African LMICs, the literature shows a common trend of limited formal education among vendors, minimal training opportunities, and poor implementation of food safety policies, all of which hinder the translation of knowledge and attitude into safe food handling practices.

In Kenya, numerous studies have investigated the KAP of RTE meat handlers, highlighting critical gaps that pose public health risks. A study conducted in Nairobi revealed that while some food handlers had basic knowledge of food safety, there were gaps in attitudes and practices, leading to unsafe food handling behaviors (Mathenge *et al.*, 2017). Poor practices such as lack of temperature control has been reported in all stages of food handling of most hotels in Nairobi county (Mosota, 2023). A study done by Kaugi and others (2023) to determine the effect of training on knowledge, attitude and practices of food handlers in a boarding school in Embu county reported a 19% increase in knowledge, 18% increase in good attitude and 30% increase in good hygiene handling practices after training. Showing that training could have a significant impact on improved positive attitude and practices. In an attempt to find the relationship between attitude knowledge and practices on food contamination in hotels within Nairobi county, Kinyua (2024) reported that food handling practices such as touching foods after handling money, fondling of body parts without handwashing, and lack of pretreatment of drinking water were significantly associated with foods and water contamination (P-value<0.05).

A study by Omondi *et al.* (2021) in Nairobi found that although most RTE meat handlers were aware of foodborne pathogens like *Salmonella* and *E. coli*, less than 30% adhered to basic food safety practices such as wearing gloves or maintaining cold chain storage. Gikonyo *et al.* (2022) identified that low training uptake, economic constraints, and lack of consistent inspections significantly contributed to poor hygiene practices in urban butcherries. Similarly, a study by Muli *et al.* (2020) across peri-urban areas of

Machakos and Kitui counties revealed that many RTE vendors held positive attitudes toward food safety, but their knowledge was either outdated or based on informal learning. According to Mwangi *et al.* (2023), food handlers working in informal settings rarely underwent medical screening or certification, and personal hygiene was often neglected due to poor facilities. Furthermore, Oloo *et al.* (2021) emphasized that enforcement of public health regulations is sporadic, especially in informal markets, making it difficult to translate knowledge and attitudes into safer practices. These findings underscore the pressing need for comprehensive food safety training programs, stronger regulatory enforcement, and improved sanitation facilities such as hand washing sinks, to support safe handling of RTE meat in Kenya.

## **2.2 Occurrence of *Escherichia coli* in Raw and Ready-to-Eat Beef and Chicken Meat**

*Escherichia coli* is a rod-shaped, Gram-negative member of the Enterobacteriaceae family. It is a gut commensal of warm-blooded mammals, including humans. They are one of the most highly adapted and successful facultative anaerobes of the human gut microflora (Sweeney *et al.*, 1996). *Escherichia coli* is a ubiquitous bacterium which has frequent exposure to a variety of antimicrobials in the gastrointestinal (GI) tract, aimed at treating other pathogens. It is thought to act as a reservoir for AMR genes which may transfer resistance to other zoonotic or commensal organisms (Aarestrup, 2015); as such it would be a good indicator of resistance.

*Escherichia coli* is also the most comprehensively studied bacterium due to the relative ease of genetic manipulation and controlled growth in laboratory settings. They are relatively easy to isolate and investigate, and have been the subject of numerous studies which have highlighted the issue of increasing prevalence of drug-resistance, they are also a major contaminant of carcasses during slaughtering of animals (Stopforth *et al.*, 2006; Kabiru *et al.*, 2015). For these reasons, *E. coli* was chosen as a model organism in this study. Pathogenic *E. coli* cause clinical syndromes including diarrhoeal disease, urinary tract infections and sepsis/meningitis.

Coliforms and *E. coli* are used as faecal indicators, and their presence in meat often suggests poor sanitation, or faecal contamination. According to Codex Alimentarius (CA) and KEBS standards, *E. coli* in raw meat should not exceed 100 CFU/g, ( $2 \log_{10}$

CFU/g) and coliforms must remain below  $2.5 \log_{10}$  CFU/g (FAO/WHO, 2021; KEBS, 2021). Internationally, the European Union (EU) Regulation 2073/2005 sets process hygiene criteria, recommending that coliforms should not exceed  $2.5 \log_{10}$  CFU/g, and *E. coli* below  $2.0 \log_{10}$  CFU/g in fresh meat (EFSA, 2020). The United States Department of Agriculture (USDA, 2021) provides similar standards, emphasizing zero tolerance for pathogenic *E. coli* O157:H7 in raw meat. Codex Alimentarius recommends regular microbial testing to ensure food safety and compliance, particularly in LMICs (FAO/WHO, 2021).

In Kenya, the Kenya Bureau of Standards (KEBS) outlines acceptable microbial limits under KS EAS 82:2017 for beef and KS EAS 847:2017 for poultry. It recommends a maximum of  $5.0 \log_{10}$  CFU/g for Total coliform counts and *E. coli* not to exceed  $2 \log_{10}$  CFU/g (KEBS, 2021). These standards reflect an effort to align local benchmarks with international food safety requirements. However, enforcement remains weak, particularly in informal retail markets. The presence of antimicrobial-resistant *E. coli* in raw meat is not yet explicitly addressed in KEBS standards, although regional discussions on integrating AMR testing are ongoing (MoH Kenya, 2022). These regulatory frameworks serve as critical benchmarks for monitoring microbial contamination, yet gaps persist in their application, especially in resource-limited settings.

Pathogenic *E. coli* are grouped into extraintestinal *E. coli* causing diarrhoea. Among the bacterial pathogens, diarrheagenic *E. coli* (DAE) group is the main cause of globally epidemic and endemic diarrhoea in human, because they harbour virulence elements that are responsible for causing disease in human (Campos et al., 2004; Kaper et al., 2014). DAE strains are grouped into six pathotypes which includes enteropathogenic *E. coli* (EPEC) enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusely-adherent *E. coli*. (Shabana et al., 2013).

EPEC causes infantile diarrhea by forming characteristic attaching-and-effacing (A/E) lesions on intestinal epithelial cells. It uses bundle-forming pili (BFP) encoded on the pEAF plasmid for initial localized adherence, then deploys a type III secretion system (T3SS) to translocate effector proteins (e.g. EspA, EspB, EspD) that trigger actin

polymerization beneath the bacterium and efface microvilli. Intimin (encoded by *eae*) mediates intimate binding to the host cell. The global regulator *Ler* controls expression of the *LEE* (locus of enterocyte effacement) operons. EPEC strains tightly regulate virulence in response to environmental cues (e.g. stress, pH) to optimize colonization (Deborah & Frankel, 2005).

EHEC (a subset of Shiga toxin-producing *E. coli* or STEC) combines A/E lesion formation with potent toxin production (Shiga toxins, *Stx1* and/or *Stx2*). After intestinal colonization via the *LEE*-encoded T3SS and intimin, EHEC synthesizes and releases Shiga toxins, which translocate into the bloodstream to damage vascular endothelial cells, notably in the kidney, triggering hemolytic uremic syndrome (HUS). The toxin inhibits ribosomal function in host cells, inducing apoptosis. Antibiotic exposure may induce prophage-mediated *Stx* release via the bacterial SOS response, exacerbating disease (Nguyen & Sperandio, 2012).

EPEC is a leading cause of traveler's diarrhea and childhood diarrhea in developing countries. Its virulence rests on colonization (via colonization factors, CFs, such as CFA/I, CS antigens) that mediate adhesion to small intestinal epithelium, followed by secretion of at least one of two enterotoxins: the heat-labile toxin (LT) and/or the heat-stable toxin (ST). LT acts by elevating intracellular cAMP, while ST increases cGMP, both leading to chloride and water secretion into the intestinal lumen (secretory diarrhea). Additional autotransporter toxins and adhesins may augment virulence (Mirhoseini *et al.*, 2018).

EIEC causes a syndrome similar to *Shigella*, with invasion of the colonic epithelium and an inflammatory, often bloody, colitis. It carries a large invasion plasmid (pINV) encoding a type III secretion system that injects effectors to mediate host cell invasion, actin-based intracellular movement, intercellular spread, and evasion of host defenses. Unlike *Shigella*, EIEC retains some metabolic capacity and flagella, but the core invasive machinery is highly similar. The pathogenic process includes intracellular replication and induction of host inflammation and epithelial damage (Miri *et al.*, 2017).

EAEC is associated with persistent and acute diarrhea, especially in children and immunocompromised hosts. Its hallmark is an aggregative ("stacked brick") adherence pattern mediated by aggregative adherence fimbriae (AAF) under control of the

transcriptional regulator *AggR*, carried on the *pAA* plasmid. EAEC also produces a suite of toxins (e.g. EAST1, Pet, Pic) and hemolysins, and can form biofilms on mucosal surfaces, resisting clearance. It induces mucosal inflammation, disrupts tight junctions, and stimulates mucin secretion. Genomic heterogeneity and plasmid variability complicate its virulence repertoire (Meza-Segura *et al.*, 2020).

DAEC is defined by a diffuse adherence pattern on epithelial cells (HEp-2/HeLa). Many DAEC strains express Afa/Dr adhesins, which bind receptors such as decay-accelerating factor (DAF), and may trigger receptor-mediated signaling and cytoskeletal changes. Some express virulence effectors like the Sat autotransporter toxin, and in some cases the *pks* genomic island producing colibactin. DAEC strains may modulate cytokine responses (e.g. suppressing IL-8 secretion) to subvert host defenses. However, their exact pathogenic mechanisms remain less clearly understood and are likely heterogeneous across strains (Servin, 2014).

In Kenya, retail surveys consistently report substantial *E. coli* contamination of raw meats, with studies around Nairobi's high-density vending areas showing frequent isolation from raw red meat, highlighting cross-contamination risks along informal value chains (Koech *et al.*, 2024). Kenya-wide retail sampling detected *E. coli* in poultry underscoring the burden of enteric bacteria in raw meat sold in towns and cities (Muinde *et al.*, 2023). Although a lot of research in Kenya emphasise hygiene indicators and antimicrobial resistance, molecular data increasingly document DEC reservoirs in food animals and household environments: a Nairobi/Kiambu case-control study detected EPEC and EAEC among children and food-animal interfaces, underscoring spill over potential into meat value chains (Yeda *et al.*, 2024). Together, these Kenyan findings point to frequent *E. coli* contamination of raw beef and chicken, with emerging evidence for DEC gene carriage in the broader meat ecosystem and a need to expand routine pathotype genotyping at butchereries levels (Koech *et al.*, 2024; Muinde *et al.*, 2023; Yeda *et al.*, 2024).

When it comes to RTE meat local research consistently detect *E. coli*, indicating post-process contamination risks. In a Nairobi slum survey of cooked chicken portions, Birgen *et al.*, (2020) reported frequent *E. coli* recovery and unsatisfactory hygiene indicators, underscoring the potential for DEC survival or re-contamination after

cooking in informal settings. More broadly, Kenyan street-food studies highlight food-handler and vending-site hygiene gaps that plausibly enable DEC re-entry into cooked meats (Ronald *et al.*, 2023).

In other African countries pathotype-resolved studies are more common. In Namibia, a 2020 abattoir-to-retail survey found STEC (stx1/stx2) in raw beef products, with isolates harbouring virulence genes typical of EHEC lineages (Nehoya *et al.*, 2020). South African farm-to-retail investigations identified both STEC and non-STEC O157 in beef and poultry chains, characterising *stx/ea*e profiles and additional virulence determinants relevant to EHEC (Madoroba *et al.*, 2022). Complementary retail studies in South Africa detected STEC in raw beef and ready-to-eat meats, highlighting persistence beyond slaughter (Onyeka *et al.*, 2020). Collectively, these African data show that STEC/EHEC are the most frequently typed DEC in meat, while reports of ETEC, EPEC, and EAEC are emerging but remain under-quantified in many surveys that still prioritise indicator *E. coli* or O157 alone.

Other reports document several PCR-resolved studies to profile pathotypes directly in RTE meats. In Lagos, Nigeria, Fayemi *et al.* (2021) found shiga-toxigenic *E. coli* (STEC) serogroups in both fresh beef and locally processed RTE meat products, with virulence genes (stx1/stx2) and notable antimicrobial resistance profiles, evidencing a tangible EHEC/STEC risk in cooked, ready-to-eat beef sold in urban markets (Fayemi *et al.*, 2021). South African retail monitoring also documents STEC occurrence at the point of sale, including RTE beef items, aligning with the inference that cross-contamination along slicing/serving equipment is a key driver of positivity in cooked products (Onyeka *et al.*, 2020). In Egypt, qPCR surveys of RTE meat products have repeatedly identified STEC in post-process foods such as luncheon meats and cooked sausages, with stx genes detected in finished product (Mokhtar & Karmi, 2021).

Evidence from high-income settings generally shows low (but non-zero) detection of STEC in RTE foods, consistent with effective lethality and HACCP controls, with residual risk due to post-cook contamination. The EU One Health Zoonoses surveillance for 2022 reported 1.1% positivity for STEC across RTE foods tested, including 1.0% in meat and meat products, versus higher positivity in non-RTE items.

This could highlight cross-contamination as the plausible route for RTE meats (EFSA & ECDC, 2023).

## **2.3 Antibiotics, Antimicrobial Resistance Mechanisms and Patterns of *Escherichia coli* in Animal and Human Health**

### **2.3.1 Antibiotics in Animal Husbandry and Human Population**

Antibiotics are used as agents of disease treatment and prevention whenever they occur. Microorganisms naturally adapt to their environment to enhance longevity and ability to thrive in the constant changing environment. At the same time there are activities by value chain actors predisposing meat to contamination with pathogenic drug resistant microorganisms. These include; poor hygiene, self-medication (Neuhaus *et al.*, 2001), inappropriate drugs prescription (Martínez *et al.*, 2015; Holmes *et al.*, 2016), overuse of antibiotics, inaccurate diagnosis, extensive agricultural application of antibiotics and presence of few antibiotic alternatives (Munita & Arias, 2016; MacGowan & Macnaughton, 2017).

Antibiotics can be classified based on their mode of action and chemical structure. Their action against microorganisms occur through different mechanisms, these include; interference with cell wall synthesis (cephalosporins, penicillin, monobactams, carbapenems, bacitracin, vancomycin, cycloserine) and cytoplasmic membrane structure (polymyxins, daptomycin). Prevention of folic acid metabolism (trimethoprim, sulphonamides), interference with protein synthesis like tRNA (puromycin, mupirocin), 30s inhibitor (tetracycline, streptomycin, gentamicin, kanamycin, amikacin, nitrofurans, spectinomycin), 50s inhibitor (erythromycin, chloramphenicol, clindamycin, lincomycin). Their action against bacteria is mainly through five mechanisms. Interference with cell wall synthesis (Levin & Angert, 2015), inhibition of protein synthesis (Krause *et al.*, 2016), inhibition of nucleic acid synthesis (Cole *et al.*, 2019), inhibition of metabolic pathways (Fernández-Villa *et al.*, 2019) and cell membrane function inhibition (Trimble *et al.*, 2016; Lerminiaux & Cameron, 2019).

Food animal husbandry uses a lot of antibiotics. According to European Surveillance of Veterinary Antimicrobial Consumption participating Countries (ESVAC), of 1.5 tonnes of active microbials, 98% of these are used in animal production with and over

84% used as feed additives (ESVAC, 2019). Poultry production has been reported to be the largest user in antibiotics followed by pigs and cattle. In Kenya the most dispensed antibiotics for animal production have been reported to be tetracyclines, beta-lactams, sulphonamides and macrolides while human health the most dispensed antibiotics were reported to be beta-lactams, fluoroquinolones and metronidazole (Muloi *et al.*, 2016; Kemp *et al.*, 2019). Majority of antibiotics used in animal production are also used in human medicine including; beta-lactams, macrolides, aminoglycosides, tetracyclines, sulphonamides, quinolones, lincosamides, peptides, oxazolidinones, pleuromutilins, amphenicol and di-aminopyrimidines. Antibiotics such as isoniazid is exclusively used in human medicine (treatment of tuberculosis) while flavophospholipol and ionophores are exclusively used in animals due to their toxicity in humans. This overlap of antibiotic use in human medicine and veterinary makes it such that if a microorganism was resistant to one antibiotic it would be a risk to both populations (WHO, 2020).

The use of antibiotics as part of animal feeds is rooted in the ability of the antibiotics to enhance growth through improved feed conversion, better digestion, accelerated food absorption in the ileum and disease prevention (Mehdi *et al.*, 2018; Lee *et al.*, 2019). Broad spectrum antibiotics have recorded to have higher sales, these include Tetracyclines and streptomycin (Muloi *et al.*, 2016, Kemp *et al.*, 2019). *E. coli* is mainly treated by cephalosporins (mainly beta lactams) and fluoroquinolones (especially ciprofloxacin). Broad spectrum antibiotics are also used in treatment of *E. coli* associated infections such as tetracyclines, aminoglycosides and sulphonamides and polymixins.

### **2.2.2 Antibiotic Resistance Mechanisms**

Antimicrobial resistance is one of the most pressing global health challenges, threatening the effective treatment of bacterial, viral, parasitic, and fungal infections. Antibiotic resistance, which refers specifically to resistance against antibacterial drugs, can be expressed naturally or acquired when microorganisms are exposed to antimicrobial agents. Microorganisms develop resistance either through intrinsic genetic traits or by acquiring external genetic material. Horizontal gene transfer, which occurs via transformation, conjugation, or transposition, plays a crucial role in the spread of resistance genes between microorganisms (Watkins & Bonomo, 2016). In

addition, spontaneous mutations in chromosomal DNA can alter bacterial physiology and lead to antimicrobial resistance.

There are four widely recognized mechanisms of antibiotic resistance (Figure 1): limitation of drug uptake, modification of the drug target, inactivation of the drug, and active drug efflux (Mahtab *et al.*, 2021). Gram-positive and Gram-negative bacteria differ significantly in their ability to employ these mechanisms. Gram-positive bacteria, lacking the outer polysaccharide layer, are generally limited in their ability to block drug uptake or perform drug efflux. Conversely, Gram-negative bacteria possess an additional protective outer membrane and thus can utilize all four mechanisms, making them particularly difficult to control and eradicate (Levin & Angert, 2015; David, 2017).

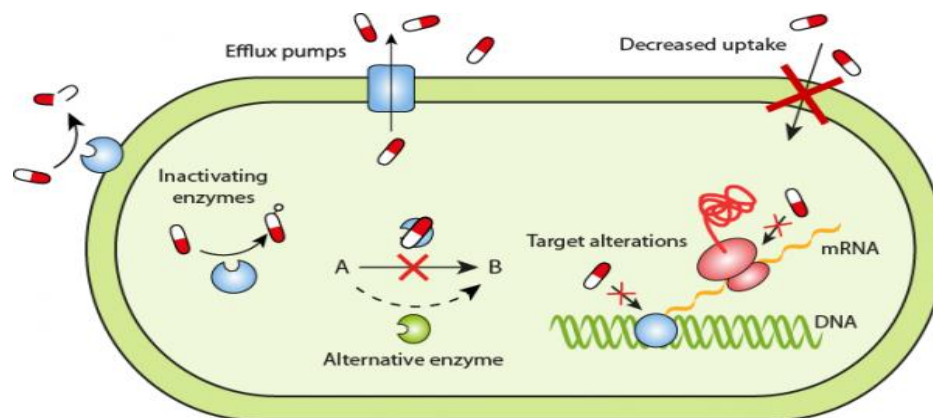


Figure 1: Mechanism of Antibacterial Resistance (Source: Levin & Angert, 2015)

Resistant bacterial strains, or genes encoding resistance, may also be transferred to humans through different routes. Transmission from hospital environment has received more attention compared to other routes such as animal to human and environmental such as water and soil (Kemp, 2021). Food value chains have been the least explored when it comes to tracing antimicrobial resistance transfer routes from minimal empirical data (WHO, 2022). Resistance genes can be transmitted to the normal flora of the consumer without causing an infection and spread to pathogenic microbes. The resistant bacteria can potentially cause infections later on and spread to other people (David, 2017).

The transfer of resistant bacterial strains and genes to humans occurs through multiple routes, including clinical, community, agricultural, and environmental pathways. The hospital environment has been the most studied and remains a major hotspot for the emergence and spread of multidrug-resistant (MDR) pathogens due to high antibiotic use and frequent patient contact (Kemp, 2021). However, other important routes, such as animal-to-human transmission through direct contact, food consumption, and environmental exposure through contaminated water and soil, are increasingly being recognized. Despite this, food value chains remain underexplored in empirical research, even though they may represent significant reservoirs and transmission pathways for AMR.

When resistant bacteria enter the human body through food or environmental exposure, they may not immediately cause disease. Instead, resistance genes can be integrated into the commensal microbiota, which serves as a reservoir for future dissemination to pathogenic bacteria (David, 2017). Such silent carriage represents a major public health risk, as resistance traits may persist undetected until triggered by infection or antibiotic treatment. Once resistance is established in the microbiota, it can spread between individuals and communities, amplifying the challenge of infection control.

Beyond the clinical setting, agriculture and food production systems play an important role in the evolution and dissemination of antibiotic resistance. The use of antibiotics as growth promoters, prophylactics, and therapeutics in livestock has contributed to the selection of resistant bacteria within animal microbiota (Marshall & Levy, 2011). Resistant organisms can contaminate meat during slaughter, processing, and handling, and subsequently be transferred to humans via food consumption. Even when foodborne bacteria are nonpathogenic, their resistance genes can be horizontally transferred to human pathogens, perpetuating the AMR cycle.

The World Health Organization (2022) emphasizes that food chains are one of the least studied yet most significant drivers of AMR dissemination, particularly in LMICs where food safety infrastructure and surveillance systems are often weak. In such contexts, consumers may be exposed to resistant bacteria through undercooked meat, raw vegetables irrigated with contaminated water, or unpasteurized dairy products. The asymptomatic carriage of resistant bacteria increases the potential for community-level

transmission, particularly in densely populated urban areas with limited sanitation facilities.

In addition to microbial and environmental dynamics, socioeconomic and behavioral factors play an important role in the spread of AMR. Inappropriate prescribing practices, self-medication, and poor adherence to treatment regimens contribute significantly to resistance development in humans (O'Neill, 2016). In LMICs, antibiotics are often available over the counter without prescription, exacerbating misuse. Similarly, limited veterinary regulation facilitates the misuse of antibiotics in animal husbandry. These challenges underscore the importance of coordinated global, regional, and local interventions.

Several strategies have been proposed to curb the spread of AMR. First, prudent use of antibiotics in both human and veterinary medicine is paramount. Antimicrobial stewardship programs have been shown to reduce inappropriate prescribing and improve treatment outcomes (Baur *et al.*, 2017). Second, improving hygiene, sanitation, and infection prevention measures in hospitals, communities, and food systems is critical to reduce bacterial transmission. Third, surveillance systems are essential to monitor resistance patterns and detect emerging threats, particularly in under-researched sectors such as food chains and environmental reservoirs. Finally, investment in research and development of new antibiotics, vaccines, and alternative therapies remains crucial to stay ahead of evolving resistance.

### **2.3.2 Antibiotic Resistance Patterns of *E. coli* to Different Antibiotics**

The antimicrobial resistance profile of *E. coli* against several antibiotics is different between countries, locations and sample type as reported by some systematic reviews (Luria, 2018; Moctar *et al.*, 2020; Paudyal *et al.*, 2017). Resistance against high priority clinically important (HPCI) antimicrobials has been reported in several studies around the globe (WHO, 2023). In Japan Wang *et al.*, (2017) demonstrated that *E. coli* isolated from raw meat was resistant to tetracyclines (49%), nalidixic acid (28%), ampicillin (24%) trimethoprim and sulfamethoxazole (20%) and cephalexin (18%) (Wang *et al.*, 2017b). Another study in China focusing on *Shiga* toxin producing *E. coli* (STEC) and enteropathogenic *E. coli* (ETEC) reported these pathotypes to be resistant to tetracycline (8%), chloramphenicol (8%), ampicillin (4%), ceftazidime (4%) and

trimethoprim-sulfamethoxazole (4%) (Shen *et al.*, 2022). These variations across countries can be explained by differences in antibiotic regulation policies, patterns of antibiotic usage in livestock production, laboratory methodologies used for testing, and levels of surveillance. Countries with stricter antimicrobial stewardship programs often report lower resistance prevalence, while nations with unregulated veterinary antibiotic use continue to see high resistance levels in foodborne pathogens. Furthermore, cultural dietary habits and preferences may influence which meat types are more commonly consumed, thereby shaping the scope of exposure to resistant strains (Founou *et al.*, 2018).

Antibacterial resistance of *E. coli* has been reported to be to be highest in food animals with cattle taking the lead from all studies reviewed (Founou *et al.*, 2018). From the analysis by Founou and others, majority of studies in Africa on AMR were done at farm level on live animals while market samples constituted 5% of all available publications at the time (Founou *et al.*, 2018). According to Moctar *et al.*, (2020), *E. coli* ABR in animal products has an average MDR rate of 47% against HPCIA. Meta-analysis for African countries reported that of all research on ABR and AMR the highest prevalence was reported in meat and meat products (Paudyal *et al.*, 2017, Founou *et al.*, 2018, Moctar *et al.*, 2020). These findings suggest that meat and its associated production systems act as critical reservoirs of resistance genes that can spill over to human populations. The high prevalence in cattle may be linked to their intensive rearing practices, frequent exposure to antimicrobials, and the role of ruminant gut flora as an environment where resistance genes thrive. The underrepresentation of market and retail-level studies in Africa highlights a research gap, since these are the points at which consumers are directly exposed. This gap underscores the urgent need for surveillance efforts in slaughterhouses, butcheries, and informal markets where hygiene challenges may exacerbate the problem.

Antimicrobial resistant *E. coli* in Kenyan raw meat has been reported majorly in Nairobi and its environs (Odwar *et al.*, 2014, Nkiiri, 2017; Kamau *et al.*, 2021). In 2016 Nkiiri isolated *E. coli* (Shingatoxigenic) from animal carcass in slaughter houses with minimal market samples. (Nkiiri, 2017). In 2021 a research around Kenyatta University was done to identify pathogenic microorganisms and it was reported that resistance against; ampicillin, tetracycline and amikacin was highest in all *E. coli* isolates (Kamau, 2021).

In Poland *E. coli* isolated from RTE meat was resistant to Amoxicillin-clavulanate (28%), Ampicillin (20%) and Extended-beta lactams (21%) (Zarzecka *et al.*, 2021). In Japan RTE beef and poultry meat products recorded presence of *E. coli* resistant to tetracyclines, nalidixic acid, ampicillin, trimethoprim, sulfamethoxazole and cephalothin (Wang *et al.*, 2017). Ready to eat food in Italy recorded MDR of 50% without a distinction of the source of food, whether food animal or plant (Vincenti *et al.*, 2018). These findings illustrate that resistant *E. coli* are not only confined to raw meat but persist even in processed or ready-to-eat products, raising public health concerns since RTE foods bypass further cooking that could kill resistant bacteria. In addition, regional differences observed between Kenya, Poland, Japan, and Italy reveal that antimicrobial resistance in foodborne *E. coli* is truly a global phenomenon, demanding harmonized surveillance frameworks to enable comparisons and policy interventions.

In Africa, some countries have determined the resistance patterns of *E. coli* from RTE meats (Abass *et al.*, 2020; Adzitey *et al.*, 2021). *E. coli* isolated from RTE foods in Ghanaian towns showed resistance against tetracycline (94%), amoxicillin (71%), azithromycin (71%), and sulfamethoxazole (58%) (Adzitey *et al.*, 2021). Furthermore, *E. coli* isolated from RTE poultry and beef exhibited resistance against amoxicillin, trimethoprim, ciprofloxacin, azithromycin, chloramphenicol, and imipenem. In Kenya, however, studies on AMR in RTE meat are minimal. One study in Nakuru identified resistant *E. coli* in RTE meat but did not specify the animal source, which is critical in AMR testing since different livestock species are reared under varying systems of husbandry, including the use of antibiotics (Odwar *et al.*, 2014). The lack of species-specific data poses challenges in linking resistance patterns to particular production systems, making it harder to design targeted interventions. More so, informal street food markets—which are common across African cities—remain poorly studied despite being important sources of affordable meals for urban populations. Given that cooking practices and storage conditions in such informal markets may not meet food safety standards, consumers are at an increased risk of exposure to resistant bacteria. Addressing these knowledge gaps will be central to building a comprehensive understanding of AMR in Africa's food systems and mitigating its associated risks.

## **2.4 Resistance and Virulence Genes of Pathogenic *Escherichia coli***

### **2.4.1 Resistance Genes of *Escherichia coli***

Resistance to a wide range of antimicrobial classes in human and veterinary medicine has been reported to majority of our antimicrobials (Moyo *et al.*, 2023). A plethora of genes which encode for resistance against antimicrobials have been identified in *E. coli*; due to its ubiquitous presence in the human microbiome and exposure to antimicrobials consumed by human beings and animals.

#### **2.4.1.1 Resistance Against Beta-lactams**

Resistance against beta-lactams is conferred by beta-lactamase enzymes by hydrolyzing the  $\beta$ -lactam central ring – inactivating the molecule. By the end of 2009, approximately 890 unique  $\beta$ -lactamase protein sequences had been identified (Bush & Jacoby, 2010). There are currently two  $\beta$ -lactamase classification models, and these are broadly based on either the primary structure or the functional properties of the enzyme. Molecular classifications split them into classes A, B, C and D, based on the amino acid motifs (Ambler, 1980) where each class except for B hydrolyse the  $\beta$ -lactam ring via an active site serine. Class B  $\beta$ -lactamases are metalloenzymes e.g. New Delhi metallo- $\beta$ -lactamase 1 (NDM-1), and they utilise at least one active site zinc in ring hydrolysis.

The other classification model introduces functional grouping: group 1 (class C) cephalosporinases; group 2 (classes A and D) broad-and extended-spectrum  $\beta$ -lactamases and serine carbapenemases; and group 3 metallo- $\beta$ -lactamases (Bush *et al.*, 1995). The most commonly occurring plasmid-mediated  $\beta$ -lactamase types are *TEM*, which confer resistance to ampicillin (Datta & Kontomichalou, 1965); *SHV* (which can also be found on the chromosome) confers resistance to broad-spectrum penicillins (Livermore, 1995); *OXA* (almost always plasmid-mediated) which confer resistance to penicillins (some newer *OXA* can also confer resistance to cephalosporins and carbapenems (Evans & Amyes, 2014); and *CTX-M*, which preferentially hydrolyses cefotaxime (Gazouli *et al.*, 1998). These three enzymes make up much of the  $\beta$ -lactam resistance encountered in *E. coli*. These  $\beta$ -lactamases are encoded for by *bla* genes which can be found on chromosomal cassettes and plasmids (Matthew, 1979). As of 2018, there were 172 defined *CTX-M* enzymes, 223 *TEM* and 193 *SHV* types, *CTX-M-14* and *CTX-M-15* are the most dominant and clinically relevant enzymes and they have been associated with both hospital- and community-acquired infections, as well as

increasingly in animal and environmental sources (Hawkey & Jones, 2009; Bevan *et al.*, 2017).

#### **2.4.1.2 Resistance Against Tetracyclines**

There are more than fifty tetracycline resistance genes have been described in the literature and these confer resistance in three main ways: efflux (*tetM*), ribosomal protection (*tetA*) and less commonly, enzymatic inactivation (*tetX*). The primary mechanism of resistance is via increased efflux of the tetracycline molecule from the cell, which is mediated by a family of Tet proteins which are found on the cytoplasmic surface of the cell membrane; twelve transmembrane (Yamaguchi *et al.*, 1990). There are seven different groups of efflux pump, which are sorted according to their amino acid sequences; all except one tetracycline efflux pump belong to group 1, and they share almost 75% protein homology (Thaker *et al.*, 2010). Group 1 efflux proteins can be found on transposons within large plasmids and are associated with other antimicrobial and heavy metal resistances (Chopra & Roberts, 2001). Another resistance mechanism involves ribosomal protection proteins - cytoplasmic proteins Tet(O) and Tet(M) – which bind to the ribosome, blocking binding of tetracycline molecules, as well as unbinding bound tetracycline from the ribosome (Connell *et al.*, 2003). A final mechanism of tetracycline-resistance is antimicrobial inactivation. *Tet(X)* is a flavoprotein monooxygenase which hydroxylates tetracycline molecules, causing it to lose affinity for bacterial ribosomes, leading to non-enzymatic decomposition of molecules (Volkers *et al.*, 2011).

#### **2.4.1.3 Resistance Against Quinolones**

Plasmid mediated-quinolone resistance (PMQR) was first described in 1998 as conferring low-level, transferable resistance to quinolones and fluoroquinolones (Martinez-Martinez *et al.*, 1998). The *qnr* (quinolone resistance) gene and subsequent QNR proteins inhibit quinolone binding to DNA gyrase- and DNA topoisomerase-complexes (Tran & Jacoby, 2002). Of relevance to *E. coli*, plasmids carrying *qnrA* (and other *qnr* genes), *qnrB* and *qnrS* have been identified globally and have been associated with ESBL genes (on the same integron) (Poirel *et al.*, 2006). These genes have been shown to only confer low-level resistance, but they may also increase the mutation prevention concentration (Gebbru *et al.*, 2011; Hooper & Jacoby, 2015). Another plasmid-mediated mode of resistance is a type of enzyme inactivation, mediated by the

aminoglycoside acetyltransferase, *aac(6')-Ib-cr*. This can modify ciprofloxacin and reduces its activity by acetylation of the piperazinyl ring (Robicsek *et al.*, 2006). This confers low-level resistance to some fluoroquinolones, but not to certain quinolones, which lack an amino nitrogen on the piperazinyl ring, such as nalidixic acid. This AAC works synergistically with *qnrA* but has also been shown to confer low-level resistance in its absence too (Robicsek *et al.*, 2006). Plasmid-mediated quinolone and aminoglycoside resistance genes are often reported to be co-associated with genes encoding for  $\beta$ -lactamases, most predominantly *bla*CTX-M-15. These have been associated with the pandemic *E. coli* ST131 clone (Karisik *et al.*, 2006; Jiang *et al.*, 2008). Finally, a PM efflux pump, *qepA*, has been identified on plasmid pHPA in *E. coli*; it preferentially targets ciprofloxacin and norfloxacin, but not hydrophobic quinolones such as nalidixic acid (Yamane *et al.*, 2007). Other efflux pumps have been described in *E. coli*; *AcrAB-TolC* and *OqxAB* (Li *et al.*, 2000; Hansen *et al.*, 2007) are multi-drug efflux pumps which efflux chloramphenicol and trimethoprim molecules, as well as quinolones and tetracyclines (Hansen *et al.*, 2007).

#### **2.4.1.4 Resistance Against Trimethoprim**

Resistance mechanisms against trimethoprim include provision of alternative metabolic pathways, changes in the DHFR enzymes and active efflux (Flensburg & Skold, 1987; Eliopoulos & Huovinen, 2001). There are a number of DHFR enzymes, which are categorised according to their amino acid sequences, and these are encoded for by *dfr* genes carried on plasmids and transposons. The most prevalent trimethoprim resistance mechanism in *E. coli* is the acquisition of trimethoprim insensitive DHFR variant resulting in high-level trimethoprim resistance (de Crecy-Lagard, 2014).

#### **2.4.1.5 Resistance Against Sulphonamides**

In *E. coli*, sulphonamide resistance is chromosomal or plasmid-mediated. The primary mechanism for sulphonamide resistance is mutations in the dihydropteroate synthase (DHPS) gene, *folP*. As sulphonamides are analogues of PABA, substitutions of Phe (28) on *folP* reduces the affinity of sulfa-drugs to bind to the active site of DHPS which prevents the production of dihydropteroic acid. However, it also reduces the affinity of PABA itself, albeit to a lesser degree (Sköld, 2000). Plasmid-mediated resistance to sulphonamides is via dissemination of variant genes which encode for AMR DHPS enzymes (Swedberg & Skold, 1983). Originally only two genes, *sul1* and *sul2*, were

thought to confer resistance to sulphonamides – they were found in equal proportions amongst sulphonamide-resistant *E. coli* and other bacterial species due to their ability to transfer horizontally across class 1 integrons (Radstrom *et al.*, 1991). Despite *sul1* being the dominant resistance gene, more recently prevalence of *sul2* has increased amongst clinically-relevant isolates of *E. coli* in Denmark and the UK (Kern *et al.*, 2002). Both *sul1* and *sul2* share 57% homology and their origins are yet unknown (Radstrom & Swedberg, 1988). In 2003, a third gene encoding sulphonamide-resistant DHPS was discovered in *E. coli* amongst pathogenic strains amongst pigs in Switzerland, *sul3* (Perreten & Boerlin, 2003). As recently as 2017, a fourth mobile sulphonamide gene, *sul4*, and is described as having potential impact on both humans and animals (Razavi *et al.*, 2017).

#### **2.4.2 Virulence Genes Associated with Most Common *E. coli* Pathotypes**

Pathotypes are identified according to the specific combination of virulence factors they carry. There are numerous different virulence determinants attributed to pathogenic *E. coli*, including adhesins, invasins, motility/chemotaxis assistance, toxins, antiphagocytic surface properties, defences against bactericidal or immune responses and genetic attributes. Adhesins are a group of proteins which are involved in the attachment of bacteria to non-biological (plastic and steel) and biological (intestinal wall) surfaces (Klemm & Schembri, 2000). Usually these proteins form distinct structures, called fimbriae which allow *E. coli* to attach to mannose receptors, resist shear force and obtain nutrients. Invasins allow invasive pathotypes of *E. coli* to invade and multiply within cells e.g. EIEC replication inside epithelial cells.

Various toxins are produced by *E. coli* – these have effects on several eukaryotic processes. Common toxins include heat labile enterotoxin (LT), heat-stable enterotoxin a (STa) and heat-stable enterotoxin b (STb) (Sears & Kaper, 1996), Shiga toxin (Stx) (Kaper & O'Brien, 1998) and cytolethal distending toxin (CDT) (De Rycke & Oswald, 2001). Siderophores are high-affinity iron-chelating compounds which allow bacteria to acquire iron from their immediate environment (even where iron concentrations are low) for various microbial systems. Enterobactin is an example of a siderophore found in Gram-Negative bacteria such as *E. coli* (Harris *et al.*, 1979). Some pathogenic *E. coli* have K antigens, which are capsular polysaccharides that decrease binding of antibodies to the bacteria, and reduce the ability of phagocytes to recognize and engulf the bacterial

cells (Van Dijk *et al.*, 1979). Combinations of these virulence factors pertain to a specific pathotypes. Some virulence genes for particular pathotypes are in the Table 1.

Table 1: Combination of virulence genes in pathogenic *Escherichia. coli*

Pathotype	Symptoms of Infection	Virulence Gene
ETEC	Rapid onset of watery diarrhoea	Adhesins <i>cfaB, cooA, cs3, cssA, etpA, etpB</i> Toxins <i>eltA-B, estIa, StII</i>
DAEC	Urinary Tract infection, pregnancy complications, Diarrhoea in children <5 years old.	Adhesins <i>(Afa/Dr)</i> <i>afaE-I, afaE-III, daaE, draE, draE2</i>
EIEC	Diarrhoea /bacillary dysentery Haemolytic Uremic Syndrome	Actin-based Motility ( <i>icsA/virG</i> ), Endotoxin ( <i>gtrA-B, gtrII</i> ), Iron uptake ( <i>iucaA-D, iutA</i> ), Protease ( <i>Pic</i> ), Toxin ( <i>setIA-B, senB, stxA-BX</i> ), Pathogenicity islands (SHI-1-3, SRL)
EAEC	Traveller's diarrhoea, Haemolytic Uremic Syndrome, Persistent diarrhoea	Adherence AAFs ( <i>aafA-D, agg3A-D, aggA-D, R</i> ) Dispersin ( <i>aap, aatA-D, P</i> ) Toxins ( <i>astA, pet, pic, setIA</i> )
EHEC	Watery diarrhoea, haemorrhagic colitis	Adherence ( <i>yagV, ecpE, yagW/ecpD, efa1, eae, paa, toxB</i> ), Ironuptake ( <i>chuA, chuS, chuU, chuW</i> ) Protease ( <i>espP, stcE</i> ) Regulation ( <i>Ler</i> ) Toxin ( <i>hlyA-D stx1A/B, stx2A/B</i> ) Pathogenicity island ( <i>LEE</i> )
EPEC	Diarrhoea in children <5 years old.	Adherence ( <i>bfpA-L, eaeA, lifA/efa1, paa</i> ) Protease ( <i>espC</i> ) Regulation ( <i>Ler</i> ) Toxin ( <i>cdtA-C, east1</i> ) Pathogenicity islands ( <i>EspC island, LEE</i> )

## 2.5 *Escherichia coli* Characterization through Antibacterial Sensitivity Testing and Genotyping

### 2.5.1 Antimicrobial Sensitivity Testing

Antimicrobial susceptibility testing (AST) methods are used to confirm susceptibility to chosen empirical antimicrobial agents, or to detect resistance in individual bacterial isolates (Tenover, 2017). Further, ASTs can help to identify isolates with defined resistance mechanisms of major interest to public health (for example, ESBL

producers) (Ezadi *et al.*, 2019). Several different AST methods are available for use by clinical microbiology laboratories: broth dilution tests, antimicrobial gradient method, and disk diffusion test. Of the three methods, disk diffusion test is the widely used in the vast majority of clinical laboratories in LMICs, because it is simple and standardized (Wanger & Chávez, 2021). In this method, the zones of growth inhibition around each of the antimicrobial disks (dispensed onto bacteria-containing agar plates) are measured to the nearest millimetre. The diameter of the zone is related to the susceptibility of an isolate and those values translated to categories of susceptible, intermediate, or resistant using the latest tables published by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2020) or European Committee on Antimicrobial Susceptibility Testing (EUCAST).

### **2.5.2 Genotyping**

Genotypic methods are increasingly being employed to identify specific antimicrobial resistance (AMR) genes or genetic mutations using molecular or genomic approaches, most of which are DNA-based and involve amplification or sequencing. Techniques such as polymerase chain reaction (PCR) and microarrays have been widely applied to generate antimicrobial susceptibility profiles in bacterial pathogens (Kockum, *et al.*, 2023). Polymerase Chain Reaction (PCR) as a technique amplifies a specific DNA sequence exponentially through repeated thermal cycling—denaturation, annealing, and extension—using a thermostable DNA polymerase (e.g., Taq) and sequence-specific primers (Eggertsson *et al.*, 2017). Variants like real-time PCR allow quantification, while reverse transcription PCR (RT-PCR) enables amplification from RNA templates. However, PCR's accuracy depends heavily on primer design, thermal cycling conditions, and avoiding contamination (Wanger & Chávez, 2021).

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3.1 Study Area**

The study was carried out in selected urban centres in Meru, Embu and Tharaka-Nithi counties (Figure 2). These counties are characterised with rapidly urbanising towns with a high demand for animal products to support the growing population. Meru is on the foot of Mt. Kenya. Meru is found between latitudes 37° West and 38° East and between longitudes 0°6' North and 0°1' South. The county has an estimated total population of 1,535,635 (KNBS, 2019). Tharaka-Nithi County is also located in the former Eastern province and divided into five administrative counties. It lies between latitude 00° 07' and 00° 26' South and between longitudes 37° 19' and 37° 46' East (County Government of Tharaka-Nithi, 2018). Based on the Kenya National Bureau of Statistics report, the population is estimated at 393,177 (KNBS, 2019). Embu County is located approximately between latitude 00° 8' and 00°50' South and longitude 37° 3' and 37° 9' East. It has a population of 608,599 persons. Livestock production is one of the economic activities carried out in this region (KNBS, 2019), with food animals ranging from chicken, cows, goats and pigs. Since majority are small scale farmers, there is a close proximity between human and food animals posing a corridor for microbial resistance transfer between the two populations through their environment. This could later be felt as a consequence on disease outbreak in the human food value chain.

#### **3.2 Research Design**

A cross-sectional survey design was carried out to identify the effect of knowledge of retailers in the meat value chain, their attitudes and practices, which could act as risk factors to contamination of raw and RTE meat with *E. coli*. For microbiological analysis, a nested design was adopted, with towns nested within counties, and butchereries and RTE establishments sampled within these towns. This approach accounted for potential clustering effects at both the county and town levels, allowing for a more precise assessment of contamination patterns. For microbiological analysis, a nested design was adopted where the butchereries and RTE establishment were nested within towns.

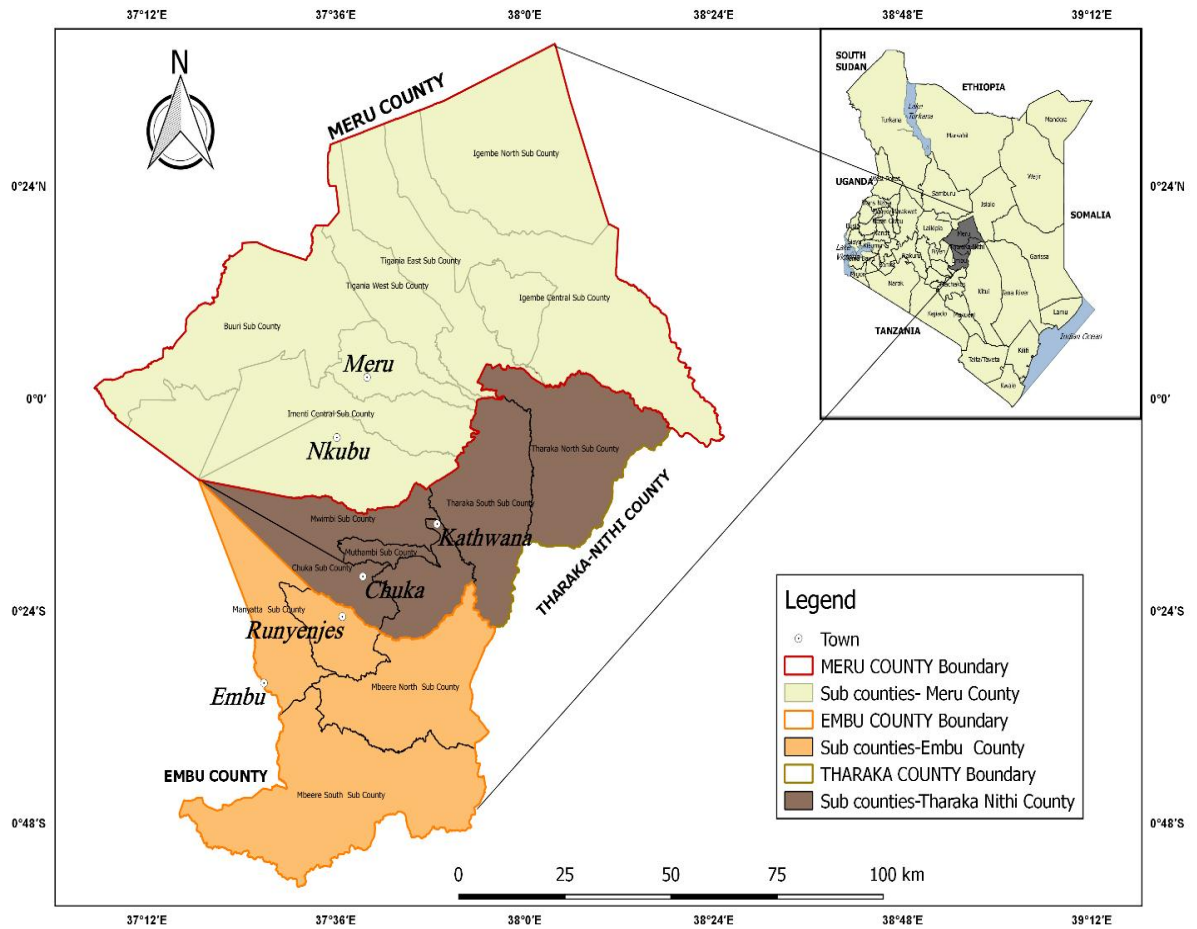


Figure 2: Map of Meru Embu and Tharaka Nithi Counties (Source: GIS Smart draw, LLC 2023)

### 3.3 Target population, Sample size and Sampling Procedure

#### 3.3.1 Target Population

The study purposively targeted major urban centres within Meru, Embu, and Tharaka-Nithi counties, specifically Meru town, Embu town, and Chuka town, due to their high concentration of raw and ready-to-eat (RTE) meat outlets. These towns were selected because their central business districts (CBDs) experience high human traffic and host the largest number of establishments dealing in meat products, making them strategic locations for assessing potential contamination risks.

Two distinct populations were considered in the study. The first comprised 151 registered butcheries across the three counties, which specialized in the sale of raw beef and chicken. The second population consisted of 148 hotels and restaurants that prepared and sold ready-to-eat (RTE) beef and chicken products. By targeting both raw meat retailers and RTE outlets, the study was able to capture the entire value chain of

beef and chicken meat, ranging from primary retail points to final consumer access points.

### 3.3.2 Sample Size Determination

The sample size determination was based on Krejcie and Morgan's (1970) for both raw and RTE populations.

$$n = \frac{X^2 \times N \times P(1 - P)}{(d^2 \times (N - 1)) + (X^2 \times P \times (1 - P))}$$

where; n = desired sample size

$X^2$  = Chi square value of 3.841 for 95% CI

N = Population size = 151

d = degrees of accuracy set at 0.05 for 95% CI

P = Population proportion (0.5)

The sample size for butcheries was determined as;

$$n = \frac{3.841 \times 151 \times 0.5(0.5)}{[(0.05^2 \times (150 - 1)) + (3.841 \times 0.5 \times (0.5))]}$$

$$n = \frac{(134.435)}{(0.3725) + 0.96025} = 109$$

$$n = 109$$

Thus, a sample size of 109 of butcheries was used (Table 2).

The sample size for hotels and restaurants was determined as,

$$\frac{3.841 \times 148 \times 0.5(0.5)}{[(0.05^2 \times (148 - 1)) + (3.841 \times 0.5 \times (0.5))]}$$

$$n = \frac{(142.117)}{(0.3675) + 0.96025} = 107$$

$$n = 107$$

n = Thus, a sample size of 107 of hotels and restaurants was used.

Cluster random sampling was used with towns as cluster in this study (Table 2).

Table 2: Sampling Frame for Raw Meat Retailers at Butcheries in Embu, Meru and Chuka Towns

County	Town	Population in the CBD	Sample size (n)
<b>Registered butcheries</b>			
Tharaka - Nithi	Chuka	36	26
Meru	Meru	71	51
Embu	Embu	44	32
Total		151	109
<b>Registered RTE meat retailers</b>			
Tharaka-Nithi	Chuka	47	34
Meru	Meru	51	37
Embu	Embu	50	36
Total		148	107

CBD (Central Business District), RTE (Ready-to-eat)

### 3.3.3 Sampling Procedure

Cluster random sampling was employed to select participants from both raw meat retailers (butcheries) and ready-to-eat (RTE) meat outlets (hotels and restaurants). For each of the three counties (Meru, Embu, and Tharaka-Nithi), the main urban centre (Meru town, Embu town, and Chuka town, respectively) was treated as a distinct cluster.

### 3.4 Research Tool

Semi-structured questionnaires were developed following Essential Food safety requirements of the (WHO, 2018) with some modifications to suit the context of the study (Appendix I and II). The questionnaire's validity and reliability were tested before being administered to the respondents.

#### 3.4.1 Pilot Study

Questionnaires were pretested and administered to raw meat handlers (butcheries) and RTE meat retailers in Kirinyaga County. Pre-testing the questionnaires was done as per Wilson (2010) who recommended 10% of the expected sample size to assess the clarity, relevance, and reliability of the questions. Feedback from the pre-test was used to refine the questionnaire's wording, structure, and sequencing before the main survey.

### **3.4.2 Validity and Reliability of the Research Tool**

#### **3.4.2.1 Validity of the Research Tool**

To determine the validity of the questionnaire, the university supervisors, peers, and experts in the area of Food science carefully checked the questionnaire to improve its content and construct validity.

#### **3.4.2.1 Reliability of the Research Tool**

Cronbach alpha coefficient was used to test questionnaire's reliability. In the pilot study, the alpha coefficient obtained was  $\alpha = 0.85$ . Therefore, the instrument was reliable for use in the study. Avakol & Dennick (2011) reported that Cronbach's alpha values of 0.7–0.8 are considered acceptable, 0.8–0.9 indicate good reliability, and values  $\geq 0.9$  are deemed excellent. The pilot study's Cronbach's alpha of 0.85 indicates a high level of internal consistency, reflecting good reliability.

### **3.5 Data Collection**

#### **3.5.1 Determination of Risk Factors Associated with Handling of Raw Beef and Chicken Meat in Butcheries within Embu, Meru and Tharaka Nithi Counties**

Data collection was done between May and September 2024. A semi-structured questionnaire was used to collect data based on the Essential Food Safety Requirement by WHO. The information collected were; bio data, food handling practices related to food safety and quality, personnel hygiene, finally, on water and sanitation quality, (Appendix 1). The questionnaires were administered using face-to-face approach to give the interviewer an opportunity to make observations. From each selected establishment, one personnel member was interviewed during the survey. In establishments where questionnaires (109) were administered, meat samples were also collected for microbiological analysis. Each establishment served as a single sampling unit, ensuring that both the interview and sample collection were conducted within the same location.

#### **3.5.2 Determination of Knowledge Attitude and Practices of Ready-to-eat Beef and Chicken Meat in Embu, Meru and Tharaka Nithi Counties**

A questionnaire with a set of questions was developed in the following categories to be administered to RTE (107) beef and chicken meat retailers; demographic characteristics, knowledge, attitude and practices related to handling and safety of RTE

foods in the establishment (Appendix 2). The questionnaire had four sections namely; demographic characteristics of the respondents, food safety knowledge questions (16 questions), food safety attitude questions (14) and food safety practices questions (13).

### **3.5.3 Determination of Coliforms, *Escherichia coli* and Antimicrobial Resistant *Escherichia coli***

Meat samples weighing about 100 g were collected from raw (109) and RTE (107) beef and chicken meat establishments. These were transferred to an airtight sterile zip-lock bags, well labelled and put in a cool-box at 4<sup>o</sup> and transported to Chuka University Food Technology laboratory for microbiological analysis. Administration of questionnaires and sample collection took between 15 to 30 minutes, the approximate time between sample collection and arrival at the lab was 4 hours. In the laboratory, the meat samples were blended using sterile peptone water in a ratio of 1:9 and blended using a Stomacher ® 400 circulator lab blender (United Kingdom) for 500 and 300 rpm. This formed 10<sup>-1</sup> dilution and was later serially diluted to 10<sup>-3</sup>.

Violet Red Bile Agar (VRBA) and Tryptone bile agar with glucuronide (TBX) OXOID were prepared separately. A 41.5g of VRBA was mixed with 1litre of distilled water in a flask. The mixture was then heated to boil in a microwave. To prepare 1 litre of TBX solution, 36.6 g of the TBX powder was suspended in 1 litre of distilled water, it was heated to dissolve, then autoclave at 121°C for 15 minutes for sterilization. After sterilization, it was cooled to 45-50°C in a water bath.

Another TBX media was prepared to contain cefotaxime at 0.25mg/L (CTX – Sigma-Aldrich Darmstadt, Germany) which is the epidemiological MIC for the antibiotic. One millilitre from each diluent was plated on 20ml of the three different media types in duplicate. Incubation was done at 35<sup>o</sup> C for 24 hours. From the VRBA media, pink to red colonies were counted as positive for coliforms. In the TBX media, blue-green colonies were recorded as positive *E. coli*. The same interpretation was used in TBX media containing antibiotic. From the TBX plates (without antibiotic) with positive *E. coli* growth, four colonies were randomly taken from each quarter of the plate and proceeded to DNA extraction and investigation for the presence of pathogenic *E. coli* genes. Plates with less than four colonies were all picked for, confirmation of *E. coli*, DNA extraction and investigation for presence of *E. coli* virulence and resistance genes.

Confirmation of *E. coli* isolates was done through the IMVIC method (Huang *et al.*, 1997). To determine if the bacterium produced the enzyme tryptophanase and can break down tryptophan into indole, the indole test was carried out. Tryptophan containing broth was inoculated with the suspected *e. coli*. The test was considered positive when a red layer formed on the top layer of the tube indicating indole production. Methyl red test was carried out to detect the ability of bacteria to produce stable mixed acids (such as lactic acid, acetic acid, and succinic acid) from glucose fermentation. Suspected colonies were inoculated in a tube containing Methyl red Voges- Proskauer (MR- VP) broth with a methyl red indicator. The result was considered positive when the broth turned red indicating presence of stable acids and a pH of 4.4 or less. After that a Voges- Proskauer test was done which sought to detect the production of acetylmethylcarbinol (butanediol), a neutral product of glucose fermentation. Using the MR- VP broth the suspected colonies were incubated at 37 ° C for 24 hours with added Barrit's reagent and potassium hydroxide. Appositive result would indicate presence of reddish-brown colonies. Finally, a citrate test was carried out to seek if the bacteria could use citrate as its sole source of energy. The procedure involved inoculating colonies into Simmons' citrate agar containing bromothymol blue as a pH an indicator and incubated at 37 ° C for 24. A positive result would give a change of colour to blue indicating utilization of citrate. For *e. coli* positive colonies, the results were ccolonies that scored Indole +, Methyl +, Voges + and citrate -, were considered positive for *E. coli*.

### **3.5.3.1 Extraction of Bacterial DNA**

A total of 120 colonies (5 from each of the six towns) of *E. coli* DNA was extracted using an extraction kit - Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research Company, USA), following the manufacturer's instructions. The extraction was as follows; 200 µL of the overnight colony broth was transferred into DNase free 2 mL microcentrifuge tubes, centrifuged at 15,000 rpm for 10 min and the supernatant decanted. The pellet was re-suspended in 200 µL sterile distilled water; vortexed and the cells was lysed by boiling on AccuBlock (Digital dry bath, Labnet, Staffordshire, UK) for 15 min at 100<sup>0</sup> C and thereafter centrifuge at 15,000 rmp for 5 min. DNA-containing supernatants was transferred into another DNase free microcentrifuge tubes and were stored at -20<sup>0</sup> C for further analysis. The detailed steps included; β-mercapto-

ethanol (0.5% v/v) was added to the Genomic Lysis Buffer (e.g. 500 µL into 100 mL) after precipitation formed in DNA Pre-Wash Buffer, it was warmed 30–37 ° C and invert to resuspend. Bacterial cells were harvested and placed on the cell pallet into a ZR Bashing Bead tube and bashing buffer added at 20 µl. the tubes were secured in a horizontal vortex adapter and beat beaten for 3 minutes and centrifuged for 1min at 10,000 rpm. The clear supernatant was transferred to a fresh tubes where genomic lysis buffer was added and mixed thoroughly. The mixture was loaded into a Zymo-Spin IICR column and centrifuged. The flow through was discarded. The remaining was washed with g-DNA Wash Buffer twice. DNA elution was done into clean microcentrifuge tubes and a DNA elution buffer was used at 50 µL directly into the column matrix. This was left to stand for 1 minute and then centrifuged at 7,000rpm. The DNA concentration was measured using spectrophotometry and a purity of A260/280, A260/230 was accepted

### 3.5.3.2 Molecular Characterisation of *Escherichia coli* and *Escherichia coli* Pathotypes

A reference strain of *E. coli* (16S rRNA, 147 bp) was used as a quality control organism in this study (*E. coli* ATCC25922). Pathotyping of the confirmed *E. coli* isolates was performed using PCR technique (Kockum *et al.*, 2023), employing specific primers for the detection of key virulence genes from 120 isolates.

Table 3: Pathogenic *Escherichia coli* target primers

Primer Sequence (3'-5')	<i>E. coli</i> , and pathotypes	Targeted genes	Base pair
F-GAACGTTGGTTAATGTGGGGTAA R-ACGCGTGGTTACAGTCTTGCG	<i>E. coli</i>	<i>uidA</i>	147
F-GAACGTTGGTTAATGTGGGGTAA R-TATTCACCGGTTCGGTTATCAGT	DAEC	<i>daaE</i>	542
F-AGCTCAGGCAATGAACTTTGAC R-TGGGCTTGATATTCCGATAAGTC	EIEC	<i>virF</i>	618
F-CACAGGCAACTGAAATAAGTCTGG R-ATTCCCATGATGTCAAGCACTTC	EAEC	<i>aafIII</i>	378
F-TCAATGCAGTTCCGT TATCAGTT R-GTAAAGTCCGTTACCCCAACCTG	EPEC	<i>eae</i>	482
F-CAGTTAATGTGGTGGCGAAGG R-CACCAGACAATGTAACCGCTG	EHEC	<i>stxI</i>	348
F-GCACACGGAGCTCCTCAGTC R-GCCACACAAAGCTCCTCAGT	ETEC	<i>stIII</i>	129

Source: Igwaran *et al.* (2018)

The *virF* gene for enteroinvasive *E. coli* (EIEC), *aafIII* gene for enteroaggregative *E. coli* (EAEC), *daaE* gene for diffusely adherent *E. coli* (DAEC), *eae* gene for

enteropathogenic *E. coli* (EPEC), *stx1* gene for enterohaemorrhagic *E. coli* (EHEC) and *stII* gene for enterotoxigenic *E. coli* (ETEC), as listed in Table 3 above.

Each reaction mixture (25 µl) consisted of 5 µl of 10× reaction buffer (Tris HCl pH 8.3, 0.1% w/v gelatin, 1 mM EDTA and 0.5g/ml Boric Acid), 3 µl of template DNA, 0.5 µl of each primer (the primers were used at a final concentration of 100 M), 3 µl 5 mM dNTP mixture (at a concentration of 100 µl each), and 1.25 µl of Taq polymerase (3 U/l). The remaining volume of the reaction mixture was nuclease-free water. The EPEC and DAEC primers were multiplexed and the amplification was carried out using a Tianlong PCR Thermocycler with thermal cycling conditions of an initial denaturation at 95<sup>0</sup> C for 5 min, followed by 34 cycles of denaturation at 94<sup>0</sup> C for 45 s, annealing at 55<sup>0</sup> C for 30 s, extension at 72<sup>0</sup> C for 1 minute, and with a final extension at 72<sup>0</sup> C for 10 minutes and a cooling at 4<sup>0</sup> C. Finally, PCR products were separated in a horizontal equipment system by running on a 1.5% (w/v) agarose gel containing 0.5 g/ml ethidium bromide for 55 min at 110 V using 1XTAE buffer (40 mM Tris, 1 mM EDTA, and 20 mM glacial acetic acid, pH 8.0). Their molecular weight was estimated by comparing them with a 50 bp DNA molecular weight marker (Solis BioDyne, Tartu, Estonia) for the following bp 428 (EPEC - *eae*) and 542 (DAEC - *daaE*). The resolved PCR products were visualized and photographed under UV light trans-illuminator (ALLIANCE 4.7) molecular Imager Gel Doc system.

The STEC and EAEC primers were also multiplexed with master mix products as stated above. These had different thermocycler conditions of initial denaturation at 95<sup>0</sup> C for 5 min, followed by 34 cycles of denaturation at 94<sup>0</sup> C for 45 s, annealing at 55<sup>0</sup> C for 1 minute, extension at 72<sup>0</sup> C for 1 minute, and with a final extension at 72<sup>0</sup> C for 10 minutes and a cooling at 4<sup>0</sup> C. The DAEC and ETEC were also multiplexed with master mix conditions maintained but different PCR conditions as initial denaturation at 95<sup>0</sup> C for 3 min, followed by 35 cycles of denaturation at 94<sup>0</sup> C for 1 minute, annealing at 55<sup>0</sup> C for 1 minute, extension at 72<sup>0</sup> C for 1 minute, and with a final extension at 72<sup>0</sup> C for 5 minutes and a cooling at 4<sup>0</sup> C. The PCR products were also separated in agarose gel as described above with a 50bp ladder. The above procedures were adopted from Gugsá *et.al.*, 2022, manufacturer's instructions with some modifications in the PCR conditions by the author. For ETEC and EIEC, no

multiplexing was done. The procedure followed the one described above while the annealing temperatures were those provided by the manufacturer.

### **3.5.4 Determination of Antibiotic Susceptibility Patterns of *Escherichia coli* and Resistance Genes**

Sixteen antibiotics from nine groups commonly used in animal husbandry, human medicine and also listed as High Priority Clinically Important Antibiotics (HPCIA) by WHO (Dadgostar, 2019) were used to test for susceptibility. Penicilins (Amoxyl (AMX 30 µg), Ampicillin (AMP 10 µg), methicillin (MET 5 µg)), cephalosporins (CTX cefotaxime (30 µg), cefpodoxime (CPD 30 µg), ceftriaxone (CTR 30 µg)), tetracyclines (tetracycline (TET 30 µg), doxycycline (DO 30 µg), minocycline (MI 30 µg)), aminoglycosides (amikacin (AK 30 µg), gentamycin (GEN 10 µg)), polymyxin (colistin (CL 10 µg)), sulphonamide (cotrimazole (Sulfamethoxazole/trimethoprim COT 23.5/1.25 µg)), lincosamide (lincomycin (L 2 µg)), macrolide (erythromycin (E 15 µg)) and chloramphenicol (chloramphenicol (C 15 µg)).

A bioassay was carried out where a single colony will be picked from TBX (Oxoid) and emulsified in 3 millilitres of physiological saline solution to make a suspension equivalent to 0.5 McFarland turbidity standards (Bacterial turbidity standard prepared by dissolving barium chloride into 1m sulphuric acid to attain a turbidity of 0.5 which is equivalent to  $1.5 \times 10^8$  CFU/ml (Remel, Kansas, USA). A fresh sterile swab was dipped into the suspension and spread evenly over the Muller Hinton agar (Oxoid, UK). The inoculum was allowed to soak in agar and dry for 5-10 min.

Antibiotics discs were thereafter dispensed onto agar by use of a disc dispenser commonly known as Kirby-bauer disc diffusion method (Hudzicki, 2009). *Escherichia coli* ATCC 25922 (a common susceptible strain) was used as a quality control strain. Agar was incubated for 16 h at 37<sup>0</sup>C and examined for presence or absence of zones of inhibition which were read and recorded as recommended by the Clinical Laboratory Standard Institute (CLSI, 2020). The results from antimicrobial sensitivity tests were classified as intermediate, resistant and susceptible according to CLSI document M100S (Appendix 3). Multiple antibiotic resistance (MAR) were calculated as  $MAR = a/b$ . where 'a' represents number of antibiotics the isolate is resistant to, and, 'b' represents total number of antibiotics (Lima *et al.*, 2017).

### 3.5.4.2 PCR Amplification and Visualisation of Antibiotic Resistance Genes

DNA extraction on the multidrug resistant colonies were extracted as explained in 3.5.3.1. Eleven types of antibiotic resistance genes (*bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA</sub>, *bla*<sub>CTX-M-15</sub>, *mcr-1*, *sul1*, and *tet A*) were targeted using primers sequences obtained from literature (Ng *et al.*, 2001; Knapp *et al.*, 2010; Liu *et al.*, 2020; Kemp *et al.*, 2020; Saenz *et al.*, 2021). PCR was performed under the specified conditions, with double distilled water serving as a negative control, using both forward and reverse primers (Table 6).

For *bla*<sub>TEM</sub> gene, the pre-denaturation was at 94° C for 6 minutes, denaturation at 94° C for 45 seconds, annealing at 55° C 30 seconds extension at 72° C for 45 seconds for 34 cycles and final extension at 72° C for 6 minutes. The *bla*<sub>CTXM-15</sub> gene PCR conditions were 95° C for 5 minutes, denaturation at 95° C for 1 minute, annealing at 50° C 1 minute, extension at 72° C for 1 minute for 34 cycles and final extension at 72° C for 10 minutes.

*Bla*<sub>OXA</sub> gene the pre-denaturation was at 95° C for 5 minutes, denaturation at 94° C for, 1 minute, annealing at 50° C 1 minute, extension at 72° C for 1 minute for 35 cycles and final extension at 72° C for 10 minutes. For *Bla*<sub>SHV</sub> gene the pre-denaturation was at 95° C for 3 minutes, denaturation at 94° C for, 1 minute, annealing at 50° C 1 minute, extension at 72° C for 1 minute for 34 cycles and final extension at 72° C for 10 minutes.

All the products were cooled to 4° C. the controls for the β -lactam genes were *Klebsiella Pneumoniae* 78578 (CTXM positive), *Klebsiella Pneumoniae* 885 (OXA and SHV positive) and *K. Pneumoniae* 722(TEM and CTXM positive). The *mcr1*, *sul1* and *dfra1* ARGs were multiplexed under the following thermocycler conditions; pre-denaturation was at 94° C for 5 minutes, denaturation at 94° C for, 30 seconds, annealing at 58° C 30 seconds, extension at 72° C for 40 seconds for 35 cycles and final extension at 72° C for 5 minutes and a final cooling at 4° C. The *tetA* and *sul1* genes were also multiplexed at the pre-denaturation was at 94° C for 6 minutes, denaturation at 94° C for 45 seconds, annealing at 55° C 30 seconds extension at 72° C for 45 seconds for 34 cycles and final extension at 72° C for 6 minutes. Visualisation was done as explained in 3.5.3.2 with a 1kb ladder used in separation. Gel images were printed for analysis and saved using UVIPromV (UVItec, Cambridge, UK). The primer sequences are as shown in Table 4.

Table 4:Primer Sequences for Antibiotic Resistance Genes

Resistance genes	Antibiotic structure that the gene is resistant to	Primer Sequence (3'-5')	Amplicon size (bp)	Source
<i>Bla</i> <sub>TEM</sub>	βlactams	F-GAACGTTGGTTAATGTGGGGT R-ACGCGTGGTTACAGTCTTGCG	365	Knapp <i>et. al.</i> , 2010
<i>Bla</i> <sub>SHV</sub>	βlactams	F-GAACGTTGGTTAATGTGGGGT R-TATTCACCGGTCGGTTATCAG	885	Kemp <i>et. al.</i> , 2020
<i>Bla</i> <sub>OXA</sub>	βlactams	F-AGCTCAGGCAATGAAACTTTG R-TGGGCTTGATATTCCGATAAG	813	Muloi <i>et. al.</i> , 2016
<i>Bla</i> <sub>CTX-M15</sub>	βlactams	F-CACAGGCAACTGAAATAAGTG R-ATTCCCATGATGTCAAGCACT	996	Knapp <i>et. al.</i> , 2010
<i>Tet A</i>	Tetracycline	F: GCTACATCCTGCTTGCCTTC R: CATAGATCGCCGTGAAGAGG	210	Ng <i>et.al.</i> , 2001
<i>sul1</i>	Sulfonamide	F: TGGTGACGGTGTTCCGGCATTG R: CGAGGGTTTCCGAGAAGGTG	789	Saenz <i>et.al.</i> , 2004
<i>Mcr1</i>	Colistin	F: AGTCCGTTTGTCTTGTGGC R: AGATCCTTGGTCTCGGCTTG	309	Liu <i>et. al.</i> , 2020

### 3.6 Data Analysis

The survey data from raw meat retailers checked and edited for completeness, clarity and consistency. The data was coded in Ms-Excel before being analysed using R-version 4.5.0. Descriptive statistics were used to summarize demographic characteristics and hygiene-related practices. Chi-square tests were conducted to assess associations between categorical variables across counties. To identify key predictors of critical hygiene practices, binary logistic regression was performed using demographic variables, age, gender, education level and work experience, as independent variables. The dependent variables were species separation during storage and equipment/premise disinfection, both of which are highly sensitive to microbial contamination risks. The level of statistical significance was set at  $\alpha = 0.05$ . A heatmap was generated in R using the ggplot2 package to visualise the distribution intensity of meat types across counties, with darker shades representing higher retail frequency, thereby enabling quick identification of dominant and regionally variable meat types.

For KAP analysis of RTE meat retailers, the level of knowledge of the food handlers was determined by scoring. For each correct answer- Yes- 2 points, for wrong ones - No- 1 point, and for Unsure - 0 point. Based on the response to 16 knowledge questions, the scores could vary from 0 to 32. The range of 0 to 32 was determined and divided by three to give an approximate number used in creating the categories. The three

categories were: scores between 0 to 11 were classified as poor, 12 to 22 as neutral, and 23 to 32 as satisfactory. For 14 attitude questions, the answers were pointed from 1 to 5 where 1 refers to strongly disagree and 5 strongly agree. Total scores could differ from 14 to 70: These categories created were 14 -32 for negative, 33 – 51 neutral and 52– 70 satisfactory. For 13 practice questions, the answers were; Yes (2) and No (1). The scores for practice could range from 13 to 26. The categories developed were; negative (13 -17), neutral (18-22) and satisfactory (23 -26) (ul Haq *et al.*, 2012; Fariba *et al.*, 2018, Wangmo *et al.*, 2021). The scoring criteria is as shown in the Table 5.

The data was coded and analysed using STATA version 15.0. The analysis included; percentages and frequencies. Mean and standard deviation was calculated to determine the scores and their categories (Poor, neutral, and satisfactory). Kruskal- Wallis test and Mann Whitney U-test were used to derive mean ranks and p-values in determining the effect of demographic characteristics on food safety knowledge, attitude and practices at 0.05 significant level.

Table 5: Scoring Criteria for Assessing Knowledge, Attitude and Practices of Ready-to-eat Meat Retailers

Food Safety Section	Number of Questions	Range	Criteria	Interpretation
Knowledge	16	0 -32	0-11	Poor
			12 -22	Neutral
			23 -32	Satisfactory
Attitude	14	14 - 70	14 -32	Poor
			33-51	Neutral
			52 -70	Satisfactory
Practices	13	13 -26	13 -17	Poor
			18 -22	Neutral
			23 -26	Satisfactory

Data from total coliform counts, *E. coli* counts, antibiotic resistant *E. coli* counts were subjected to analysis of variance (ANOVA) as implemented in SAS version 9.4 (SAS 2013) to determine the variation among counties, towns and sample types. Qualitative data was analysed using goodness of fit Chi-square test. The significant means were separated using least significant difference (LSD) at 5% significance level ( $\alpha = 0.05$ ). The data obtained from microbial analysis were analysed using the following model.

$$Y_{ijk} = \mu + a_i + \beta_j + (a\beta)\gamma_{(ij)} + e_{ij}$$

Where;

$Y_{ijk}$  represents the observed response (*E. coli* counts) for the  $i^{\text{th}}$  type of meat, the  $j^{\text{th}}$  county, and the  $ab^{\text{th}}$  Interaction effect.

$\mu$  represents the overall mean.

$\alpha_i$  represents the effect of the  $i^{\text{th}}$  county.

$\beta_j$  represents the effect of the  $j^{\text{th}}$  type of meat

$\alpha\beta_{(ji)}$  represents the effect of interaction.

$\varepsilon_{ijk}$  represents the error term

Prevalence of antibiotic resistant isolates, virulence and resistance genes was calculated using this formula;

Percentage prevalence

$$= \frac{\text{Number of colonies positive for the particular trait}}{\text{Total number of isolates subjected to the test}} \times 100$$

### **3.7 Ethical Consideration**

A recommendation letter from Chuka university Ethical consideration committee (Appendix 4) and research clearance and permit from National Commission of Science and Technology (NACOSTI) was sought (Appendix 5). Consent was also sought from all raw and RTE meat retailers for permission to participate in the study. Handling of *E. coli*, being a potential biological hazard, followed the laid down microbiological safe procedures to prevent environmental contamination.

## CHAPTER FOUR

### RESULTS

#### 4.1 Demographic, Operational and Hygiene Practices Within Butcherries in Meru Embu and Tharaka-Nithi Counties

##### 4.1.1 Demographic Characteristics of Butchery Personnel

This subsection presents the demographic characteristics of butchery personnel across the three counties, including gender, age, education level and work experience. The findings showed that the majority of respondents were male (94% (102)), with female respondents accounting for only 6.4% (7) (Table 6). Meru county had the highest proportion of male participants (98% (50)), while Embu had the highest proportion of females (13% (4)). Regarding age distribution, the largest proportion of respondents were aged 31–40 years (39% (42)), followed by those aged 21–30 years (29% (32)). Only 8.3% (9) were over 50 years, and none were below 20 years. In terms of education, nearly half had attained secondary level education (49% (53)), while 37% (40) had post-secondary qualification. A small proportion had completed primary education (13% (14)), and 1.8% (2) had no formal education. With respect to work experience, the majority had 3–5 years (47% (51)), followed by 1–3 years (28% (31)) and less than 1 year (25% (27)).

Table 6: Demographic characteristics of personnel working at butcherries (N = 109)

Characteristic		Overall N = 109	Tharaka- Nithi N = 26	Embu N = 32	Meru N = 51
Gender	Male	102 (94%)	24 (92%)	28 (88%)	50 (98%)
	Female	7 (6.4%)	2 (7.7%)	4 (13%)	1 (2.0%)
Age	21-30	32 (29%)	8 (31%)	13 (41%)	11 (22%)
	31-40	42 (39%)	10 (38%)	10 (31%)	22 (43%)
	41-50	26 (24%)	5 (19%)	5 (16%)	16 (31%)
	Above 50	9 (8.3%)	3 (12%)	4 (13%)	2 (3.9%)
Education level	Non-Formal	2 (1.8%)	0 (0%)	1 (3.1%)	1 (2.0%)
	Primary level	14 (13%)	4 (15%)	4 (13%)	6 (12%)
	Secondary level	53 (49%)	13 (50%)	13 (41%)	27 (53%)
	Post-secondary	40 (37%)	9 (35%)	14 (44%)	17 (33%)
Experience	Less than 1 year	27 (25%)	5 (19%)	5 (16%)	17 (33%)
	1 <3 years	31 (28%)	7 (27%)	13 (41%)	11 (22%)
	3 – 5 years	51 (47%)	14 (54%)	14 (44%)	23 (45%)

#### 4.1.2 Types and Distribution of Meat Sold in Surveyed Butcheries

The study focused on beef and chicken, the most commonly consumed sources of animal protein in the study area, while also mapping the availability of other meat types in urban butcherries. The results showed that beef was the most widely retailed meat across all counties, with Meru recording the highest number of outlets selling beef only (29.7% (41) of all surveyed butcherries), followed by Embu (15.2% (21)) and Tharaka Nithi (12.3% (17)) counties (Figure 3). Chicken was also common but was rarely sold as a standalone product; it was most often offered alongside beef, particularly in Embu (7.2% (10)) and Tharaka Nithi (5.8% (8)) counties, compared to only 2.2% (3) in Meru County. Outlets selling chicken exclusively were few, found only in Meru (2.2% (3)) and Tharaka Nithi (1, 0.7%) counties. Butcherries selling beef with other meats (excluding chicken) were recorded in all counties but were most common in Meru County (2.9% (4)). Other meats, such as goat and pork, were available in small quantities and typically complemented beef sales.

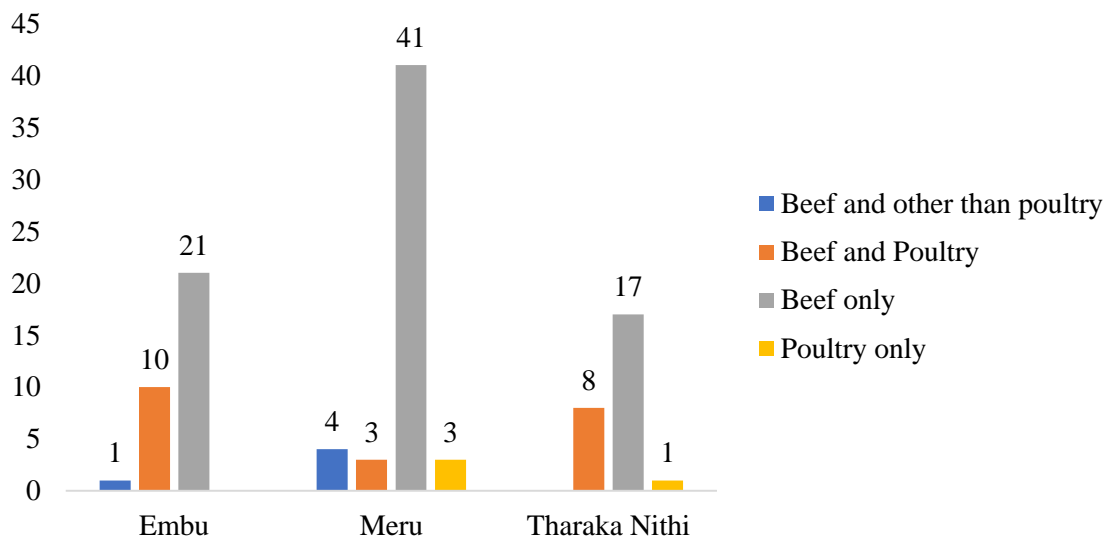


Figure 3: Distribution of butcherries by meat type sold in Meru, Embu, and Tharaka Nithi counties, showing counts and percentages of the total surveyed outlets (n = 109).

The results in Figure 4 present a heatmap showing the distribution intensity of different meat types retailed across butcherries in Tharaka-Nithi, Embu, and Meru counties. The visual intensity reflects relative frequency, with darker shades indicating higher availability. Beef emerged as the dominant meat type across all three counties, with Meru County exhibiting the highest frequency. Chicken was also widely sold, though typically in combination with beef, rather than as a standalone offering. Goat meat was present in Embu and Meru counties, while completely absent in Tharaka-Nithi County.

Pork was least common, recorded only in Meru County. These patterns suggest a regional variation in meat preference and supply, with beef maintaining a central role in urban meat consumption, while other meats like pork and goat are more localized or secondary in distribution.

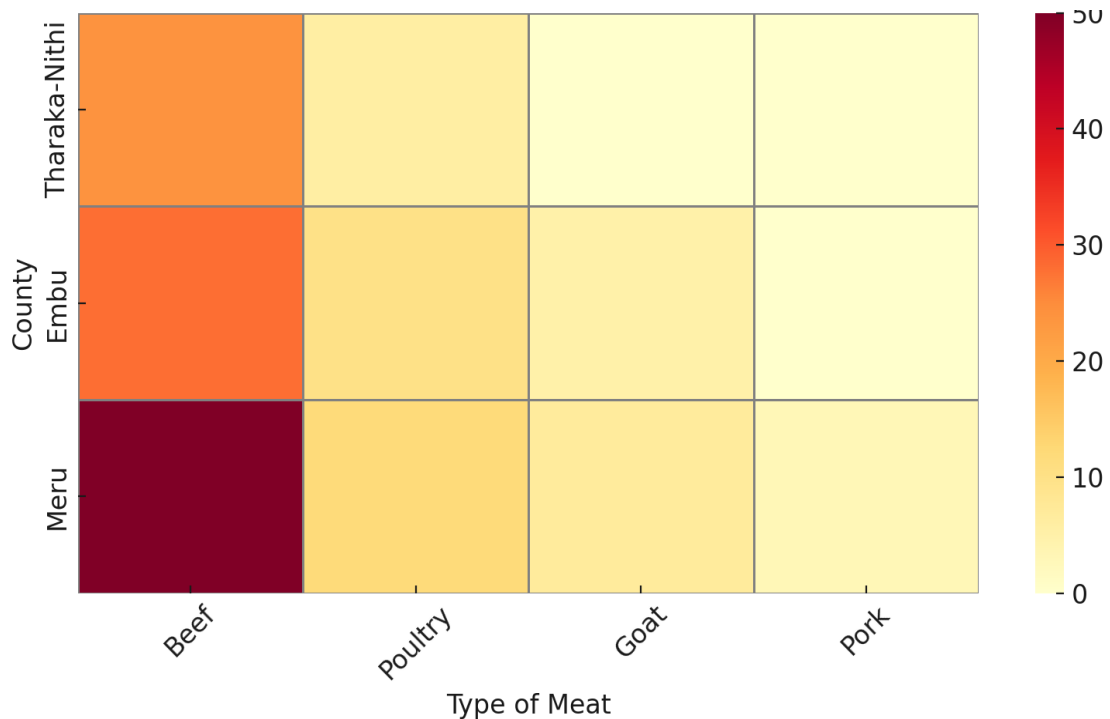


Figure 4: Heatmap showing the distribution of meat types sold across counties

#### 4.1.3 Sources and Procurement Practices for Beef and Chicken Meat Sold in Butcheries

The findings of the study revealed that the majority of beef retailers bought their meat from licensed slaughterhouses. In some cases, particularly in Meru County, retailers procured live animals directly from farmers in Isiolo town. However, in all such cases, the animals were subsequently taken to official slaughterhouses for inspection and certification by veterinary officers before slaughter and distribution to butcheries.

In contrast, chicken sourcing showed variation across counties, though the number of sourcing channels was limited. Statistical analysis revealed significant differences in sourcing practices ( $\chi^2 = 24.712$ ,  $P = 0.0004$ ) (Table 7). The majority of chicken retailers, especially in Tharaka-Nithi County (90% (9)), sourced directly from farmers. This

preference was also common in Meru (62.5% (10)) and Embu (53.8% (7)), with the remaining retailers in each county obtaining poultry from local markets.

Table 7: Sources of chicken meat sold by counties

County	Response	Frequency	Percent	Total	X <sup>2</sup> , P -value
Embu	Directly from farmers	7	17.9	13 (33.4%)	24.712, 0.0004
	Market	6	15.4		
Meru	Directly from farmers	10	25.6	16 (41%)	
	Market	6	15.4		
Tharaka- Nithi	Directly from farmers	9	23.5	10 (25.6%)	
	Market	1	2.1		

N = 39, ( $\alpha = 0.05$ )

#### 4.1.4 Time to Complete Sale and Associated Storage Conditions of Meat in Butcheries

The duration between the reception of meat in butcheries and its complete sale was evaluated to assess turnover rates and potential public health risks. Meat is a highly perishable commodity due to its rich nutrient profile and high moisture content, which creates a favourable environment for microbial growth, particularly under warm storage conditions. The findings of this study revealed that most butcheries (71.56% (78)) sold their meat within 2–3 days of arrival, while 26.61% (29) sold it within one day or less (Figure 5). Only 1.83% (2) of butcheries reported having meat remaining for sale beyond three days. These findings indicate relatively fast turnover; however, they also highlight potential food safety concerns, especially for outlets lacking refrigeration or adequate hygiene practices. Continuous monitoring of meat storage duration is crucial, particularly in informal retail settings where cold chain infrastructure may be limited or absent.

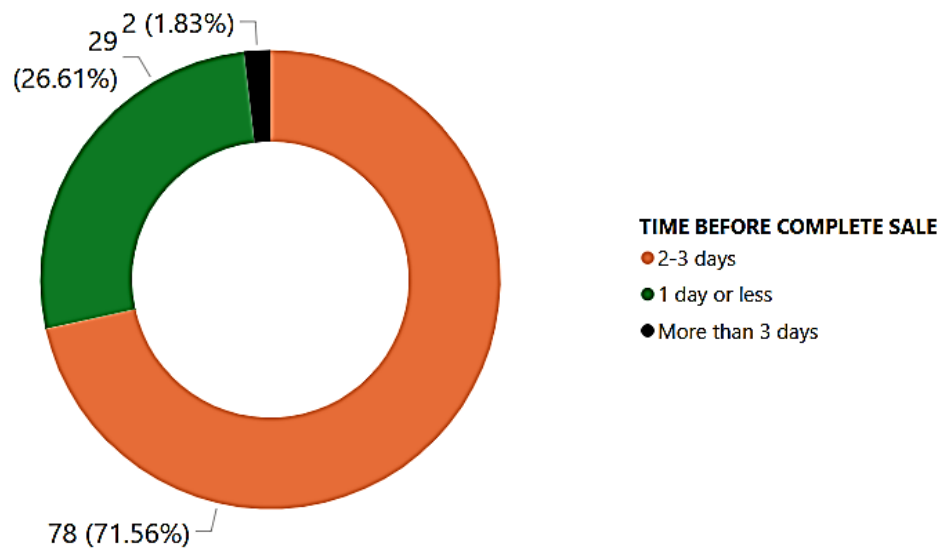


Figure 5: Distribution of butcheries by time taken to sell meat after arrival

#### 4.1.5 Overnight Meat Storage Practices and Species Separation

Butcheries in Embu County used refrigeration more (15.5 % (17), compared to only 6.2% (7) in Meru and 3.1% (1) in Tharaka-Nithi. To assess food safety practices in butcheries, the study examined overnight meat storage methods of meat, focusing on temperature control and species separation. Majority of butcheries stored meat at room temperature (76.85%, 83), typically by hanging it on hooks, rather than under refrigeration, while only 23.15% (26) stored refrigerated overnight (Table 8). There were statistically significant differences ( $\chi^2 = 16.175$ ,  $P = 0.0003$ ) refrigeration across counties. Specie separation was done by only 12.8% (15) while 87.2% (94) never separated during storage.

Table 8: The relationship between overnight meat storage methods by counties (n= 109)

Counties		Frequency	Percent	Total N	X <sup>2</sup> , P-value
Embu	Refrigerate	17	15.5	32 (31%)	16.175, 0.0003
	Room temperature (Hanging on hook)	17	15.5		
Meru	Refrigerate	7	6.2	51(46.4%)	
	Room temperature (Hanging on hook)	44	40.2		
Tharaka-Nithi	Refrigerate	1	3.1	26 (22.7%)	
	Room temperature (Hanging on hook)	22	20.6		

$\alpha = 0.05$

#### 4.1.6 Predictors of Species Separation Practices in Butcherries

A binary logistic regression analysis was used to identify factors influencing whether butcherries practiced species separation during meat storage. The dependent variable was species separation (1 = Yes, 0 = No), while predictor variables included gender, age, education level and work experience. The results showed that age and level of education were statistically significant predictors ( $P < 0.05$ ) predictors of species separation (e.g., chicken and beef) during storage, while gender and work experience were not (Table 9). Compared to personnel aged 20–30 years, those above 50 years had significantly lower odds of practicing species separation (OR = 0.14, 95% CI: 0.03–0.65,  $P = 0.016$ ). This suggests that younger operators in this dataset were more likely to apply separation practices, contrary to the assumption that older butchers might be more cautious.

Table 9: Binary Logistic Regression of Factors Associated with Species Separation During Meat Storage

Characteristics	Predictors	Odds Ratios	Std. Error	95% CI for Odds Ratio	Z-value	P-value
Constant 'Female'	(Intercept)	244.96	319.76	0.11–1.25	0.01	0.990
Reference 'Age 20- 30'	Gender [Male]	0.00	0.00	0.01–1.91	-0.01	0.989
	Age31- 40	0.40	0.29	0.09–1.57	-1.27	0.202
Reference 'Less than 1 year'	Age 41-50	1.17	0.85	0.27–4.82	0.22	0.829
	Age [Above 50]	0.14	0.11	0.03–0.65	-2.42	0.016
Reference 'Non-formal education'	Experience1-3 Years	0.64	0.35	0.22–1.85	-0.81	0.419
	Experience3-5 Years	1.32	0.85	0.38–4.83	0.44	0.661
Reference 'Non-formal education'	Education [Primary]	5.15	2.99	1.71–16.97	2.82	0.005
	Education [Secondary]	10.17	6.44	3.15–38.68	3.66	<0.001

Observations = 109,  $\alpha = 0.05$

$R^2$  Tour = 0.75

Education had a strong positive association with species separation. Butchers with primary education were 5.15 times more likely ( $P = 0.005$ ), and those with secondary education were 10.17 times more likely ( $P < 0.001$ ) to practice separation compared to those with no formal education. This highlights the role of education in fostering hygienic meat handling practices. The model explained a substantial proportion of the variation in the data (Tjur's  $R^2 = 0.75$ ), indicating good predictive power.

#### 4.1.7 Premise and Equipment Hygiene Practices

##### 4.1.7.1 Frequency of Cleaning Meat Handling Equipment, Storage Units and Premises

The results of this study showed that meat handling equipment cleaning was generally frequent across all counties, with Embu recording the highest proportion of respondents (62.5%, 67) cleaning more than once daily. However, differences between counties were not statistically significant ( $\chi^2 = 1.598$ ,  $P = 0.450$ ) (Table 10). In contrast, meat storage equipment cleaning showed marked differences. Daily cleaning was most common in Tharaka-Nithi (70.0%, 16) and Meru (67.5%, 35), but substantially lower

in Embu (43.5%, 15). This variation was highly significant ( $\chi^2 = 19.485$ ,  $P = 0.0006$ ), indicating notable disparities in storage hygiene practices.

For premise cleaning, Tharaka-Nithi (65.4%, 15) and Meru (58.8%, 30) most frequently cleaned once daily, whereas Embu had a higher proportion cleaning twice daily (46.9%, 18) (Table 10). The differences across counties were statistically significant ( $\chi^2 = 16.865$ ,  $P = 0.0021$ ), suggesting variability in overall hygiene rigor. Overall, while basic hygiene practices are present across all counties, differences in frequency, especially in storage and premise cleaning, may have implications for meat safety, particularly where cleaning is less frequent.

Table 10: Cleaning Frequency of Meat Handling Equipment and Butchery Premises by County (N 109,  $\alpha = 0.05$ )

Counties				X <sup>2</sup> , P - value
Hygiene Practice	Embu	Meru	Tharaka-Nithi	
Meat handling equipment cleaning frequency (%)				
Once daily	37.5	47.06	53.9	
More than Once	62.5	52.94	46.15	1.598, 0.450
Meat storage equipment cleaning frequency (%)				
Daily	43.5	67.5	70.0	19.485,
Weekly	47.8	20.0	20.0	0.0006
Cannot tell	8.7	12.5	10.0	
Butchery/Premise cleaning frequency (%)				
Once daily	18.7	58.8	65.4	
Twice daily	46.9	21.6	23.1	16.865, 0.0021
When dirty	34.4	19.6	11.5	

Disinfection is a crucial sanitation step in ensuring the safety of meat products and preventing cross-contamination in butcheries. This study assessed the prevalence of disinfection practices among meat retailers in Embu, Meru, and Tharaka-Nithi counties. The results revealed that disinfection was least common in Tharaka-Nithi County, where only 19.23% (4) of respondents reported practicing equipment and premises disinfection. In contrast, Embu (40.63%, 14) and Meru (43.14%, 22) reported higher levels of sterilization, though still below 50%, indicating room for improvement in all counties (Figure 6).

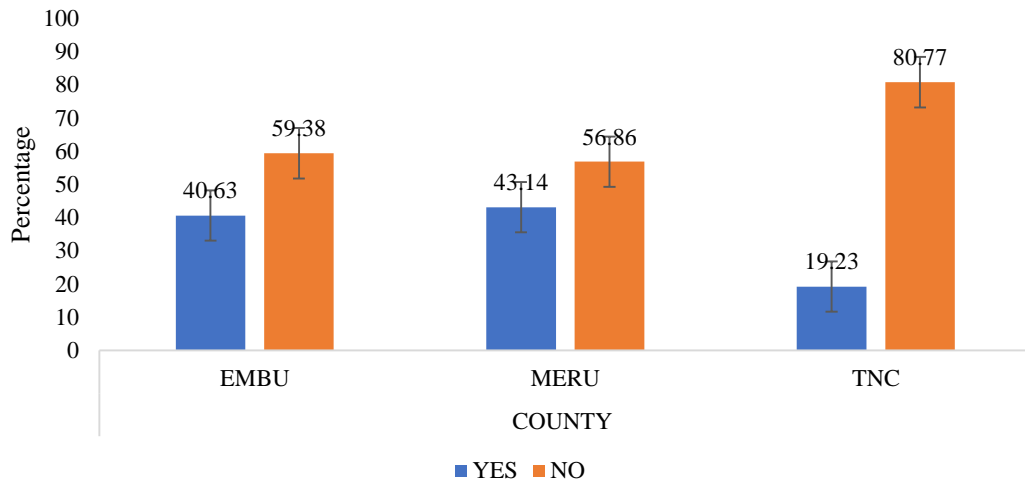


Figure 6: Percentage of butcheries practicing equipment and premise disinfection in Embu, Meru, and Tharaka-Nithi Counties, where, YES = Disinfects and NO = Does not disinfect; Error bars represent 95% Confidence intervals

To identify demographic factors influencing sterilization practices in butcheries, a binary logistic regression analysis was conducted (Table 11). The model assessed the impact of gender, age, work experience, and education level on the likelihood of using sterilization for equipment and premises. The logistic regression analysis revealed that age was the only statistically significant predictor of sterilization practices in butcheries. Compared to respondents aged 21–30 years (reference group), those aged 31–40 years had almost four times higher odds of practicing sterilization (OR = 3.94, 95% CI: 1.07–15.87,  $P = 0.044$ ), while those aged 41–50 years had over six times higher odds (OR = 6.68, 95% CI: 1.73–28.88,  $P = 0.007$ ). In contrast, gender, work experience, and education level were not statistically significant predictors. Although individuals with 3–5 years of work experience demonstrated relatively high odds of practicing sterilization (OR = 4.05), this association was borderline and did not achieve statistical significance ( $P = 0.064$ ). The model’s Tjur’s  $R^2$  value of 0.214 indicates modest explanatory power, suggesting that while age is an important determinant, other unmeasured factors may also play a role in influencing sterilization behaviour.

Table 11: Binary Logistic Regression Analysis of Predictors of disinfection Practices in Butcherries

Characteristics	Disinfection predictors	Odds Ratios	Std. Error	95% CI for Odds Ratio	Z-value	P-value
Reference 'Female'	(Intercept)	2.91	4.13	(0.23–83.93)	0.75	0.452
	Gender [Male]	0.42	0.53	(0.02–3.75)	0.69	0.492
Reference Age '21 – 30'	Age 31-40	3.94	2.69	(1.07–15.87)	2.01	0.044
	Age 41-50	6.68	4.74	(1.73–28.88)	2.68	0.007
	Age [Above 50]	0.70	0.48	(0.18–2.69)	-0.52	0.603
Reference Experience 'Less than 1 year'	Expereince1-3 Years	0.74	0.39	(0.26–2.11)	-0.56	0.575
	Experience 3-5 years	4.05	3.05	(1.02–21.21)	1.85	0.064
	Education [Primary]	0.45	0.27	(0.13–1.46)	1.31	0.191
	Education [Secondary]	0.75	0.46	(0.23–2.47)	0.47	0.641

Observations = 109

$R^2$  Tjur = 0.214, ( $\alpha = 0.05$ )

#### 4.1.8 Environment, Hygiene, Water Supply and Sanitation Facilities

Access to safe clean water, adequate sanitation and proper waste disposal is critical for maintaining hygiene and food safety in meat retail environments. This study assessed the availability and source of water, sanitation facilities, and waste disposal systems in butcherries across Embu, Meru, and Tharaka-Nithi counties.

Water source distribution varied significantly across counties ( $\chi^2 = 17.408$ ,  $P = 0.00016$ ), indicating a statistically significant difference at the 1% significance level (Table 12). Treated municipal water was the primary source in all counties, with the highest proportion reported in Meru (88.2%, 45), followed by Tharaka-Nithi (84.6%, 20) and Embu (81.3%, 28). River water use was reported exclusively in Meru (11.8%, 6) and Tharaka-Nithi (7.7%, 1), while water vendors were more common in Embu (18.8%, 6) and Tharaka-Nithi (7.7%, 1), but absent in Meru.

Regarding in-premise facilities, over half of the butcherries in each county had sinks within the premises, with Meru recording the highest proportion (78%, 40). These

differences were not statistically significant ( $P = 0.229$ ). The presence of toilets within the vicinity of the butcheries was highest in Embu (75%, 24), followed by Meru (69%, 35) and Tharaka-Nithi (65%, 17), with no significant difference between counties ( $P = 0.717$ ). For waste disposal or drainage systems located within approximately 100 meters of the butchery, Tharaka-Nithi reported the highest availability (62%, 6), followed by Meru (59%, 30) and Embu (53%, 17). Although these differences were not statistically significant ( $P = 0.7955$ ), they point to regional disparities in basic infrastructure that could influence hygiene and food safety practices.

Table 12: Environmental Hygiene, Water Source, Sanitation, and Waste Disposal By County

Environment, water and sanitation factors		Embu (%(n))	Meru (%(n))	Tharaka-Nithi (%(n))	X <sup>2</sup> , P - Value
Water source	Municipal	81.3 (26)	88.2 (45)	84.6 (22)	17.408, 0.00016
	Vendors	18.8(6)	0 (0)	7.7 (2)	
	River	0 (0)	11.8 (6)	7.7 (2)	
Sink within Premise	Yes	53 (17)	78 (40)	69 (18)	2.946, 0.229
	No	47 (15)	22 (11)	31 (8)	
Toilet in vicinity	Yes	75 (24)	69 (35)	65 (17)	0.683, 0.717
	No	25 (8)	31 (16)	35 (9)	
Waste disposal/drainage in vicinity	Yes	53 (17)	59 (30)	62 (6)	0.4576, 0.7955
	No	47 (15)	41 (21)	38 (1)	

N = 109, ( $\alpha = 0.05$ )

#### 4.1.9 Personnel Hygiene Compliance and Enforcement of Government Regulations

In Kenya, the enforcement of food safety and hygiene standards falls under the mandate of the Ministry of Public Health implemented through county governments. A key role is mandate is to ensure that all food handlers undergo regular medical examinations and hold valid food handler's certificates, as stipulated by the Public Health Act (Government of Kenya, 2016).

In the surveyed butcheries across Embu, Meru, and Tharaka-Nithi counties, 100% (109) of raw meat handlers possessed valid food handler's certificates, indicating full compliance with this legal requirement (Table 13). However, adherence to the

recommended three-month interval for medical check-ups was inconsistent. In Embu, 50% (16) of workers had undergone a medical check-up within the past three months, compared to 40% (20) in Meru and 38% (10) in Tharaka-Nithi. The remainder had check-ups between three and six months prior, with a small proportion reporting examination more than six months ago. These differences were not statistically significant ( $P = 0.6667$ ), suggesting a need for stronger enforcement of routine health monitoring.

Government hygiene inspections were reported to occur mainly on a monthly basis, 91% (29) in Embu, 86% (44) in Meru, and 88% (23) in Tharaka-Nithi, while weekly inspections were rare across all counties. Training of butchery personnel was also evaluated as part of hygiene enforcement and capacity building. Most workers had received training, either through formal courses or informal/apprenticeship arrangements (Figure 7). Training content primarily focused on meat cutting techniques, sanitation procedures, and personal hygiene practices within the butchery environment.

Table 13: Compliance with Government Food Safety and Hygiene Regulations among Butcherries by County

Response		Embu	Meru	TNC	$X^2$ , P -value ( $\alpha = 0.05$ )
		% (n)			
Food handler's medical check-up	Yes	100 (32)	100 (51)	100 (26)	3.0806, 0.0143
	No	0 (0)	0 (0)	0 (0)	
Last medical check-up	Less than 3 months	50 (16)	40 (20)	38 (10)	4.0734, 0.6667
	3-6 Months	41 (13)	44 (22)	50 (13)	
	More than 6 months	9 (6)	12 (6)	12 (3)	
	Once only	0 (0)	4 (2)	0 (0)	
Govt inspection frequency	Weekly	9 (3)	7 (14)	3 (12)	11.1846, 0.6715
	Once a month	91 (29)	86 (44)	88 (23)	

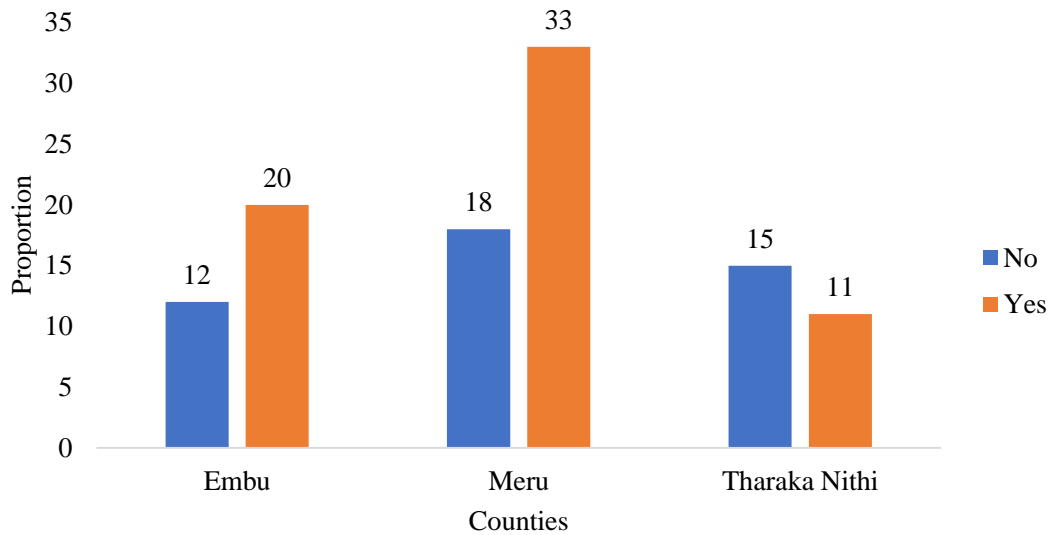


Figure 7: Proportion of trained butchery personnel with formal or informal hygiene and meat-handling training by county. Where, yes = has formal/informal training and No = has neither formal nor informal training.

#### 4.1.10 Personnel and Money Handling

Personnel hygiene and financial transaction practices in meat-handling environments play a critical role in maintaining food safety standards. Handling money while simultaneously managing raw meat can facilitate microbial transfer and increase contamination risk. This study assessed payment methods, the use of designated cashiers and the wearing of protective clothing among butchery personnel in Embu, Meru, and Tharaka-Nithi (TNC) counties.

The findings of showed that the majority of butcheries in all three counties accepted both cash and mobile money payments, 97% (48) in Meru, 94% (24) in TNC, and 96% (25) in Embu (Table 14). Use of only cash or only mobile money was uncommon. However, differences in payment methods were not statistically significant ( $\chi^2 = 3.301$ ,  $P = 0.5088$ ).

When assessing whether butcheries used a separate cashier to handle money transactions, variations across counties were more pronounced. In Meru, 56% (18) of butcheries reported having a designated cashier, compared to 35% (9) in Embu and 33% (17) in TNC. However, this difference was also not statistically significant ( $\chi^2 = 4.759$ ,  $P = 0.0926$ ). Consistent use of protective clothing is essential not only for

safeguarding meat handlers but also for reducing the risk of cross-contamination during meat preparation and sales.

Table 14: Personnel and money handling practices by County ( $\alpha = 0.05$ )

Response		Meru	Tharaka-Nithi	Embu	X <sup>2</sup> , P -value
	Response	%(n)			
Payment Method	Cash only	3 (1)	4 (2)	0 (0)	3.301, 0.5088
	Mobile Money only	0 (0)	2 (1)	4 (1)	
	Both	97 (48)	94 (24)	96 (25)	
Separate Cashier	Yes	56 (18)	33 (9)	35 (17)	4.759, 0.0926
	No	44 (14)	67 (34)	65 (17)	

N = 109

In terms of personnel attire, the results highlights that use of protective clothing (such as aprons, gloves, or hairnets) was not consistently practiced across the counties. Meru County had the highest proportion of individuals wearing protective clothing (50%, 28), while TNC had the lowest at 31% (9) (Figure 8). Wearing protective clothing is crucial not only for the safety of meat handlers but also for preventing cross-contamination of meat products.

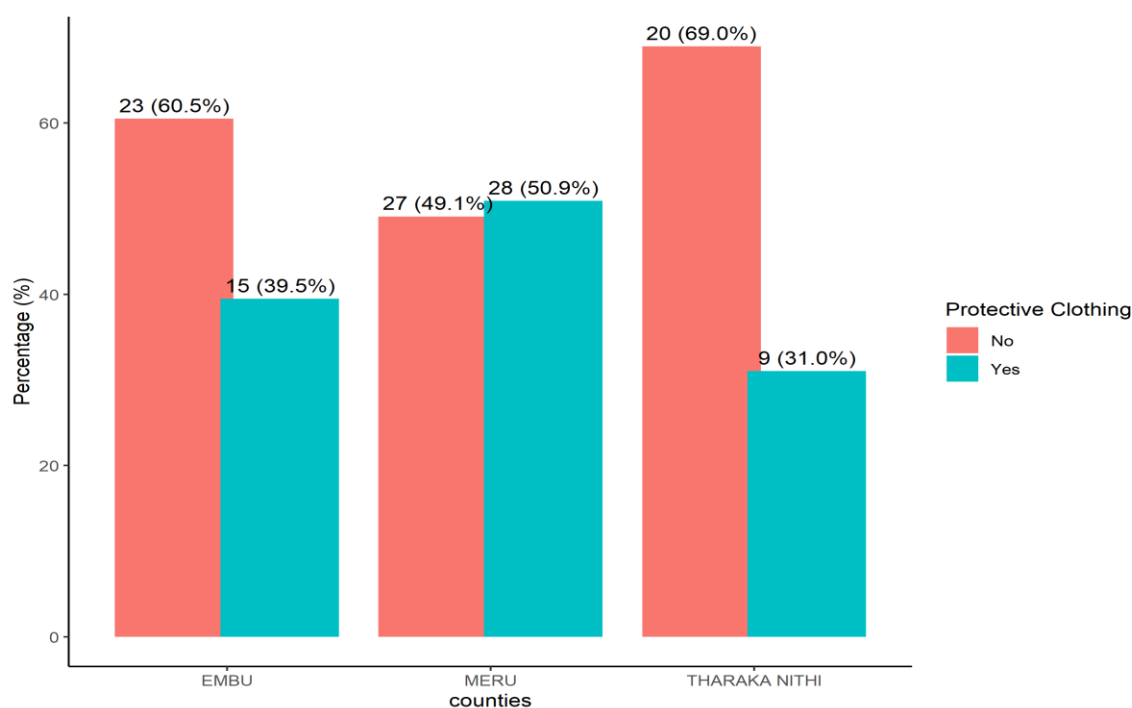


Figure 8: Proportion of butchery personnel wearing protective clothing

Where, Yes = have protective clothing and No = does not have protective clothing

## **4.2 Evaluation of Food Safety Knowledge, Attitudes, and Practices of Ready-to-Eat Meat Retailers as Affected by their Demographic Characteristics**

### **4.2.1 Demographic Characteristics of Ready-to-Eat Meat Retailers**

Across the three counties males constituted a slightly higher proportion (53.3%, 59) than females (46.7%, 57) (Table 15). The highest proportion of males was recorded in Tharaka-Nithi County (55.5%, 20), while the lowest was in Embu County (52.9%, 18). Age distribution showed that the 21–30 age group was most represented overall (24.3%, 26), with the highest proportions in Tharaka-Nithi (27.8%, 10) and Meru County (27.0%, 10). The least represented age category was those above 50 years (15.0%, 16), particularly low in Tharaka-Nithi (8.3%, 3). The youngest group (18–20 years) was least represented in Meru (8.1%, 3) but relatively high in Tharaka-Nithi (25%).

In terms of work experience, retailers with less than one year of experience formed the largest group (38.3%), with Tharaka-Nithi recording the highest proportion (41.7%). Those with over five years' experience were the least common overall (25.3%). Education levels were dominated by secondary education (41.1%), most prevalent in Tharaka-Nithi (47.2%). College education was the second most common (34.6%) and was relatively consistent across counties. Primary education was least common overall (13.1%), highest in Embu (17.7%, 6) and lowest in Meru (8.1%). University education accounted for only 11.2% (12) of respondents, with Tharaka-Nithi having the lowest representation (5.6%, 9).

Regarding food safety training, 41.1% (44) of respondents reported having received formal training, with Tharaka-Nithi recording the highest proportion (47.2%, 17) and Embu the lowest (17.7%, 6). Similarly, 44.6% (48) of respondents held a valid food handlers' certificate, with Tharaka-Nithi again recording the highest proportion (69.4%, 25) and Meru the lowest (40.5%, 15). Over half of respondents (54.2%, 58) also reported selling raw meat alongside RTE meat products. This practice was most common in Embu (55.9%, 19) and least common in Meru (37.8%, 14).

Table 15: Socio-demographic characteristics of retailers of ready-to-eat beef and chicken meat

Demographic Characteristics	Category	N (%) Total	Meru	Embu	TNC
Gender	Male	57 (46.7)	19 (51.4)	18 (52.9)	20 (55.5)
	Female	59 (53.3)	18 (48.6)	16 (47.1)	16 (44.4)
AGE	18-20	19 (17.8)	3 (8.1)	7 (20.6)	9 (25)
	21 -30	26 (24.3)	10 (27.0)	6 (17.7)	10 (27.8)
	31 -40	25 (23.4)	7 (18.9)	10 (29.4)	8 (22.2)
	41 -50	21 (19.4)	9 (24.3)	6 (17.7)	6 (16.7)
	Above 50	16 (15.0)	8 (21.6)	5 (14.7)	3 (8.3)
Experience	<1 Year	41 (38.3)	13 (35.1)	13 (38.2)	15 (41.7)
	1 - 5 Years	39 (36.5)	14 (37.9)	12 (35.3)	13 (36.1)
	> 5 Years	27 (25.3)	10 (27.0)	9 (26.5)	8 (22.2)
Education	Primary	14 (13.1)	3 (8.1)	6 (17.7)	5 (13.9)
	Secondary	44 (41.1)	16 (43.2)	11 (32.4)	17 (47.2)
	College	37 (34.6)	14 (37.8)	11 (32.4)	12 (33.3)
	University	12 (11.2)	4 (10.8)	6 (17.7)	2 (5.6)
Training	Yes	44 (41.1)	18 (48.7)	28 (82.4)	17 (47.2)
	No	63 (58.9)	19 (51.4)	6 (17.7)	19 (52.8)
Food Handlers Certificate	Yes	48 (44.6)	15 (40.5)	19 (55.9)	25 (69.4)
	No	59 (55.1)	22 (59.5)	15 (44.1)	11 (30.6)
Also sells Raw Meat	Yes	58 (54.2)	14 (37.8)	19 (55.9)	16 (44.4)
	No	49 (45.8)	23 (62.2)	15 (44.1)	20 (55.6)

TNC (Tharaka Nithi County), N= 106

#### 4.2.2 Food Safety Knowledge Attitude and Practices of Ready-to-Eat Meat Retailers

On knowledge of food safety, most participants (77.5%, 83) knew what foodborne diseases are, while only 8 (7.5%) did not, and 16 (15.0%) were unsure (Table 16). Similarly, 66.4% (71) were aware of how these diseases could be transmitted, though 11.2% (12) lacked awareness and 22.4% (24) were unsure. A large majority (79.4%, 85) recognized that raw meat quality affects ready-to-eat meat quality, with 5 (4.7%) disagreeing and 15.9% (17) unsure. Knowledge of spoilage signs in meat or chicken was high, with 86 (80.4%) affirming awareness, 7 (6.5%) denying it, and 13.1% (14) unsure. About two-thirds (72, 67.3%) understood that cooked meat can be contaminated by surfaces previously used for raw meat, though 28.0% were unsure. Hand hygiene knowledge varied where only 47 (43.9%) believed hand sanitizers are effective as part of cleaning after handling raw meat. About 94 (87.9%) respondents recognized that unhygienic food handling can cause contamination, with only 3.7% (4) disagreeing.

Knowledge of safe storage practices was mixed: 53 (49.5%) knew cooked meat should be stored at specific temperatures, while 45 (42.1%) were unsure.

Table 16: Food safety knowledge of ready-to-eat beef and chicken meat retailers (N=106)

Knowledge Questions	Yes	No (N (%))	Unsure
1. Do you know what food borne diseases are?	83 (77.5)	8 (7.5)	16 (15.0)
2. Are you aware of how foodborne diseases can be transmitted?	71 (66.4)	12 (11.2)	24 (22.4)
3. Do you know that the quality of your raw meat affects the quality of ready-to-eat meat?	85 (79.4)	5 (4.7)	17 (15.9)
4. Do you know of any signs accompanied by spoiled meat?	86 (80.4)	7 (6.5)	14 (13.1)
5. Can cooked meat be contaminated by surfaces previously used for raw meat e.g. cutting boards?	72 (67.3)	5 (4.7)	30 (28.0)
6. Are hand sanitizers effective in killing most germs after handling raw meat?	47 (43.9)	20 (18.7)	40 (37.4)
7. When you handle food unhygienically can it cause food contamination with pathogenic microorganisms?	94 (87.9)	4 (3.7)	9 (8.4)
8. Is there a specific temperature at which cooked meat should be stored at?	53 (49.5)	9 (8.4)	45 (42.1)
9. Do you know how long cooked meat can safely remain unrefrigerated?	45 (42.1)	20 (18.7)	42 (39.3)
10. Can ready-to-eat meat be infected by flies and rodents?	94 (87.9)	2 (1.9)	11 (10.3)
11. Can improperly reheated cooked meat cause foodborne illness?	71 (66.4)	14 (13.1)	22 (20.6)
12. Do you know if meat can become contaminated even after thorough cooking?	70 (65.4)	18 (16.8)	19 (17.8)
13. Are you aware of the diseases commonly caused by contaminated beef or chicken (e.g., <i>Salmonella</i> , <i>E. coli</i> )?	58 (54.2)	21 (19.6)	28 (26.2)
14. Can using untreated water to wash meat or utensils cause contamination?	74 (69.2)	9 (8.4)	24 (22.3)
15. Do you know the importance of using clean gloves or tongs when serving meat?	83 (77.6)	5 (4.7)	19 (17.8)
16. Do you know of any government guidelines on handling ready-to-eat meat?	59 (55.1)	25 (23.4)	23 (21.5)

A large proportion (87.9%, 94) acknowledged that ready-to-eat meat could be contaminated by flies and rodents, with just 2 (1.9%) disagreeing. Additionally, 66.4% (71) recognized the risk from improperly reheated meat. About 65% (70) of respondents acknowledged that contamination can still occur after thorough cooking from other sources like utensils and non-portable water among other environmental factors. Over

half of the RTE meat retailers (54.2%, 58) were aware of diseases caused by contaminated meat such as *Salmonella* and *E. coli*, though 26.2% (28) were unsure. Most respondents (69.2%, 74) understood that untreated water could contaminate meat or utensils, and 83 (77.6%) knew the importance of clean gloves or tongs. Finally, knowledge of government guidelines on ready-to-eat meat handling was generally moderate (55.1%, 59). Generally, the highest awareness was on hygiene-related risks (over 80%, 86 for several questions), while the lowest knowledge was on safe storage duration, use of hand sanitizers, and food borne disease pathogen awareness.

#### **4.2.3 Food Safety Attitude of Ready-to-Eat Beef and Chicken Meat Retailers**

On food safety attitudes, a majority of respondents valued core hygiene practices. For instance, 30.8% (33) agreed and 0.5% (5) strongly agreed that washing hands before handling ready-to-eat meat is an important practice (Table 17). Wearing protective clothing was rated as important by 33.6% (36) of respondents who agreed and 41.1% who strongly agreed, with only 2 (1.9%) strongly disagreeing. The importance of following public health officers' recommendations was also high, with 42.1% (45) agreeing and 44.9% (48) strongly agreeing.

Hand hygiene was supported by over 50% (54) agreeing and strongly agreeing that using soap and clean water each time is necessary. On meat safety practices, 46.7% (50) agreed and 39 (36.5%) strongly agreed that covering food prevents contamination. Additionally, 40 (37.3%) agreed and 52 (48.6%) strongly agreed that spoiled meat should be disposed of rather than sold at a discount the following day (Table 18).

Training was positively perceived by 34 (31.9%) agreeing and 48.6% (52) strongly agreeing that they would attend free food safety training, only 2% (2) disagreed. The necessity to get regular training received neutral responses where 42% (45) neutral on the practice with only 18% (20) strongly agreeing to it. The responsibility of ensuring food safety should be a shared responsibility between the seller and the consumer of the RTE meat but only 41.1% (45) that it is a shared responsibility. Some (24%, 26) retailers shifted the blame to the consumer to be fully responsible for food safety without them being involved. Confidence in personal hygiene practices was not high, with 35.5% (38) agreed while 19.6 % (21) were neutral on the effectiveness of their practices in ensuring food safety.

Table 17: Food safety attitudes of ready-to-eat beef and chicken meat retailers

Attitude Questions	Responses (N (%))				
	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
1. I believe washing hands before handling ready-to-eat meat is very important.	5 (4.7)	4 (3.7)	11 (10.3)	33 (30.8)	5 (0.5)
2. Maintaining a clean selling environment helps prevent diseases.	54 (50.5)	16 (15.0)	13 (12.2)	12 (11.2)	12 (11.2)
3. It is acceptable to reheat cooked meat only when a customer asks.	1 (0.9)	2 (1.9)	8 (7.5)	49 (45.8)	47 (43.9)
4. I feel that regular training on food hygiene is necessary.	11 (10.3)	12 (11.2)	45 (42.1)	19 (17.8)	20 (18.7)
5. Wearing protective clothing (e.g., aprons, gloves) while handling meat is important.	2 (1.9)	4 (3.7)	21 (19.6)	36 (33.6)	44 (41.1)
6. I am confident that my current hygiene practices are enough to prevent foodborne illnesses.	3 (2.8)	11 (10.3)	21 (19.6)	38 (35.5)	34 (31.8)
7. Selling meat in open-air conditions significantly affect its safety.	12 (11.2)	10 (9.4)	27 (25.2)	37 (34.4)	21 (19.6)
8. Using soap and clean water every time I wash my hands is always necessary.	11 (10.3)	15 (14.0)	12 (11.2)	18 (16.8)	51 (47.7)
9. It is important to follow public health officers' recommendations about meat hygiene.	1 (0.9)	2 (1.9)	11 (10.3)	45 (42.1)	48 (44.9)
10. Food safety is a shared responsibility between the seller and the customer.	1 (0.9)	7 (6.5)	16 (15.0)	44 (41.1)	39 (36.5)
11. Covering food is a good way to prevent contamination.	1 (0.9)	3 (2.0)	14 (13.1)	50 (46.7)	39 (36.5)
12. I would attend food safety training if offered for free.	1 (0.9)	1 (0.9)	19 (17.8)	34 (31.9)	52 (48.6)
13. Disposing of spoiled meat is better than selling it at a discount.	8 (7.5)	1 (0.9)	6 (5.6)	40 (37.3)	52 (48.6)
14. It is the customer's job to ensure meat is safe before eating.	1 (0.9)	9 (8.4)	35 (32.7)	26 (24.3)	36 (33.6)

N = 106

#### 4.2.4 Food Safety Practices of RTE Beef and Chicken Meat Retailers

These results show that most ready-to-eat meat retailers reported good hygiene practices in certain areas, though notable gaps remain (Table 18). High compliance was observed in key hand hygiene measures. Almost all respondents (91.6%, 98) washed their hands with soap before handling meat, and an even larger proportion (98.1%, 105) washed hands after using the toilet before returning to food handling. Covering meat to protect it from dust and flies was also widely practiced (92.5%, 99). Hygiene practices such as using safe, treated water for cleaning (87; 81.3%), daily cleaning of storage or display equipment (73.8%, 79), and washing/disinfecting equipment and utensils (77; 71.9%) were highly practiced. Similarly, 72.0% (77) reheated meat kept for over two hours before sale, and 71.0% (76) stored unsold cooked meat in under refrigeration.

Moderate compliance was noted for glove use during meat handling where only 58.9% (63) were involved in the practice. The act of discarding leftover meat at day's end of business day was carried out by 58.9% (63) only posing a public health risk by the remaining 41.1% (44) who keep the leftovers for sale the next day. Food safety risk was evident in equipment separation and temperature control. Only 56 (52.3%) used separate knives and boards for raw and cooked meat, while 46.7% (50) monitored the temperature of stored cooked meat.

Table 18: Food safety hygiene practices of ready-to-eat beef and chicken meat retailers (N= 106)

Practice Questions	Yes (N (%))	No (N (%))
1. Do you wash your hands with soap before handling meat	98 (91.6)	9 (8.4)
2. Do you wash your hands after using the toilet before handling food again	105 (98.1)	2 (1.9)
3. Do you sanitize your hands at any moment	59 (55.1)	48 (44.9)
4. Do you use separate equipment (knives, boards) for raw and cooked meat	56 (52.3)	51 (47.7)
5. Do you wash and sterilize your equipment and utensils	77 (71.9)	30 (28.0)
6. Do you store unsold cooked meat in a refrigerator or cooler	76 (71.0)	31 (29.0)
7. Do you check the temperature of stored cooked meat	50 (46.7)	57 (53.3)
8. Do you cover meat to protect it from dust and flies	99 (92.5)	7 (6.5)
9. Do you clean your storage or display equipment daily	79 (73.8)	28 (26.2)
10. Do you ensure the water used for cleaning is safe and treated	87 (81.3)	20 (18.7)
11. Do you wear gloves when handling ready-to-eat meat	63 (58.9)	44 (41.1)
12. Do you reheat meat before sale when it has been kept for more than 2 hours	77 (72.0)	30 (28.0)
13. Do you discard leftover meat at the end of the day	63 (58.9)	44 (41.1)

#### 4.2.5 Relationship between Socio-Demographic Characteristics and Food Safety Knowledge, Attitude and Practices

The analysis exploring associations between socio-demographic characteristics with KAP scores on food safety had several statistically significant relationships ( $P < 0.05$ ) as shown in Table 19. Education level was strongly associated with food safety knowledge ( $P = 0.0001$ ). Mean scores increased progressively from primary education

(13.9 ± 9.2) to university (28.3 ± 6.2), indicating higher education correlates with greater knowledge. Possession of a food handling certificate (P = 0.0116) and attendance at food handling training (P < 0.0001) were also linked to higher knowledge scores; trained respondents scored (25.6 ± 7.6) versus (19.5 ± 7.4) untrained. Retailers who also sold raw meat had higher knowledge (25.5 ± 7.0) than those who did not sell both (22.1 ± 8.4; P = 0.0016). Town-level differences were significant (P = 0.0282), with Nkubu recording the highest mean score in knowledge (27.5 ± 4.1) and Chuka the lowest (19.1 ± 5.7).

Table 19: Relationship between socio-demographic characteristics and ready-to-eat meat retailers' knowledge, attitude, and practice scores regarding food safety in handling and processing

		Food Safety Knowledge			Food Safety Attitude		
		Mean ± SD	Mean Rank	P-value	Mean ± SD	Mean Rank	P-value (α = 0.05)
Gender	Female	23.4 ± 7.6	55.0	0.7549	52.5 ± 7.1	58.7	0.1440
	Male	22.9 ± 8.6	53.1		49.8 ± 7.8	49.9	
Age	18 -20	25.2 ± 5.8	60.7	0.6379	53.1 ± 5.7	65.6	0.0524
	21- 30	23.8 ± 8.6	58.0		50.0 ± 4.4	50.0	
	31 - 40	22.6 ± 7.9	50.8		53.7 ± 7.3	63.1	
	41- 50	22.6 ± 8.1	52.5		48.8 ± 6.5	40.8	
	Above 50	21 ± 9.8	46.6		49.0 ± 2.6	49.8	
Experience	< 1 year	23.1 ± 7.7	52.9	0.7164	51.3 ± 6.5	56.2	0.8455
	1 - 5 years	22.3 ± 9.1	52.2		51.0 ± 6.2	52.5	
	> 5 years	24.3 ± 7.0	58.2		50.6 ± 0.6	52.9	
Education Level	Primary	13.9 ± 9.2	23.5	0.0001	50.2 ± 6.2	56.6	0.5163
	Secondary	23.1 ± 6.1	50.4		50.9 ± 6.4	51.6	
	College	24.9 ± 7.6	61.6		51.2 ± 6.7	52.0	
	University	28.3 ± 6.2	79.3		51.8 ± 14	65.9	
Food Handling Certificate	Yes	24.9 ± 7.0	60.8	0.0116	51.3 ± 8.1	56.4	0.3771
	No	20.9 ± 8.7	45.6		50.6 ± 7.0	51.1	
Food Handling training	Yes	25.6 ± 7.6	66.0	0.0000	52.5 ± 8.8	60.7	0.0080
	No	19.5 ± 7.4	36.8		48.9 ± 4.7	44.5	
Also sells raw Meat	Yes	25.5 ± 7.0	58.6	0.0016	52.3 ± 9.5	59.8	0.0768
	No	22.1 ± 8.4	45.3		49.9 ± 5.3	49.1	
County	Meru	25.4 ± 5.0	58.6	0.1978	49.9 ± 5.4	44.3	0.003
	Embu	22.1 ± 11	57.0		49.2 ± 2.8	68.6	
	TNC	21.8 ± 7.0	46.5		54.1 ± 1.7	50.2	

Table 19: (Continued)

		Food Safety Practices		P -value, ( $\alpha = 0.05$ )
		Mean	Mean Rank	
Gender	Female	22.1 $\pm$ 2.8	49.2	0.2990
	Male	22.6 $\pm$ 3.5	55.4	
Age	18 -20	23.1 $\pm$ 2.4	23.1	0.7934
	21- 30	22.7 $\pm$ 2.8	22.7	
	31 - 40	22.0 $\pm$ 3.2	22.4	
	41- 50	22.2 $\pm$ 3.7	22.2	
	Above 50	21.5 $\pm$ 4.0	21.5	
Experience	< 1 year	21.5 $\pm$ 3.0	44.4	0.0974
	1 - 5 years	22.9 $\pm$ 3.1	57.6	
	> 5 years	22.8 $\pm$ 3.4	57.5	
Education Level	Primary	20.1 $\pm$ 2.8	32.4	0.0006
	Secondary	21.7 $\pm$ 3.1	45.3	
	College	23.4 $\pm$ 3.0	63.0	
	University	24.3 $\pm$ 2.1	70.8	
Food Handling Certificate	Yes	22.7 $\pm$ 3.2	55.9	0.2110
	No	22.0 $\pm$ 3.1	48.4	
Food Handling training	Yes	23.6 $\pm$ 2.6	64.0	<0.0001
	No	20.4 $\pm$ 3.0	34.9	
Also sells raw Meat	Yes	23.4 $\pm$ 2.7	61.6	0.0042
	No	21.5 $\pm$ 3.3	44.7	
County	Meru	22.4 $\pm$ 2.9	53.4	0.0053
	Embu	23.6 $\pm$ 3.2	63.9	
	TNC	21.1 $\pm$ 3.1	40.2	
Towns	Meru	22.4 $\pm$ 2.8	53.8	0.0001
	Nkubu	23.2 $\pm$ 2.4	57.7	
	Embu	24.0 $\pm$ 3.3	68.6	
	Runyenjes	22.6 $\pm$ 2.7	52.5	
	Chuka	19.0 $\pm$ 2.2	22.2	
	Ndagani	23.0 $\pm$ 2.7	57.1	

Significant associations were found with food handling training ( $P = 0.0080$ ), county ( $P = 0.003$ ), and town ( $P = 0.0001$ ). Training improved attitudes ( $52.5 \pm 8.8$ ) compared to no training ( $48.9 \pm 4.7$ ). Embu County had the highest mean rank (68.6), indicating more positive attitudes, while Meru had the lowest (44.3). At town level, Embu town recorded the highest score ( $54.9 \pm 13.7$ ), while Chuka had the lowest ( $47.3 \pm 3.1$ ). Education level was significantly related to food safety practices ( $P = 0.0006$ ), with university-educated retailers scoring highest ( $24.3 \pm 2.1$ ) and those with primary education lowest ( $20.1 \pm 2.8$ ). Training had a strong effect ( $P < 0.0001$ ), as trained respondents reported better practices ( $23.6 \pm 2.6$ ) than untrained ( $20.4 \pm 3.0$ ). Selling raw meat alongside RTE products was linked to better practice scores ( $P = 0.0042$ ).

County differences were significant ( $P = 0.0053$ ), with Embu having the highest practice score ( $23.6 \pm 3.2$ ) and TNC the lowest ( $21.1 \pm 3.1$ ). Town-level variations ( $P = 0.0001$ ) showed Embu town leading ( $24.0 \pm 3.3$ ) and Chuka trailing ( $19.0 \pm 2.2$ ). Gender, age, and experience were not significantly associated with any of the KAP domains ( $P > 0.05$ ), although younger groups (18–20 years) tended to have slightly higher knowledge and attitude mean ranks. The most consistent predictor of higher KAP scores was participation in food handling training, which significantly improved KAP. Education also played a substantial role, particularly in knowledge and practices. Geographic differences, both at county and town level, showed significant differences in KAP, suggesting targeted interventions may be more effective than a general approach.

#### **4.2.6 Mean Scores of Knowledge Attitude and Practices of Ready-to-eat Beef and Chicken Meat Retailers in Different Counties**

The findings of this study reveal notable variations in food safety knowledge, attitudes, and practices (KAP) among RTE beef and chicken meat handlers in Tharaka-Nithi, Meru, and Embu Counties (Table 20). For knowledge, Meru recorded the highest mean score ( $25.4 \pm 5.1$ ), interpreted as satisfactory, while Tharaka Nithi ( $21.8 \pm 6.9$ ) and Embu ( $22.1 \pm 11.0$ ) both showed neutral knowledge levels. Notably, Embu had the widest score range (0–32), indicating substantial disparities among handlers in the same county.

In terms of attitude, Embu scored the highest mean ( $54.1 \pm 11.7$ ) with a satisfactory interpretation, reflecting generally positive perceptions toward food safety. Tharaka Nithi also demonstrated a satisfactory attitude ( $50.0 \pm 4.8$ ), whereas Meru, despite relatively high scores ( $49.1 \pm 2.8$ ), was interpreted as neutral. This suggests that although Meru handlers had moderate attitudes, they may lack full commitment to best food safety practices. For practices, Embu again led with the highest mean ( $23.6 \pm 3.2$ ), achieving a satisfactory interpretation. Meru ( $22.4 \pm 2.9$ ) and Tharaka Nithi ( $20.9 \pm 3.1$ ) both fell into the neutral category, pointing to gaps in actual application of safe handling measures despite some knowledge and attitude strengths. Generally, Embu County demonstrated the strongest performance, excelling in attitude and practices, while Meru excelled in knowledge but lagged in attitude and practices. Tharaka Nithi showed

consistently neutral scores across knowledge and practices, with only attitudes reaching satisfactory levels

Table 20: Mean scores of ready-to-eat meat handlers' food safety knowledge, attitude and practices in Embu, Meru and Tharaka Nithi counties (N = 106)

	County	Mean	SD	Minimum Score	Maximum Score	Interpretation (Based on Mean)	P > F
Knowledge	Tharaka Nithi	21.8	6.9	8	31	Neutral	0.0367
	Meru	25.4	5.1	11	32	Satisfactory	0.0037
	Embu	22.1	11.0	0	32	Neutral	0.6594
Attitude	Tharaka Nithi	50.0	4.8	40	58	Satisfactory	0.0001
	Meru	49.1	2.8	44	57	Neutral	0.1489
	Embu	54.1	11.7	14	70	Satisfactory	0.5443
Practices	Tharaka Nithi	20.9	3.1	15	26	Neutral	0.0000
	Meru	22.4	2.9	16	26	Neutral	0.0512
	Embu	23.6	3.2	15	26	Satisfactory	0.2474

The overall assessment of food safety among RTE meat handlers across the three counties shows mixed performance (Table 21). Knowledge recorded a mean score of  $23.1 \pm 8.0$ , with scores ranging from 0 to 32, interpreted as satisfactory, indicating a generally acceptable understanding of food safety concepts despite some respondents scoring very low. Attitude towards food safety had a mean score of  $51.0 \pm 7.6$  (range 14–70), interpreted as neutral, suggesting that while some handlers value safe food handling, others may lack strong commitment or motivation to consistently apply good practices. Practices scored a mean of  $22.3 \pm 3.2$ , ranging from 15 to 26, also interpreted as neutral, reflecting inconsistencies between knowledge and actual behaviour. Overall, while knowledge levels are relatively satisfactory, the neutral ratings in both attitude and practices point to a need for targeted interventions, especially in translating knowledge into consistent, positive attitudes and safe handling behaviours in the RTE meat retail sector.

Table 21: Overall comparison on level of food safety knowledge attitude and practices in the three study counties

	Mean	SD	Minimum Score	Maximum score	Interpretation
Knowledge	23.1	8.0	0	32	Satisfactory
Attitude	51.0	7.6	14	70	Neutral
Practices	22.3	3.2	15	26	Neutral

### 4.3 Microbial Contamination Levels of Raw Beef and Chicken Meat with Coliforms, *Escherichia coli* and Antibiotic Resistant *Escherichia coli*

All raw beef and chicken meat were contaminated with coliforms and *E. coli* (prevalence of 100% (109)). The test of the fitted model indicated that it was statistically adequate ( $P < 0.05$ ) in explaining the linear relationship between coliform counts, *E. coli* and AR *E. coli* from raw beef and chicken meat and the factors considered (counties, towns, and sample types) (Appendix 6). A significant difference ( $P < 0.05$ ) was observed between coliform counts *E. coli* and AR *E. coli*, towns, and sample types (Appendix 6). Further analysis of the main effects revealed that counties, towns, and sample types each had a significant influence ( $P < 0.05$ ) on coliform counts *E. coli* and AR *E. coli* (Appendix 7).

The highest total coliform counts (TCC) were recorded in chicken meat from Meru Town (8.32  $\text{Log}_{10}$  CFU/g), followed closely by chicken from Ndagani & Mitheru (8.18  $\text{Log}_{10}$  CFU/g) (Table 22). In contrast, the lowest TCC values were observed in beef from Nkubu Town (3.23  $\text{Log}_{10}$  CFU/g) and Runyenjes Town (3.71  $\text{Log}_{10}$  CFU/g). For *E. coli*, the highest counts were in chicken from Embu Town (5.69  $\text{Log}_{10}$  CFU/g) and chicken from Meru Town (4.64  $\text{Log}_{10}$  CFU/g), while the lowest were in beef from Ndagani & Mitheru (1.40  $\text{Log}_{10}$  CFU/g). Antimicrobial-resistant *E. coli* counts followed a similar trend, peaking in chicken from Meru Town (3.22  $\text{Log}_{10}$  CFU/g) and Nkubu (2.70  $\text{Log}_{10}$  CFU/g), but were minimal in beef from Ndagani and Mitheru (0.47  $\text{Log}_{10}$  CFU/g) and Embu Town (0.68  $\text{Log}_{10}$  CFU/g). Proportions of AR *E. coli* relative to total *E. coli* were highest in chicken from Meru Town (69.40%) and Nkubu (62.94%), as well as beef from Nkubu (58.22%), indicating substantial antimicrobial resistance risks.

Overall, all raw meat samples (100%, 109) exceeded the maximum permissible limit for TCC (2.5  $\text{Log}_{10}$  CFU/g), and more than 80% (87) surpassed the acceptable limit for *E. coli* (2  $\text{Log}_{10}$  CFU/g). The results clearly identify raw chicken meat, particularly from Meru County, as the most contaminated and potentially hazardous to consumers. The findings raise serious public health concerns for populations in Meru, Embu and Tharaka Nithi counties.

Table 22: Mean Microbial Counts ( $\text{Log}_{10}$  CFU/g) of Raw Beef and Chicken Meat in Different Counties and Towns (N= 109)

Category	Characteristics	TCC	<i>E. coli</i>	AR <i>E. coli</i>	% AR <i>E. coli</i> of <i>E. coli</i>
Meat	Chicken	7.35 ± 1.21 a	4.21 ± 0.31 a	2.08 ± 0.12 a	49.41
	Beef	4.90 ± 0.41 b	2.94 ± 0.21 b	1.01 ± 0.01 b	34.35
Counties	TNC	6.28 ± 1.02 a	3.21 ± 0.03 b	1.55 ± 0.05 a	48.29
	Meru	5.35 ± 0.05 b	3.37 ± 0.04 a	1.41 ± 0.02 a	41.84
	Embu	5.35 ± 0.06 b	3.23 ± 0.03 b	0.96 ± 0.04 b	29.72
Town	Chuka	6.45 ± 0.06 a	3.75 ± 0.34 b	1.84 ± 0.02 a	49.07
	Meru	6.16 ± 0.61 b	3.84 ± 0.33 a	1.15 ± 0.01 b	29.95
	N & M	6.00 ± 0.32 c	2.28 ± 0.23 e	1.06 ± 0.00 b	46.49
	Embu	5.96 ± 0.25 c	3.79 ± 0.41 ab	1.00 ± 0.01 b	26.39
	Runyenjes	4.68 ± 0.22 d	2.60 ± 0.24 d	0.89 ± 0.02 b	34.23
	Nkubu	4.38 ± 0.15 e	2.82 ± 0.14 c	1.02 ± 0.02 b	36.17

Means followed by the same letter in a column within a category are not significantly different at 5% probability level. TCC (Total coliform counts), TNC (Tharaka-Nithi county), N & M (Ndagani and Mitheru)

The prevalence of antimicrobial-resistant (AR *E. coli*) relative to total *E. coli* counts was highest in chicken meat from Meru Town (69.4%, 75), while the lowest was observed in beef from the same town (Table 23). Across the region, chicken meat consistently exhibited significantly higher mean counts of AR *E. coli* (2.08  $\text{Log}_{10}$  CFU/g) compared to beef (1.01  $\text{Log}_{10}$  CFU/g) ( $P < 0.05$ ), with Tharaka Nithi County recording the highest overall AR *E. coli* load (1.55  $\text{Log}_{10}$  CFU/g).

Table 23: Mean microbial counts ( $\text{Log}_{10}$  CFU/g) of raw beef and chicken meat by county, town, and meat source, and the percentage of antimicrobial-resistant (AR) *E. coli* (N = 109).

Meat Source	County	Town	TCC	<i>E. coli</i>	AR <i>E. coli</i>	% AR <i>E. coli</i> of <i>E. coli</i>
Chicken	Meru	Meru	8.32 ± 0.81 <sup>a</sup>	4.64 ± 0.44 <sup>b</sup>	3.22 ± 0.21 <sup>a</sup>	69.40
	TNC	N&M	8.18 ± 0.41 <sup>a</sup>	3.83 ± 0.31 <sup>d</sup>	2.10 ± 0.11 <sup>b</sup>	54.83
	Meru	Nkubu	7.34 ± 0.72 <sup>b</sup>	4.29 ± 0.41 <sup>c</sup>	2.70 ± 0.21 <sup>a</sup>	62.94
	Embu	Embu	7.19 ± 0.71 <sup>b</sup>	5.69 ± 0.55 <sup>a</sup>	1.64 ± 0.03 <sup>bcd</sup>	28.82
	TNC	Chuka	6.84 ± 0.66 <sup>c</sup>	2.32 ± 0.23 <sup>g</sup>	1.36 ± 0.02 <sup>d</sup>	58.62
	Embu	Runyenjes	6.62 ± 0.61 <sup>d</sup>	3.87 ± 0.33 <sup>d</sup>	1.42 ± 0.01 <sup>cd</sup>	36.69
Beef	TNC	Chuka	6.31 ± 0.61 <sup>e</sup>	4.26 ± 0.41 <sup>c</sup>	2.01 ± 0.02 <sup>bc</sup>	47.18
	Meru	Meru	5.73 ± 0.51 <sup>f</sup>	3.68 ± 0.41 <sup>e</sup>	0.74 ± 0.01 <sup>f</sup>	20.11
	Embu	Embu	5.29 ± 0.53 <sup>g</sup>	2.77 ± 0.21 <sup>f</sup>	0.68 ± 0.00 <sup>f</sup>	24.55
	TNC	N&M	4.76 ± 0.44 <sup>h</sup>	1.40 ± 0.01 <sup>i</sup>	0.47 ± 0.01 <sup>f</sup>	33.57
	Embu	Runyenjes	3.71 ± 0.31 <sup>i</sup>	1.97 ± 0.02 <sup>h</sup>	0.63 ± 0.01 <sup>f</sup>	31.98
	Meru	Nkubu	3.23 ± 0.33 <sup>j</sup>	2.25 ± 0.03 <sup>g</sup>	1.31 ± 0.01 <sup>de</sup>	58.22

Means followed by the same letter in a column are not significantly different at 5% probability level. TCC (Total coliform counts), TNC (Tharaka Nithi County), N&M (Ndagani and Mitheru).

Town-level comparisons showed that Chuka Town had the greatest contamination (1.84 Log<sub>10</sub> CFU/g), followed by Meru Town (1.15 Log<sub>10</sub> CFU/g), while Runyenjes recorded the lowest (0.89 Log<sub>10</sub> CFU/g) (Table 23). These findings highlight that chicken meat as the predominant source of AR *E. coli* contamination in the region, with important public health implications.

#### **4.4 Prevalence and Microbial Quality Assessment of Ready-to-Eat Beef and Chicken Meat**

Chicken meat samples showed higher TCC contamination compared to beef across most towns in prevalence (Figure 9). Chicken meat samples had consistently high TCC, with peaks in Chuka (90%, 19) and Nkubu (90%, 14). This was followed by N&M (80%, 8) and Embu (83%, 16), while Meru (70%, 18) and Runyenjes (71%, 11) showed the lowest. The prevalence of TCC in beef was highest in Ndagani & Mitheru (90%, 9) and Meru Town (85%, 21), followed by Chuka (80%, 17), Nkubu (70%, 7), Embu Town (75%, 11), and lowest in Runyenjes (60%, 10). In the prevalence of *E. coli* chicken meat generally exhibited higher *E. coli* contamination than beef in most towns, except in Meru where beef was higher. For chicken meat higher prevalence was recorded in Chuka (70%, 14) and Embu (60%, 12), followed by Ndagani, Mitheru and Runyenjes (50%, 10) while Nkubu (30%, 6) and Meru (40%, 8) had the lowest positive samples with *E. coli*.

Antibiotic resistant *E. coli* was harboured more in chicken samples compared to beef samples with hot spots recorded in N&M, Embu and Nkubu. For beef prevalence of AR *E. coli* was highest in Nkubu (20%, 17) and Chuka (20%, 17), followed closely by Embu (16%, 13) and Runyenjes (14%, 12). Lower values were seen in N&M (15%, 13) and Meru (10%, 9). Chicken meat on the other hand had AR *E. coli* peaking in N&M (20%, 17) and Embu (20%, 17), followed by Nkubu (19%, 5) and Runyenjes (17%, 4). Lower levels were observed in Chuka (10%, 2) and Meru (10%, 2). Overall the prevalence of TCC, *E. coli* and AR *E. coli* for RTE beef was 77%, 48% and 16% while for chicken meat it was; 81%, 50% and 17% respectively.

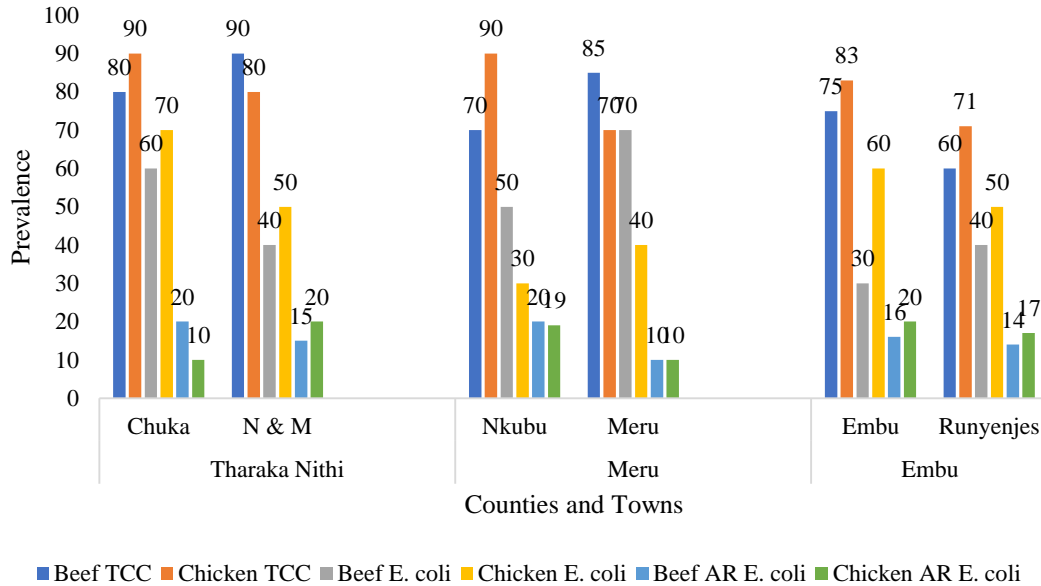


Figure 9: Prevalence of Total Coliform Counts (TCC), *Escherichia coli* (*E. coli*) and Antibiotic Resistant *E. coli* isolated from ready-to-eat beef and chicken meat in different counties. (N&M = Ndagani and Mitheru)

The test of the fitted model indicated that it was statistically adequate ( $P < 0.05$ ) in explaining the linear relationship between coliform counts, *E. coli* and AR *E. coli* from ready-to-eat beef and chicken meat and the factors considered (counties, towns, and sample types) (Appendix 8). A significant interaction ( $P < 0.05$ ) was observed between coliform counts *E. coli* and AR *E. coli*, towns, and sample types (Appendix 9). Further analysis of the main effects revealed that counties, towns, and sample types each had a significant influence ( $P < 0.05$ ) on coliform counts *E. coli* and AR *E. coli* (Appendix 9).

The microbial quality assessment of ready-to-eat (RTE) meat showed clear differences between beef and chicken across towns and counties (Table 24). Overall, beef samples exhibited significantly higher total coliform counts (TCC:  $4.19 \text{ Log}_{10} \text{ CFU/g}$ ) and *E. coli* contamination ( $1.99 \text{ Log}_{10} \text{ CFU/g}$ ) compared to chicken ( $2.41$  and  $0.75 \text{ Log}_{10} \text{ CFU/g}$ ), respectively ( $P < 0.05$ ). At the town level, the highest TCC was observed in Embu ( $4.23 \text{ Log}_{10} \text{ CFU/g}$ ), while the lowest was in Ndagani and Mitheru ( $2.15 \text{ Log}_{10} \text{ CFU/g}$ ). Similarly, *E. coli* contamination peaked in beef from Meru ( $2.24 \text{ Log}_{10} \text{ CFU/g}$ ) and was lowest in chicken from Chuka ( $1.11 \text{ Log}_{10} \text{ CFU/g}$ ).

In contrast, antimicrobial-resistant *E. coli* (AR *E. coli*) was more prevalent in chicken ( $0.26 \text{ Log}_{10} \text{ CFU/g}$ ; 34.67%) than in beef ( $0.18 \text{ Log}_{10} \text{ CFU/g}$ ; 9.05%) ( $p < 0.05$ ).

The highest AR *E. coli* counts were recorded in chicken from Embu (0.36 Log<sub>10</sub> CFU/g), whereas beef samples showed a maximum of 0.24 Log<sub>10</sub> CFU/g in Meru. Notably, the lowest resistance levels were detected in chicken from Ndagani and Mitheru (0.01 Log<sub>10</sub> CFU/g; 0.81%). These findings indicate that while beef generally carried higher overall bacterial loads in RTE form, chicken posed the greater risk as a reservoir of antimicrobial-resistant *E. coli*, with geographical variation strongly influencing the contamination burden.

Table 24: Mean microbial counts (Log<sub>10</sub> CFU/g) of ready-to-eat beef and chicken meat by county, and town (N = 106).

Category	Characteristics	TCC	<i>E. coli</i>	AR <i>E. coli</i>	% AR <i>E. coli</i> of <i>E. coli</i>
Type of sample	Beef	4.19 ± 0.34 a	1.99 ± 0.01 a	0.18 ± 0.01 b	9.05
	Chicken meat	2.41 ± 0.22 b	0.75 ± 0.00 b	0.26 ± 0.02 a	34.67
Counties	Meru	4.09 ± 0.31 a	1.96 ± 0.02 a	0.19 ± 0.01 b	9.69
	Embu	3.88 ± 0.31 b	1.67 ± 0.05 b	0.23 ± 0.02 a	13.77
	TNC	2.88 ± 0.18 c	1.15 ± 0.02 b	0.12 ± 0.01 c	10.43
Towns	Embu	4.23 ± 0.39 a	1.88 ± 0.01 b	0.36 ± 0.03 a	19.15
	Meru	4.19 ± 0.41 b	2.24 ± 0.02 a	0.19 ± 0.01 b	8.48
	Nkubu	3.92 ± 0.39 c	1.47 ± 0.03 c	0.19 ± 0.01 bc	12.93
	Runyenjes	3.42 ± 0.33 d	1.40 ± 0.12 d	0.21 ± 0.02 b	15.00
	Chuka	3.23 ± 0.23 e	1.11 ± 0.03 f	0.17 ± 0.02 c	15.32
	N & M	2.15 ± 0.22 f	1.23 ± 0.02 e	0.01 ± 0.00 d	0.81

Means followed by the same letter in a column per category are not significantly different at 5% probability level. TNC (Tharaka Nithi County), N&M (Ndagani and Mitheru), TCC (Total coliform counts), AR *E. coli* (Antimicrobial Resistant *E. coli*)

The microbial quality assessment of RTE meat revealed marked differences between beef and chicken across towns, counties and meat source (Table 25). Among beef samples, the highest total coliform count (TCC) was observed in Nkubu Town, Meru County (5.66 Log<sub>10</sub> CFU/g), followed closely by Meru Town (5.12 Log<sub>10</sub> CFU/g), while the lowest was recorded in Ndagani & Mitheru (2.29 Log<sub>10</sub> CFU/g). In contrast, chicken samples generally exhibited lower TCC values, ranging from 3.89 Log<sub>10</sub> CFU/g in Embu Town to 1.27 Log<sub>10</sub> CFU/g in Meru Town.

*Escherichia coli* contamination followed a similar trend, with beef samples showing the highest levels in Meru Town (2.87 Log<sub>10</sub> CFU/g) and the lowest in Meru Town chicken (0.22 Log<sub>10</sub> CFU/g). Notably, antimicrobial-resistant *E. coli* (AR *E. coli*) was consistently higher in chicken than in beef. The greatest AR *E. coli* load was detected

in chicken from Embu Town (0.67 Log<sub>10</sub> CFU/g; 48.55% of total *E. coli*), while beef from Ndagani and Mitheru recorded the lowest (0.01 Log<sub>10</sub> CFU/g; 0.65%). Other chicken sources, including Chuka (0.38 Log<sub>10</sub> CFU/g; 44.19%) and Nkubu (0.12 Log<sub>10</sub> CFU/g; 38.71%), also exhibited high proportions of resistant isolates. Overall, these results indicate that while beef generally carried higher bacterial loads in RTE form, chicken was the predominant source of antimicrobial-resistant *E. coli*, with Embu Town posing the greatest contamination risk.

Table 25: Mean microbial counts (Log<sub>10</sub> CFU/g) of ready-to-eat beef and chicken meat by county, town and type of meat (N = 106)

Meat Source	County	Town	TCC	<i>E. coli</i>	AR <i>E. coli</i>	% AR <i>E. coli</i> of <i>E. coli</i>
Beef	Meru	Nkubu	5.66 ± 0.51 a	2.23 ± 0.09 b	0.23 ± 0.02 c	10.31
	Meru	Meru	5.12 ± 0.44 b	2.87 ± 0.38 a	0.24 ± 0.01 c	8.36
	Embu	Embu	4.39 ± 0.35 c	2.11 ± 0.27 c	0.22 ± 0.03 cd	10.43
	TNC	Chuka	3.53 ± 0.21 e	1.19 ± 0.04 f	0.11 ± 0.00 ef	9.24
	Embu	Runyenjes	3.22 ± 0.60 f	1.52 ± 0.06 d	0.21 ± 0.02 cd	13.82
	TNC	N&M	2.29 ± 0.29 g	1.54 ± 0.05 d	0.01 ± 0.00 g	0.65
Chicken	TNC	Chuka	2.29 ± 0.22 g	0.86 ± 0.07 g	0.38 ± 0.03 b	44.19
	TNC	N&M	1.80 ± 0.11 h	0.50 ± 0.02 h	0.02 ± 0.00 g	4.00
	Meru	Nkubu	1.32 ± 0.01 i	0.31 ± 0.01 i	0.12 ± 0.01 e	38.71
	Meru	Meru	1.27 ± 0.02 j	0.22 ± 0.00 j	0.08 ± 0.01 f	36.36
	Embu	Embu	3.89 ± 0.41 d	1.38 ± 0.04 e	0.67 ± 0.05 a	48.55
	Embu	Runyenjes	3.84 ± 0.28 d	1.16 ± 0.03 f	0.20 ± 0.01 d	17.24

Means followed by the same letter in a column are not significantly different at 5% probability level. TNC (Tharaka Nithi County), N&M (Ndagani and Mitheru), TCC (Total coliform counts), AR *E. coli* (Antimicrobial Resistant *E. coli*)

#### 4.5 Antibiotic Sensitivity Profiles of *Escherichia. coli* Isolated from Raw and RTE Beef and Chicken Meat

##### 4.5.1 Antibiotic Susceptibility Profile of *Escherichia. coli* isolated from Raw and Ready-to-Eat Beef Samples

The antibiotic susceptibility profile of *Escherichia coli* isolates from raw beef in Eastern Kenya revealed varying levels of resistance and intermediate susceptibility to different antibiotics (Figure 10). The results reveal that all raw meat isolates (100%, 60) were resistant to amoxicillin (AMX 30), with no intermediate susceptibility recorded. High levels of resistance were also observed for methicillin (MET 10; 63.33%), ampicillin (AMP 10; 53.33%), cefpodoxime (CPD 10; 56.67%), and ceftriaxone (CTR 30; 60%).

Moderate resistance was noted against tetracycline (TE 30) at 40% (24), doxycycline (DO 30) at 26.67% (16), and minocycline (MI 30) at 63.33% (38). In contrast, resistance to gentamicin (GE 10) and chloramphenicol (C 15) was low, at 6.67% (4) and 0%, respectively. Ciprofloxacin (CL 10) also exhibited minimal resistance (3.33%, 2). Sulfamethoxazole-trimethoprim (COT 25) showed 53.33% (32) resistance, while resistance to erythromycin (E 15) and lincomycin (L 2) stood at 13.33% (8) and 0%, respectively. Intermediate susceptibility varied across antibiotics, with notable values for AMP (20%, 12), MET (20%, 12), CPD (23.33%, 14), CTR (40%, 24), TE (46.67%, 28), DO (60%, 36), MI (13.33%, 8), AK (53.33%, 31), GE (76.67%, 46), CL (66.67%, 40), L (83.33%, 50), and E (60%, 36). Overall, the results demonstrate widespread resistance to  $\beta$ -lactam antibiotics (e.g., amoxicillin, ampicillin, ceftriaxone), moderate resistance to tetracycline derivatives, and relatively low resistance to aminoglycosides (gentamicin) and fluoroquinolones (ciprofloxacin).

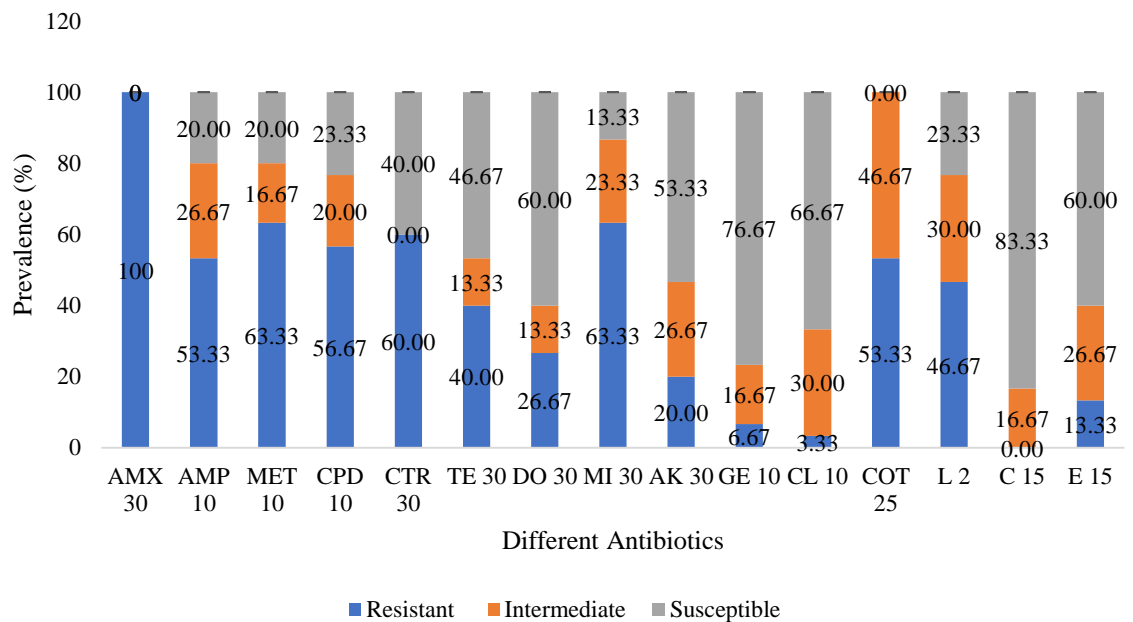


Figure 10: Prevalence of antibiotic resistance among *Escherichia coli* isolates obtained from raw beef in Eastern Kenya. AMX (amoxyl), TE (Tetracycline), MI (Minocycline), CTR (cotrimazole), L (licomycin), MET (methicillin), CPD (cefepodoxime), CTR (ceftriaxone), DO (doxycycline), AK (Amikacin), COT (cotrimazole), E (erythromycin), CL (colistin), GE (gentamicin), C (chloramphenicol)

The antibiotic susceptibility profiles of *E. coli* isolates recovered from ready-to-eat (RTE) beef in Eastern Kenya revealed varying levels of resistance, intermediate susceptibility, and susceptibility to different antibiotics (Figure 11). Among all antibiotics, amoxicillin, exhibited the highest resistance, with 100% (60) of isolates

resistant and none showing intermediate or susceptible responses. Moderate resistance was observed against ampicillin (10 µg; 40%) and methicillin (10 µg; 37%), both of which displayed high intermediate susceptibility rates (around 40–45%). Similarly, cephalosporins such as cefpodoxime (30 µg) and ceftriaxone (30 µg) recorded resistance levels of 33–40%, with substantial proportions of isolates showing intermediate responses (up to 50%, 30).

Tetracyclines demonstrated varied resistance: tetracycline (30 µg) and doxycycline (30 µg) showed relatively low resistance (13–20%, 12), while minocycline (30 µg) exhibited a higher resistance (33%, 20). Nonetheless, intermediate susceptibility was frequent in this group, ranging from 30 – 47% (18- 28), pointing toward a gradual shift toward resistance.

Aminoglycosides, amikacin (30 µg) and gentamicin showed low resistance (10 - 15%) and high susceptibility, with over 50% (30) of isolates fully susceptible, suggesting continued therapeutic potential. Colistin (10 µg), a critical last-line polymyxin, exhibited only 10% (6) resistance, with more than half (57%, 34) of isolates were susceptible.

Cotrimoxazole (25 µg) and lincosamide (2 µg) showed moderate resistance rates of 22 – 28% (17). Chloramphenicol (15 µg) and erythromycin (15 µg) demonstrated the lowest resistance levels ( $\leq 10\%$ ), with susceptibility exceeding 60% (36). These findings highlight that while  $\beta$ -lactams and cephalosporins face considerable resistance, aminoglycosides, colistin, and chloramphenicol remain largely effective against *E. coli* in RTE beef.

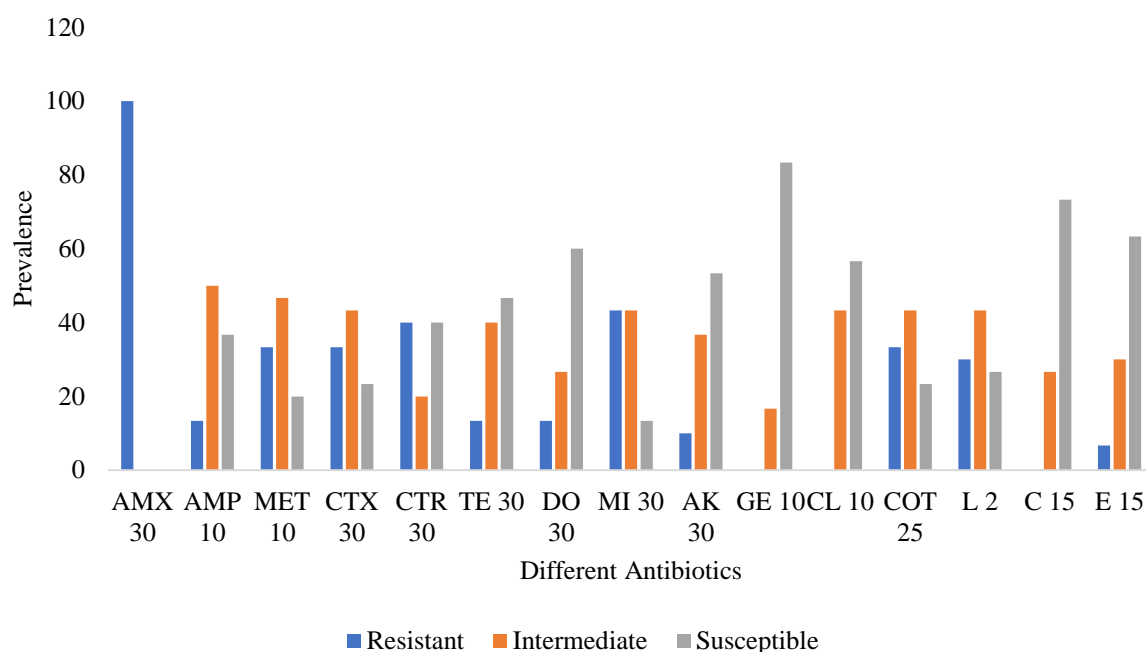


Figure 11: Antibiotic resistance, intermediate susceptibility, and susceptibility profiles of *Escherichia coli* isolates recovered from ready-to-eat beef in eastern Kenya. AMX (amoxyl), TE (Tetracycline), MI (Minocycline), CTR (cotrimazole), L (licomycin), MET (methicillin), CPD (cefepodoxime), CTR (ceftriaxone), DO (doxycycline), AK (Amikacin), COT (cotrimazole), E (erythromycin), CL (colistin), GE (gentamicin), C (chloramphenicol)

#### 4.5.2 Antibiotic Susceptibility Profile of *Escherichia. coli* Isolated from Raw and ready-to-Eat Chicken Meat

The antibiotic susceptibility profile of *Escherichia coli* isolates from raw chicken meat in Eastern Kenya revealed distinct resistance, intermediate susceptibility, and susceptibility patterns (Figure 12). High resistance levels were against methicillin (MET, 10  $\mu$ g) and ampicillin (AMP, 10  $\mu$ g) at 80% (48), followed by amoxicillin (AMX 30  $\mu$ g) at 66.67% (40). Third-generation cephalosporins also showed elevated resistance, with cefepodoxime (CPD, 10  $\mu$ g) and ceftriaxone (CTR, 30  $\mu$ g) both at 60% (36). Moderate resistance was recorded for tetracycline (TE, 30  $\mu$ g) at 50% (30), doxycycline (DO, 30  $\mu$ g) at 66.67% (40), and cotrimoxazole (COT, 25  $\mu$ g) at 63.33% (18).

Lower resistance levels were detected for minocycline (MI, 30  $\mu$ g) at 36.67% (22), amikacin (AK, 30  $\mu$ g) at 26.67%, (16) gentamicin (GE, 10  $\mu$ g) at 16.67% (10), and colistin (C, 15  $\mu$ g) at 16.67% (10). chloramphenicol (CL, 10  $\mu$ g) exhibited moderate resistance at 60% (36), whereas erythromycin (E, 15  $\mu$ g) and lincomycin (L, 2  $\mu$ g)

showed comparatively low resistance rates at 23.33% (14) each. Intermediate susceptibility was highly across antibiotics, with notable values observed for AMP (20%, 12), CPD (30%, 18), CTR (20%, 12), TE (33.33%, 20), DO (10%, 6), MI (40%, 24), AK (53.33%, 32), GE (66.67%, 40), CL (0%), COT (20%, 12), L (63.33%, 38), C (66.67%, 40), and E (36.67%, 22). Overall, *Escherichia coli* isolates from raw chicken meat in Eastern Kenya exhibited high resistance to  $\beta$ -lactams and cephalosporins, moderate resistance to tetracyclines and sulphonamides, but relatively low resistance to aminoglycosides and colistin, with intermediate susceptibility varying widely across antibiotics

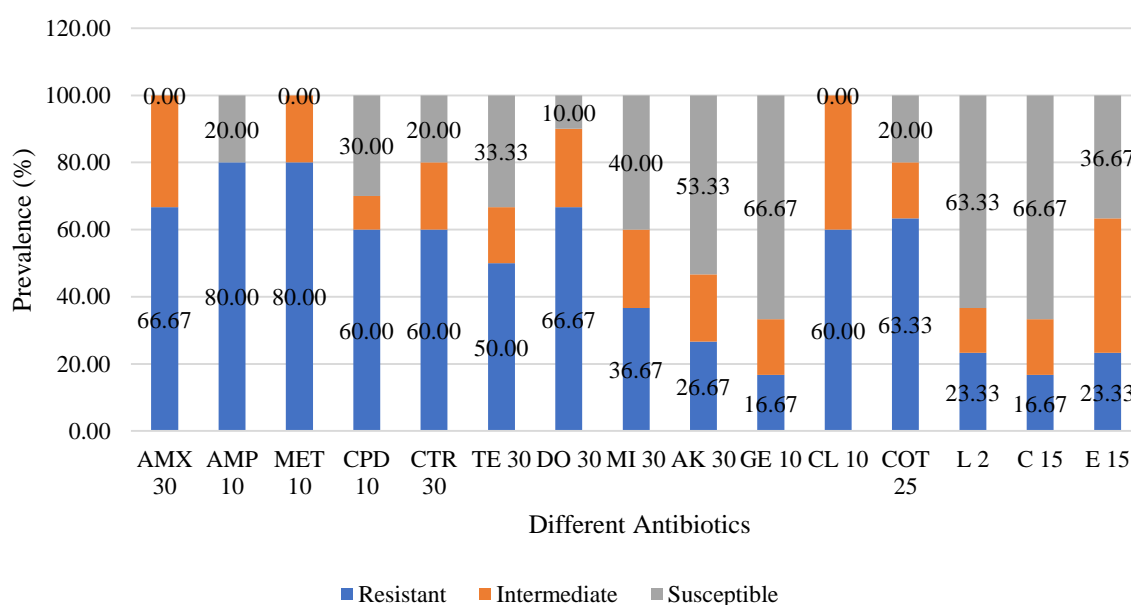


Figure 12: Prevalence of antibiotic resistant *Escherichia coli* isolated from raw chicken meat in Eastern Kenya. AMX (amoxyl), TE (Tetracycline), MI (Minocycline), CTR (cotrimazole), L (licomycin), MET (methicillin), CPD (cefpodoxime), CTR (ceftriaxone), DO (doxycycline), AK (Amikacin), COT (cotrimazole), E (erythromycin), CL (colistin), GE (gentamycin), C (chloramphenicol)

The *E. coli* isolates exhibited widespread antimicrobial resistance, with markedly high prevalence across several agents, particularly those listed by the WHO as Highest Priority Critically Important Antimicrobials (HPCIA) in RTE chicken meat (Figure 13). Resistance was notably high against gentamicin (86.7%, 52), erythromycin (80%, 48), tetracycline (66.7%, 40), doxycycline (66.7%, 40), and ciprofloxacin (66.7%, 40). Moderate resistance levels were observed for chloramphenicol (66.7%, 40), lincomycin (L, 66.7%, 40), cotrimoxazole (66.7%, 40), ceftriaxone (66.7%, 40), cephalixin

(66.7%, 40), and minocycline (76.7%, 46). In contrast, comparatively lower resistance was seen against ampicillin (26.7%, 16) and amikacin (46.7%, 27). Amoxicillin showed no resistance, with isolates being almost entirely susceptible. Overall, the data indicate that *E. coli* isolates from Chicken exhibit multidrug resistance patterns, with alarming levels of resistance to several frontline and critically important antibiotics, highlighting the potential risk to both veterinary and public health.

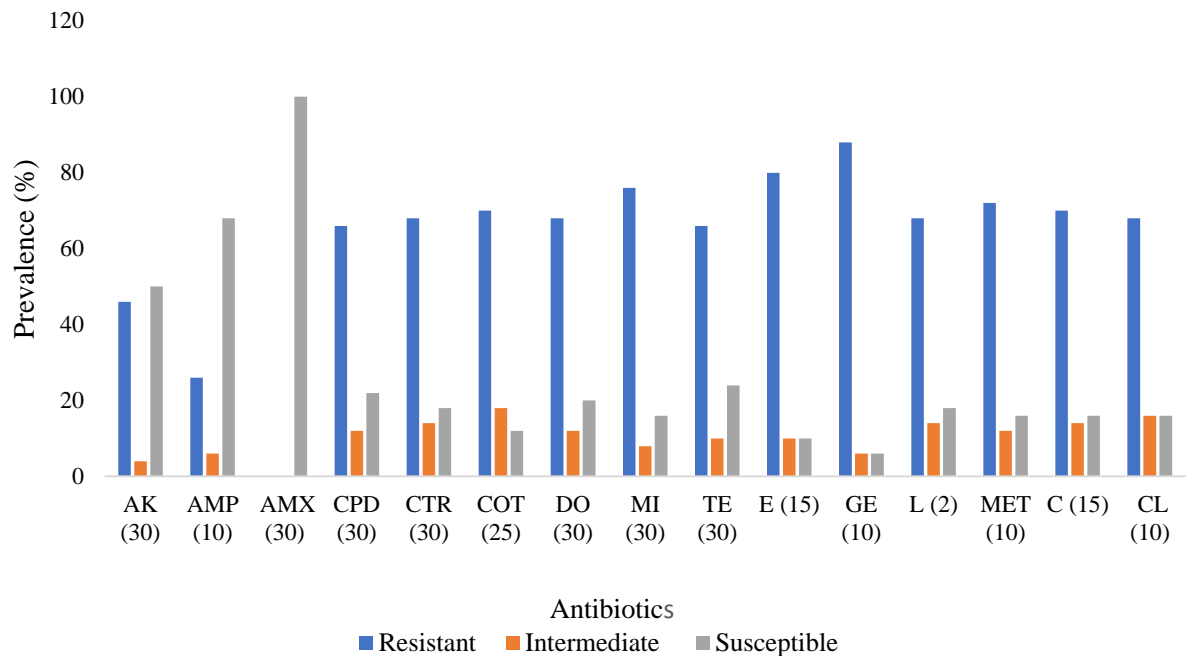


Figure 13: Antibiotic resistant pattern of escherichia coli isolated from ready-to-eat chicken meat in eastern kenya. AMX (amoxyl), TE (Tetracycline), MI (Minocycline), CTR (cotrimazole), L (licomycin), MET (methicillin), CPD (cefepodoxime), CTR (ceftriaxone), DO (doxycycline), AK (Amikacin), COT (cotrimazole), E (erythromycin), CL (colistin), GE (gentamycin), C (chloramphenicol)

#### 4.6 Multiple Antibiotic Resistance of *Escherichia coli* Isolates from Raw and RTE Beef and Chicken Meat

Multiple Antibiotic Resistance (MAR) (Number of antibiotics microorganism is resistant to divided by total number of antibiotics tested) index values of *E. coli* isolates from RTE beef, RTE chicken meat, raw beef, and raw chicken meat across 15 antibiotics had a negative trend for raw chicken meat (Table 26). For RTE beef, MAR prevalence ranged from 3 to 7 colonies, with MAR indices between 0.20 and 0.47, indicating moderate resistance levels. The RTE chicken showed slightly higher variation. Raw beef isolates exhibited MAR counts between 4 and 9 colonies, with

MAR indices from 0.27 to 0.60, indicating greater exposure to multiple antibiotics. Raw chicken meat showed the highest resistance, with MAR colonies ranging from 4 to 11 and MAR indices between 0.27 and 0.73, showing significant antimicrobial exposure and potential public health concern. The findings show that raw chicken meat harboured the highest multidrug resistance and MAR index values, followed by raw beef, RTE chicken, and RTE beef, highlighting raw meats as the greatest reservoirs of antibiotic-resistant *E. coli*.

Table 26: Multiple Antibiotic Resistance Index of *Escherichia coli* Isolates from different samples

	RTE Beef (N =30)	RTE Chicken (N=30)	Raw Beef (N =30)	Raw Chicken (N= 30)
Antibiotics tested	MAR Index	MAR Index	MAR Index	MAR Index
15	0.20	0.20	0.27	0.60
15	0.27	0.40	0.40	0.47
15	0.33	0.33	0.33	0.67
15	0.47	0.20	0.47	0.27
15	0.27	0.40	0.53	0.40
15	0.20	0.33	0.33	0.53
15	0.40	0.20	0.27	0.53
15	0.40	0.27	0.33	0.73
15	0.33	0.27	0.47	0.33
15	0.33	0.40	0.53	0.40
15	0.20	0.40	0.40	0.40
15	0.20	0.20	0.47	0.40
15	0.27	0.27	0.40	0.73
15	0.20	0.27	0.53	0.47
15	0.40	0.33	0.53	0.47
15	0.33	0.27	0.27	0.53
15	0.27	0.53	0.27	0.47
15	0.20	0.20	0.33	0.60
15	0.27	0.53	0.33	0.33
15	0.20	0.40	0.33	0.53
15	0.47	0.20	0.47	0.53
15		0.40	0.33	0.67
15		0.40	0.40	0.40
15		0.33	0.53	0.27
15		0.27	0.53	0.47
15		0.20	0.40	0.53
15		0.20	0.27	0.53
15			0.27	0.60
15			0.40	0.47
15			0.60	0.33

Generally, *E. coli* isolates exhibited resistance to between 3 and 11 antibiotics, reflecting both moderate and extensive resistance profiles (Table 27). In raw beef, resistance was most frequent against six antibiotics (n = 7), particularly the combination of amoxicillin (AMX), ampicillin (AMP), metronidazole (MET), cefpodoxime (CPD), tetracycline (TE), and cotrimoxazole (COT), followed by five-antibiotic resistance patterns (n = 6). Although less common, some isolates showed broader resistance to seven (n = 4), eight (n = 6), or nine (n = 1) antibiotics. Raw chicken isolates displayed wider resistance spectra, with resistance to six antibiotics (n=5) as well as higher frequencies at seven (n = 6), eight (n = 7), ten (n = 3), and even eleven (n = 1) antibiotics, underscoring chicken meat as a stronger reservoir of extensively resistant strains. In contrast, isolates from RTE beef and RTE chicken exhibited lower resistance levels, limited to 3–6 antibiotics, and none exceeded this range, suggesting comparatively lower antimicrobial pressure in ready-to-eat meats. The multi-drug resistance ranged between 31% to 50%. The MAR index was between 0.2 to 0.6 with isolates from raw chicken meat samples recording higher values of 0.33 to 0.6. This was followed by raw beef isolates at 0.27 – 0.53 and RTE beef isolates at 0.2 to 0.47.

Table 27: Resistance Patterns of *Escherichia coli* Isolates from Raw and Ready-to-eat Beef and Chicken

Classes of Antibiotics	Resistant Pattern	Source and Number of Colonies			
		Raw Beef	Raw Chicken meat	RTE Beef	RTE Chicken meat
3	AMX, TE, MI	0	0	6	8
4	AMX, AMP, TE, MI	5	2	5	5
5	AMX, MET, CTR, MI, L	6	2	4	4
6	AMX, AMP, MET, CPD, TE, COT	7	5	3	7
7	AMX, AMP, MET, CPD, CTR, DO, L	4	6	2	0
8	AMX, AMP, MET, CPD, CTR, AK, COT, L	6	7	0	2
9	AMX, AMP, MET, CTR, TE, DO, COT, L, E	1	3	0	0
10	AMX, AMP, CPD, TE, MI, GE, CL, COT, C, E	0	3	0	0
11	AMX, AMP, CTR, TE, DO, AK, COT, C, CL, E, GE	0	1	0	0

RTE (Ready-to-eat), AMX (amoxyl), TE (Tetracycline), MI (Minocycline), CTR (cotrimazole), L (licomycin), MET (methicillin), CPD (cefpodoxime), CTR (ceftriaxone), DO (doxycycline), AK (Amikacin), COT (cotrimazole), E (erythromycin), CL (colistin), GE (gentamycin)

#### **4.7 Prevalence of Antibiotic Resistance Genes of *Escherichia coli* Isolated from Raw and RTE Beef and Chicken Meat in Embu Meru and Tharaka-Nithi County**

The PCR products were visualized against markers and the following visuals were generated (Figure 14). Overall, raw meat consistently exhibited higher prevalence rates of all ARGs tested compared to RTE meat. For  $\beta$ -lactam resistance genes, *bla*<sub>TEM</sub> was detected in approximately 20% (12) of raw samples versus 12% (7) in RTE samples. The gene *bla*<sub>SHV</sub> showed the highest prevalence among  $\beta$ -lactam genes in raw meat (22%, 7) and was markedly lower in RTE meat (6%, 4). The *bla*<sub>OXA</sub> gene was present in 13% (8) of raw and 6% (4) of RTE samples, while *bla*<sub>CTX-M-15</sub> recorded the lowest prevalence in both categories, at around 7% (2) in raw and 5% (2) in RTE meats. The colistin resistance gene (*mcr-1*) was identified in 13% (8) of raw meat samples, compared to only 5% in RTE products. The sulphonamide resistance gene *sul1* was prevalent in 18% (11) of raw samples but reduced to 10% in RTE meat. Among all genes tested, the tetracycline resistance gene *tetA* had the highest overall prevalence, detected in 23% (14) of raw meat and 17% (10) of RTE meat.

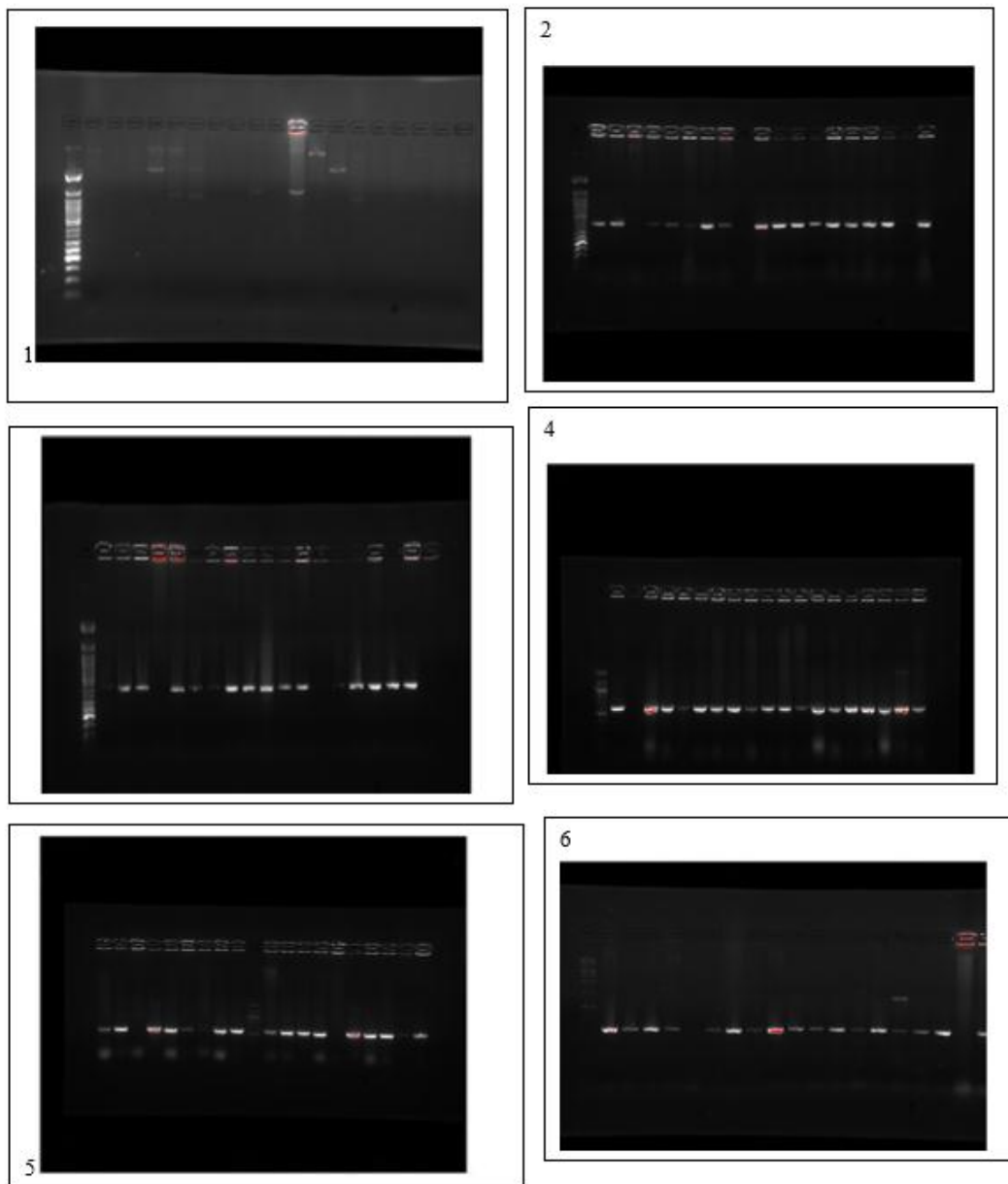


Figure 14: PCR products visualisation for presence of resistance genes 1(*Bla<sub>TEM</sub>*), 2(*Bla<sub>SHV</sub>*), 3(*Bla<sub>OXA</sub>*) 4(*Bla<sub>CTXM-15</sub>*), 5(*mcr-1*), 6(*sul-1* and *Tet A*)

The data show a clear reduction in ARG prevalence from raw to RTE meat across all categories, likely due to the effects of processing and cooking in eliminating or reducing resistant bacteria. However, the persistence of notable levels in RTE meat indicates incomplete elimination of resistance determinants and possible post-processing contamination.

Comparison between counties showed that raw beef, Meru recorded the highest prevalence, notably *bla<sub>SHV</sub>* (40%, 4), *bla<sub>TEM</sub>* (30%, 3), *sul1* (30%, 3), and *tetA* (30%, 3) (Figure 22). Tharaka Nithi and Embu showed lower levels, with *bla<sub>TEM</sub>* and *bla<sub>OXA</sub>* at 20% (2) in Tharaka Nithi, and *bla<sub>SHV</sub>*, *sul1*, and *tetA* at 20% (2/10) in Embu Counties. For raw chicken, Meru again showed high prevalence, particularly in *tetA* (40%, 4/10) and *bla<sub>SHV</sub>* resistance genes (30%, 3). Tharaka Nithi had generally low levels (10–20%, 1-2), while Embu’s highest was *bla<sub>TEM</sub>* (30%, 3). In raw beef, Meru recorded the highest prevalence, notably *bla<sub>SHV</sub>* (40%, 4), *bla<sub>TEM</sub>* (30%, 3), *sul1* (30%, 3), and *tetA* (30%, 3) (Figure 15). Tharaka Nithi and Embu showed lower levels, with *bla<sub>TEM</sub>* and *bla<sub>OXA</sub>* at 20% (2) in Tharaka Nithi, and *bla<sub>SHV</sub>*, *sul1*, and *tetA* at 20% (2) in Embu Counties. For raw chicken, Meru again showed high prevalence, particularly in *tetA* (40%, 4) and *bla<sub>SHV</sub>* resistance genes (30%, 3). Tharaka Nithi had generally low levels (10–20%, 1-2), while Embu’s highest was *bla<sub>TEM</sub>* (30%, 3).

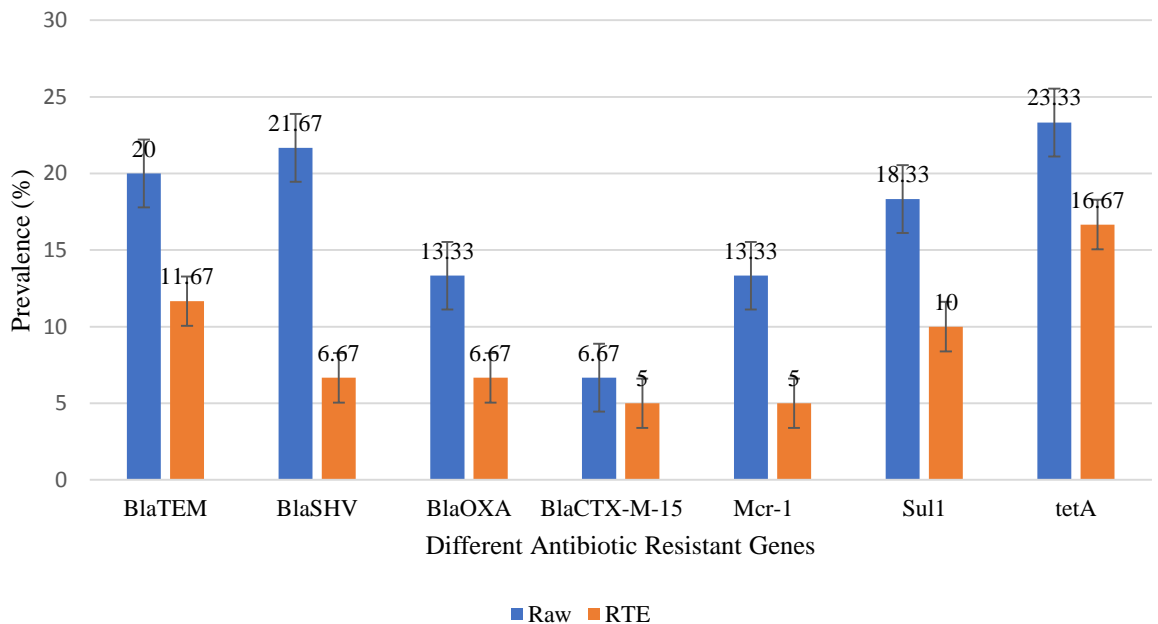


Figure 15:Prevalence of BlaTEM, BlaOXA, BlaCTXM15, Mcr-1, Sul1 and tet A resistance genes of Escherichia coli in raw and ready-to-eat raw meat samples

In RTE beef, Tharaka Nithi had *tetA* and *mcr-1* at 20%, while Embu recorded *bla<sub>CTX-M-15</sub>* at 20% (2). Meru’s highest in RTE beef was *bla<sub>TEM</sub>* at 20% (2). For RTE chicken, Meru showed elevated *tetA* (30%, 3) and *bla<sub>SHV</sub>* (20%, 2). Tharaka Nithi recorded *tetA* gene at 20% (2) and low levels of other genes. Embu’s RTE chicken had very low

prevalence, with all genes  $\leq 10\%$  (1). Overall, raw meats exhibited higher ARG prevalence than RTE meats, with Meru county showing the highest contamination levels across both beef and chicken. The resistance genes *tetA*, *bla<sub>SHV</sub>*, and *bla<sub>TEM</sub>* were the most widespread genes, while *bla<sub>CTX-M-15</sub>* and *mcr-1* were less frequent (Table 28).

Table 28: Distribution of Antibiotic Resistance Genes in *Escherichia coli* isolates Expressed as Percentage Prevalence (with number of Positive Isolates) Across Raw and Ready-to-eat beef and Chicken Meat in Three Counties

County	Sample type	Source	<i>Bla<sub>TE</sub></i>	<i>Bla<sub>SH</sub></i>	<i>Bla<sub>OX</sub></i>	<i>Bla<sub>CTX-M-15</sub></i>	<i>Mcr-I</i>	<i>SulI</i>	<i>tetA</i>
			M	V	A	15	I		
Tharaka Nithi	RAW	Beef	20 (2)	10 (1)	20 (2)	30 (3)	20 (2)	20 (2)	30 (3)
		Chicken	20 (2)	10 (1)	10 (1)	0 (0)	10 (1)	10 (1)	10 (1)
	RTE	Beef	10 (1)	10 (1)	10 (1)	0 (0)	20 (2)	10 (10)	20 (2)
		Chicken	0 (0)	0 (0)	10 (1)	0 (0)	10 (1)	10 (0)	20 (2)
Embu	RAW	Beef	10 (1)	20 (2)	10 (1)	0 (0)	10 (1)	20 (2)	20 (2)
		Chicken	30 (3)	20 (2)	10 (1)	0 (0)	10 (0)	20 (2)	10 (1)
	RTE	Beef	10 (1)	10 (1)	0 (0)	20 (2)	0 (0)	10 (1)	10 (1)
		Chicken	10 (1)	0 (0)	10 (1)	0 (0)	0 (0)	0 (0)	10 (1)
Meru	RAW	Beef	30 (3)	40 (4)	20 (2)	10 (1)	10 (1)	30 (3)	30 (3)
		Chicken	20 (2)	30 (3)	10 (1)	0 (0)	20 (2)	10 (1)	40 (4)
	RTE	Beef	20 (2)	0 (0)	0 (0)	10 (1)	0 (0)	10 (1)	10 (1)
		Chicken	20 (2)	20 (2)	10 (1)	0 (0)	0 (0)	20 (2)	30 (3)

RTE (Ready-to-eat)

#### 4.8 Molecular Characterisation of Virulence Genes of Drug Resistant Pathogenic *Escherichia coli* in Raw and Ready-to-Eat Beef and Chicken Meat

All *E. coli* colonies (240 (120 from raw meats and 120 from RTE meats)) were characterised for the presence of different *E. coli* virulence genes. The characterisation targeted different markers *Uida gene*, *eae*, *DaaE*, *stII*, *VirF*, *stx* and *aafII* as shown in figures 17, 18, 19, 20 and 21. All (240) *E. coli* isolates from raw and ready-to-eat (RTE) beef and chicken were positive for at least one virulence gene. The *uidA* gene was amplified in all isolates at 147 bp, confirming their identity as *E. coli*. Pathotype-specific genes were detected as follows: *eae* (EPEC) at 482 bp, *daaE* (DAEC) at 542 bp, *stII* (ETEC) at 125 bp, *stxI* (STEC) at 348 bp, and *virF* (EIEC) at 618 bp. These results indicate the presence of multiple pathogenic *E. coli* types across both raw and RTE meat samples. These findings underscore the potential public health risk posed by pathogenic *E. coli* contamination in both raw and ready-to-eat meat products. These findings imply that both raw and ready-to-eat meat can serve as reservoirs for diverse pathogenic *Escherichia coli* strains, increasing the risk of foodborne infections. They also highlight the need for stringent hygiene practices and proper cooking to prevent transmission to consumers.

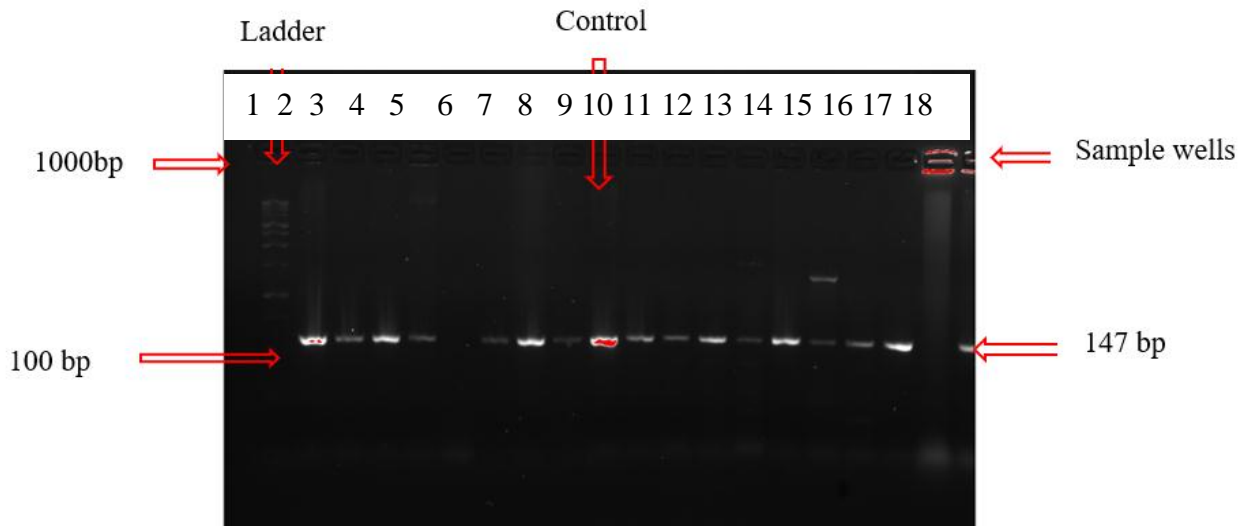


Figure 16: Polymerase chain reaction amplification of the *uidA* gene (147 bp) in *Escherichia coli* isolates.

Lane M: 100 bp DNA ladder; Lane 5: Negative control (no marker); Lane 9: Positive control (*Uida*); Remaining lanes show *uidA* amplification in test samples.



Figure 17: Polymerase chain reaction amplification of *eae* (EPEC, 482 bp) and *daaE* (DAEC, 542 bp) virulence genes in *Escherichia coli* isolates.

Lane 1: 100 bp DNA ladder; Lane 10: positive control for EPEC (EPEC marker); Lane 20: negative control (no marker); remaining lanes show amplification in test samples.

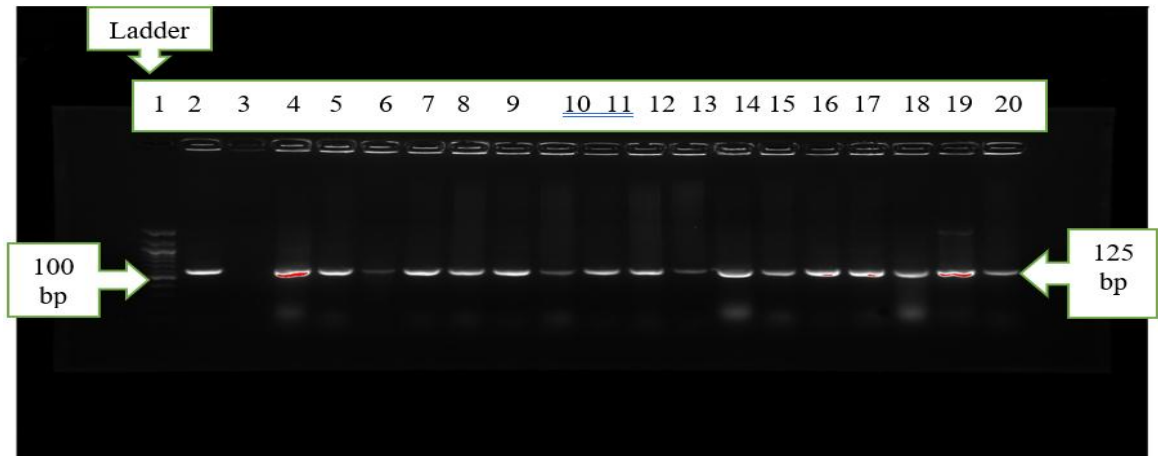


Figure 18: Polymerase chain reaction amplification of the *stII* virulence gene (ETEC, 125 bp) in *Escherichia coli* Isolates

Lane 1: 100 bp DNA ladder; Lane 3: negative control (no marker); Lane 4: positive control (ETEC control marker); Lanes 2–20: test samples showing amplified *stII* gene.

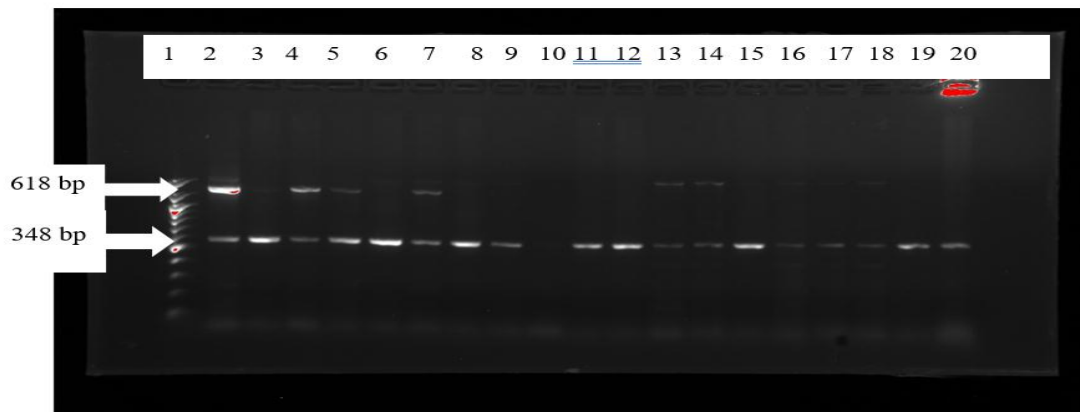


Figure 19: Polymerase chain reaction amplification of *stx1* (STEC, 348 bp) and *virF* (EIEC, 618 bp) virulence genes in *Escherichia coli* isolates.

Lane M: 50 bp DNA ladder; Lane 2: positive control (STEC marker, EIEC marker); Lane 10: negative control (no marker); remaining lanes show amplification in test samples.

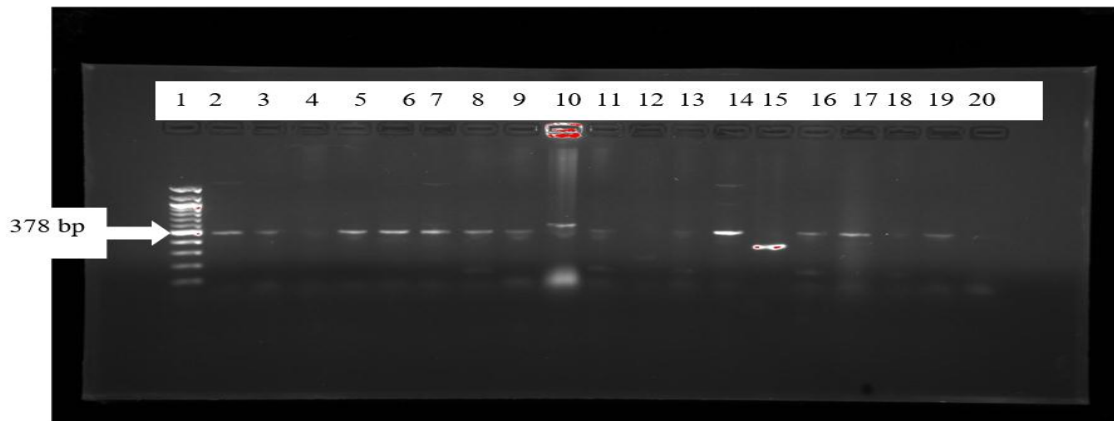


Figure 20: Polymerase Chain Reaction Amplification of the *aafII* Virulence Gene (EAEC, 378 bp) in *Escherichia coli* isolates.

Lane 1: DNA ladder; Lane 2: positive control (EAEC positive marker); Lane 4: negative control (no marker); remaining lanes show amplified *aafII* in test samples.

The distribution of diarrheagenic *E. coli* (DEC) pathotypes across raw and ready-to-eat (RTE) meat samples is presented in Figure 21. All six major DEC classes were detected in both raw and RTE meat, though prevalence was consistently higher in raw meat samples. Among raw meat isolates, the highest prevalence was recorded for Shiga toxin-producing *E. coli* (STEC) at 51% (61), followed by enteropathogenic *E. coli* (EPEC) at 46.7% (56), enterotoxigenic *E. coli* (ETEC) at 45% (54), and enteroaggregative *E. coli* (EAEC) at 43.3% (51). Diffusely adherent *E. coli* (DAEC) and enteroinvasive *E. coli* (EIEC) were detected at lower frequencies, accounting for 33.3% (39) and 26.7% (32), respectively.

In contrast, RTE meat samples exhibited lower overall prevalence across all pathotypes. STEC remained the most common at 33.3% (40), followed by ETEC (35%, 42), EPEC (30%, 36), EAEC (28.3%, 34), DAEC (26.7%, 32), and EIEC (23.3%, 28). The comparative trend clearly demonstrates that raw meat harboured a higher diversity and burden of virulent *E. coli* strains compared to RTE products. However, the presence of DEC pathotypes in RTE samples is of particular concern, as it indicates incomplete elimination of pathogenic strains during food processing and preparation, posing a direct risk to consumers. These findings highlight the public health significance of meat as a reservoir for diarrheagenic *E. coli* and reinforce the need for enhanced hygienic handling, rigorous cooking practices, and stricter regulatory monitoring to mitigate potential foodborne infections.

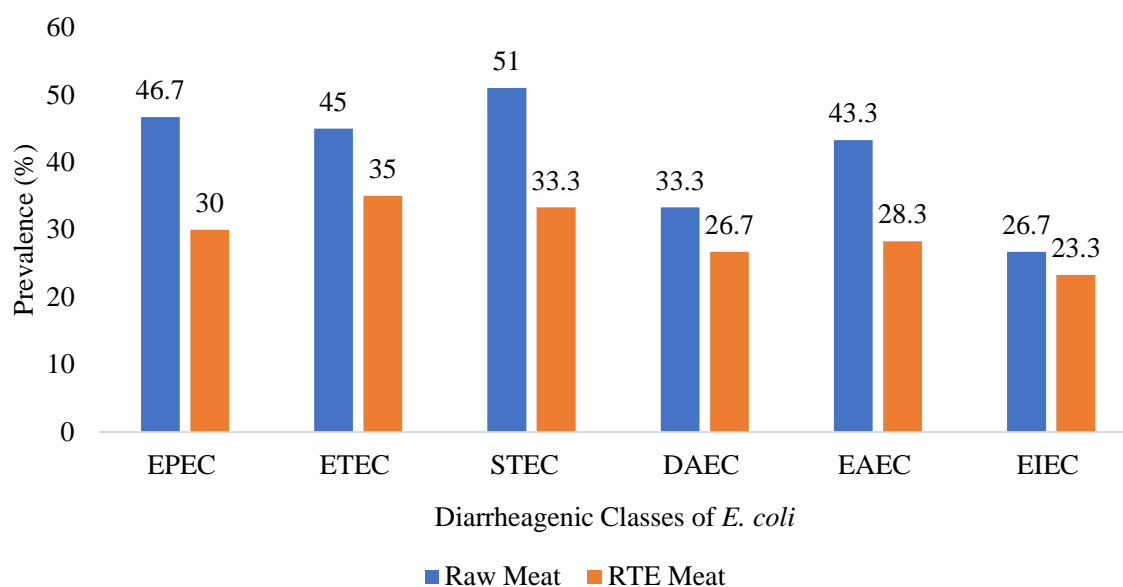


Figure 21: Prevalence of Diarrheagenic Escherichia coli Pathotypes in raw and Ready-to-eat Meat Samples from Eastern Kenya.

The distribution of *E. coli* virulence-associated genes across raw beef, raw chicken, ready-to-eat (RTE) beef, and RTE chicken meat is presented in Figure 22. All six targeted virulence markers (*virF*, *aafII*, *daaE*, *stx1*, *stII*, and *eae*) were detected in varying proportions across the different sample types, with raw chicken meat consistently exhibiting the highest prevalence for most genes. The *virF* gene (invasive plasmid antigen, EIEC marker) was most prevalent in raw chicken meat (70%, 84), followed by raw beef (36.7%, 44), and equally lower in RTE beef and RTE chicken meat (both 23.3%, 28). The *aafII* gene (EAEC marker) was also dominant in raw chicken (56.7%, 68), moderate in raw beef (30%, 36) and RTE chicken (36.7%, 44), and lowest in RTE beef (20.6%, 25).

For the *daaE* gene (DAEC marker), raw chicken meat recorded the highest prevalence (40%), followed by RTE chicken (30%, 18), while raw beef (26.7%, 16) and RTE beef (23.3%, 14) showed comparatively lower proportions. Similarly, the *stx1* gene (STEC marker) was most frequent in raw chicken (63.3%, 38), moderate in RTE chicken (30%, 18) and RTE beef (23.3%, 14), and lowest in raw beef (26.7%, 16). The *stII* gene (ETEC marker) displayed a similar pattern, with highest prevalence in raw chicken meat (56.7%, 34), followed by raw beef (33.3%, 20), RTE beef (23.3%, 14), and lowest in RTE chicken meat (16.7%, 10). For the *eae* gene (EPEC marker), raw chicken meat

again had the highest prevalence (53.3%, 32), followed by RTE chicken (43.3%, 25), raw beef (40%, 24), and RTE beef (16.7%, 10).

Overall, raw chicken meat exhibited the highest burden of virulence genes across all categories, suggesting it is a major reservoir of pathogenic *E. coli* strains. Notably, the persistence of these genes in RTE chicken and beef samples highlights the potential risk of transmission to consumers despite processing and preparation. These findings underscore the public health implications of inadequate hygienic handling and insufficient cooking, and call for stricter food safety measures to curb the dissemination of virulent *E. coli* through the meat value chain.

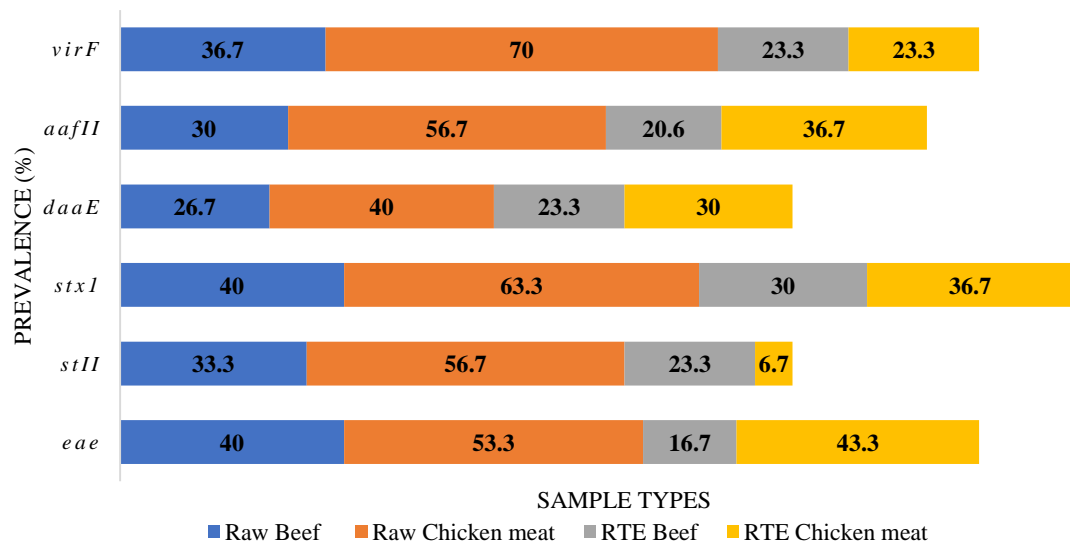


Figure 22: Prevalence of *Escherichia coli* virulence genes across raw beef, raw chicken, ready-to-eat (RTE) beef, and RTE chicken meat samples from Eastern Kenya.

The prevalence of the diarrheagenic *E. coli* pathotypes varied across counties and sample types (Table 29). Among all detected virulence markers, the Shiga toxin gene (STEC's, *stxI*) was the most dominant, particularly in raw chicken from Meru County, where it was present in 90% (9/10) of isolates. High prevalence of *stxI* was also observed in raw beef (50%, 30) and RTE chicken (50%, 30) in Tharaka Nithi, as well as in raw chicken from Embu (50%, 30) and Tharaka Nithi (50%, 30), underscoring the widespread distribution of STEC across both raw and RTE meat products. In addition to STEC, other diarrheagenic pathotypes were also frequent in Meru County's raw chicken, which exhibited high contamination levels of ETEC (*stII*, 80%, 16/20), EPEC

(*eaeA*, 70%, 14), and EIEC (*virF*, 90%, 18). Notably, this made Meru County's raw chicken the most heavily contaminated sample type, harbouring multiple virulence determinants simultaneously. Similarly, raw chicken in Embu and Tharaka Nithi counties showed elevated levels of EIEC (60%, 12/20) and ETEC (50%, 10/20), further confirming raw chicken as a major reservoir of pathogenic *E. coli*.

Table 29: Distribution of *Escherichia coli* Virulence Genes Expressed as Percentage (with number of Positive Isolates) across meat types and Counties (N= 120)

Sample type	Counties	% Virulent Factors					
		<i>eaeA</i>	<i>stII</i>	<i>stx1</i>	<i>daaE</i>	<i>aafII</i>	<i>virF</i>
Raw Beef	Tharaka Nithi	50 (5)	30 (3)	50 (5)	40 (4)	40 (4)	30 (30)
	Embu	40 (4)	30 (3)	30 (3)	20 (2)	20 (2)	40 (4)
	Meru	40 (4)	40 (4)	40 (4)	20 (2)	30 (3)	40 (4)
Raw Chicken	Tharaka Nithi	50 (5)	50 (5)	50 (5)	30 (3)	60 (6)	60 (6)
	Embu	40 (4)	40 (4)	50 (5)	20 (2)	50 (5)	60 (6)
	Meru	70 (7)	80 (8)	90 (9)	70 (7)	60 (6)	90 (9)
RTE Beef	Tharaka Nithi	20 (2)	10 (1)	40 (4)	30 (3)	30 (3)	20 (2)
	Embu	0 (0)	20 (2)	20 (2)	20 (2)	20 (2)	20 (2)
	Meru	30 (3)	40 (4)	30 (3)	20 (2)	10 (1)	30 (3)
RTE Chicken	Tharaka Nithi	50 (5)	50 (5)	40 (4)	50 (5)	40 (4)	20 (2)
	Embu	40 (4)	40 (4)	30 (3)	30 (3)	30 (3)	20 (2)
	Meru	40 (4)	50 (5)	40 (4)	10 (1)	40 (4)	30 (3)

By comparison, RTE meat samples showed lower overall prevalence of virulence genes, although contamination was still evident. In Tharaka Nithi, RTE chicken carried relatively high proportions of *stII* (50%, 30), *eae* (50%, 30), and *daaE* (50%, 30), while RTE beef showed lower levels, with most genes detected at  $\leq 40\%$  prevalence. Embu and Meru RTE beef and chicken samples exhibited the lowest contamination rates, with some markers (e.g., *eae* in Embu RTE beef) being completely absent. The least contaminated samples overall were RTE beef and RTE chicken from Meru County, which showed only 10% (12) prevalence of DAEC.

In general, these results indicate that raw chicken meat, particularly from Meru County, had the highest burden of virulence genes, suggesting it represents the greatest risk for dissemination of diarrheagenic *E. coli*. Conversely, RTE products were comparatively less contaminated, though their detection of virulent markers demonstrates incomplete pathogen inactivation during processing and preparation. Collectively, these findings highlight both the county-level differences in contamination and the need for improved food safety measures targeting raw chicken in particular.

## CHAPTER FIVE

### DISCUSSION

#### 5.1 Demographic, Operational, and Hygiene Practices of Butcheries in Meru Embu and Tharaka-Nithi Counties

This study revealed that the meat retail industry is highly male-dominated, with 94% of respondents being men. These findings are consistent with those by Koech *et al.* (2024) and Chepkemoi *et al.* (2016) and across other African countries, including Ghana, Ethiopia, South Africa, and Uganda (Asati *et al.*, 2024; Siluma *et al.*, 2023; Nabwiire *et al.*, 2023; Kanko *et al.*, 2023). The recurrent pattern highlights entrenched gender roles and socio-cultural norms that restrict women's involvement in the sector. This gender imbalance may exacerbate antimicrobial resistance (AMR) risks. Conversely, restricting women's roles in decision-making can increase exposure and resistance risks in livestock systems. Addressing this gap requires gender-sensitive interventions, including targeted training programs, supportive institutional policies, and empowerment initiatives. Promoting women's equitable participation in meat retailing is therefore not only a matter of social inclusion but also a critical strategy for mitigating AMR within food systems.

The majority of raw meat sellers in this study were between 30 - 40 (39%). This is consistent with other findings in Kenya showing that most of raw meat handlers fall between 35 and 50 years (Chepkemoi *et al.*, 2016, Kunyanga *et al.*, 2021, Koech *et al.*, 2024). This trend suggests that meat retailing is physically demanding, attracting predominantly middle-aged men rather than younger populations. Education was found to significantly influence hygiene practices, with more educated individuals demonstrating better compliance with food safety standards. Similar patterns have been reported in Uganda and Nigeria (Kehinde *et al.*, 2020, Nabwiire *et al.*, 2023). These findings highlight the need for regular, targeted training programs to enhance food safety practices within meat establishments, particularly among less educated operators.

From the study, the most common red meat is beef. On the other hand, the main source of white meat is chicken which is mainly sold within the butcheries as well. In this study beef sold alone was highest in all butcheries. This was followed by beef together with chicken and finally beef with meat from other species such as goats and sheep. However, the sale of chicken meat alone was very rare (less than 5%) in all three

counties. The chicken meat value chain was not structured between the farmer to the butchery. This was unlike beef where the cattle had to be slaughtered at slaughterhouses before distribution to butcheries. Chicken meat value chain is non-structured and could be a source of contamination to the meat value chain. There is lack of veterinary inspection during slaughter unlike in beef where the cattle are slaughtered in designated slaughterhouses with supervision from veterinary officers. This was noted as big gap which should be looked into in the spread and contamination of chicken meat by sickly birds which is a rapidly growing industry (Gulati & Juneja, 2023)

Meat is a highly perishable protein source and has a very short shelf life, especially when not refrigerated (Johansson *et al.*, 2020). From the current study it is evident that meat could actually take more than three days in a butchery. For most of the establishments the meat was sold within 36 to 48 hours. In another study in Uganda, only 10% of meat in butcheries was sold within 1 day (Nabwiire *et al.*, 2023) According to the Kenyan Standards (KS EAS 1190: 2023), slaughterhouse products should be frozen or refrigerated. It is evident that regulations are set by the government but very little compliance is observed within the butcheries. There is a need to have strict follow ups and possible fines to those who do not comply with these regulations.

Without refrigeration, microbial multiplication could rise above the levels set by KEBS and other standards which is set at 316 CFU/g for coliforms (Commission regulation 2005; NDVQPH, 2010). With only 25% of butcheries installed with refrigerators, the risk is very high. Some studies have reported that refrigerators in butcheries available are not more than 11% in Nairobi and 39% in Isiolo (Chepkemoi *et al.*, 2016). In Uganda, refrigeration systems were recorded in 26% (Nabwiire *et al.*, 2023) of butcheries while in Nigeria 42% of butcheries reported to refrigerate meat (Kehinde *et al.*, 2020). This trend is alarming and could be a possible hotspot for foodborne diseases causes. Microorganisms are temperature specific (McMeekin *et al.*, 2018), and therefore refrigeration is among the first step in trying to control microbial multiplication and possible outbreak of foodborne diseases.

Separation of species and different types of meat play a major role in controlling cross-contamination (Braley *et al.*, 2022). Therefore, having beef and chicken meat in close proximity with each other will cause contamination of all types of meat with a wide

range of microorganism. This is because the different sources of the animals are also accompanied by different types of microbes (Dsani *et al.*, 2020). Separation of species is therefore an important step in controlling possible zoonotic bacteria between species. In the current study, separation was only done in 12.8 % of the butcherries. Separation of species was influenced by age, where individuals above 50 years were likely to separate the meats. This could be due to more experience and knowledge on food safety accrued with the more exposure and possible training than younger individuals. And also, more educated personnel were likely to separate species compared to those with lesser level of education. This difference was significant ( $p = 0.001$ ).

At the butchery the equipment used in handling and storing meat such as knives, sharpening tools, refrigerators, hooks and cutting boards were considered as those likely to breed microorganisms due to their frequency of contact with meat. Their cleaning frequency is critical since sales occur at unprecedented intervals between one customer and another. The Meat Control Cap 356 under the meat control regulations of 1973, state that meat handling equipment should be washed with soap and water and undergo disinfection with hot water. One of the methods in controlling microorganisms is through physical removal. Cleaning removes most microorganisms and should be followed by disinfection for microbial safety (Urban-Chmiel *et al.*, 2025). This was not the case in this study and should be a recommendation during cleaning to the local butcherries. This is because Meat contact surfaces have had pathogenic microorganisms (*E. coli*, *Staphylococcus aureus* and *Salmonella*) isolated from them in other studies them (Hiko *et al.*, 2025, Kenaw *et al.*, 2024, Kanko *et al.*, 2023).

When it comes to premises, the regulations are that the floor and walls should be cleaned at least once daily followed by disinfection (US 736: 2019 Section 7.5). Premise where food is being sold as a major factor in food safety. Hiko *et al.*, (2025) isolated pathogenic microorganisms from the butchery floors. The cleaning frequency was different in all study sites while daily cleaning was common in TNC while twice - daily cleaning was common in Embu County. Overall, it can be concluded that cleaning practiced was a well-established practice among butcherries in the study area with 100% of the respondents doing daily cleaning and sometimes twice per day. Previous studies in Kenya generalized cleaning practice with no differentiation between the equipment and the premise, (Chepkemoi *et al.*, 2016, Kunyanga *et al.*, 2019). In all the above

studies cleaning was done daily in 100% of the butcheries. Since the results show that the younger individuals are likely to carry out disinfection, it is recommended that this population should be hired to clean the butcheries or contracted to carry out the hygiene practices within the butcheries.

The Kenyan Standard (KS EAS 1190: 2023) state that water used for cleaning of meat and equipment should be potable. The water provided by the government piping system (Municipal) is usually treated and potable. The certainty of water quality from vendors could not be determined and river water carries with it a lot of environmental pathogens which is a possible source of contamination (Njuguna *et al.*, 2020). A significant number of butcheries draw water from municipal sources (Over 80%) per county. This practice could be among the steps encouraged towards improvement of hygiene in the establishment. Portable water will enhance even personnel hygiene practices like hand washing and hand washing sinks in the premise which is already at over 50% according to this study.

Waste disposal sites and toilets are useful in managing environmental and human waste. In this study, the proximity of toilets to a point where they could become sources of contamination was considered. Open waste disposals within vicinity could act as sources of cross contamination especially with flies (AL-Wabel *et al.*, 2022). We reported that not less than 60% establishments had toilets within the vicinity while more than 50% had waste disposal sites/ and draining facilities too close to the butchery. The toilets could act as sources of contamination if not well maintained, the same goes to waste disposal sites. These need to be well regulated since none is mentioned in the meat control act CAP 456 of Kenya.

The government is responsible for ensuring that the set-out regulations are being followed to the letter by the raw meat handlers. This is usually done by public health officers who are mandated to do frequent inspection of these premises. This study reported that all butcheries had at one point been visited by a public health officer. It is also a requirement that food handlers are tested and given food handlers certificate if they pass the test. In this study all the participating butcheries personnel had food handlers' certificate in all three counties. Medical certificates should be renewed after every 3 months and not later than 6 months (Government of Kenya, 2016). In this study

majority of the certificates were reviewed in the last 3- 6 months in all three counties, however, some food handlers had not gone for medical check-up for a period exceeding six months (33%) while others never reviewed after getting the first food handlers' certificate (4% in Meru county only). These results indicate a disconnect between legal information and implementation from the government side (Kimindu *et al.*, 2024).

Training of food safety during handling is essential to maintain a hygienic environment and reduce cross contamination (Government of Kenya, 2016). Training both formal and informal were considered crucial in the current study and there was a positive trend. Training has a positive impact on personnel practices such as wearing of protective clothing (hair nets, water proof gumboots, gloves, nose masks and bright aprons) which scored poorly in the current study. Money handling could be a possible source of contamination when personnel do not wash hands between meat handling and money. Although this is not a recommendation in the Kenyan standard, it should be included in the legislation. However, with the increase in mobile money transfer in Kenya (Ngugi *et al.*, 2010) money contact is expected to be low. On the downside, the personnel might need to check payment messages in the phone, and therefore the phone might become the next source of meat contaminant. Either way this study, recommends separate cashiers per establishment.

## **5.2 Evaluation of Food Safety Knowledge, Attitudes, and Practices of Ready-to-Eat Meat Retailers**

The study having more men than women (53%, 56) is contrary to the cultural practice that women are more conversant with the kitchen and thus more should be in RTE establishment where a lot of cooking and serving occurs (Letuka *et al.*, 2021). These results are similar to those of Mwove, (2025), who reported that men were the majority gender in street vended foods in Meru Town. A study in Ethiopia reported even higher proportion of males at 97.2% involvement in the trade (Abunna *et al.*, 2022). These results are contrary to those of Gichunge *et al.*, (2023) where female were the majority participants in RTE establishments around a campus environment in Kenya. This shows that men are actively taking up roles that were traditionally left for women in order to benefit economically.

Secondary level education attainment was very common among the participants (41.1%) which was different to studies in Ethiopia where most retailers of RTE foods had primary level of education (Abunna *et al.*, 2022; Gameda *et al.*, 2025). Other Kenyan studies have given similar results of secondary education personnel being the majority in Meru (Mwove, 2025) and Nairobi (Obinda *et al.*, 2021). This could be affected by the economic milestones which are different from one country to another. Similarly, those with university level of education were very few showing that, individuals with low level of education had fewer job opportunities and therefore opted for short term employment among the RTE meat establishments.

Work experience in terms of years varied with most having a less than one year on job experience (38.3%). This could be explained by the fact that RTE establishment could be a short-term employment space. The results are different from those of Obinda *et al.* (2021) who reported that the majority of personnel in RTE businesses and been working for 1-5 years in Nairobi. Nairobi being the capital city could have a stable cash flow compared to towns like Meru, Embu and Chuka which have lower economic activities compared to Nairobi City. The age group of many people working in RTE establishment were people over 50 years old and majority were between 21-30 at 26% and 31 – 40 25% giving an accumulative of 51% of the age group between 21-40. The industry is labour intensive and would be attractive to the youthful population compared to the older individuals. These results are similar to those reported by Mwove *et al.*, (2020) in Thika and (Obinda *et al.*, 2021) in Nairobi.

Training is an important aspect in food safety and public health issues within food distribution (Soon *et al.*, 2020). Untrained personnel were the majority in the study (58.9%) posing a public health risk. The report is similar to that of Gameda *et al.*, (2025) who reported a higher percentage of non-trained personnel involvement in Ethiopia. However, this was different from a study done in Nairobi which found out that a high percentage were had some form of training if food safety handling practices (Obinda *et al.*, 2022). The Kenyan bylaw expect individuals involved in food trade to have some form of food safety training, however this is not a consideration to the business people in the sector.

It is mandatory for all food handlers to have medical certificates which should be renewed every 3 months (Government of Kenya, 2016). Medical certificates were only with 48% of the respondents. Most towns (Embu and Meru) also scored low in medical certificate (49% and 47%). It is only Chuka where the medical certificate was highly reported at 82.4% (90). This could be due to the instability in employment retention among the personnel since the majority were only employed for less than a year this could include months below three.

One of the five world health recommendations towards ensuring food safety is the separation of raw and ready-to-eat food and associated utensils (WHO, 2018). This study reported that over 50% of RTE (61) meat establishment also sold raw meat. This is a public health hazard especially when the level of food safety practices is low as reported. The mix in raw and RTE meat establishment is rising since entrepreneurs would like to control most of the value chain (raw and RTE nodes). The same scenario was reported in Nairobi where butcheries were reported to also prepare RTE meat for sale (Koech *et al.*, 2024). This setup is likely to cause cross contamination of pathogenic microbes from the carcass to the RTE if proper hygiene measures are missing (Haddad *et al.*, 2020).

This study revealed satisfactory food safety knowledge but only neutral attitudes and practices among RTE meat handlers. Similar findings have been reported in Kenyan, where food handlers who attended training scored significantly higher on KAP measures than their untrained counterparts (Mwove 2025). The findings of this study corroborate this, showing that trained and certified handlers, particularly in Meru, demonstrated better practices. Likewise, identified education, certification, and training as significant predictors of knowledge and practice. However, evidence from Ghana shows that even certified handlers may exhibit unsafe behaviours, such as handling meat and money with bare hands (Asati *et al.*, 2024). This is consistent with the findings of this study in Embu and Meru counties, suggesting that knowledge alone does not guarantee behaviour change. Broader systemic barriers, including weak enforcement, limited resources, and entrenched habits, may undermine translation of knowledge into safe practices. This underscores the need for integrated interventions that combine training with stronger institutional support and behaviour-focused strategies.

Other research on food safety knowledge had almost similar results. In Ghana, Asati *et al.* (2024) found that while 89% of meat sellers were aware that unhygienic handling could transmit pathogens, fewer than half understood the role of temperature in microbial growth. This mirrors the current finding where only 49.5% identified correct storage temperatures. In Ethiopia, Abunna *et al.* (2023) reported that although 94% of meat handlers had positive attitudes, 83.7% practiced unsafe handling due to poor knowledge of detailed hygiene protocols. In developed countries, studies show higher baseline knowledge but still reveal deficiencies in specific domains. A review by Infrán-Rivarola (2020) reported that food handlers in Europe and North America generally scored above 80% on awareness of foodborne disease transmission, yet only 55 – 65% correctly identified safe cooking and storage parameters. Similarly, a UK study noted that while most handlers understood cross-contamination risks, fewer than half adhered to correct refrigeration practices (Soon *et al.*, 2020). Compared with the present data, handlers in developed settings display slightly higher technical knowledge, reflecting stronger regulatory oversight and mandatory training systems. Nonetheless, gaps in practice remain universal.

In Kenya, comparable practice patterns have been reported. Ng'ang'a *et al.* (2021) observed that while over 85% of Nairobi meat handlers adhered to routine handwashing, fewer than half regularly monitored storage conditions or separated raw from cooked products. Similarly, Kimindu *et al.*, (2024) in Kajiado found that although 90% of workers maintained personal hygiene, only 49% adhered to safe meat storage and temperature control. These parallels confirm that Kenyan handlers excel in visible hygiene but fall short in critical practices requiring equipment, monitoring, or resources. The current study's finding that 71% (77) refrigerated unsold meat echoes national results, but the low proportion monitoring storage temperatures (46.7%) highlights persistent gaps in operational food safety. Other African studies report similar discrepancies. In Ethiopia, Abunna *et al.* (2023) found that although 94% of meat handlers had positive attitudes toward safety, only 43% practiced correct separation of raw and cooked meat, and temperature monitoring was rarely done. In Ghana, Asati *et al.* (2024) also documented high compliance with handwashing and covering meat, yet only about half discarded leftovers at day's end. These findings align with the present data, underscoring that while African handlers are diligent in basic

hygiene, resource-intensive or knowledge-intensive practices such as refrigeration monitoring, cross-contamination prevention, remain weak points.

On the other hand, some studies outside Africa present a stronger correlation between knowledge, attitudes, and practice than these findings. In Bangladesh, only 20% and 16.3% of meat handlers had good knowledge and practices, respectively, with training and longer working hours strongly predictive of both (Banna *et al.*, 2021). That contrasts with the current results of this study where handlers had comparatively higher knowledge and moderate practices. The difference may be as a result of varied baseline education levels in Kenya versus Bangladesh, or differing training outreach programs. A study in Ethiopia reported that most meat handlers exhibited good attitudes but still had poor practices (Abunna *et al.*, 2023). This is similar to the results obtained in Tharaka-Nithi and Meru, where attitudes were neutral or satisfactory but practice remained neutral. It suggests that negative or partial attitudes might still fail to drive safe behaviours could be possibly due to systemic barriers like inadequate infrastructure or oversight.

This study revealed satisfactory food safety knowledge but only neutral attitudes and practices among RTE meat handlers. Similar findings have been reported in Kenyan, where food handlers who attended training scored significantly higher on KAP measures than their untrained counterparts (Mwove, 2025) The findings of this study corroborate this, showing that trained and certified handlers, particularly in Meru, demonstrated better practices. Likewise, Tohonon *et al.*, (2025) identified education, certification, and training as significant predictors of knowledge and practice. However, evidence from Ghana shows that even certified handlers may exhibit unsafe behaviours, such as handling meat and money with bare hands (Asati *et al.*, 2024). This is consistent with the findings of this study in Embu and Meru counties, suggesting that knowledge alone does not guarantee behaviour change. Broader barriers, including weak enforcement, limited resources, and entrenched habits, may undermine translation of knowledge into safe practices. This underscores the need for integrated interventions that combine training with stronger institutional support and behaviour-focused strategies.

While handlers with higher education or training scored better in knowledge and practice, many may lack regular courses or practical training emphasizing hands-on behavioural change. Other studies have reported satisfactory attitude and knowledge but inadequate practices just as in this study (Abunna *et al.*, 2022; Gameda *et al.*, 2025). This means that training may cover theoretical aspects but lack competence in real-world scenarios like temperature control or causes of cross-contamination. Handlers may know the ideal practices but lack access to refrigeration, food grade sanitization equipment, calibrated thermometers, or clean storage spaces. In the practices data, temperature checks and equipment separation were among the main drivers in poor food safety practices. Retailers may face pressure to minimize spoilage and maximize profit, leading to cutting corners such as delayed reheating, extended storage without refrigeration, or dual handling of raw and cooked meat. Economic constraints may outweigh safety considerations even with awareness (Soon *et al.*, 2020). Since most of the retailers were small and medium enterprises, the cash flow could likely be a hindrance to acquiring equipment required to monitor food safety procedures such as refrigerators and thermometers.

Discrepancies across counties and towns (i.e., Embu more satisfactory at attitude and practices than Meru or Tharaka Nithi) may reflect differences in local regulatory enforcement, health inspections, and public health outreach by the local county governments. Neutral attitude scores suggest that while handlers may appreciate food safety, they might lack strong conviction about its importance. This could stem from limited perceptions of risk, if foodborne illness is not commonly experienced or tied to their practice, motivation may remain uncertain. The neutral practice scores, particularly in temperature control, cross-contamination prevention, and reheating, would accelerate the risk of foodborne disease outbreaks, especially *E. coli* (most common pathogen in meat) infections (Tohonon *et al.*, 2025). *Escherichia. coli* (particularly pathotypes) can be transmitted via contaminated beef, causing symptoms ranging from diarrhoea to haemolytic uremic syndrome. It thrives when meat is undercooked or improperly stored between 4 °C and 60 °C the “danger zone.” Without adequate monitoring, meat may remain for long. In unsafe temperature ranges conducive proliferation of pathogenic microorganisms.

### 5.3 Microbial Contamination Levels of Raw Beef and Chicken Meat with Coliforms, *Escherichia coli* and Antibiotic Resistant *Escherichia coli*

All raw meat samples were positive for, *E. coli* and AR *E. coli*. Majority of Kenyan studies on raw meat also focus on beef (Kunyanga *et al.*, 2021; Koech *et al.*, 2024; Kimindu *et al.*, 2025) than chicken meat (Kamau, 2020; Odwar *et al.*, 2014). Studies are also concentrated at the slaughterhouses with few in butcheries (personal observation). A study done by Kunyanga *et al.*, (2021) reported that prevalence of *E. coli* in beef at butcheries was 78% in Meru town, a figure slightly lower than these results. Reports on raw chicken meat with respect to *E. coli* prevalence have been reported at 88% (Kamau, 2020) and 75% (Kago *et al.*, 2015) in Nairobi and Limuru respectively.

In other countries, *E. coli* has been isolated as well in raw meat of beef and chicken origin. In Ghana a study done on raw meat samples comparing different animal species carcasses, raw beef recorded higher *E. coli* prevalence (80%) than chicken (50%) (Adzitey *et al.*, 2021). These findings are different from the ones obtained in our study where chicken meat consistently reported higher microbial counts. In Bangladesh, a LMIC just like Kenya, *E. coli* was prevalent in 78% of frozen chicken meat (Parvin *et al.*, 2020). The values are lower than in this study probably due to differences in sample handling where the freezing could have played a part in reducing the contamination levels by reducing the growth rate of *E. coli*. In Egypt the contamination levels of raw chicken meat were even lower (16%) than beef (54%) (Gwida *et al.*, 2014) compared to any other study referenced here. This could arise from stricter hygiene conditions being implemented compared to the local butcheries in Meru Embu and Thraka-Nithi Counties of Kenya. A study in USA, however, had similar results to this study since the contamination levels of raw chicken meat (83%) with *E. coli* was found to be higher than raw beef (68.9%) (Zhao *et al.*, 2012). Results show that raw meats are still a harbours of *Escherichia coli* and pose a public health risk for many countries around the globe.

The high contamination levels of raw chicken meat from this study could be due to a number of reasons. Chicken farming, particularly broiler chicken production, often involves high-density rearing conditions, which can facilitate the rapid spread of bacteria, including coliforms and *E. coli*, among the flock (Kariuki *et al.*, 2023; Woyda

*et al.*, 2023). These bacteria are commonly found in the intestinal tract of animals and can contaminate the carcass during slaughter (Ipara *et al.*, 2019). Rearing of chicken is usually done in close proximity to each other in LMICs like Kenya increasing cross contamination with these microorganisms (Onono, 2023). Broiler chicken are generally harvested between 4 to 6 weeks. At this age the birds still have a lower immunity that is not well developed to fight diseases. Their relatively shorter lifespan potentially increases their susceptibility to colonization by coliforms and *E. coli* (Abdelaziz *et al.*, 2025). In many low and middle-income countries, slaughtering processes of chicken lacks the stringent bio hygiene standards and infrastructure present compared to cattle (Ipara *et al.*, 2019; Kiambi *et al.*, 2021).

The anatomical difference could also play a role in explaining the high microbial load in chicken compared to beef. Chicken have a shorter less complicated GIT which can easily contaminate the meat during slaughter (Roobab *et al.*, 2024). Coupled with a thin subcutaneous layer which usually forms part of the skin and is not removed during slaughter compared to cattle could keep most commensal coliforms attached to the carcass unlike in beef. Moreover, chickens skin provides less of a barrier against bacterial invasion compared to the thicker skin and fat layers in beef cattle. Chicken meat has a more neutral pH and high-water activity compared to beef (Kozak *et al.*, 2022), which has a dense texture and low moisture content is likely to be less contaminated. These characteristics accelerate the growth and multiplication of coliform bacteria which grow much faster under these conditions. It therefore means that chicken meat has a shorter ambient shelf life compared to beef and should undergo immediate refrigeration after slaughter

The slaughtering process itself presents numerous opportunities for contamination. Inadequate sanitation during evisceration, defeathering, and washing can lead to the transfer of bacteria from the intestinal contents to the carcass surface. Additionally, the reuse of water for washing carcasses, if not properly treated, can become a source of cross-contamination, further spreading bacteria among the slaughtered birds (Ipara *et al.*, 2019; Kariuki *et al.*, 2023). Compared to beef processing, chicken processing lines often operate at higher speeds, which can compromise the thoroughness of cleaning and sanitation procedures. Moreover, the common practice of scalding chickens to facilitate

feather removal can create an environment conducive to bacterial growth if the water temperature is not precisely controlled (Mbai *et al.*, 2022; Kozak *et al.*, 2022).

The results showing high contamination rates of AR *E. coli* in chicken than beef could point to overuse of antibiotics in chicken farming. This could be reason for higher AR *E. coli* chicken meat than beef (Kiambi *et al.*, 2021). The high prevalence is could be driven by economic incentives, disease prevention, and growth promotion. Farmers often administer antibiotics to chicken flocks to prevent disease outbreaks in overcrowded and unsanitary conditions common in LMICs (Van Boeckel *et al.*, 2015). These prophylactic treatments help reduce losses and ensure consistent production, making them economically attractive. Additionally, sub-therapeutic doses of antibiotics are used as growth promoters to enhance feed efficiency and accelerate weight gain this practice increases their profits (Landers *et al.*, 2012; Onono, 2023). Most African countries lack strict regulations or enforcement mechanisms concerning antibiotic use in livestock hence the practice persist (O'Neill, 2016). In other reports farmers are able to access antibiotics without prescriptions, they also have limited knowledge about the long-term risks of antibiotic resistance (Kemp *et al.*, 2020).

#### **5.4 Microbial Quality Assessment of Ready-to-Eat Beef and Chicken Meat**

The contamination level of RTE chicken meat was higher than that in Beef in coliform counts, *E. coli* and antibiotic resistant *E. coli* (i.e prevalence for ; TCC (chicken meat 81%, beef 77%), *E.coli* ( chicken meat 50%, beef 48%), AR *E. coil* (chicken meat 17%, beef 16%)). Kenyan studies have previously reported the presence of coliforms and *E. coli* in RTE beef, chicken meat and meat related products. In Nakuru studies done in 2023 and 2019 reported *E. coli* 100% of samples (Ronald *et al.*, 2023) and 91% (Indago *et al.*, 2019). According to Ronald *et al.*, (2023) the range of contamination in log<sub>10</sub> CFU/g for coliform counts was in the range of 1.74 to 2,53, while *E. coli* was 1.84 to 2.56. These ranges are close to my results in RTE beef and chicken meat. Comparing to Birgen *et al.*, (2020), contamination levels of chicken meat in Nairobi, Korogocho slums was at log<sub>10</sub> CFU/g 2.22 to 2.67. This indicates that *E. coli* prevalence is still high in most RTE foods around the country jeopardizing the public health.

Other studies in LMICs have also the same trend of consistent *E. coli* in RTE foods. According to Ema *et al.*, (2022) in Bangladesh chicken products were contaminated

with *E. coli* with approximately 3.57 CFU/g. This is close to some of the reports made in Nkubu and Runyenjes town. With Ayamah *et al* (2021) in Ghana, the microbial contamination was higher with a wider range of 1.7 CFU/g to 6.02 CFU/g. This could be because the samples were strictly street vended while in this study hotels and even street vended were combined in the study. According to Ema *et al.*, (2022) in Bangladesh chicken products were contaminated with *E. coli* with approximately 3.57 CFU/g. This is close to some of the reports made in Nkubu and Runyenjes town.

With Ayamah *et al* (2021) in Ghana, the microbial contamination was higher with a wider range of 1.7 CFU/g to 6.02 CFU/g. This could be because the samples were strictly street vended while in this study hotels and even street vended were combined in the study. There have been variations where beef has higher *E. coli* counts compared to chicken in some studies. For instance, another study in Ghana has reported a prevalence of 30% in RTE beef and 20% in RTE chicken (Adzitey *et al.*, 2021). In Colombia RTE ground beef had a 53% prevalence (Amezquita-Montes *et al.*, 2015). A study from Ethiopia reported 65% prevalence in RTE minced beef (Tonjo *et al.*, 2022). Similarly, Makhtar and Karmi (2021) in Egypt also reported higher contamination rates for RTE beef (30%) than chicken meat (3%) which contradicts the findings of this study. This could be due to better poultry management practices and hygiene preparation methods.

Similar results however, have been published in Ghana where contamination level of chicken (16%) was higher than beef (8%) (Abass *et al.*, 2020). Other RTE foods of animal origin have also been reported to harbour *E. coli*. In developed countries such as Italy and Poland, *E. coli* contamination have also been reported at 71% and 10% (Vincenti *et al.*, 2018, Zarzecka *et al.*, 2021). This indicates that the *E. coli* menace in RTE foods is a global problem that should be tackled with urgency.

*Escherichia. coli* is responsible for over 70% of diarrhoeas in children under the age of 5 and majority of the population. Ready-to-eat meats are usually consumed without any further processing. Presence of *E. coli* in RTE meats should be at a minimum of 0 CFU/g. In this study 90% of the RTE samples were positive with coliforms and *E. coli*, a situation that could possibly jeopardize public health by exposing the population to possible food borne disease outbreaks. The consistent presence of *E. coli* in RTE meat

in Kenyan towns as reported in this study could be attributed to by different factors. Most importantly are hygiene conditions in handling and processing of the personnel as well. The survey done by this study on knowledge attitude and practices of RTE meat retailers reported that hygiene practices are poor irrespective of satisfactory knowledge on food safety practices (Section 4.2). Other studies have also emphasized the same (Nga'ng'a *et al.*, 2021; Obinda *et al.*, 2021; Asati *et al.*, 2024). Coupled with a large non-educated proportion, food safety information was of less importance to most retailers. This could explain the reasons why coliforms are consistently reported in RTE beef and chicken meats in the study area and possible in other reported regions as well. This gap is made wider by lack of training on food safety handling. The trainings are sometimes non-structured and only given as a by the way during induction into the work place by the owner/employers

Equipment are also major sources of contamination of the RTE meat. This is because they come into contact with the meat frequently during cutting and serving. Studies have reported that equipment could contaminated with bacteria could contaminate food and the personnel handling the meat if not well washed and sterilized (Adzitey *et al.*, 2021). disinfection of equipment is not a common practice among food handlers in LMICs. Water quality is a major contributor to contamination of RTE meat (Castro *et al.*, 2024). Non-portable water used in the washing of utensils is likely to contaminate RTE food (Ronald *et al.*, 2023). limited access to potable water, which is essential for cleaning and sanitation throughout the food production and handling process could jeopardize the meat microbial safety.

Among the three towns, Meru is the oldest and has a higher urban population compared to all the towns. In this study it has recorded the highest microbial counts in terms of total coliform counts and *E. coli* counts. This could be due to the fact that with a larger urban population, the town is overpopulated and therefore sanitation facilities are overstretched unable to provide basic hygiene services (Adzitey *et al.*, 2021). Cross contamination could also be high due to the high population of people constantly moving from one part of the city to another acting as microbial carriers within the town. Chuka town has recorded the least in AR *E. coli* and *E. coli*. Chuka, Ndagani and Mitheru represented Tharaka Nithi county which has a lower population compared to Embu and Meru County. Translating this to urban population, the numbers could also

be smaller. This could explain the lower contamination rates in Tharaka-Nithi county compared to Meru and Embu County.

Antimicrobial resistant *E. coli* in RTE meat is significantly lower than in raw meat. This could be explained by the heat treatment that the meat has undergone destroying most of the bacteria which would otherwise be present in raw meat (Abass *et al.*, 2020). Meat processing involves extreme changes of temperature during boiling, frying or even grilling. Most of the bacteria present when the meat was raw would probably die. In cases where the meat is highly contaminated, then in its raw state it would introduce a considerable number of bacteria in the handling environment and utensils (Adzitey *et al.*, 2020). The human hands handling the meat, the cutting board and even the cutting equipment would get contaminated. When proper cleaning and sanitation is not done, then the initial bacterial load distributed between the human hands, cutting boards and knives would cause recontamination of the meat after it has been prepared. This cycle of contamination is also likely to occur in instances where raw and RTE meat is sold together too. When hygiene conditions are poor, then it does differ whether raw and RTE meat is sold together. Raw meat could still be a source of contamination to RTE meat in unsanitary environments.

New business establishment which combine sale of raw and RTE meat are becoming very common in Kenyan towns. This is likely due to the fact that business owners would like to benefit from controlling most parts of the value chain. A survey which was part of this study, reported that over 50% (4.1) butcheries are integrated with hotels. Another study in Nairobi also reported the same (Koech *et al.*, 2024) This practice poses a risk to cross contamination from raw and ready to eat. Given that unsatisfactory hygiene practices are reported in the area the risk becomes even more. Sanitation and hygiene of equipment, personnel and premises is not up to standard and therefore this practice should be discouraged or practiced with utmost hygiene practices.

The government has regulations under KEBS on food service hygiene practices (KS 2573: 2015 and DKS 2455:2024) but most of the businesses are not aware of these. Most of the hotels develop their own business plan based on the affordability of the service they can offer. This in turn means that there is no minimum standard to be adhered to and therefore make-shift kiosks and roadside hotels with improper premise

infrastructure are found among the low-income earning population. Some RTE establishment also had raw meat sale, which increases the pressure of cross contamination to the RTE foods from raw meat pathogens. Regulations for raw meat handling by KEBS has been outlined (KS EAS 1190:2023) but one regulation which considers the integrated raw and RTE establishment does not exist. On the other hand, these businesses are continuing to increase since entrepreneurs are maximising on space and opportunities within the value chain. Lack of stringent regulations is a pathway for unregulated procedures without a clear-cut guideline on what needs to be done and what doesn't.

### **5.5 Antibiotic Susceptibility Profile of *Escherichia. coli* isolated from Raw and Ready-to-Eat Beef Samples**

The data from antibiotic sensitivity patterns of *Escherichia coli* isolated from raw beef demonstrated a high prevalence of resistance to  $\beta$ -lactam antibiotics, which are critical in both veterinary and human medicine. Comparatively, resistance against high priority clinically important (HPCI) antimicrobials has been reported in several studies around the globe. In Japan Wang *et al.*, (2017) demonstrated that *E. coli* isolated from raw meat was resistant to tetracyclines (49%), nalidixic acid (28%), ampicillin (24%) trimethoprim and sulfamethoxazole (20%) and cephalexin (18%). Another study in Iran reported MAR of *E. coli* isolated from raw meat and resistance to amoxicillin (79%), trimethoprim/sulfamethoxazole (70%), ampicillin (45%), nitrofurantoin (54%), chloramphenicol (54%), streptomycin (45%), kanamycin (33%), and tetracycline (54%) (Pakbin *et al.*, 2021). In Ethiopia several studies on raw meat have reported resistance to amoxyl, cefoxazime, co-trimazole, gentamicin, third generation cephalosporins, penicillin, erythromycin and amikacin from raw minced meat and a multidrug resistance ranging from 95 to 60% (Tonjo *et al.*, 2022, Worku *et al.*, 2022, Asfaw *et al.*, 2023). Another study focusing on raw meat in Ghana reported that the isolates were resistant to erythromycin (85%), tetracyclines (73%) and ampicillin (72%). The MAR Index was between 0.13 and 1 (Adzitey *et al.*, 2020) values slightly lower than my findings (0.2 – 0.6)

According to several studies around the globe, *E coli* isolated from RTE meat is resistant to at least one antibiotic resulting in multidrug resistance (MAR) with a higher than 0.2 MAR index similar to this thesis. In Kenya, ready to eat red meats in Nakuru

county Kenya had *E. coli* resistance reported against; co-trimazole (80%), tetracycline (73%), streptomycin (67%), ampicillin (67%), amikacin (80%) and ciprofloxacin (100%). At the same time 14/30 of the *E. coli* isolates demonstrated multidrug resistance with a resistance index of 0.2 (Ronald *et al.*, 2023). The multidrug resistance for RTE beef in this study had a range of between 0.2 to 0.47 which is higher than that reported by Ronald *et al.*, (2023) in Nakuru. This could mean that antibiotic uses in eastern Kenyan counties was higher than in Nakuru County resulting to a lower multidrug resistance pattern. *Escherichia coli* isolated from RTE red meat in Ghanaian towns showed resistance against teicoplanin (97%), tetracycline (94%), amoxicillin (71%), azithromycin (71%), sulfamethoxide (58%). The AMR index ranged between 0.22-0.78 while the multidrug resistance was 94% (Abass *et al.*, 2020). These values are higher than the findings of this thesis where the highest AMR index was 0.47 in RTE beef. Resistance against more antibiotics could mean that the commensal bacteria is exposed to different types of antibiotics in its environment.

The resistance patterns observed suggest not only widespread and repeated exposure of *E. coli* populations to these drugs but also possible genetic exchanges through plasmids, transposons, and integrons that facilitate the spread of resistance determinants within bacterial communities (Muloi *et al.*, 2019). Conversely, aminoglycosides (gentamicin, amikacin), phenicols (chloramphenicol), and fluoroquinolones (ciprofloxacin) showed relatively low resistance rates, which may be explained by more restricted usage or higher cost that limits their overuse in veterinary settings.

These findings are significant because  $\beta$ -lactams represent some of the most widely used antibiotics worldwide, and resistance to them severely restricts therapeutic options in both clinical and agricultural settings (Dankar *et al.*, 2022). Amoxicillin, ampicillin, and cotrimoxazole are commonly used in cattle farms in Kenya (Kemp *et al.*, 2021). Their popularity is largely due to their affordability, wide availability, and broad-spectrum activity. However, widespread and unregulated access to these drugs in agrovets and informal markets means they are often administered without veterinary prescription or proper dose control, creating conditions for misuse (Moctor *et al.*, 2020). This results in sub-therapeutic dosing, incomplete treatment courses, and unnecessary prophylactic use, all of which accelerate the emergence of resistant strains. According to Rware *et al.* (2024), 92.7% of antibiotics are administered as prophylaxis, creating

strong selective pressure for resistance. This misuse and overuse of antimicrobials drive the emergence of resistant strains that can then enter the food chain, increasing exposure risk to consumers. Moreover, ampicillin, ceftriaxone, and cotrimoxazole are among the most prescribed antibiotics in human medicine in Kenya for urinary tract infections, enteric fevers, respiratory infections, and sepsis (Kemp *et al.*, 2021; Rware *et al.*, 2024).

Resistance to these drugs in foodborne *E. coli* poses a direct risk to treatment efficacy for common community- and hospital-acquired infections, as infections caused by resistant strains are often associated with longer hospital stays, higher treatment costs, and increased mortality (WHO, 2022b). The overlap of veterinary and clinical use of the same antibiotic classes magnifies the risk, since resistance selected in the agricultural environment can readily spill over into human populations, especially where hygiene and food handling practices are suboptimal. This creates a vicious cycle where antibiotics lose their effectiveness across both sectors simultaneously, leading to a crisis in both healthcare and food security (WHO, 2023b).

Third-generation cephalosporins (like ceftriaxone) are a mainstay for empirical treatment of severe Gram-negative infections in hospitals, often used before diagnostic results are available because of their broad-spectrum activity (Van Boeckel *et al.*, 2015; WHO, 2023b). Gentamicin and amikacin are aminoglycosides used for severe systemic infections in hospitals, and while retained susceptibility in this study is positive, it remains vulnerable, as aminoglycoside resistance can spread rapidly once selection pressure increases (Landers *et al.*, 2012). Some colonies in this study also reported intermediate resistance, which could still pose a risk to resistance development (6%, 20% intermediate resistance). This “intermediate” category suggests that bacteria are already developing tolerance, which could easily progress to full resistance with continued antibiotic exposure (McKernan *et al.*, 2021). Chloramphenicol, once widely used, is now rarely prescribed in humans due to toxicity concerns, but it retains utility as a reserve drug in certain typhoid and meningitis cases, especially where multidrug-resistant Gram-negatives limit treatment options (WHO, 2023a).

Such contrasts demonstrate the complex and multifactorial nature of resistance, shaped not only by local prescription habits but also by farming practices, variations in veterinary drug regulation, and even the microbiological ecology of specific regions (Van Boeckel *et al.*, 2015; Landers *et al.*, 2012). Additionally, the differences may

reflect variations in sampling approaches, laboratory methodologies, and even temporal discrepancies in resistance trends. They can also be attributed to geographical variations and regionalized antibiotic uses (Founou *et al.*, 2018; Moyo *et al.*, 2023). High resistance figures communicate an intense selective pressure exerted by frequent and unregulated use of these antibiotics, which allows resistant strains to thrive while susceptible ones are eliminated. This suggests that when antibiotics are either unavailable, expensive, or tightly controlled in certain regions, resistance trends can be reversed or slowed down, pointing to the effectiveness of regulatory measures when implemented consistently (Mocor *et al.*, 2020). However, the high resistance figures for commonly used drugs highlight a worrying trend that could compromise both animal and public health by limiting treatment options and fostering the spread of resistant pathogens through the food chain.

#### **5.6 Antibiotic Susceptibility Profile of *Escherichia coli* isolated from Raw and Ready-to-Eat Chicken Meat**

Chicken meat had high resistance and more diverse pattern of resistance compared to beef. According to a study done in Limuru with chicken meat, high resistance levels were detected for most commonly used drugs like tetracycline (75.9%) and cotrimoxazole (72.4%) (Adelaide *et al.*, 2008). Other antibiotics like ampicillin (39%), chloramphenicol (13.2%) and ciprofloxacin (19%) recorded resistance levels although they are rarely used in chicken farming. One hundred and seventeen isolates showed resistance to more than two antibiotics. The *E. coli* demonstrated 71 different multidrug resistant patterns with tetracycline/cotrimoxazole being the most common. which is higher than this finding of 9 different resistance patterns. In study done in Dagoreti in Nairobi on chicken meat reported, it was reported that resistance was found in 65.5% and multi-resistance in 37.9% of the isolates (Kikuvi *et al.*, 2013). This resistance pattern is lower than these findings of 50% in MAR.

The most common resistance was to ampicillin, streptomycin, tetracycline, sulphamethoxazole / trimethoprim, and kanamycin (42.5-11.9%) while in this study resistance was highest against amoxyl, tetracycline and amikacin. Resistance to kanamycin, sulphamethoxazole /trimethoprim, and tetracycline was significantly lower (Kikuvi *et al.*, 2013) similar to this report. According to Odwar in Nairobi with chicken carcass, 75% of the isolates were resistant to at least one of the 12 antibiotics tested

with resistance to tetracycline being the highest at 60.3% (Odwar *et al.*, 2014). These findings are similar to my findings where resistance against tetracycline was high, probably due to a lot of exposure of chicken during rearing to the tetracycline antibiotic as a feed promoter (Ngai *et al.*, 2021).

The resistance profile in the Eastern Kenya chicken meat isolates reflects antibiotic selection pressure shaped by on-farm use, retail chain practices, and weak regulatory controls. Globally and in sub-Saharan Africa, the most commonly used antibiotic classes in chicken include tetracyclines, penicillins, and sulphonamides (Woyda *et al.*, 2023). These are the same classes with high resistance in this study. This class of antibiotics are common because they are affordable, widely available, and often used for prophylaxis, metaphylaxis, or growth promotion (Onono, 2023). High intermediate categories seen in several antibiotics suggest frequent sub-therapeutic exposure and evolving tolerance that can evolve into full resistance when misuse continues. Without such oversight, both animal welfare and public health remain at risk. Importantly, interventions should emphasize farmer training, improvement of record-keeping for treatments, and enforcement of prescription-only sales of veterinary antimicrobials, which remain poorly implemented in many Kenyan counties (Ipara *et al.*, 2019; Kiambi *et al.*, 2021; Mbai *et al.*, 2022).

High-priority critically important (HPCI) antibiotics for human medicine include third-generation cephalosporins, fluoroquinolones, macrolides, and certain aminoglycosides. This study shows notable resistance to cefpodoxime and ceftriaxone (third-generation cephalosporins). Use of third-generation cephalosporins and fluoroquinolones in Chicken, especially off-label, selects for ESBLs and resistance, creating a human health risk because these drugs are essential for severe human infections (Moyo *et al.*, 2023). Studies in Kenya and neighboring countries have documented veterinary and farm-level access to such drugs informally under limited veterinary oversight, facilitating selection of HPCI resistance in chicken (Muinde *et al.*, 2023; Muloi *et al.*, 2025).

The dominant phenotypes of multidrug resistance patterns featured  $\beta$ -lactams (amoxicillin/ampicillin), tetracycline, and trimethoprim-sulfamethoxazole, with frequent co-resistance to first/third-generation cephalosporins and co-trimoxazole. These patterns mirror Kenyan evidence that chicken and meat value-chains are

important reservoirs of MAR *E. coli* (Muloi *et al.*, 2025). These could be driven by excess antibiotic use, informal market hygiene gaps, and slaughterhouse cross-contamination. A study in Nairobi on retail meats and chicken commodities reported high resistance to tetracycline, ampicillin and co-trimoxazole, closely similar to these findings (Muinde *et al.*, 2023). Slaughterhouse studies in western Kenya further show environmental circulation of resistant *E. coli*, explaining why both raw and RTE products can share resistance signatures when handling and evisceration controls are weak (Hamilton *et al.*, 2024). Kenyan butchereries frequently report unsafe handling and inadequate sanitation; such practices plausibly sustain RTE contamination in unhygienic cooking environments. Moreover, Kenyan chicken studies consistently report high phenotypic resistance to ampicillin, tetracycline and co-trimoxazole closely similar to these results (Ipara *et al.*, 2019; Mbai *et al.*, 2022; Onono, 2023; Gulati & Juneta, 2023).

Comparatively across Africa, beef and poultry systems report similar MAR patterns. Ghanaian raw-meat surveys found ESBL-producing and MAR *E. coli* with prominent resistance to  $\beta$ -lactams and tetracycline, consistent with AMX/AMP/cephalosporin co-resistance in this study (Dsani *et al.*, 2020). Ethiopian retail-beef also documents high MAR burdens, reflecting regional parallels in antimicrobial access, veterinary oversight, and sanitation along slaughter and retail nodes (Woldu, 2024). Taken together, the evidence demonstrates how resistance patterns are shaped not only by drug exposure but also by cross-contamination pathways that connect farms, slaughterhouses, retail chains, and consumer kitchens. Public health policies must therefore integrate both antimicrobial use reduction and stringent food hygiene regulations to break the resistance cycle effectively.

### **5.7 Prevalence of Resistance Genes of Pathogenic Drug Resistant *Escherichia. coli* in Raw and Ready-to-Eat Beef and Chicken Meat**

Antimicrobial resistance genes have emerged as a major global health threat, with the food chain increasingly recognized as a critical transmission pathway. Tetracycline resistance genes such as *tetA* are widely reported in livestock and meat because tetracyclines have been heavily used historically in animal production and remain widely accessible in many low- and middle-income country (LMIC) settings (Moyo *et al.*, 2023; Moctor *et al.*, 2020). ESBL genes (*bla*<sub>CTX-M</sub> family, *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub>, *bla*<sub>OXA</sub>)

are commonly carried on mobile plasmids in Enterobacterales and frequently detected in raw meat across settings because of selection in the farm environment and contamination at slaughter and retail.

In Kenya presence of resistance genes in RTE meat is minimal compared to studies in carcasses at the slaughterhouse and with raw meat (Personal observation). Resistant genes of *E. coli* have been reported in RTE meat in Nakuru (Ronald *et al.*, 2023). The identified ARGs were *Sul1* (100%), *bla<sub>TEM</sub>* (50%) and *tetA* (100%). These values are higher than our findings where the prevalence of *Sul1*, *bla<sub>TEM</sub>* and *tetA* in RTE meat was 10%, 11.7% and 16.7% respectively. These differences could be due to different antibiotic use between the regions. Resistance genes of *E. coli* have been isolated locally in different samples such as raw milk (Ngaywa *et al.*, 2019), animal droppings at the farm (Langata *et al.*, 2019), waste water (Wawire *et al.*, 2021) and even human faeces (Kipkorir *et al.*, 2016). The environmental samples in those studies could act as contamination sources for raw and RTE meat under poor hygiene handling conditions. In Nairobi raw meat at butchereries were reported to harbour ESBL such as *bla<sub>TEM</sub>* (65%), *bla<sub>CTXM</sub>* (44%), *bla<sub>OXA</sub>* (33%) and *sul1* (46.7%) (Chepkemei *et al.*, 2025). The values are also higher compared to findings in this study which recorded values ranging between 23.3% and 5% in prevalence of the ARGs under study. These genes could be responsible for slowing down treatment among patients. A study done in six hospitals in Kenya reported the prevalence of ESBL with prevalence of over 90% in some ARGs (*bla<sub>CTXM</sub>* (95.6%), *bla<sub>TEM</sub>* (95.6%), *bla<sub>SHV</sub>* (21.7%)) (Muriuki *et al.*, 2022).

In Mozambique an LMIC country several ARG from *E. coli* have been isolated from street foods and water. The prevalence of the ARGs in the water was higher than those in RTE foods indicating that water could be a contaminant of RTE foods with *E. coli* containing resistance genes. In the RTE foods reported was *bla<sub>TEM</sub>* (15.8%), *bla<sub>SHV</sub>* (23.7%), *bla<sub>OXA</sub>* (0%), *tetA* (71.4%) and *Sul1* (0%) (Salamandane *et al.*, 2022). The values reported by Salamandane *et al.*, (2022) are almost within the range of my findings except for *tetA* which was at 71.4% compared to 23.3%. Tetracycline has been reported to be one of the antibiotics used as a growth promoter in poultry farming (Moyo *et al.*, 2023). This could mean that the use of tetracycline in Mozambique is higher than in local eastern farms of Kenya. Studies from South Asia and other African LMICs report comparable ARG patterns in meats. Research in Bangladesh and Pakistan

found high prevalence of *tetA* and various  $\beta$ -lactamase genes in raw meat and poultry, often at higher rates than in RTE products, and documents the presence of *mcr* variants in animal and food samples as documented in a recent regional review (Chilanga *et al.*, 2025).

In other countries outside the continent, prevalence of ESBL and other ARG are also being reported in meat value chain products (raw and RTE). In Bangladesh, Hossain *et al* (2024) reported the presence of *sul1* (21.8%), *tetA* (43.5), *Bla<sub>TEM</sub>* (0%) and *bla<sub>SHV</sub>* (0%) in raw meat samples. The values are lower compared to this study likely due to strict antibiotic use or high hygiene in the country. From developed countries such as China, antibiotic resistance genes such as *mcr1* (colistin resistant) have been isolated from poultry farms, raw and RTE meat and meat products. Colistin being a last resort antibiotic its resistance genes could mean lack of treatment if transferred to the human microbiome. According to Li *et al.*, (2024) *mcr1* was identified in 9 *E. coli* isolates out of 200 in poultry farm samples. In raw retail meat of China *mcr1* has also been isolated in 5% of the samples (Wang *et al.*, 2024).

In contrast, high-income countries often report lower overall prevalence of some ARGs in retail meats, though ESBLs and tetracycline genes remain detectable in surveillance programs. According to the European Union Food safety Authority (EFSA & ECDC, 2023) reports that strict regulations on antibiotic use in livestock and established hygiene controls reduce, but do not eliminate, ARGs in meats; notable exceptions occur where specific farming practices or importation introduce ARGs Genomic studies in Europe and North America also highlight complex resistance pattern overlap between human and animal *E. coli* strains, but tend to show lower *mcr* prevalence compared with LMIC reports. The presence of these resistance genes could be due to a number of drivers. First of all, on-farm antibiotic use where animals are exposed to antibiotics during husbandry. Routine therapeutic, prophylactic and growth-promotion antibiotic use in livestock selects for resistant bacteria; tetracyclines, penicillins /  $\beta$ -lactams and sulphonamides are commonly used and match the genes detected (*tetA*, *bla<sub>TEM</sub>*, *sul1*) (Moctor *et al.*, 2020; Moyo *et al.*, 2023; Chilanga *et al.*, 2025).

Horizontal gene transfer through Mobile genetic elements (plasmids, transposons) facilitate dissemination of ARGs among the gut bacteria such as *E. coli* (Founou *et al.*,

2018). Cross contamination may occur during slaughter from animal GIT, during transport, at butcheries and during food preparation which could contribute to spread and persistence of ARG-carrying organisms in the food value chain. Environmental factors such as manure, contaminated water, or sanitation gaps may introduce ARGs into the food chains; inadequate wastewater treatment amplifies this cycle and creates an environmental ARG pools (Salamandane *et al.*, 2022). For RTE meats, post cooking processes could lead to acquisition of resistant bacteria through the food handlers, contaminated utensils and storage of RTE foods at sub-optimal temperatures.

### **5.8 Prevalence of Virulence Genes of Pathogenic Drug Resistant *Escherichia. coli* in Raw and RTE Beef and Chicken Meat**

These results indicate a prevalence of Diarrhoeagenic *E. coli* in raw and RTE meat which is similar to other studies in Ghana, Bangladesh, China, Japan and Egypt (Baloch *et al.*, 2017, Mkhtar & Karmi, 2021, Wang *et al.*, 2017, Beshiru *et al.*, 2022). The distribution of the pathotypes is however different from one study to the other probably due to geographical differences. In this study STEC was the most prevalent pathotype while Beshiru and others (2022) in Nigeria recorded ETEC as the most prevalent pathotype in RTE foods (Fayemi *et al.*, 2021). The results could also be different since apart from meat and meat products, fruits and vegetables were also sampled and that was not the same for this work. According to Wang *et al.*, (2017), STEC was the least prevalent pathotype of *E. coli* while EAEC recorded high prevalence of 62% followed by EPEC at 16% in Japan. Similarly, Poultry products also scored the highest prevalence of diarrheagenic *E. coli* which is also similar to the findings of this study. The low prevalence of STEC could be attributed to improved hygiene practices in the markets of Osaka Japan compared to the LMICs towns like the ones in Kenya. Bolach *et al.*, (2017) also did not find presence of STEC in the RTE foods in China but only reported EIEC. Mokhtar and Kirmi in Egypt reported presence of STEC and EPEC in meat and meat samples such as hamburgers, sausages, minced meat and fried chicken.

Contamination of food with DEC is mainly from environmental factors and poor hygiene. According to Ogumbo *et al.*, (2024) who together with KEMRI reported that Eastern Kenya is leading in diarrheagenic diseases, also in the same report reiterated that the main source of human contamination with DEC is from poor sanitation and hygiene. Pathogenic *E. coli* is spread to the environment from human and animal faeces

(Okumu *et al.*, 2025). *Escherichia coli* is a normal microbial flora in the animal GIT and is the first one to colonize an infant's GIT. It is beneficial in depleting Oxygen and encouraging the growth of anaerobic beneficial bacteria in the GIT, it also produces vitamins a relationship referred to as commensal by most literature (EFSA & ECDE, 2023). On the downside, some pathogenic strains may also occur together with the commensal variants and be shed into the environment during defecation. This makes warm blooded animals the main reservoirs of pathogenic *E. coli*.

Surface water runoff, poor sewer systems and lack of frequent hand washing especially after visiting the toilet is among the main sources of environmental contamination with *E. coli* (Brito-Junior *et al.*, 2025). In this study (4.1) some butchereries had close proximity to waste disposal sights and open sewer systems. Some establishment also lacked hand washing stations while others reported to use water from unknown sources (vendors) and even rivers. Studies have been able to isolate pathogenic *E. coli* from river water, service stations, equipment and personnel. (Salamandane *et al.*, 2022). The same study collected samples from hands of personnel, kitchen utensils and cookerries in RTE food stations and reported that they were highly contaminated with pathogenic *E. coli* especially EAEC. Therefore, contamination of Food is likely to be facilitated by contaminated equipment in food service stations.

The pathotypes studied in this work each has a characteristic virulent factor which is responsible for the symptom of diarrhoea. For STEC the virulent factor *stx* (1 and 2) are responsible for the production of shiga-toxin. This toxin is responsible for early onset of diarrhoea (watery or bloody) in individuals especially children under the age of 5 which still have low immunity (Madoroba *et al.*, 2022; Okumu *et al.*, 2025). It is also associated with haemorrhagic colitis, haemolytic uremic syndrome, and thrombotic thrombocytopenic purpura (Mokhtar & Karmi, 2021). Centre for disease control and prevention has also reported that it is very common in minced meat (CDC, 2017). The *stx1* factor was used as an indicator for the presence of STEC, STEC is considered the most pathogenic DEC which is also present in the O157:H7 strain which has developed heat resistance and can thrive at temperatures where other STECs would be destroyed (EFSA & ECDE, 2023). In this study *stx1* was present in not less than 30% of all *E. coli* isolates from raw beef and chicken meat and RTE beef and chicken meat samples. The shiga-like producing *E. coli* also contain the *stx* gene has been isolated

in many poultry farms, meat and associated RTE chicken products (Lopes *et al.*, 2023; Li *et al.*, 2024; Wang *et al.*, 2024). These STEC have mainly been isolated from meat, meat products, raw milk, cattle faeces and have been categorised as the main mediums to human infections in some Kenyan studies (Muinde *et al.*, 2023; Okumu *et al.*, 2025). The hemolytic uremic syndrome (HUS) causes a 5% mortality rate and survivors are highly likely to remain with chronic kidney illness (CDC,2017).

The *stII* gene in enterotoxigenic *E. coli* code for the production of heat stable enterotoxin which causes fluid secretion in the intestines leading to diarrhoea. The symptom is common in travellers and children (Barito-Juniro *et al.*, 2025). Since this was the only gene identified in the procedure, it could be assumed that other virulent factors of ETEC are present in the isolate. Virulent factors in ETEC are primarily based on colonization (CFs) and toxin production factors. The colonisation factors help the bacteria to adhere to receptors on the intestinal cell walls before they start to produce toxins. Enterotoxin factors are mainly ST and LT. the ST factors are responsible for the production of heat stable enterotoxins while the LT code for the production of heat labile enterotoxins (Pakbin *et al.*, 2021). The prevalence of *stII* in this study was 45% in raw meat and 35% in RTE meats. The *stII* gene mainly causes diseases in calves and pigs and is only found in human as a contamination from animal sources (Li *et al.*, 2024). Its presence in our RTE meat samples indicate that there could be contamination from sick calves or pigs which share the environment with the human in the vicinity where the meat is being prepared.

The virulent gene *eaeA* in EPEC is responsible for intimate attachment on the epithelial cells (Pakbin *et al.*, 2021). The intimin gene is required for full virulence to occur. In this study *eaeA* gene was found in 46% in raw meat and 30% in RTE meat. Its presence in raw meat is explained by cross contamination during slaughter process while RTE is contaminated from the non-portable water, poorly cleaned cutlery or unwashed dirty hands contaminated before handling of food (Li *et al.*, 2024). Other virulent factors occur in EPEC and are suspected to be present in the isolates positive for *eae A* gene. In Egypt the *eae* gene was isolated from raw beef, cooked beef and raw milk (Mokhtar and Kirmi, 2021), indicating that its highly prevalent in food of animal source especially cattle.

The virulent gene in EAEC *aafII* is responsible for its adherence to the epithelial wall in a pattern commonly referred to as a stacked brick pattern (Hunt, 2010). It therefore plays a major role in the pathogenesis of the bacteria. EAEC is characterised by other virulent factors such as toxin production. Apart from diarrhoea, EAEC is responsible for intestinal inflammation, childhood malnutrition and growth impairment. In Nairobi, Mukuru Kwa Njenga settlement, EAEC was isolated from stools of children with close contact with animals, the study concluded that food animals are the main reservoirs to transmission to children (Okumu *et al.*, 2025). Due to the frequent hand to mouth behaviour, children are highly likely to be infected by the pathogen. For meat, contamination occurs mainly during skinning of the animal and removal of the gut contents. If poor hygiene proceeds throughout the value chain pathogenesis will be enhanced.

The *virF* gene is a critical virulence regulator in Enteroinvasive *Escherichia coli* (EIEC). This gene is located on a large virulence plasmid known as *pINV*. It encodes an AraC-like transcriptional activator that initiates the expression of other key virulence genes, such as *virB*, which in turn activates the invasion plasmid antigen (*ipa*) genes (Boukli-Hacene, 2024). These *ipa* genes are directly responsible for enabling EIEC to invade and multiply within human intestinal epithelial cells and subsequent cell-to-cell spread, leading to inflammation and mucosal damage (Pakbin *et al.*, 2021). Clinically, EIEC infections caused by *virF*-positive strains manifest as gastrointestinal illness the symptoms include watery or bloody diarrhoea, fever, abdominal pain, tenesmus (a painful urge to defecate), and general discomfort. These effects are a direct result of the cellular invasion and tissue destruction driven by the *virF*-regulated virulence system (Eltai *et al.*, 2020). The severity of illness can vary from mild diarrhoea to more serious inflammatory colitis, particularly in vulnerable populations such as children, the elderly, and immunocompromised individuals. While EIEC is more commonly transmitted through the faecal-oral route via contaminated water. In this study (4.1), majority of the establishment obtained water from municipal water system, which is treated. A number (14%) were still getting water from uncertain vendors and rivers, the risk for contamination with EIEC virulent gene therefore still exists in the area.

In this study the *daaE* gene of diffusively adherent *E. coli* (DAEC) had a prevalence of 33% in raw meat and 27% in RTE meats, the least prevalence was reported in chicken

compared to beef. Naturally the *daaE* gene is mainly associated with cattle unlike poultry populations (Igbokwe *et al.*, 2025). The *daaE* gene encodes a subunit of the *Dr* family of adhesins, specifically the F1845 fimbrial adhesin, which mediates diffuse adherence to epithelial cells. The *daaE* protein enables the bacteria to bind to the host cell surface receptor, facilitating the colonization of the intestinal mucosa (Pakbin *et al.*, 2025). This interaction is crucial for initial attachment and subsequent colonization, thereby promoting the persistence of the organism in the gut and contributing significantly to its pathogenic potential. Infections caused by DAEC strains harbouring the *daaE* gene are typically associated with recurrent diarrhoea (Yada *et al.*, 2024). The symptoms include mild to moderate watery diarrhoea, abdominal cramps, low-grade fever and dehydration. Unlike other diarrheagenic *E. coli* such as EIEC or EHEC, DAEC does not invade epithelial cells or produce classical toxins like Shiga toxin, but rather causes damage through sustained adherence and possibly by inducing pro-inflammatory responses. The chronic colonization facilitated by *daaE*-mediated adhesion may also disrupt the epithelial barrier and modulate host immune responses, contributing to prolonged gastrointestinal symptoms (Eltai *et al.*, 2020). Main contamination source is indicated as faecal oral route. With the current study, contaminated meat value chain is also a driver of spread within the environment.

## CHAPTER SIX

### SUMMARY CONCLUSION AND RECOMMENDATIONS

#### 6.1 Summary

The study profiled butcheries across Embu, Meru, and Tharaka-Nithi counties, revealing diverse socio-demographic characteristics of meat handlers, with most being male, aged 31–40 years, and possessing at least secondary education. Operationally, butcheries varied in size, meat types sold, and sourcing patterns, with a majority relying on local suppliers. While disinfection of equipment and premises was generally practiced, binary logistic regression identified age as the only statistically significant predictor ( $p < 0.05$ ): meat handlers aged 31–40 years had nearly four times higher odds (OR = 3.94) and those aged 41–50 years had over six times higher odds (OR = 6.68) of practicing disinfection compared to those aged 21–30 years. Compliance with mandatory food handler's certification was 100% across counties; however, adherence to the recommended three-month medical check-up interval was inconsistent. Government hygiene inspections were typically conducted monthly. Most butcheries accepted both cash and mobile money payments, though few had separate cashiers, potentially increasing cross-contamination risks. Use of protective clothing was inconsistent, ranging from 31% in TNC to 50% in Meru, highlighting gaps in personal hygiene practices.

Analysis of socio-demographic factors revealed that education level, possession of a food handling certificate, and participation in food safety training were strongly associated with higher knowledge, attitude, and practice (KAP) scores among RTE meat handlers ( $p < 0.05$ ). Knowledge scores increased progressively with education, from primary ( $13.9 \pm 9.2$ ) to university level ( $28.3 \pm 6.2$ ). Trained handlers scored significantly higher in knowledge ( $25.6 \pm 7.6$ ), attitudes ( $52.5 \pm 8.8$ ), and practices ( $23.6 \pm 2.6$ ) than untrained counterparts. Selling raw meat alongside RTE products was also linked to better KAP. County and town-level variations were evident; Embu consistently recorded the highest attitude ( $54.1 \pm 11.7$ ) and practice ( $23.6 \pm 3.2$ ) scores, while Meru led in knowledge ( $25.4 \pm 5.1$ ). Tharaka-Nithi recorded lower scores in all domains. Notably, Embu had the widest range in knowledge scores (0–32), indicating disparities among handlers. Gender, age, and work experience showed no significant associations with KAP ( $p > 0.05$ ). Overall, food safety training emerged as the most

consistent predictor of higher KAP scores, underscoring its critical role in improving food safety compliance among RTE meat retailers.

There was microbial quality assessment of raw beef and chicken meat which revealed widespread contamination, with chicken consistently exhibiting higher microbial loads than beef. Total coliform counts in chicken were highest in Meru Town (8.32 Log<sub>10</sub> CFU/g) and Ndagani & Mitheru (8.18 Log<sub>10</sub> CFU/g), while the lowest levels were found in beef from Nkubu (3.23 Log<sub>10</sub> CFU/g) and Runyenjes (3.71 Log<sub>10</sub> CFU/g). *E. coli* counts followed a similar pattern, with chicken from Embu Town recording the highest levels (5.69 Log<sub>10</sub> CFU/g) and beef from Ndagani & Mitheru the lowest (1.40 Log<sub>10</sub> CFU/g). Antimicrobial-resistant (AR) *E. coli* were more prevalent in chicken, peaking in Meru Town (3.22 Log<sub>10</sub> CFU/g). Beef showed relatively lower loads, with the lowest values in Embu (<0.70 Log<sub>10</sub> CFU/g). The proportion of AR *E. coli* relative to total *E. coli* was highest in chicken from Meru (69.40%) and Nkubu (62.94%). Overall, 100% of samples exceeded the permissible TCC limits and over 80% surpassed acceptable *E. coli* limits, highlighting significant food safety risks.

The microbial quality assessment of ready-to-eat beef and chicken meat revealed varying contamination levels across counties and towns. Beef samples generally exhibited higher TCC than chicken, with the highest in Nkubu (5.66 Log<sub>10</sub> CFU/g) and Meru Town (5.12 Log<sub>10</sub> CFU/g), while the lowest was in Ndagani & Mitheru (1.80 Log<sub>10</sub> CFU/g). In contrast, chicken had significantly lower TCC values, ranging from 3.89 Log<sub>10</sub> CFU/g in Embu Town to as low as 1.27 Log<sub>10</sub> CFU/g in Meru Town. For *E. coli*, beef samples recorded higher contamination at 2.87 Log<sub>10</sub> CFU/g in Meru Town, while the highest in chicken was 1.54 Log<sub>10</sub> CFU/g in Ndagani & Mitheru. However, antimicrobial-resistant *E. coli* (AR *E. coli*) was more prominent in chicken, particularly in Embu Town (0.67 Log<sub>10</sub> CFU/g), compared to beef, which reached a maximum of 0.24 Log<sub>10</sub> CFU/g. Proportionally, chicken had a higher percentage of resistant isolates (34.67%) than beef (9.05%), highlighting a greater risk of resistance transmission through chicken meat. Geographically, Embu Town recorded the highest overall resistance levels, while Ndagani & Mitheru showed the lowest. These findings emphasize that although RTE beef carries higher general bacterial loads, RTE chicken poses a greater antimicrobial resistance risk, underscoring critical food safety concerns.

The antibiotic susceptibility profile of *Escherichia coli* isolated from raw and RTE beef showed varying resistance pattern. In raw beef resistance was highest against amoxicillin (100%), followed by ceftriaxone (60%), methicillin (63.33%), minocycline (63.33%), cefpodoxime (56.67%), ampicillin (53.33%), and sulfamethoxazole-trimethoprim (53.33%). Moderate resistance was noted against tetracycline (40%) and doxycycline (26.67%), while low resistance was observed against gentamicin (6.67%), ciprofloxacin (3.33%), erythromycin (13.33%), and lincomycin (0%). Notably, chloramphenicol recorded no resistance. Intermediate susceptibility was frequent, particularly with doxycycline (60%), gentamicin (76.67%), ciprofloxacin (66.67%), and lincomycin (83.33%), indicating emerging resistance trends. For RTE beef, amoxicillin also recorded complete resistance (100%), with no susceptible or intermediate isolates. Ampicillin and methicillin showed moderate resistance (40% and 33%, respectively) but high intermediate susceptibility (42–45%). Cephalosporins displayed resistance ranging between 28–33%, with 30–50% intermediate susceptibility. Colistin retained efficacy, showing only 10% resistance and 57% susceptibility. Lower resistance rates were recorded for cotrimoxazole (28%), chloramphenicol (8%), erythromycin (5%), and lincosamide (22%), most of which had susceptibility above 60%.

The antibiotic susceptibility profile of *Escherichia coli* isolates from raw and RTE chicken meat in Eastern Kenya demonstrated significant resistance trends. High resistance was observed against methicillin and ampicillin (80% each), amoxicillin (66.67%), and third-generation cephalosporins, cefpodoxime and ceftriaxone (60%) each in raw chicken meat. Tetracycline (50%), doxycycline (66.67%), and cotrimoxazole (63.33%) also exhibited moderate to high resistance. Chloramphenicol recorded 60% resistance, while minocycline (36.67%), amikacin (26.67%), gentamicin (16.67%), colistin (16.67%), erythromycin (23.33%), and lincomycin (23.33%) showed comparatively lower resistance. Intermediate susceptibility was common across several antibiotics, with high values in gentamicin (66.67%), amikacin (53.33%), minocycline (40%), and lincomycin (63.33%), reflecting a potential shift towards resistance if selective pressure persists. In RTE chicken, resistance levels were markedly higher. Lincomycin showed the highest prevalence at 96.7%, followed by erythromycin (93.3%), tetracycline (90%), and ciprofloxacin (83.3%), all categorized as WHO Highest Priority Critically Important (HPCI) antimicrobials. This suggests significant

antimicrobial exposure in poultry production, raising concerns over treatment efficacy in both veterinary and human medicine. Lower resistance was noted in gentamicin (36.7%) and chloramphenicol (20%), indicating retained effectiveness of these drugs. The high resistance burden, especially in RTE chicken, underscores the risk of transmitting multidrug-resistant strains to consumers.

The analysis of multiple antibiotic resistance (MAR) indices of *Escherichia coli* from raw and ready-to-eat (RTE) beef and chicken meat revealed widespread resistance with notable differences across sample types. Ready-to-eat beef isolates showed moderate resistance, RTE chicken isolates exhibited slightly higher resistance, with MAR colonies between 3–8 and MAR values up to 0.53, indicating increased exposure to multiple antibiotics. Resistance was more pronounced in raw meats. Raw beef isolates with MAR indices of 0.27–0.60, most frequently showing resistance to six antibiotics, especially  $\beta$ -lactams, cephalosporins, tetracyclines, and cotrimoxazole. Raw chicken isolates demonstrated the highest resistance burden, MAR indices up to 0.73. Several isolates resisted up to ten or eleven antibiotics, highlighting chicken meat as a stronger reservoir of multidrug-resistant strains.

This study examined the occurrence and drivers of antimicrobial resistance genes (ARGs) such as *tetA*, *blaCTX-M*, *blaTEM*, *blaSHV*, and *mcr* variants in raw and ready-to-eat (RTE) meats. Tetracycline resistance genes remain widespread due to the historical and continued use of tetracyclines in livestock, especially in low- and middle-income countries (LMICs). Extended-spectrum  $\beta$ -lactamases (ESBLs) were frequently detected, often carried on mobile plasmids, with higher prevalence in raw meat compared to RTE products, though cross-contamination and poor hygiene sustained detection in RTE samples. Kenyan studies revealed frequent detection of *blaTEM* and *tetA* in raw meats, Key drivers could include on-farm antibiotic misuse, cross-contamination at slaughter, poor sanitation in butcheries, environmental spread through contaminated water and manure, and handling lapses in RTE food preparation. These ARGs pose major health risks, including transfer to human gut flora and reduced treatment options due to ESBL and *mcr-1*.

Amplification using PCR confirmed the presence of virulence genes associated with diarrheagenic *Escherichia coli* (DEC) pathotypes across raw and ready-to-eat (RTE)

meat samples. Key virulence markers detected included *eae* (EPEC), *daaE* (DAEC), *stII* (ETEC), *stx1* (STEC), *virF* (EIEC), and *aafII* (EAEC). Overall, STEC (*stx1* gene) emerged as the most prevalent pathotype, accounting for 51% of isolates in raw meat, while EIEC recorded the lowest prevalence (23.3%) in RTE meat. A consistent trend was observed where higher contamination in raw meat corresponded with higher prevalence in RTE meat, highlighting cross-contamination along the value chain. Raw chicken meat was the most contaminated sample type, with EIEC (70%) and STEC (63.3%) showing the highest detection rates. ETEC exhibited the lowest prevalence (6.7%) in chicken, though DAEC and ETEC were disproportionately represented in raw chicken compared to other meats. Raw beef was moderately contaminated, with *stx1* at 40% and *daaE* at 26.7%. RTE chicken showed higher contamination with pathogenic, drug-resistance genes compared to RTE beef substantiating its role as a critical vehicle for resistant *E. coli*. Differences across counties suggest varying antibiotic use and hygiene practices influence prevalence

## **6.2 Conclusion**

This study highlights critical socio-demographic and operational dynamics that could affect hygiene and food safety in butcheries across Embu, Meru, and Tharaka-Nithi counties. While disinfection practices were generally observed, age emerged as the strongest predictor of compliance. Food handler certification was universal, though medical check-up adherence remained inconsistent. Protective clothing use and cashier separation were notably weak, increasing cross-contamination risks. Education level, food safety training, and certification were strongly associated with higher knowledge, attitude, and practice (KAP) scores, with Embu outperforming other counties. Overall, training stood out as the most significant factor influencing compliance, underscoring its role in safer meat handling in the whole meat value chain.

The microbial assessment of raw and ready-to-eat (RTE) meats revealed widespread contamination, with chicken consistently harboring higher microbial and antimicrobial-resistant (*E. coli*) loads compared to beef. Raw chicken, particularly from Meru and Embu, exceeded permissible limits, posing significant food safety risks. Although RTE beef demonstrated higher general bacterial counts, RTE chicken carried proportionally more resistant isolates, indicating a greater potential for antimicrobial resistance transmission. Geographical variations highlighted Meru and Embu as hotspots, while

Ndagani & Mitheru recorded the lowest contamination levels. Overall, the findings underscore the dual risks of poor hygiene and rising resistance, especially from chicken meat.

The antibiotic susceptibility profile of *E. coli* from raw and ready-to-eat (RTE) meats revealed widespread resistance, with raw beef and chicken exhibiting particularly high resistance to  $\beta$ -lactams and tetracyclines. Amoxicillin resistance was universal across all samples, while chloramphenicol and gentamicin retained partial efficacy. RTE chicken posed the greatest concern, showing alarmingly high resistance to WHO Highest Priority Critically Important antimicrobials, including lincomycin, erythromycin, tetracycline, and ciprofloxacin. Multiple antibiotic resistance (MAR) indices were highest in raw chicken, confirming it as a key reservoir of resistant strains, with significant risks for public health and treatment effectiveness.

Also, antimicrobial resistance genes are widespread in raw and RTE meats. Tetracycline and ESBL resistance determinants are particularly prevalent, driven by indiscriminate antibiotic use, cross-contamination, and environmental reservoirs. While RTE meats generally show reduced prevalence due to cooking, re-contamination during handling and poor hygiene sustain risks. The presence of clinically significant genes such as *blaTEM* and *mcr-1* underscores the public health threat, as resistant bacteria or their genes may reach consumers.

PCR analysis confirmed the widespread presence of diarrheagenic *E. coli* (DEC) pathotypes in both raw and ready-to-eat (RTE) meats, with six key virulence genes identified. Shiga toxin-producing *E. coli* (STEC, stx1) was the most dominant, particularly in raw meats, while entero-invasive *E. coli* (EIEC) showed the lowest prevalence in RTE samples. Raw chicken emerged as the most contaminated matrix, harbouring especially high levels of STEC and EIEC, while RTE chicken carried greater pathogenic and resistant strains than RTE beef. Cross-contamination from raw to RTE products underscores gaps in hygiene, handling, and value-chain practices, amplifying public health risks.

### **6.3 Recommendation of the Study**

- i. Enhance routine training, enforce medical check-up intervals, strengthen protective clothing use, and improve hygiene infrastructure to ensure safer meat retail.
- ii. Routine surveillance of antibiotic residues and usage along the entire meat value chain
- iii. Development of appropriate policies for informal meat markets
- iv. Transform hygiene practices by embracing latest technologies that are environmentally friendly
- v. Implement stricter hygiene controls, strengthen antimicrobial resistance monitoring, and enforce HACCP-based practices to minimize contamination and resistance risks in meat.
- vi. Strengthen antibiotic stewardship, regulate veterinary drug use, encourage hygiene in in the food value chain, and intensify surveillance to curb multidrug-resistant *E. coli* in meat.
- vii. Enforce strict hygiene protocols, improve handling practices, and implement targeted surveillance to minimize pathogenic *E. coli* transmission through meat products. Adopt One Health interventions, regulate antibiotic use, enforce hygiene across value chains, expand surveillance, and promote public awareness on antimicrobial resistance risks.

### **6.4 Recommendations for Further Studies**

- i. Conduct studies with broader spectrum of microorganisms such as *Salmonella spp*, *Listeria spp*, *Campylobacter spp*, *Staphylococcus aureus* etc.
- ii. Introduce more food of animal source like milk and eggs in future studies.
- iii. Carry out whole genome sequencing in microbial characterisation.
- iv. Apart from meat samples analysis, introduce environmental samples such as swabs of walls, floors, hands and even water used in butcheries and hotels

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## APPENDICES

### Appendix 1: Raw Meat Retailers Questionnaire

**Introduction:** I am a student from Chuka University, studying practices related to raw meat contamination in Lower Eastern Kenya. Your participation will be highly appreciated.

**Consent to participate in research:** Participation is entirely voluntary. Nevertheless, if you choose to participate, you will be contributing to the body of knowledge on raw meat handling practices in relationship with risks to contamination with pathogenic microorganisms. In case any questions are asked that you would rather not respond to, you may request that we skip or entirely stop the interview.

**Confidentiality:** Your responses will be kept completely confidential by the researchers. No names or personal information will be published by the researchers. The data collected will only be used to give an understanding of the raw meat handling practices that pose as risks to contamination with pathogenic microorganisms.

Questionnaire number..... Date of interview .....

Interviewer's Name.....

County.....Sub County.....

Town.....Location: Latitude..... Longitude.....

#### A. BIO DATA

1. Gender a) Male b) Female
2. Age:
  - 1) Below 20 [ ] 2) 20-30 [ ] 3) 30-40 [ ] 4) 40-50 [ ] 5) 50 -60 [ ] 6) Above 60 [ ]
3. Level of Education of the butchery personnel
  - a) No formal education [ ]
  - b) Primary school (In-complete) [ ]
  - c) Primary School (Complete) [ ]
  - d) Secondary school (In-complete) [ ]
  - e) Secondary school (Complete) [ ]
  - f) College Education/ Vocational training [ ]
  - g) University Education [ ]
  - h) Other.....
4. How long have you worked in this Butchery
  - a) Less than 1 [ ] b) 1 - 5 [ ] c) Above 5
5. Is the business registered by the County government
  - a) Yes [ ] b) No [ ]
6. Income per day
  - a) Less than 1000 b) Between 1000- 5000 c) Above 5, 000

## **B. FOOD SAFETY**

1. Which type of meat do you sell  
a) Beef  b) Chicken  c) Goat  d) Pork  e) Others
2. Do you source from licensed abattoirs or directly from farmers
  - i. Beef a) Yes  b) No
  - ii. Goat a) Yes  b) No
  - iii. Chicken a) Yes  b) No
  - iv. Pork a) Yes  b) No
3. When purchasing meat, what do you consider  
a) Low cost  b) Quality (Freshness) c) Other consideration.....
4. When receiving meat do you check for any defects? A) Yes  b) No
5. If Yes Which parameters, do you look for first  
a) Colour  b) Off-odour  c) Texture  d) Others....
6. How long does it take to sell all meat bought in a particular day  
a) 1 day or less b) 2-3 days c) More than 5 days
7. If b or c above, how do you store your meat?  
a) Refrigeration  b) No refrigeration  c) Other Methods....
8. Do you store raw meat separately according to species? a) Yes  b) No
9. How frequently do you clean your meat storage equipment?  
a) Weekly b) Bi-weekly C) Once- a month d) Cannot tell the frequency
10. How do you clean?  
a) Soap and water b) water only c) Other methods .....
11. Do you sterilize the meat storage equipment? a) Yes  b) No
12. If Yes, which method do you use? a) Hot water b) Chemical based c) Sun rays
13. Have public health officials inspected this premise during your tenure? Yes  b) No
14. If yes, when was the last time they came a) < 1yr , b)1-2yrs, c) >2 yrs, d) Never

## **C. WATER QUALITY**

1. Where do you draw water for use at the premise?  
a) Municipal taps b) River c) Water vendors
2. Are you certain if the water is treated? a) Yes  b) No

## **D. PERSONAL HYGIENE**

1. Have you ever attended any training on hygiene handling of raw meats? a) Yes   
b) No
2. Have you ever gone for a medical check up? a) Yes  b) No
3. How do you receive payment? A) Mpesa  b) Cash  c) Both
4. If b or c above Do you have a separate cashier handling money? a) Yes  b) No
5. If no, do you wash your hands between handling meat and money a) Yes b) No

## **Appendix 2: Food Safety Knowledge Attitude and Practices of Ready-to-Eat Beef and Chicken Meat Retailers**

**Introduction:** I am a student from Chuka University, who is studying practices related to ready-to-eat meat contamination with pathogenic microorganisms in Lower Eastern Kenya. Your participation will be highly appreciated.

**Consent to participate in research:** Participation is entirely voluntary. Nevertheless, if you choose to participate, you will be contributing to the body of knowledge on ready-to-eat meat handling practices in relationship with risks to contamination with pathogenic microorganisms. In case any questions are asked that you would rather not respond to, you may request that we skip or entirely stop the interview.

**Confidentiality:** Your responses will be kept completely confidential by the researchers. No names or personal information will be published by the researchers. The data collected will only be used to give an understanding of the ready-to-eat-meat handling practices that pose as risks to contamination with pathogenic microorganisms.

Questionnaire number..... Date of interview .....

Interviewer's Name.....

County..... Sub County.....

Town.....

Location: Latitude..... Longitude.....

	<b>Demographic Characteristics</b>	<b>Responses</b>
1	County	Embu, Meru, TNC
2	Town	Embu, Runyenjes, Meru, Nkubu, Chuka, Ndagani & Mitheru
3	Gender	Male, Female
4	Age	Below 20, 21- 30, 30-40, 41-50, Above 50
5	Experience	Less than 1 year, 1 -5 years, Above 5
6	Level of Education	Primary, Secondary, College, University
7	Food Handlers certificate	Yes, No
8	Food handling training	Yes, No
9	Also sells raw meat	Yes, No
	<b>Knowledge Section</b>	
1	Do you know what food borne diseases are?	Yes, No

2	Are you aware of how foodborne diseases can be transmitted?	Yes, No
3	Do you know that the quality of your raw meat affects the quality of ready-to-eat meat?	Yes, No
4	Do you know of any signs accompanied by spoiled meat or poultry?	Yes, No
5	Can using the same knife for raw and cooked meat without washing it leads to cross-contamination?	Yes, No
6	Are hand sanitizers effective in killing most germs after handling raw meat?	Yes, No
7	When you handle food unhygienically can it cause food contamination with pathogenic microorganisms?	Yes, No
8	Is there a specific temperature at which cooked meat should be stored at?	Yes, No
9	Do you know how long cooked meat can safely remain unrefrigerated?	Yes, No
10	Can ready-to-eat meat be infected by flies and rodents?	Yes, No
11	Can improperly reheated cooked meat cause foodborne illness?	Yes, No
12	Do you know if meat can become contaminated even after thorough cooking?	Yes, No
13	Are you aware of the diseases commonly caused by contaminated beef or poultry (e.g., Salmonella, E. coli)?	Yes, No
14	Can using untreated water to wash meat or utensils cause contamination?	Yes, No
15	Do you know the importance of using clean gloves or tongs when serving meat?	Yes, No
16	Do you know of any government guidelines on handling ready-to-eat meat?	Yes, No
	<b>Attitude Section</b>	
1	I believe washing hands before handling ready-to-eat meat is very important.	Strongly Disagree, Disagree, Neutral, Agree, Strongly Agree
2	Maintaining a clean selling environment helps prevent diseases.	Strongly Disagree, Disagree, Neutral, Agree, Strongly Agree
3	It is acceptable to reheat cooked meat only when a customer asks.	Strongly Disagree, Disagree, Neutral, Agree, Strongly Agree
4	I feel that regular training on food hygiene is unnecessary.	Strongly Disagree, Disagree, Neutral, Agree, Strongly Agree
5	Wearing protective clothing (e.g., aprons, gloves) while handling meat is important.	Strongly Disagree, Disagree, Neutral, Agree, Strongly Agree
6	I am confident that my current hygiene practices are enough to prevent foodborne illnesses.	Strongly Disagree, Disagree, Neutral, Agree, Strongly Agree

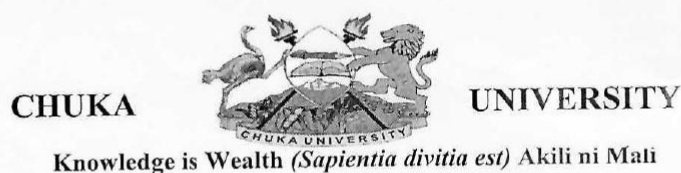
7	Selling meat in open-air conditions does not significantly affect its safety. N	Strongly Disagree, Disagree, Neutral, Agree, Strongly Agree
8	Using soap and clean water every time I wash my hands is not always necessary. N	Strongly Disagree, Disagree, Neutral, Agree, Strongly Agree
9	It is important to follow public health officers' recommendations about meat hygiene.	Strongly Disagree, Disagree, Neutral, Agree, Strongly Agree
10	Food safety is a shared responsibility between the seller and the customer.	Strongly Disagree, Disagree, Neutral, Agree, Strongly Agree
11	Covering food is a good way to prevent contamination.	Strongly Disagree, Disagree, Neutral, Agree, Strongly Agree
12	I would attend food safety training if offered for free.	Strongly Disagree, Disagree, Neutral, Agree, Strongly Agree
13	Disposing of spoiled meat is better than selling it at a discount.	Strongly Disagree, Disagree, Neutral, Agree, Strongly Agree
14	It is the customer's job to ensure meat is safe before eating.	Strongly Disagree, Disagree, Neutral, Agree, Strongly Agree
	<b>Practice Section</b>	
1	Do you wash your hands with soap before handling meat	Yes, No
2	Do you wash your hands after using the toilet before handling food again	Yes, No
3	Do you sanitize your hands at any moment	Yes, No
4	Do you use separate equipment (knives, boards) for raw and cooked meat	Yes, No
5	Do you wash and sterilize your equipment and utensils	Yes, No
6	Do you store unsold cooked meat in a refrigerator or cooler	Yes, No
7	Do you check the temperature of stored cooked meat	Yes, No
8	Do you cover meat to protect it from dust and flies	Yes, No
9	Do you clean your storage or display equipment daily	Yes, No
10	Do you ensure the water used for cleaning is safe and treated	Yes, No
11	Do you wear gloves when handling ready-to-eat meat	Yes, No
12	Do you reheat meat before sale when it has been kept for more than 2 hours	Yes, No
13	Do you discard leftover meat at the end of the day	Yes, No

**Appendix 3: Clinical and Laboratory Standard Institute Criteria for Interpreting Antibiotic Sensitivity Results**

Antibiotic class	Antimicrobial (abbreviation)	Disc Content mcg	Resistant (R)	Intermediate (I)	Susceptible (S)
Penicillins	Amoxyl (AMX)	30	≤ 20	21 – 28	≥ 29
	Ampicillin (AMP)	10	≤ 13	14 –16	≥ 17
	Methicillin (MET)	5	≤ 10	11–14	≥ 15
Cephalosporins	Cefotaxime (CTX)	30	≤ 22	23–27	≥ 28
	Cefpodoxime (CPD)	10	≤ 17	18–20	≥ 21
	Ceftriaxone (CTR)	30	≤ 14	15–17	≥ 18
Tetracycline	Tetracycline (TE)	30	≤ 14	15–18	≥ 19
	Doxycycline (DO)	30	≤ 10	11–13	≥ 14
	Minocycline (MI)	30	≤ 14	15–18	≥ 19
Aminoglycosides	Amikacin (AK)	30	≤ 14	15–16	≥ 17
	Gentamicin (GE)	10	≤ 14	13–14	≥ 15
Polymixins	Colistin (CL)	10	≤ 11	12–13	≥ 14
Sulfonamides	Cotrimazole (COT)	25	≤ 11	12–16	≥ 17
	Sulfameth/trimeth (23.5/1.25)				
Lincosamide	Lincomycin (L)	2	≤ 14	15–18	≥ 19
Chloramphenicol	Chloramphenicol (C)	15	≤ 12	13–17	≥ 18
Macrolide	Erythromycin (E)	15	≤ 13	14–17	≥ 18

Source: M100S (CLSI, 2020)

## Appendix 4: Ethics Review Committee Approval Letter



### CHUKA UNIVERSITY INSTITUTIONAL ETHICS REVIEW COMMITTEE

Telephones: 020-2310512/18

Direct Line: 0772894438

Email: [info@chuka.ac.ke](mailto:info@chuka.ac.ke)

P. O. Box 109-60400, Chuka

Website: [www.chuka.ac.ke](http://www.chuka.ac.ke)

19<sup>th</sup> March, 2024

**REF: CUIERC/NACOSTI/495**

**TO: Orwa Joy Deborah Atieno**

**RE: Factors Encoding Prevalence and Genetic Profiling of Multidrug-Resistant Pathogenic *Escherichia coli* Isolated from Beef and Poultry Meat**

This is to inform you that *Chuka University IERC* has reviewed and approved your above research proposal. Your application approval number is *NACOSTI/NBC/AC-0812*. The approval period is 19<sup>th</sup> March, 2024 – 19<sup>th</sup> March, 2025.

This approval is subject to compliance with the following requirements:


- i. Only approved documents including (informed consents, study instruments, MTA) will be used
- ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by *Chuka University IERC*.
- iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to *Chuka University IERC* within 72 hours of notification
- iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to *Chuka University IERC* within 72 hours
- v. Clearance for export of biological specimens must be obtained from relevant institutions.
- vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
- vii. Submission of an executive summary report within 90 days upon completion of the study to *Chuka University IERC*.


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

Yours sincerely

**Dr. Benjamin Kanga**  
SECRETARY


## Appendix 5: NACOSTI Research Permit

  
**REPUBLIC OF KENYA**

  
**NATIONAL COMMISSION FOR  
SCIENCE, TECHNOLOGY & INNOVATION**

RefNo: **989690** Date of Issue: **30/April/2024**


**RESEARCH LICENSE**




**This is to Certify that Ms. DEBORAH JOY ORWA of Chuka University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Embu, Meru, Tharaka-Nithi on the topic: FACTORS ENCODING PREVALENCE AND GENETIC PROFILING OF MULTI-DRUG RESISTANT PATHOGENIC ESCHERICHIA COLI ISOLATED FROM BEEF AND POULTRY MEAT for the period ending : 30/April/2025.**

License No: **NACOSTI/P/24/34614**

**989690**  
Applicant Identification Number

  
Director General  
**NATIONAL COMMISSION FOR  
SCIENCE, TECHNOLOGY &  
INNOVATION**

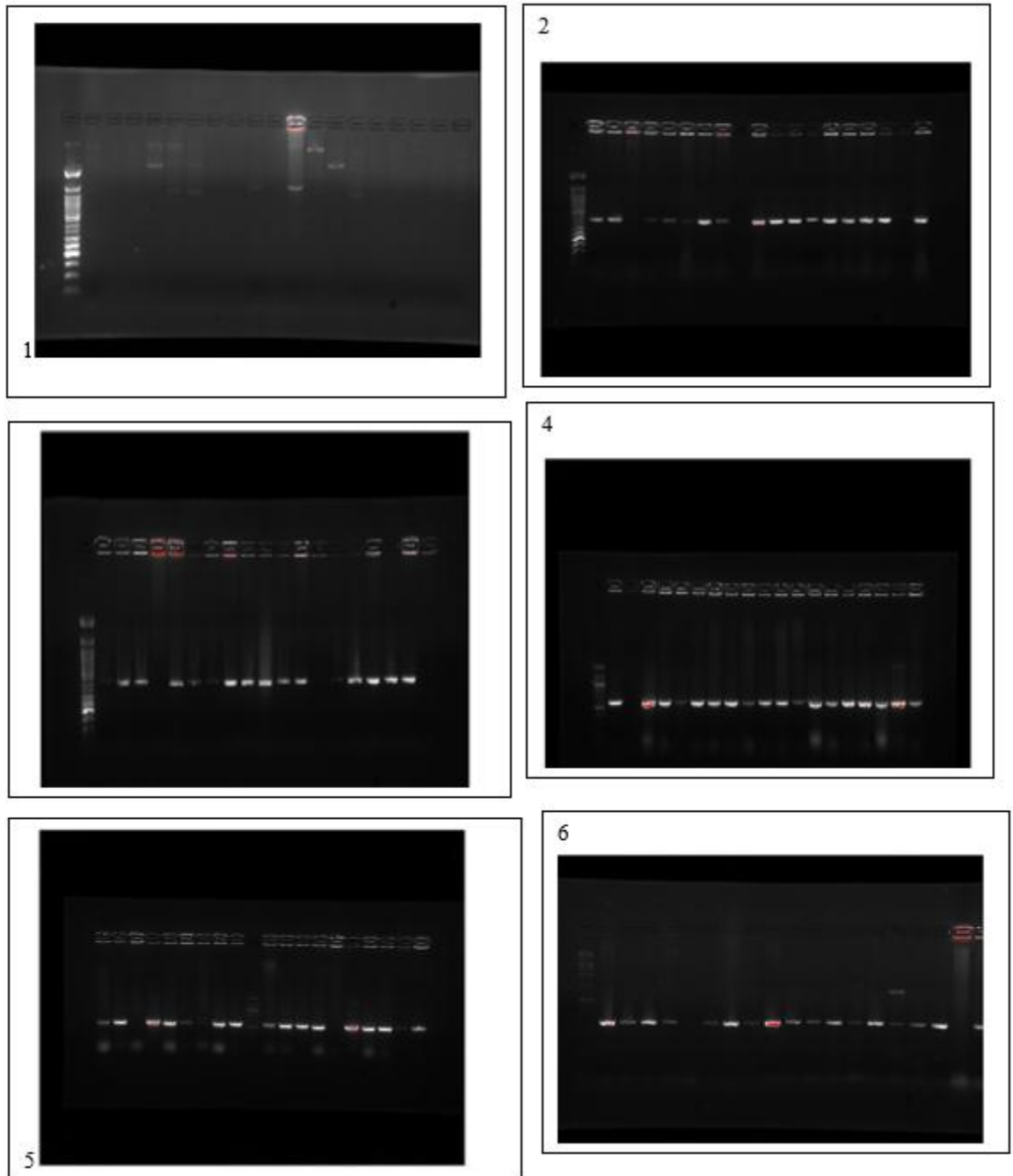
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**See overleaf for conditions**

## Appendix 6: Resistance Genes PCR Products visuals



PCR products visualisation for presence of resistant genes 1(Bla Tem), 2(Bla SHV), 3(Bla OXA) 4(Bla CTXM-15), 5(mcr -1), 6(sul-1 and Tet A).

**Appendix 7: Test of Model Adequacy for Total Coliform Counts (TCC), *Escherichia coli* (*E. coli*) and antibiotic Resistant *E. coli* (AR *E. coli*) Isolated from Raw Meats**

Variable	Source of variation	of DF	Sum squares	Mean squares	F-Value	p - value
TCC	Model	35	274.74	7.85	319.4	<0.0001
	Error	87	2.138	0.025		
	Corrected Total	122	276.87			
<i>E. coli</i>	Model	35	150.69	4.31	296.05	<0.0001
	Error	87	1.2652	0.015		
	Corrected Total	122	151.96			
AR <i>E. coli</i>	Model	35	74.05	2.12	5.54	<0.0001
	Error	87	33.22	0.38		
	Corrected Total	122	107.27			

**Appendix 8: Analysis of Variance for Effect of Counties, Towns and Sample Type (Beef/Chicken) on Total Coliform Counts (TCC), *Escherichia coli* (*E. coli*) and Antibiotic Resistant *E. coli* (AR *E. coli*) Isolated from Raw Meats**

Variable	Source of variation	DF	Sum squares	Mean squares	F-Value	p - value
TCC	County * Town	5	79.91	15.98	9.49	<0.0001
	County * Town * Sample type	11	274.12	24.92	1004.07	<0.0001
<i>E. coli</i>	County * Town	5	43.30	8.66	9.33	<0.0001
	County * Town * Sample type	11	150.01	13.65	816.49	<0.0001
AR <i>E. coli</i>	County * Town	5	15.87	3.171	4.06	0.002
	County * Town * Sample type	11	67.51	6.13	17.14	<0.0001

**Appendix 9: Test of Model Adequacy for Total Coliform Counts (TCC), *Escherichia coli* (*E. coli*) and Antibiotic Resistant *E. coli* (AR *E. coli*) Isolated from Ready-to-eat Meats**

Variable	Source of variation	of DF	Sum squares	Mean squares	F-Value	p - value
TCC	Model	29	186.38	6.43	2670.06	<0.0001
	Error	75	0.18	0.002		
	Corrected Total	104	186.57			
<i>E. coli</i>	Model	29	68.84	2.374	120.7.69	<0.0001
	Error	75	0.147	0.002		

	Corrected Total	104	69.99			
AR <i>E. coli</i>	Model	29	2.14	0.0739	75.63	<0.0001
	Error	75	0.074	0.00098		
	Corrected Total	104	2.22			

**Appendix 10: Analysis of Variance for Effect of Counties, Towns and Sample Type (Beef/Chicken) on Total Coliform Counts (TCC), *Escherichia coli* (*E. coli*) and Antibiotic Resistant *E. coli* (AR *E. coli*) Isolated from Ready-to-eat Meats**

Variable	Source of variation	DF	Sum squares	Mean squares	F-Value	p - value
TCC	County * Town	5	42.053	8.41	5.70	<0.0001
	County * Town * Sample type	11	186.32	16.94	6595.14	<0.0001
<i>E. coli</i>	County * Town	5	18.77	3.75	7.4	<0.0001
	County * Town * Sample type	11	68.79	6.25	31117.53	<0.0001
AR <i>E. coli</i>	County * Town	5	0.8700	0.174	12.8	0.002
	County * Town * Sample type	11	2.132	0.194	215.4	<0.0001