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**ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE**

**MSc THESIS ON
A ONE HEALTH ASSESSMENT OF ANTIMICROBIAL USE AND ASSOCIATED
RESISTANCE IN ESCHERICHIA COLI ALONG THE BEEF VALUE CHAIN
IN AKAKI KALITY SUB CITY, ADDIS ABABA, ETHIOPIA**

**BY:
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**A THESIS SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY,
PARASITOLOGY AND POULTRY HEALTH, IN PARTIAL FULFILLMENT OF
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LIST OF ABBREVIATIONS AND ACRONYMES

| | |
|-----------|--|
| AMR | Antimicrobial resistance |
| AMR | Antimicrobial use |
| AMS | Antimicrobial stewardship |
| AST | Antimicrobial susceptibility test |
| ATCC | American culture collection |
| AAU-CVM | Addis Ababa University College of veterinary medicine |
| BPW | Buffered peptone water |
| CLSI | Clinical laboratory standards institute |
| EHEC | Enter hemorrhagic <i>Escherichia coli</i> |
| ESBL | Extended-spectrum beta-lactamase |
| FAO | Food and agriculture organization |
| GDP | Gross domestic product |
| GLASS | Global antimicrobial resistance surveillance system |
| HUS | Hemolytic uremic syndrome |
| ISO | International organization for standardization |
| KAP | Knowledge, Attitude, and Practice |
| MALDI-TOF | Matrix-assisted laser desorption/ionization–time of flight |
| MDR | Multidrug resistance |
| OH | One health |
| OIE | World organization for animal health |
| SSA | Sub Saharan Africa |
| STATA | Data Statistics (software) |
| STEC | Shiga toxin-producing <i>Escherichia coli</i> |
| TTP | Thrombotic thrombocytopenic purpura |
| US\$ | United nation dollar |
| WHO | World health organization |

ABSTRACT

Improper use of antimicrobials (AMU) in food animals contributes the emergence of antibiotic resistant bacteria, which subsequently disseminate through the food chain between animals and humans, posing significant threats to public health and food security. This study aimed to assess AMU and antimicrobial resistance (AMR) profile of *Escherichia coli* (*E. coli*) and identify potential risk factors for resistance along the beef value chain in Akaki Kality sub city, Addis Ababa. A cross-sectional study design was employed from November 2025 to May 2026. Data collection included laboratory analysis, farmers' knowledge, attitudes, and practices (KAP) regarding AMU and AMR, and assessment of hygienic practice among meat handlers. Statistical analysis of Fisher's exact test was used to analyze KAP data, while Chi-square and logistic regression for laboratory findings. This study showed that, overall 50%, 46.2% and 42.3% respondents have poor knowledge, attitude, and practice toward AMU and AMR, respectively. Low educational level, small farm size, shorter experience in beef fattening, limited training and lack of access to AMU and AMR information were risk factor of poor KAP of farmers. Of 663 samples examined, 176 (26.5%) were positive for *E. coli* and 19(2.9%) for *E. coli* O157: H7. *E. coli* isolates were recovered from beef farms 31(18.7%), abattoir 88(34.2%), and butcher shops 57(23.8%), while *E. coli* O157:H7 isolates were detected in farms 7(4.2%), abattoir 9(3.5%), and butcher shops 3(1.3%). Antimicrobial susceptibility testing against 12 antibiotics revealed that *E. coli* isolates were most susceptible to chloramphenicol 16(69.6%), imipenem 13(56.5%), and ciprofloxacin 13(56.5%), whereas *E. coli* O157:H7 isolates were most susceptible to, imipenem 13(68.4%), chloramphenicol 12(63.2%) and amoxicillin clavulanate 11(57.9%). Multidrug resistance was observed in 17(73.9%) *E. coli* and 16 (84.2%) *E. coli* O157:H7 isolates. In general, the study revealed challenges in proper AMU, hygiene practices, and high prevalence of microbial contamination along the beef value chain. These findings collectively emphasize the need for integrated One Health interventions addressing antimicrobial stewardship, farm biosecurity, meat hygiene, and AMR surveillance.

Keywords: *Antimicrobial resistance, Antimicrobial use, Beef value chain, Escherichia coli, Food safety, One Health*

1. INTRODUCTION

1.1. Background

Misuse of antimicrobials in livestock farming, especially within the beef value chain, is a significant cause for the development of resistant bacteria. These bacteria can harbor resistance genes that transfer horizontally to other pathogenic bacteria, thereby complicating treatment options in humans (Zackariah *et al.*, 2025). The contaminated meat products, environmental runoff from farms, and improper hygiene practices facilitate the spread of resistant bacteria along the food chain, from farm to fork (Gruel *et al.*, 2021). Global AMU in animal food production is a major driver of AMR. Numerous developed countries report widespread AMU and AMR in animals. As a global antimicrobial consumption in food producing animal estimates to rise by 67% in 2030 if no urgent actions taken (Van Boeckel *et al.*, 2015).

In Sub-Saharan Africa (SSA), livestock farming contribute to a crucial role in the socio-economic and nutritional landscape. A pooled result of systematic review and meta-analysis in food producing animals and food products across 16 SSA countries shows 61.6% of AMR and 9.1% of multidrug resistance (MDR) respectively. This resistance pattern is frequently observed in *E. coli* among cattle's (Hassen *et al.*, 2026). Therefore, the dissemination of AMR within the beef value chain is a serious concern. However, the widespread and often indiscriminate use of antimicrobials in beef production, combined with weak regulatory frameworks, inadequate veterinary services, and unsustainable farming practices, has created an environment conducive to the emergence of AMR in livestock and beef products (Musuka *et al.*, 2025).

The beef value chain includes numerous phases, containing production, slaughter, processing, distribution, and intake. Antimicrobial resistant bacteria can contaminate at these stages, which can pose risk on health of human and food safety. This resistance can then be transmitted through the beef value chain, from farm to table, posing significant risks to consumers and complicating the treatment of foodborne and other infectious illnesses (Azabo *et al.*, 2022; Rekwot *et al.*, 2022).

In several developing countries antibiotic use in animal production dramatically increasing, which is due to growing need for animal protein and a shift in farming practices. Misuse of antimicrobials in veterinary medicine and their use as growth promoters, aggravating AMR due to the occurrence of residues of antibiotic in byproducts of animal such as meat and in their excretory products (Burki, 2018; Tufa *et al.*, 2023). This contributes exertion of selective pressure, promoting the survival and proliferation of resistant bacteria like extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* (Kimera *et al.*, 2020).

Escherichia coli have emerged as one of the most essential bacterial species in the study of AMR and foodborne infections. It is both a commensal organism and a common pathogen, with certain serotypes such as *E. coli* O157:H7 causing severe human illnesses (Sarba *et al.*, 2023). The majority of outbreaks involving a large number of illnesses have been linked to eating undercooked or cross- contaminated prepared foods. Foods mostly of bovine origin, such as beef meat and beef burgers, as well as unpasteurized milk, are the most frequently associated to *E. coli* outbreaks (Mesele and Abunna, 2019).

At the national level, Ethiopian meat and meat products continue to provide a problem risk-based food safety management, since it is difficult to establish the extent of contamination by *E. coli* and other pathogens due to limited surveillance and laboratory capacity (Mekonnen *et al.*, 2024). Foodborne illnesses are frequently underreported, and Ethiopia is no exception. Because of lack of strict monitoring of foodborne pathogens in meat and meat products hampers the country's ability to accurately assess contamination levels (Eliyas, 2023). The meat industry, including slaughterhouses and butcher shops, is highly susceptible to *E. coli* contamination, leading to spoilage and food-borne infections (Ma *et al.*, 2022).

AMR poses a growing threat to public health, food safety and animal production system in Ethiopia. Even though the recognized importance of the beef sector, there is limited evidence linking AMU patterns, bacterial resistance profiles, and cross- sectoral transmission pathways along the beef value chain (Debebe *et al.*, 2022 ; Nastasijevic *et al.*, 2024).

Many research studies shows AMR profiles and prevalence in *E. coli* in meat collected from abattoir and butcher shop. However, most of these studies have been narrow in scope, focusing on either abattoir and butcher shop samples, without systematically comparing isolates along the full beef value chain. As far as my knowledge concerned and reviewed literature there is no study conducted on beef value chain specifically on the environment surrounding beef farm. More data needs to be collected from farm, slaughterhouses and butcher shops to determine the prevalence of *E. coli* in the meat value chain and this information needs to be made available to the public. Even though One Health approach was promoted in AMR monitoring, its practical implementation remains limited (Sebsibe and Asfaw, 2020). Therefore this study significantly contributes in assessing AMU practices and resistance patterns in *E. coli* along the beef value chain within study area. Additionally helps to generate evidence-based data which may support for national AMR action plans and policies. The findings improve antimicrobial stewardship practices among livestock farmers and veterinarians in the study area.

1.2. Objectives

1.2.1. General objective

To assess KAP of farmers for AMU and the prevalence of AMR in *E. coli* along the beef value chain in Akaki Kality sub city using one health approach.

1.2.2. Specific objectives

- To isolate *E. coli* (including O157:H7) from beef meat, animal, environmental, and human samples.
- To determine AMR profiles of *E. coli* isolates including their multidrug resistance pattern.
- To assess farmers' knowledge, attitudes, and practices (KAP) regarding AMU and AMR.
- To assess hygienic measures of meat handlers.

2. LITERATURE REVIEW

2.1. The one health concept and its relevance to antimicrobial resistance

One health approach emphasizes the health of animal, human and environment are interdependent and that health is one inseparably linked to health of others. Therefore it's a unifying approach aims to sustainably balance and optimize those health (Kniel *et al.*, 2018). AMR spreads through interconnected pathways linking humans, animals, and the environment. Through horizontal gene transfer, potential pathogens can acquire antimicrobial resistance genes that can subsequently be spread between human, animal, and environmental reservoirs. The OH approach is crucial to our understanding of the complex mechanisms and epidemiology of AMR (Despotovic *et al.*, 2023).

Pharmaceuticals for veterinary use, aquaculture and agriculture present an important source of pharmaceuticals entering the environment, with frequent disposal of animal dung and waste to the environment or used as agricultural fertilizer. With irrational AMU in agriculture and livestock farming, there are concerns about the spread of resistant pathogen from animals to humans and the environment, contributing to the reservoir of antimicrobial resistance genes. The lack of well-regulated farm waste disposal systems and excessive and unregulated use of antibiotics as prophylactics and growth promoters in food production and animal farming act as the major contributors in this perspective (Asaduzzaman *et al.*, 2022; Hossein and Ripanda, 2025).

As a result of so many antimicrobials used in medicine and animal husbandry, they are constantly released into the environment. This damages the ecosystem and encourages the development and spread of antimicrobial resistance. The high number of antimicrobial resistance genes and bacteria raises the possibility that pathogenic bacteria in the ecosystem will develop resistance and expands the window of opportunity for human contact with AMR pathogens (Parker *et al.*, 2024).

The recipients of antimicrobial therapy release antimicrobial residues, resistant bacteria, and resistance genes in waste products. Through improper disposal of antimicrobials they enter municipal waste water, These are reduced but not removed during wastewater and manure treatment, they enter surface waters, soils, recreational parks, wildlife, and fields where animals graze and crops are grown for human and animal consumption (Kim and Cha, 2021; Allel *et al.*, 2024). One Health interdisciplinary approach to surveillance and implementation of programs, policies, and research play a key role in mitigating AMR nationally as well as globally (Fujita *et al.*, 2022). In 2015, world Health Organization (WHO) launched the Global Antimicrobial Resistance Surveillance System (GLASS), a collaborative effort to standardize AMR surveillance with the aim to inform policies and infection prevention strategies (WHO, 2022).

Ethiopian strategic plan on the Antimicrobial Resistance Surveillance System, a standardized, laboratory-based surveillance system and one of the first national efforts to combat AMR, which is revised more with particular attention to one health platform (Ethiopia Antimicrobial Resistance Surveillance Annual Report, 2020). However currently, national AMR surveillance in Ethiopia is primarily focused on humans, and there remains a knowledge gap of AMR trends across animals and the environment (Melese *et al.*, 2025).

2.2. Antimicrobial use and mismanagement in livestock production

The use of antimicrobials, including antibiotics, serves as a vital tool for maintaining animal health through disease prevention and treatment. To meet demand for animal protein antimicrobials are used routinely to maintain their health and productivity. In the global livestock production antimicrobials are used for metaphylaxis by treating all animals in a group when some show disease symptoms and for prophylaxis purposes. They are serve as growth promotion in specific situations (Shallcross and Davies, 2014; Matheou *et al.*, 2025). The improper use of antimicrobials through misuse, overuse and incorrect application methods leads to the development and spread of AMR in bacterial populations (Abate and Birhanu, 2025).

The misuse of antimicrobials also practiced in many low and middle income countries including Ethiopia, because of weak veterinary supervision and insufficient public knowledge and weak

regulatory control (Woldemichael *et al.*, 2025). The annual animal antibiotic consumption worldwide amounts to reach between 63,000 and 106,000 tonnes while tetracycline, β -lactams, sulfonamides, macrolides and trimethoprim represent the most frequently used antibiotic classes (Van Boeckel *et al.*, 2015 ; Abate and Birhanu, 2025).

In livestock production systems, antimicrobials are often administered not only for therapeutic purposes but also prophylactically and, in some cases, as growth promoters. Such practices exert selective pressure on microbial populations, promoting the emergence of resistant strains (Fujita *et al.*, 2022). A scoping broader African AMR studies also highlights that many countries are understated in surveillance, but the trend of misuse of antimicrobials is prevalent (Gahimbare *et al.*, 2024). In Ethiopia, tetracycline, β -lactams, and aminoglycosides are among the frequently used antibiotic classes in cattle (Gemedo *et al.*, 2020). Empirical administration without veterinary prescription is common, as livestock owners often self-prescribe or purchase antimicrobials directly from pharmacies or informal sellers (Gemedo *et al.*, 2023).

Another KAP study in Ethiopia reported that 44% of farmers had used antimicrobials in recent years; antibiotics constituted 21%. Many farmers had very limited knowledge and unfavorable attitudes toward AMR and residues (Kallu *et al.*, 2024). Also another KAP studies show that many farmers (94 %) had poor knowledge about AMU, residues and resistance; even though some had good behaviour scores, improper AMU practices continued, (for instance, 80 % good behaviour but still misuse) (Tufa *et al.*, 2023).

2.2.1. Key mismanagement issues

The contributing factor of AMR in the beef value chain, were categorized into four themes: (i) antimicrobial use and misuse, (ii) production practices and animal health management, (iii) market factors and informal beef trade, and (iv) environmental and waste management factors. AMR in SSA's beef value chain is shaped by various patterns related to AMU, production practices, environmental factors, and socio-economic conditions. A notable pattern is the overuse and misuse of antimicrobials (Musuka *et al.*, 2025).

The improper management of AMU occurs when farmers do not follow the correct dosage and withdrawal period guidelines. Research from Bahir Dar and Debre Tabor shows that most cattle farms in these areas fail to follow withdrawal time guidelines which results in higher chances of antimicrobial residue contamination in beef products (Agmas and Adugna, 2018). The consumption of meat and milk containing antimicrobial residual pose public health hazards while fostering bacterial resistance in the human gut microbiome. Smallholder and pastoralist farmers in Ethiopia use antimicrobials through untested methods based on their personal experience and peer advice instead of following proper diagnostic procedures and sensitivity tests (Gemeda *et al.*, 2020). The use of antimicrobials for disease prevention in healthy animals and growth promotion exists in specific areas of Ethiopia although this practice remains less documented (Tufa *et al.*, 2024).

Circulation of substandard or counterfeit veterinary drugs in many settings, further exacerbate the problem due to inadequate drug concentrations and reduced therapeutic efficacy (Lemma *et al.*, 2025). The lack of antimicrobial stewardship and surveillance mechanisms within Ethiopia's veterinary sector is another major concern. Most veterinary clinics work without antimicrobial sensitivity testing services, leading to repetitive empirical prescriptions (Fujita *et al.*, 2022). Inadequate record-keeping and limited laboratory infrastructure constrain national AMR monitoring. Thus, the potential for resistant pathogens to spread along animal, human, and environmental interconnection is amplified, which to severely constrain control efforts (Gemeda *et al.*, 2021).

2.2.2. The role of policy and regulation in addressing antimicrobial resistance

Epidemiological surveillance systems have been launched for AMR, targeting to promote collaborations directed at the well-being of human and animal health and the balance of the ecosystem globally (Salam *et al.*, 2023). In response to AMR, several global health organizations and governments have taken action to combat this issue. To addressing this growing crisis strict regulatory frameworks and Policy design, including coordinated governance across stake holder and also participation of stakeholder and cross-sectoral coordination is essential (Ferraz, 2024).

Implementing strict regulatory measures to ensure antimicrobials are distributed only with valid prescriptions is fundamental to reduce over use and misuse in agricultural sector (Musuka *et al.*, 2025). Governments and international bodies should bolster regulatory oversight, conduct regular inspections, and establish quality assurance protocols for pharmaceutical manufacturing and distribution to compact circulation of counterfeit drugs. Strengthening legal frameworks and international cooperation ensures that only high-quality drugs reach consumers, reducing the risk of resistance development. Implementing strict policy and regulation in agricultural sector which limit the use of antimicrobial's to therapeutic purposes, promoting vaccination, and improving animal husbandry practices (Van Boeckel *et al.*, 2015).

2.3. Antimicrobial Stewardship

Antimicrobial stewardship (AMS) in animal health refers to the coordinated efforts that promote the responsible and evidence based use of antimicrobial agents to maintain animal health, ensure effective treatment of infections, and minimize the emergence and spread of AMR. In livestock production systems, AMS emphasizes the use of antimicrobials only when clinically indicated, selection of appropriate antimicrobial agents, administration of correct dose and duration, and adherence to veterinary guidance. Antimicrobials are important tools for controlling infectious diseases in food producing animals; however, inappropriate practices such as unnecessary prophylactic use, empirical treatment without diagnosis, incorrect dosage, and use for growth promotion can create selective pressure that favors survival and multiplication of resistant bacteria (Shallcross and Davies, 2014; Fujita *et al.*, 2022). Therefore, improving AMS in livestock requires integration of veterinary services, disease prevention strategies, vaccination, improved biosecurity, proper hygiene, and farmer awareness to reduce unnecessary antimicrobial exposure and preserve antimicrobial effectiveness (Abate and Birhanu, 2025).

In developing countries, including Ethiopia, effective AMS in livestock production remains challenging due to limited veterinary supervision, inadequate farmer knowledge, weak regulatory enforcement, and easy access to antimicrobials without professional prescription. Studies conducted in Ethiopia have reported that livestock owners commonly obtain and administer

antimicrobials based on previous experience, advice from non-professionals, or direct purchase from pharmacies, which increases the risk of inappropriate AMU and development of resistance (Gemedo *et al.*, 2020; Gemedo *et al.*, 2023; Woldemichael *et al.*, 2025). Furthermore, poor compliance with treatment guidelines, failure to observe withdrawal periods, and limited AMU records may contribute to antimicrobial residues in animal products and dissemination of resistant bacteria through the food chain. In the One Health context, AMS requires collaboration among animal health professionals, farmers, public health sectors, and environmental stakeholders to promote responsible AMU and reduce transmission of resistant organisms between animals, humans, and the environment (Fujita *et al.*, 2022; Tufa *et al.*, 2023).

2.4. Mechanisms of antimicrobial resistance and MDR in *E. coli*

AMR occurs when microorganisms acquire the ability to survive or multiply in the presence of antimicrobial agents that were previously effective against them. In *Escherichia coli*, resistance can develop through genetic changes such as chromosomal mutations or acquisition of resistance genes through horizontal gene transfer mechanisms, including conjugation, transformation, and transduction. These mechanisms enable bacteria to exchange resistance determinants and facilitate rapid dissemination of AMR within bacterial populations (Burki, 2018; Ferraz, 2024). The major mechanisms of AMR in *E. coli* include enzymatic inactivation of antibiotics, modification of antimicrobial target sites, reduced permeability of the bacterial cell membrane, and activation of efflux pumps that remove antimicrobial agents from bacterial cells. Among these mechanisms, production of antibiotic-inactivating enzymes such as β -lactamases is one of the most important mechanisms responsible for resistance against β -lactam antibiotics, including penicillins and cephalosporins (Dallenne *et al.*, 2010 ; Garg ., 2022). The emergence and spread of ESBL producing *E. coli* is a major concern because these organisms can hydrolyze extended-spectrum cephalosporin and limit therapeutic options in both veterinary and human medicine (Chekole *et al.*, 2025).

In livestock production systems, antimicrobial exposure creates selective pressure that favors the survival and multiplication of resistant *E. coli* strains.

Cattle can serve as important reservoirs of resistant bacteria because AMR *E. coli* present in the intestinal tract may be shed through feces and contaminate farm environments, water sources, equipment, carcasses, and meat products. Resistant bacteria and resistance genes can subsequently spread between animals, humans, and the environment through direct contact, food consumption, and environmental pathways, highlighting the importance of a One Health approach for AMR control (Fujita *et al.*, 2022; Despotovic *et al.*, 2023).

MDR refers to the resistance of bacterial isolates to at least one antimicrobial agent in three or more antimicrobial classes. MDR in *E. coli* has become a major public health concern because resistant strains can persist in livestock environments and potentially transfer resistance genes to other pathogenic bacteria. The development of MDR is often associated with inappropriate AMU, repeated exposure to antibiotics, poor AMS, and inadequate infection prevention practices. In food animal production, MDR *E. coli* can move along the beef value chain from farms to slaughterhouses and retail markets through fecal contamination, poor hygiene practices, and cross-contamination during processing. Therefore, monitoring MDR patterns of *E. coli* isolates provides important information about antimicrobial pressure, transmission pathways, and potential risks to food safety and public health (Gemedu *et al.*, 2023; Hassen *et al.*, 2026).

2.5. Antimicrobial resistance: global and local perspectives

Globally, antimicrobials are widely prescribed in the livestock industry to treat and prevent infections. Global and local trends indicate the rising incidence of AMU in livestock and food producing animals (Aslam *et al.*, 2024). This surge is primarily due to the growing number of animals raised for food and a shift in farming practices, which is predicted to double antimicrobial consumption in livestock sector and food producing animals in developing countries by 2030 (Tufa *et al.*, 2023). However AMU in livestock production, as indicated in various studies, is considered an important driver of AMR and has been widely documented globally. This contributes to emergence of AMR to animals and transmission to humans are through direct contact with livestock, food chain and direct environmental exposure (Fujita *et al.*, 2022).

AMR has severe consequences, threatening the treatment of infectious diseases and increasing morbidity, mortality, and healthcare costs worldwide. Mortality associated with infections with resistant bacteria is increasing. In 2019, around 1.27 million people died, in 2022, 5 million deaths were occurred due to drug resistant infections, the number is projected to reach 10 million deaths annually by 2050 (Salam *et al.*, 2023). Even though antibiotic resistance prevalence in animals is not as well quantified as in humans, livestock, companion animals, and even wildlife serve as reservoirs for MDR strains (Ferraz, 2024). AMR also poses economic challenges, leading to at least US\$ 3.4 trillion annually by 2030 of GDP drop and increasing by 24 million the number of those living in extreme poverty. 100 trillion US\$ economic losses will be caused due to AMR, by 2050 if no urgent actions are taken (Melese *et al.*, 2025). AMR undermines the ability to protect health across all interconnected domains (human, animal, environmental), creating a state of uncertainty about the future (WHO, 2014).

Worldwide, increasing rates of AMR in *E. coli* have been observed with a high level of resistance to β -lactams, tetracycline's, and Fluoroquinolones. In Africa, surveillance capacity remains limited, however evidence shows high resistance levels in *E. coli* isolates from humans, animals, and environmental sources (Gahimbare *et al.*, 2024). Resistance to first line antibiotics such as ampicillin, tetracycline, and streptomycin exceeds 70-80%, with the emergence of MDR strains and ESBL producers being increasingly reported (Gemedà *et al.*, 2023; Abey *et al.*, 2024). The situation in Ethiopia reflects these trends, with studies documenting high resistance rates in *E. coli* from livestock and meat, coupled with the detection of ESBL-producing strains that compromise treatment options. The lack of a widespread national surveillance system hinders accurate assessment but emphasizes the urgent need for coordinated action (Gemedà *et al.*, 2021).

2.6. Regulatory framework and national action plan on Antimicrobial resistance

Ethiopia has developed a series of national strategies and regulatory frameworks to address AMR through a One Health approach involving human health, animal health, food systems, and environmental sectors.

The country developed its first AMR prevention and containment strategy in 2011 and continued strengthening its response through subsequent national action plans. The Third Ethiopian One Health Strategic Plan for AMR Prevention and Containment (2021–2025) emphasized five major strategic areas, including increasing AMR awareness, strengthening AMR surveillance and research, improving infection prevention and control, optimizing AMU, and strengthening multisectoral collaboration and governance. The plan recognized that AMR is driven not only by inappropriate AMU in humans but also by antimicrobial exposure in animals, food production systems, and environmental contamination. Therefore, implementation of AMR control requires collaboration among the Ministry of Health, Ministry of Agriculture, environmental authorities, regulatory institutions, research organizations, and other stakeholders (Fujita *et al.*, 2022; Asaduzzaman *et al.*, 2022).

Ethiopia launched the fourth national action plan for the prevention and containment of AMR (NAP 4.0, 2026–2030) as a continuation of previous efforts to strengthen AMR control using a One Health framework. The NAP 4.0 focuses on strengthening integrated AMR surveillance, improving laboratory and diagnostic capacity, promoting AMS enhancing infection prevention and control, increasing public awareness, strengthening antimicrobial regulation, and improving coordination among human, animal, and environmental health sectors. The plan particularly recognizes the importance of addressing AMR in food-producing animals because inappropriate AMU, weak veterinary oversight, and poor biosecurity practices may contribute to the emergence and transmission of resistant bacteria through the food chain (MoH, 2026). In livestock production systems, implementation of this framework requires responsible AMU, veterinary supervision, improved farm management, and surveillance of resistant organisms such as *Escherichia coli*. However, challenges remain, including limited AMR surveillance data from animal and environmental sectors, inadequate laboratory capacity, and gaps in enforcement of antimicrobial regulations (Fujita *et al.*, 2022; Abate and Birhanu, 2025). Strengthening One Health implementation under NAP 4.0 is therefore essential to reduce antimicrobial misuse, control the spread of MDR bacteria, and protect animal health, food safety, and public health in Ethiopia (WHO, 2025).

2.7. *Escherichia coli* and *E. coli* O157:H7

Escherichia coli is facultative anaerobic, in the Enterobacteriaceae family, genus *Escherichia* and species *coli*. Gram-negative rod shaped non-spore-forming, flagellate, bacteria. It colonizes the intestines of human and warm-blooded animals, this referred to as its primary habitats where the bacterium lives as a commensal (Gambushe *et al.*, 2022). Although most of the strains are commensal and non-pathogenic, some pathogenic variants (including Shiga toxin-producing *E. coli* (STEC) strains such as *E. coli* O157:H7 are linked to severe foodborne diseases (Gugsa *et al.*, 2022). The majority of nonpathogenic *E. coli* strains are not harmful but they act as reservoirs for ARGs that may transfer to pathogenic species and they are genetically diverse and capable of acquiring resistance via plasmids, integrons, or transposons (Lim *et al.*, 2010).

Pathogenic *E. coli* is an important zoonotic pathogen that can be linked to infectious diseases in animals and humans. Its strains are grouped according to virulence factors into the following variants: enterohemorrhagic, enteropathogenic, enterotoxigenic, enteroaggregative, and enteroinvasive types. Of those, *E. coli* O157:H7 is particularly significant due to its low infectious dose and potential to cause hemorrhagic colitis and hemolytic uremic syndrome in humans. In cattle, the organisms tend to colonize at the recto-anal junction without inducing clinical signs, thus cattle are considered to be the primary reservoir for human infection (Abey *et al.*, 2024; Chekole *et al.*, 2025).

Infection caused by pathogenic *E. coli* spread by fecal contamination. It can also be found due to contamination during food animal slaughter, but in most cases, it is found in soil, water, and food due to fecal contamination, and those environments are referred to as secondary habitats which are becoming an ecological issue of significance (Gambushe *et al.*, 2022). Humans are not maintenance host for this bacteria but the disease can also be transmitted from person to person and contribute to the spread of the disease during outbreaks. On the other hand, the main sources of infection in cattle are drinking water, feeds, and the animal's environment (Debebe *et al.*, 2022).

2.7.1. *E. coli* and food borne disease

Foodborne diseases are great public health and well-being concerns of individuals and countries across the world. Specially, developing countries are largely susceptible to food-borne infections which cost billions of dollars in medical care and social costs (Moawad *et al.*, 2017). Studies indicated that each year, 1 out of 10 people get ill from microbial food contamination, resulting in 600 million illnesses, 420, 000 deaths, and the loss of 33 million healthy years of life globally. Systemic review of 16 articles from 21 countries in Africa showed that the estimated global burden of *E. coli* O157:H7 is 2,801,000 acute illnesses, 3890 cases of hemolytic uremic syndrome, and 230 deaths annually. It often follows the consumption of contaminated foodstuffs, especially from animal products such as meat from infected animals or carcasses contaminated with pathogenic bacteria including *Escherichia coli* (Tadese *et al.*, 2021; Abunna *et al.*, 2023).

Since its first description in 1982, *Escherichia coli* O157: H7 has emerged as an important global zoonotic food and water-borne pathogen, it infects all age groups of humans and the pathogen is noted for its severe consequences following infection, low infective dose, and acid resistance. It produces serious illnesses in humans such as hemorrhagic colitis, HUS, and TTP (Pal and Mahindra, 2016 ; Amente *et al.*, 2022). At global level 2, 801, 000 acute illnesses annually, with an incidence rate of 43.1 cases per 100,000 persons per year caused due to EHEC O157:H7. Among those, a total of 10,200 cases of STEC infections occur in Africa with an incidence rate of 1.4 cases per 100,000 people per year (Havelaar, 2015). In food industry the cost lost as a result of recalls, destroyed food, control measures, and lost demand associated to loss of consumer confidence is estimated to be in the billions of dollars. Factors such as temperature, pH, salt, and water activity can influence on the survival and growth of *E. coli* O157:H7 in food. The optimal temperature for growth of *E. coli* O157:H7 is approximately 37°C (98.6 °F) and the organism will not grow at temperatures below 8°C to 10°C (46°F to 50°F) or above 44°C to 45°C. Sometimes it survives freezing, with some decline in the concentration of *E. coli* O157:H7 (Mengistu and Hiko, 2022).

Therefore it is concern to public health on a global scale and is found in a wide variety of foodstuffs including raw meat and meat products, milk, yogurt, water, salad vegetables fruits, fruit juices and cider. As a result of *E. coli* O157:H7 carriage in cattle, beef and dairy products often become contaminated and serve as the source of infection in outbreaks of *E. coli* O157:H7. Beef including ground beef, roast beef, steak and salami are vehicles for food-borne transmission of *E. coli* O157:H7 (Mesele and Abunna, 2019). After ingestion typical incubation period of *E. coli* O157:H7 is three to four days and the illness usually lasts 5–10 days. Life-threatening complications, some victims, particularly in young, may lead to develop HUS. This may cause permanent loss of kidney function (Martorelli *et al.*, 2017). The habit of consuming raw or undercooked meat is one of the factors that exacerbate the transmission of foodborne pathogens including *E. coli* O157:H7 in Ethiopia.

Sufficient heating of meat kills these organisms. However, consumption of raw or undercooked beef in the form of “kitfo” (minced raw beef mixed with a chili powder-based spice blend and a clarified butter infused with herbs and spices), “leb-leb” (under cooked), “gored-gored” (cuts of raw meat with butter and pepper), and “Kurt”(raw beef consumed with hot pepper and mustard) is common cultural practices in Ethiopia (Tadese *et al.*, 2021).

2.7.2. AMR in *E. coli* and relevance to beef value chain

E. coli also serves as a key indicator organism for AMR monitoring. It can obtain resistance via horizontal gene transfer or chromosomal mutations. Many studies suggest that widespread nature of AMR in *E. coli* isolated from beef and beef products (Messele *et al.*, 2022). In South Africa, study found that *E. coli* isolates from formal and informal meat sectors were resistant to tetracyclines, aminoglycosides, and cephalosporins, with MDR profiles against up to ten drugs and genes such as *aadA*, *strA*, *ampC*, *tetB*, and *sulI* detected. Comparable findings across other African countries reveal systemic weaknesses in drug regulation and slaughter hygiene (Jaja *et al.*, 2020). Preliminary evidence from Ethiopia shows alarming resistance patterns with varying rates of prevalence. Also, molecular analysis performed on Ethiopian isolates detected ESBL genes and plasmid-mediated resistance traits (Bedane *et al.*, 2024).

The identification of Shiga toxin genes (stx1 and stx2) in both cattle products and humans has raised concerns about the zoonotic nature of *E. coli* O157: H7. These results highlight the importance of integrated surveillance across the food value chain (Gugsa *et al.*, 2022).

Ruminants, especially cattle and sheep, are the major reservoirs for *E. coli* O157:H7. Cattle are the primary reservoirs of *E. coli* O157:H7 and consumption of beef and beef products are identified as major sources of foodborne transmission (Gambushe *et al.*, 2022). The beef value chain can be infiltrated with *E. coli* contamination at various points, including slaughter and evisceration, through meat processing and retail handling. Cross-contamination from carcasses to equipment and workers can also allow the spread of resistant *E. coli* strains (Mekonnen *et al.*, 2024). Eating undercooked beef or contact with contaminated surfaces may allow for the horizontal gene transfer of resistance genes to the human commensal flora, which poses an indirect threat to food safety and public health. Furthermore, abattoir and butcher workers may have increased opportunities for direct transmission due to occupation (Fujita *et al.*, 2022).

2.8. Meat Value Chain and Antimicrobial Resistance

Beef Value Chain ranging from production on farm to slaughter, retail and consumption are linked and each can act as a point for introduction and potential amplification of antimicrobial-resistant bacteria. A One Health approach is needed to evaluate AMR dynamics along this pathway as resistive microorganisms and resistance genes may flow through animals, human beings, and the environment (Gahimbare *et al.*, 2024). During production, antimicrobial use for disease prophylaxis or growth promotion may select for resistant commensal and pathogenic bacteria in the gastrointestinal tract of food animals. Shedding resistant *E. coli* in feces contaminates the pens, water sources and soil, leading to the formation of environmental contamination (Gemeda *et al.*, 2023). Also, resistant bacteria could be disseminated into soils and water bodies where they can become reservoirs due to application of manure as fertilizer.

Stress and subsequent mixing animals during transportation and lairage contribute to an additional increase in bacterial shedding and stress. Inadequate sanitation of vehicles and facilities also contribute to risk of cross-contamination populations (Abate and Birhanu, 2025). At the slaughterhouse, contamination of the carcass is mainly occurred during dehiding and evisceration. Poor sanitary practices, quality water and reuse of contaminated materials, exacerbate the situation (Agmas and Adugna, 2018). In the butcher shops and supermarkets, poor refrigeration, cross-use of knives, and no proper hand washing practices facilitate survival and spreading of bacteria. Improper handling, undercooking, or cross-contamination in kitchens can result in transmission of *E. coli* to food contact surfaces or human host (Kallu *et al.*, 2024).

Biosecurity and hygienic practices are essential preventive measures for reducing pathogen circulation and limiting unnecessary AMU within the beef production system. Farm biosecurity includes practices such as controlling animal movement, quarantining newly introduced animals, maintaining clean water and feed sources, proper manure management, vaccination, record keeping, and restricting unauthorized access to farms. Effective biosecurity reduces disease occurrence and decreases the need for antimicrobial treatment, thereby supporting AMS (Fujita *et al.*, 2022 ; Beauvais and Ivanek, 2026).

In addition, hygienic practices among animal handlers and meat workers, including regular hand washing, use of protective clothing, cleaning and disinfection of equipment, proper waste disposal, and safe carcass handling, are critical for preventing microbial contamination along the beef value chain. Poor implementation of biosecurity and hygiene measures has been reported as an important driver of foodborne pathogen transmission and AMR dissemination in livestock systems, particularly in and middle income countries where veterinary services, infrastructure, and regulatory enforcement may be limited (Gutema *et al.*, 2021; Rekwot *et al.*, 2022). Therefore, improving biosecurity and hygienic practices through a One Health approach is necessary to protect animal health, ensure food safety, and reduce the spread of AMR organisms between animals, humans, and the environment (Despotovic *et al.*, 2023).

2.8.1. Evidence from Ethiopia and Africa

Antimicrobial residues in beef meat have been reported from Ethiopia, tetracycline's and β -lactams were the most frequently encountered antimicrobials (Agmas and Adugna, 2018). In addition to the toxicological hazards, these residues may exert selective pressure on human gut bacteria and consequently expand sources of antibiotic resistance. The release of resistant bacteria or antibiotic residues present in slaughterhouse waste into the environment also plays a role in spreading these agents in water and soil (Abate and Birhanu, 2025).

Among many relevant African studies, it is well observed that foodborne AMR pathogens especially *E. coli* are highly endemic in Africa meat system although very few countries have sufficient surveillance on food value chains. Isolation of the *E. coli* O157:H7 strain that produces Shiga toxin in humans, animals, and food together with the environment has been documented in all African countries. The South African national veterinary surveillance and monitoring program for resistance to antimicrobial drugs has reported, increased AMR in *E. coli* (Beyi *et al.*, 2017; Gambushe *et al.*, 2022). In Ethiopia, a similar trend has been found in different livestock production systems, from animal feces, meat, soil and MDR *E. coli* were also found (Gemedo *et al.*, 2023). The continuity of resistant strains along the food chains highlights the importance for a combined approach in control effort and surveillance (Gahimbare *et al.*, 2024)

Table 1 summarizes the reported prevalence of *E. coli* in various studies across different locations of Ethiopia and Africa. For instance, Abera *et al.*, (2025) found overall prevalence of 81.0% in raw beef meat. Whereas Worku *et al.*, (2022) reported the lowest prevalence 2.3%. This difference might be due to hygiene practice, sample type and detection techniques.

Table 1: Reported prevalence of *E. coli* from meat and related samples in Ethiopia and Africa

| Study year | Study places | Sample type | Reported prevalence |
|-------------------------------|---|--|---|
| Abera <i>et al.</i> , 2025 | Addis Ababa and Burayu | Raw beef (butcher shop) | (81.0%) <i>E.coli</i> and (19.8%) <i>E.coli</i> O157:H7 |
| Asefa <i>et al.</i> , 2025 | Bahir Dar City | Raw meat(butcher house) | <i>E. coli</i> O157:H7: 28.4% |
| Abey <i>et al.</i> , 2024 | Gondor and Bahir Dar | Carcass swap and environmental sample | Overall <i>E. coli</i> 29.95% |
| Mokennen <i>et al.</i> , 2024 | Chelenko Eastern Ethiopia | Beef/meat value chain | Overall <i>E.coli</i> :8.6%, meat(abattoir=2.8% Meat(butcher)=21.4% |
| Abebe <i>et al.</i> ,2023 | Dessie and Kombolcha | Food of bovine origin | 54.7% (overall <i>E.coli</i> , <i>E.coli</i> O157:H7= 6.5% |
| Abunna <i>et al.</i> ,2023 | Bishoftu | Meat and meat contact surface | 3.97% <i>E. coli</i> O157:H7 |
| Ibrahim <i>et al.</i> ,2023 | Egypt | Beef meat | 28% <i>E. coli</i> |
| Debebe <i>et al.</i> ,2022 | Wolayta Sodo | Abattoir and butcher shop | <i>E.coli</i> O157:H7: Abattoir= 7.41%, butcher house= 10.12%; overall = 8.6% |
| Gugsa <i>et al.</i> , 2022 | Mekelle, Tigray | Food of bovine origin total | <i>E. coli</i> O157:H7: 14.29% |
| Gambushe <i>et al.</i> , 2022 | North Africa (review across Algeria/Morocco/Tunisia etc.) | Raw meat /marketed meat (various studies summarized) | Algeria ~7% (bovine carcasses, selected studies); Morocco <i>E. coli</i> O157:H7 ~9–11% in some meat surveys (varies by study). |
| Haile <i>et al.</i> ,2022 | Addis Ababa | Raw retail beef | <i>E. coli</i> O157:H7=3.64% |
| Worku <i>et al.</i> , 2022 | Hawasa | Raw meat and swap | ESBL <i>E. coli</i> =2.3% |
| Ajuwon <i>et al.</i> , 2021 | Moro,Nigeria | Carcass swap and environmental sample | 3.9% overall <i>E. coli</i> O157:H7 (5.6% from carcass swabs) |
| Aliyu <i>et al.</i> , 2021 | Sokoto Metropolis, Nigeria | From raw meat | 57.5% <i>E. coli</i> O157:H7 |
| Tadese <i>et al.</i> , 2021 | Ambo | Raw beef meat | <i>E. coli</i> 23.4% and 9.1% <i>E.coli</i> O157:H7 |
| Adzitey <i>et al.</i> , 2020 | Tamale,Ghana | From beef meat | 86.67% <i>E. coli</i> |
| Mengistu <i>et al</i> 2020 | Jimma | Beef meat and contact surface | 20.5% <i>E.coli</i> and 5.4% <i>E.coli</i> O157:H7 |
| Atnafie <i>et al.</i> , 2017 | Hawassa | Cattle feces, carcass and contact surface | 2.4% <i>E.coli</i> O157:H7 |
| Beyi <i>et al.</i> , 2017 | Central Ethiopia | Carcass & cutting board swabs (butcher shops and restaurant) | <i>E. coli</i> O157:H7=4.5%(carcass swabs),3.6%(cutting board swabs), overall 1.7% in butcher Shops |
| Abong'o and Momba,2009 | South Africa | Meat and meat Products | 2.8% <i>E. coli</i> O157:H7 |
| Ateba <i>et al.</i> , 2008 | South Africa | Feces of beef Animal from farm | 5.4% - 20% <i>E. coli</i> O157:H7 (varied by farm) |

2.8.2. Risk factors and drivers in the meat chain

The spread of AMR throughout the beef supply chain depends on several risk factors which include unsanitary slaughter operations, insufficient shop sanitation, no refrigeration systems, poor waste management and insufficient handler knowledge. The combination of insufficient regulatory oversight and insufficient scheduled inspections creates additional risks for contamination (Mekonnen *et al.*, 2024). The widespread presence of *E. coli* throughout the meat supply chain enables contact with intestinal or fecal matter which makes resistant *E. coli* strains an essential marker for tracking AMR in meat production. The solution to these deficiencies requires veterinary professionals, public health specialists and environmental authorities to work together (Tufa *et al.*, 2023).

A One Health strategy for the beef value chain enables comprehensive understanding of how antimicrobial resistance spreads through the system. The approach helps to identify essential control points and supports proper antibiotic use and generates data for creating evidence-based solutions (Despotovic *et al.*, 2023 ; Nastasijevic *et al.*, 2024). The fast-growing demand for meat in Ethiopia requires the implementation of AMR surveillance systems throughout livestock and food production to safeguard both human and animal wellness (Wakaso *et al.*, 2025).

Figure 1 describes the conceptual framework of this study, showing interconnections among AMU, emergence of resistant *E. coli* and associated public health risks along beef value chain. Misuse of antimicrobials in cattle production exerts selective pressure that promotes the emergence of AMR *E. coli*. These resistant bacteria can spread through three main pathways: animal pathway, environmental pathway and human pathway. Cross contamination can occur through multiple points along beef value chain linking farm, abattoir and butcher shops. Ultimately this process poses significant risks to food safety and human health. A one health response is essential to break the transmission cycle and mitigate the impact of AMR on both animal and human health (Nastasijevic *et al.*, 2024; Abate and Birhanu, 2025).

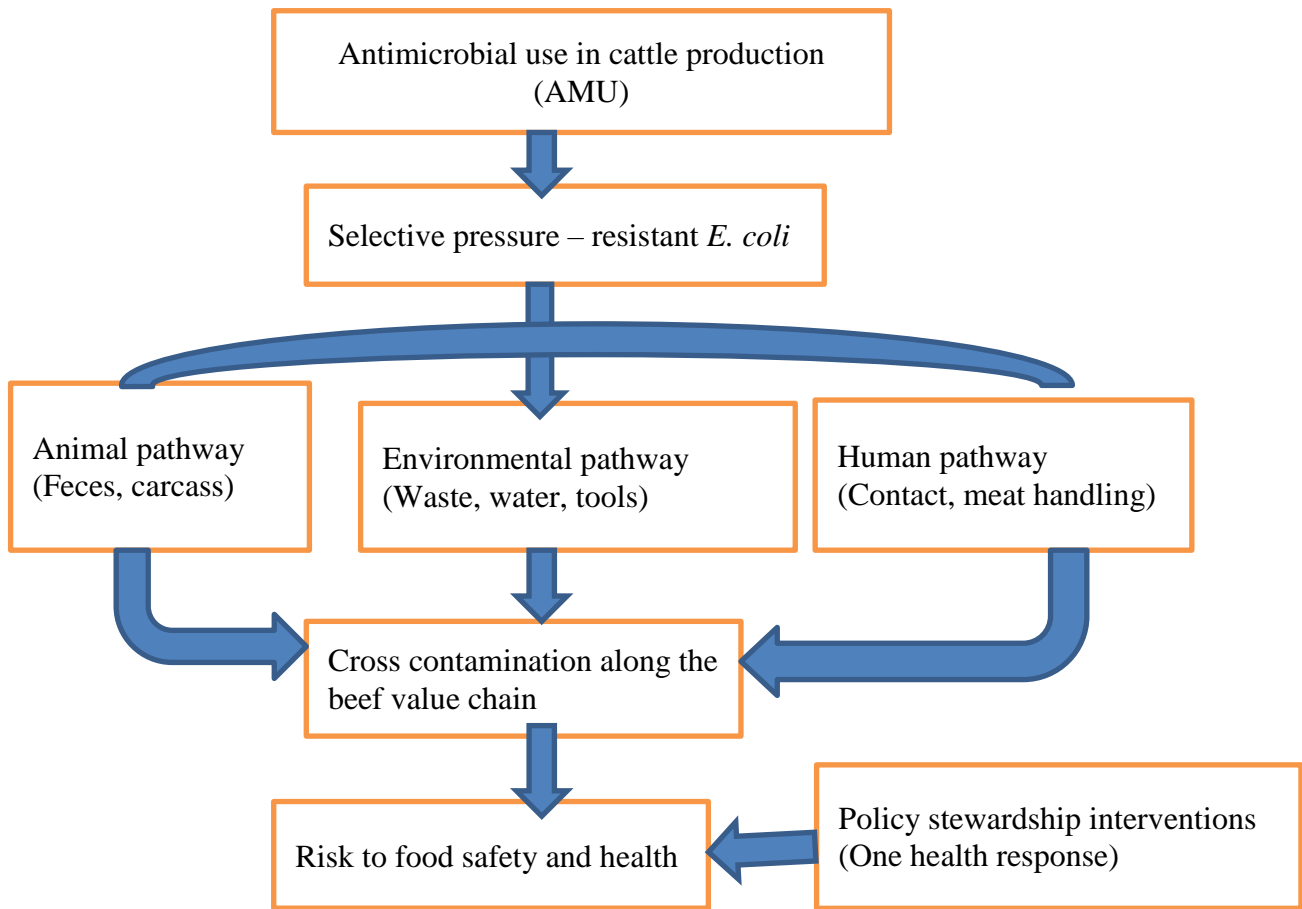


Figure 1: The framework showing interconnections among AMU and AMR adapted from (Asaduzzaman *et al.*, 2022; Fujita *et al.*, 2022; Despotovic *et al.*, 2023).

3. MATERIALS AND METHODS

3.1. Study area description

This study was conducted in Akaki Kality sub city of Addis Ababa. Akaki Kality is the second largest sub city, situated to the south of Addis Ababa city (Figure 2) in Ethiopia and approximately 20 kilometers from the city center. It has an area of 12345.4 hectare. The lowest elevation point is 2,060 meters and the maximum elevation is 2,340 meters above sea level. Akaki Kality is situated at latitude is $8^{\circ}50'–8^{\circ}57'N$ and the longitude is $38^{\circ}45'–38^{\circ}50'E$. The temperature of sub city ranges in between $17^{\circ}C$ to $20^{\circ}C$ depending on seasonal variation. Akaki Kality sub city shares boundary with Bole Sub-cities in the North, Nifas Silk Lafto Sub-cities in the Northwest and Oromia Regional States in the South and East directions. Currently it had 12 Woreda's and the population of the Sub city is close to 238,355 and its population density was 2018.6 per sq.km. The total numbers of fattening beef found in the sub city were 1639 and there was one abattoir and 315 butcher shops where found in the sub city during study period (Annual report of Akaki Kality sub-city farmers and urban Agriculture development office, 2025).

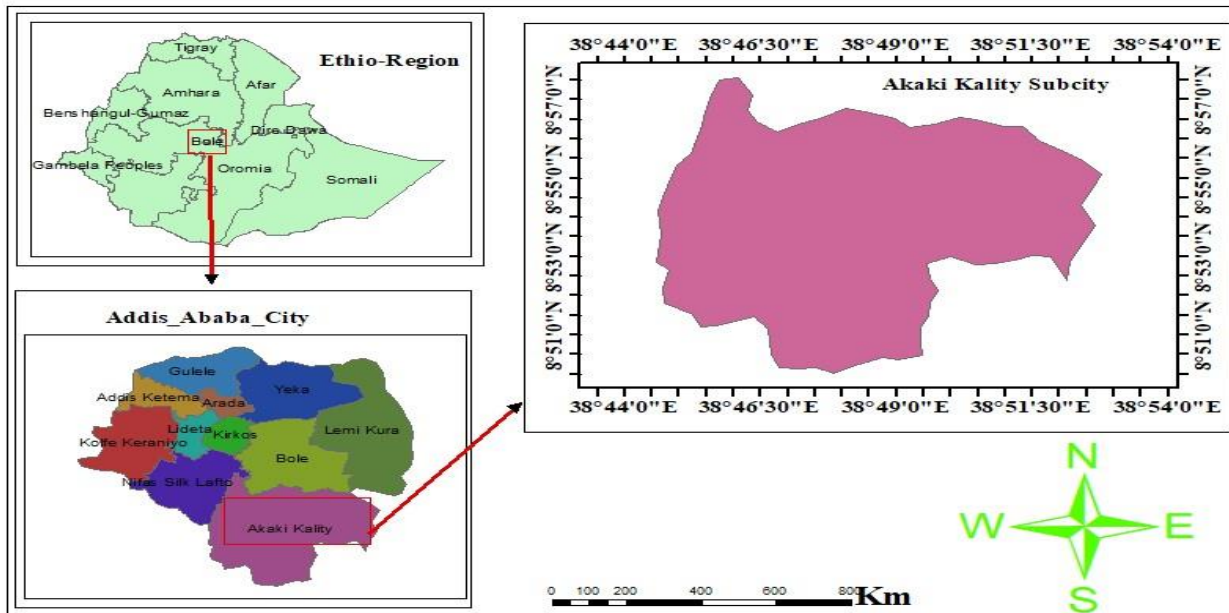


Figure 2: The Map showing the study area (ArcGis, 2026)

3.1. Study design

A cross-sectional study design was conducted from November 2025 to May 2026 to assess KAP of beef fattening farmers regarding AMU and AMR, hygienic practice of meat handlers and to determine the occurrence and antimicrobial susceptibility pattern of *E. coli* isolate respectively.

3.2. Population

3.2.1. Source population

All cattle fattening houses, meat slaughtering house, butcher shops, beef farm workers, and meat handlers working in slaughtering houses and butcher shops were the source population for this study.

3.2.2. Study population

The study populations were the selected fattening cattle and those presented for slaughter at the slaughterhouse. Watering and feeding trash at farms, meat processing and handling surfaces and materials along the value chain, including water sources, abattoir discharge, abattoir equipment (knives, axes, hook), carcass surfaces, and butcher shop environments (cutting tables, knives) were included. Hand swabs from personnel at farms, slaughterhouse and butchers, and questionnaire survey from livestock owners or workers, slaughterhouse workers and butcher shop worker were the study population.

3.3.3 Inclusion criteria

Cattle fattening houses that have greater than 5 animals, willingness of slaughterhouses and butcher shops, farmers working in cattle fattening house, and meat handlers working for at least 6 months were included in the study.

3.3.4. Study samples

Study was conducted on the total of 663 samples collected from beef value chain and 166 samples from beef farm, 257 samples from slaughterhouse and 240 samples from butcher shop.

Table 2. Type and number of sample collected along beef value chain

| Sample type | No. of samples along the beef value chain | | | |
|-------------------|---|----------|--------------|-------|
| | Beef Farm | Abattoir | Butcher shop | Total |
| Fecal carcass | 84 | 63 | - | 147 |
| Hand swab | - | 65 | 60 | 125 |
| Equipment swab | 29 | 40 | 60 | 129 |
| Water | 27 | - | - | 27 |
| Knife | 26 | 6 | - | 32 |
| Cutting board | - | 40 | 60 | 100 |
| hook | - | - | 60 | 60 |
| Axe | - | 16 | - | 16 |
| Transport vehicle | - | 13 | - | 13 |
| Abattoir drainage | - | 8 | - | 8 |
| Total | - | 6 | - | 6 |
| | 166 | 257 | 240 | 663 |

3.4. Sample size determination

The sample size was determined according to the formula given by (Thrusfield, 2007) Since there was specifically no study conducted on the title in the study area, Sample size was determined using 50% expected prevalence with 5% of marginal error, 95% confidence level.

The sample size was calculated as follows:

$$n = \frac{(Z)^2 P_{exp} (1 - p_{exp})}{d^2}$$

$$n = \frac{(1.96)^2 (0.5) (1 - 0.5)}{(0.05)^2} = 384$$

Where:

n = the required Sample size

P_{exp} = expected prevalence (50% or $P=0.5$).

Z = the value of the standard normal curve score (1.96)

d = the permissible Margin of error (the required precision) = 5%

By adding 10% nonresponse rate the final sample size was 423.

However to improve precision and reliability of study findings the sample size was further increased and a total of 663 samples were collected. From 12 woreda in Akaki Kality sub city 5 woreda (woreda 3, 4, 5, 8, & 12) were selected by lottery methods. In the selected woreda there were 70 cattle fattening farms. From these cattle fattening farms purposively 26 farms was selected and samples were collected using simple random sampling. Systematic random sampling was used to collect sample from slaughterhouse. In the selected woreda there were 150 butcher shops, from these purposively 60 butcher shops was selected and samples were collected using simple random sampling method. Sampling was conducted during operational hours, ensuring that the samples reflect typical hygiene practices. Number of farms and butcher shop were proportionally allocated to size using proportional allocation formula (Thrusfield, 2007).

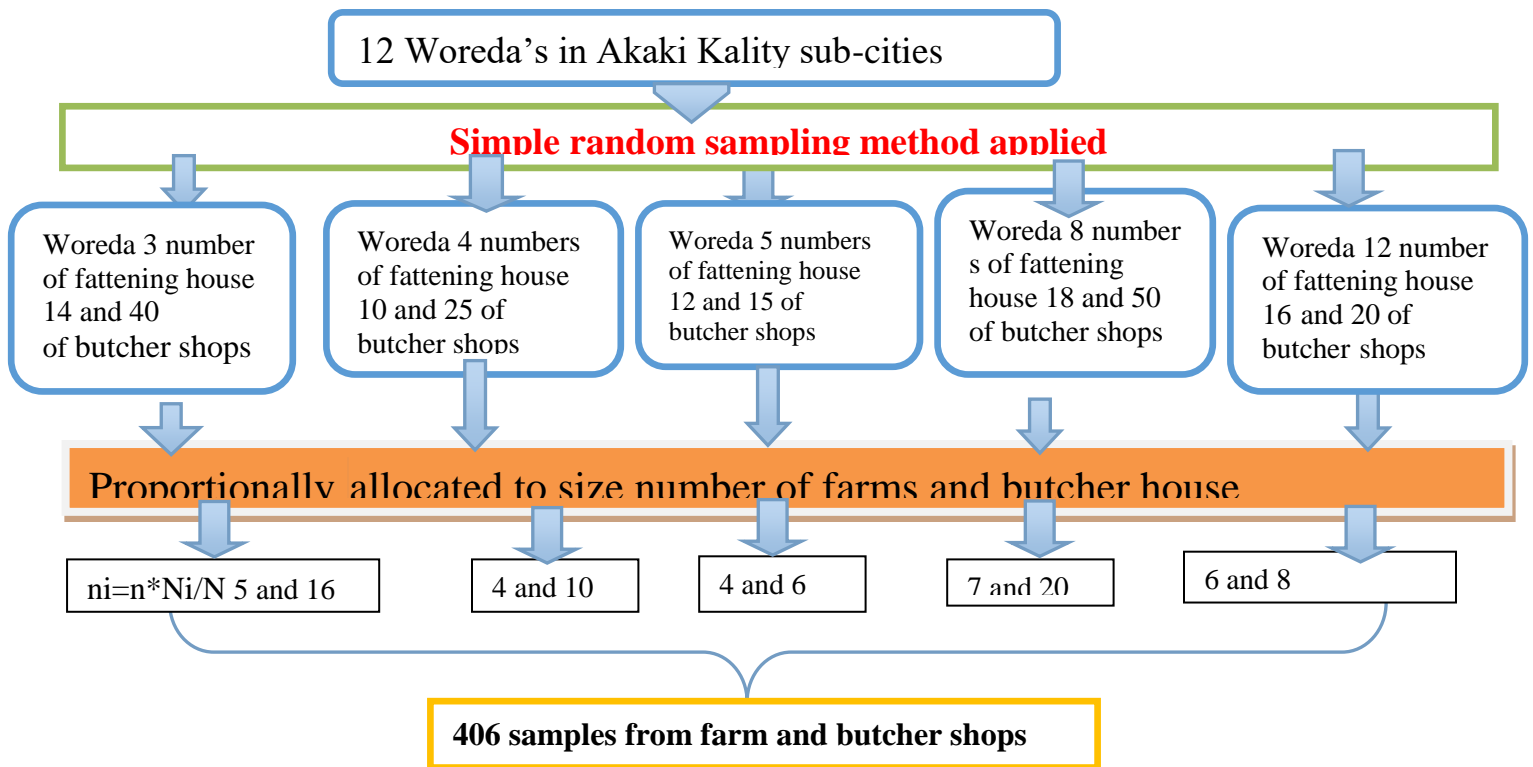


Figure 3. Schematic presentation of study design.

3.5. Sampling technique

3.5.1. Survey questionnaire and observational checklist

Structured questionnaire survey and observational check list were used as a tool to gather information on the KAP of animal handlers regarding AMU and awareness of AMR, hygiene and biosecurity practices of workers along the beef value chain process. The questionnaire was developed by referring from different studies previously done on related topics (Geta and Kibret, 2021; Gutema *et al.*, 2021; Roba *et al.*, 2023; Tufa *et al.*, 2023; Leta, 2024). The questionnaire was prepared in English and translated into the local language Amharic. The data was collected by Kobo tool apps using a face-to-face interview. On-site observational assessment (Qualitative) was done by systematically documenting the actual hygienic and handling practices, which serves as potential risk factors. Accordingly, information regarding sanitation levels, waste disposal, cross-contamination risks, and personal hygiene of handlers in abattoirs and butcher shops were collected.

3.5.2. Sample collection and transportation

A total of 147 Fecal sample was collected from the cecum of randomly selected live animals in the farm (84) and abattoir (63) before slaughtering begin then it were kept in a sterile universal bottle, labeled, transported on an ice box to the laboratory (Ajuwon *et al.*, 2021). During each visit usually once in a weak a total of 125 carcass swab was collected from abattoir (65) depending on number of animal slaughtered and from butcher shops (60). Carcass swab sample was collected by using prewetted a sterile cotton-tipped swab in 10 ml of buffered peptone water (Oxoid Ltd, Hampshire, England). After the carcass was washed pooled carcass swabs was collected from neck, breast, thorax (lateral), abdomen (flank) and rump parts of the carcasses was swabbed according to WHO guidelines (WHO, 2002). Each sampling site was swabbed approximately 100 cm², which is 10 cm horizontally and 10 cm vertically several times using separate sterile swabs. The shafts were broken, and the cotton sides of the swabs was left in the sampling bottle (Tanih *et al.*, 2015).

The samples was collected between 6:00 to 7:30 Pm, i.e., post-slaughter and during late afternoons, to minimize the microbial changes due to environmental temperatures and post-slaughter timings. It was then transported to the Microbiology laboratory of AAU-CVM under cold chain and kept in a refrigerator at 4 °C until processing.

129 Workers hand swaps was collected, the palms and fingers of both hands was swabbed horizontally and vertically by using prewetted sterile cotton swap in 10ml of peptone water. After swabbing, the shaft of the swabs was broken, and the cotton side of the swabs was kept in the labeled sampling bottle that contains 10 ml of peptone water (Abey *et al.*, 2024). The sample was then transported to the Microbiology laboratory of AAU-CVM under cold chain and kept at 4 °C until processing.

29 water samples were collected from beef farm. Midstream of water sample was collected from the container (source of water) to avoid surface debris, the water sample was also collected from the equipment where animals drink and pooled. In addition 6 water sample used for carcass washing in slaughterhouse and 6 abattoir effluent sample was also collected (Debebe *et al.*, 2022; Abey *et al.*, 2024). For each sample 25ml of water was collected by using sterile universal bottle. The collected water sample was labeled appropriately and placed in a cooler with ice packs to maintain a temperature of 4°C and transported to microbiology laboratory of the AAU-CVM for analysis within 24 hours of collection.

And also 27 equipment swab samples were collected by swabbing the feeding and drinking equipment in beef farm, knives, cutting board, axe, hook and transport vehicle in abattoir and butcher shops using a sterile cotton swab moistened in buffered peptone water and kept in the labeled sampling bottle that contains 10 ml of buffered peptone water, then transported to microbiology laboratory of the AAU-CVM with cooler ice box (Abunna *et al.*, 2023).

3.6. Isolation and characterization of *E. coli* and *E. coli* O157:H7

The detection of *E. coli* was carried out in accordance with the ISO 17604, (2005) standard protocol.

Fecal samples (1gm) was supplemented with 9 mL of buffered peptone water (Oxoid Ltd., Hampshire, England), homogenized in vortex shaker and incubated for 18–24 hours at 37°C. Similarly, all a swab sample in 10 mL of buffered peptone water (BPW) was incubated for 18–24 hours at 37°C.

After carefully mixing of the enrichment, the enriched sample was taken using bacteriological loops and was streaked primarily onto MacConkey agar (Himedia, India) and incubated at 37°C for 24 hours (Dejene *et al.*, 2022; Abunna *et al.*, 2023). On the MacConkey agar colonies with round shapes, smooth surfaces and pink color was suspected to be coliforms. Colonies were stained using Gram's stain to observe their staining and morphological characteristics. Then, suspected colonies of *E. coli* were then sub cultured on Eosin- methylene blue (EMB) (Himedia, India) agar. A single colony with a large, blue-black color and with a green metallic sheen on EMB agar was isolated and further sub cultured on nutrient agar and incubated for 24 hour at 37 °C followed by presumptive biochemical tests (indole production, methyl-red positive, Vogues- Proskauer negative, and citrate utilization negative) and fermentation of lactose and glucose using triple sugar iron agar was considered as *E. coli* (Sarba *et al.*, 2023; Abey *et al.*, 2024).

The bacteria that was presumptive as *E. coli* was sub cultured to sorbitol MacConkey agar (Oxoid Ltd., Hampshire, England) supplemented with 0.05 mg/l Cefixime and 2.5 mg/l potassium tellurite to differentiate the inability to ferment sorbitol, which is the characteristic of *E. coli* O157:H7. While other *E. coli* strains produced pink colonies, indicative of sorbitol fermentation. For Quality control *E. coli* ATCC25922 strains obtained from Ethiopian Public Health Institute, Bacteriology Unit was used (Mumed *et al.*, 2022).

Then non sorbitol fermenting colonies was sub cultured on nutrient agar and incubated at 37°C for 24 hrs for subsequent MALD-TOF technique, from confirmed *E. coli* O157:H7 isolates selected isolate was tested by latex agglutination test using latex kit (Oxoid, UK). Latex kit consists of four reagents; latex test reagent, latex control reagent, positive control and negative control reagent.

Latex test reagent contains blue latex particles coated with antibodies specific to *E. coli* O157 against *E. coli* O157:H7 antigen, positive control reagent had inactivated *E. coli* O157:H7 cells, negative control consists inactivated nonspecific *E. coli* cells whereas latex control reagent coated with rabbit globulin. The test was conducted according to manufacturer's instruction. Sample was tested after positive reaction of positive control with *E. coli* O157 latex test reagent. Then, using a sterile loop, a portion of the colony to be tested was taken and carefully mixed in the saline drop until the suspension was smooth. Then, the latex test reagent was added in a mixture and the suspension was mixed into the dry latex spots until completely suspended and spread to cover the reaction area. The test card was picked up, stirred for up to 60 seconds, and looked for agglutination under normal lighting conditions. A result was considered positive if agglutination of the latex particles occurs within 1 minute. This indicates the presence of *E. coli* O157:H7. A negative result was recorded if no agglutination occurs, and a smooth blue suspension remains after a minute (Abunna *et al.*, 2023; Manfredi *et al.*, 2023; Sarba *et al.*, 2023).

3.7. Antimicrobial susceptibility testing

The Kirby-Bauer disc diffusion method was used to determine the antimicrobial susceptibility profile of isolated *E. coli* (Mekonnen *et al.*, 2024). Accordingly, Mueller-Hinton agar plates (HiMedia, India) was prepared, and 12 commonly used antimicrobials was considered. These antimicrobial include cefoxitin (FOX, 30 µg), amoxicillin-clavulanate (AMC, 10µg), ampicillin (AMP, 10 µg), amikacin (AK, 30µg), ciprofloxacin (CIP, 5µg), cotrimoxazole (COT,30µg), tetracycline (TET, 30µg), trimethoprim (W, 5µg), chloramphenicol (CHL, 30µg), nalidixic acid (NA,30µg), aztreonam (ATM, 30µg) and imipenem (IMP, 10µg) (Sebsibe and Asfaw, 2020; Mustafai *et al.*, 2023).

Then, isolates with bacterial suspension equivalent to 0.5 McFarland standards, was uniformly streaked on Mueller Hinton agar, and plates with antimicrobial discs (Himedia) was incubated for 18 to 24 hrs. Finally, the diameter of the inhibition zone was measured using a black surface and transparent ruler.

The results was classified as sensitive, intermediate, and resistant according to Clinical laboratory standard institute (CLSI, 2025). As part of the quality control system, the standard reference strain of *E. coli* ATCC 25922 was used.

Table 3. Interpretation of disk diffusion break points for *E. coli* Antimicrobial susceptibility

| Antibiotic | Antimicrobial class | Susceptable(S) ≥ mm | Intermidiate(I) mm | Resistant (R) ≤ mm |
|-----------------------------------|---------------------|------------------------|-----------------------|-----------------------|
| Nalidixic acid (NA30µg) | Quinolone | ≥ 19 | 14-18 | ≤ 13 |
| Ciprofloxacin (Cip5µg) | Fluoroquinolone | ≥ 26 | 22-25 | ≤ 2 |
| Trimethoprim (W5µg) | Folate inhibitors | ≥ 16 | 11-15 | ≤ 10 |
| Cefoxitin (Fox30µg) | Cephalosporin | ≥ 18 | 15-17 | ≤ 14 |
| Ampicillin (Amp10µg) | Pencillin | ≥ 17 | 14-16 | ≤ 13 |
| Amoxicillin clavulanate (AmC10µg) | Beta lactam | ≥ 18 | 14-17 | ≤ 13 |
| Cotrimoxazole (Cot25µg) | Folate inhibitors | ≥ 16 | 11-15 | ≤ 10 |
| Tetracycline (TE30µg) | Tetracyclines | ≤ 15 | 12-14 | ≤ 11 |
| Aztreonam (ATM30µg) | Monobactam | ≥ 21 | 18-20 | ≤ 17 |
| Amikacin (AK30µg) | Aminoglycosides | ≥ 20 | 17-19 | ≤ 16 |
| Chloramphenicol (C30µg) | Phenicols | ≥ 18 | 13-17 | ≤ 12 |
| Imipenem(10 µg) | Carbapenems | ≥ 25 | 21-24 | ≤ 20 |

Source; (CLSI, 2025)

3.8. Data management and statistical analysis

The collected data on the assessment of KAP, hygiene and practice of meat handlers was transferred from Kobo data Collect app to Microsoft Excel dataset. Data was cleaned and validated for consistency checks to identify missing or outlier data points and verify with original records and laboratory investigation data result was entered into STATA version 14 for analysis. A P-value of less than 0.05 was considered statistically significant. Data was summarized based on the number and proportion of samples by source and sample type.

KAP of farmers was described by fisher exact test and frequency distribution. Then the prevalence of *E. coli*, *E. coli* O157:H7 and antimicrobial resistance patterns across different sample types was measured using frequency distributions, percentages, and means. For comparison of the prevalence and occurrence of *E. coli* and *E. coli* O157:H7 across sources. Chi-square was used. To measure strength of association binary logistic regression was used. For analysis of the distribution of resistance to specific antibiotics among different sample sources frequency distribution and percentage was used, the generated data was expressed and reported by using tables and graphs to compare resistance scores across groups.

3.9. Ethical consideration

Ethical clearance letter was taken from Addis Ababa University Research and Community Service offices of the College of Agriculture and veterinary medicine. A formal letter of cooperation was written to the Akaki sub cities administration. Necessary permission for data collection was taken from the city administration. Before starting the data collection, the data collector explained the purpose of the study to all the participants, and verbal consent was obtained from the study participants. The confidentiality of the data was maintained by avoiding possible identifiers such as the names of the study participants instead, the identification code numbers were used as a reference.

4. RESULTS

4.1. Findings of Questionnaire Survey

Sociodemographic characteristics of beef farm workers

In this study, a total of 108 beef fattening farm workers, abattoir, and butcher shop workers were participated. The sociodemographic characteristics of the surveyed beef fattening farmers discussed as below. Majorities (96%) of the participants was male, were between 19 and 35 years old (50%), and had a minimum of 1-4 years of livestock farming experience (50%). Most of the respondents (50%) at least attended primary school; 30.8 % completed secondary school, while 19.2 % never attended formal school. The findings on beef fattening farm indicated that the majority (61.54%) of farmers were small scale farmers. The Sociodemographic profile of respondents from beef fattening farmers was summarized in (Table 4).

Table 4. Sociodemographic data of beef fattening farmers (N = 26)

| Variable | | n(%) |
|----------------------|----------------------|-----------|
| Gender | Male | 25(96.2) |
| | Female | 1 (3.9) |
| Role in farm | Owner | 21(80.8) |
| | Worker | 5 (19.2) |
| Age | 19-35 | 13(50.0) |
| | 36-45 | 7(26.9) |
| | >45 | 6(23.1) |
| Educational Level | Can't read and write | 5(19.2) |
| | Primary School | 13(50.0) |
| | Secondary school | 8(30.8) |
| Fattening experience | 1-4 years | 8(30.8) |
| | 5-8 years | 18(69.2) |
| Farm size | Small scale | 16 (61.5) |
| | Medium scale | 10 (38.5) |

Note: n = frequency, (%) = percentage

Knowledge of beef fattening farm workers toward AMU and AMR

Overall 50 % of the respondents have poor knowledge toward AMU and AMR, While 50 % of the respondents have good knowledge depending on mean as cut off point. Most of the respondents (53.85%) were didn't know why antibiotics are given to cattle, and (92.3%) of the respondents use antimicrobials for treatment, prevention and growth promotion. Frequently tetracycline and ampicillin were used by farmers as they responded. 50.0% of the study participants do not heard about AMR. 65.38% of the respondents did not know that the use of antibiotics in farm animal without proper guidance can cause AMR. 61.54% of the respondents did not know AMR in animals have associated health risks in humans. Responses to knowledge questions related to AMU and AMR summarized in (Table 5) below.

Table 5. Knowledge of beef fattening house farm toward AMU and AMR (N = 26)

| Questions | n(%) |
|---|-------------|
| Do you know why antibiotics are given to cattle? Yes | 12(46.2) |
| Do antimicrobials is used for treatment, prevention and growth promotion? Yes | 24(92.3) |
| Do antibiotics can be used for all types of diseases in animals? No | 22(84.6) |
| Have you heard about antimicrobial resistance (AMR)? Yes | 13(50.0) |
| Do you know that the use of antibiotics in farm animal without proper guidance can cause AMR? Yes | 9(34.6) |
| Do you know AMR in animals have associated health risks in humans? Yes | 10(38.5) |
| Overall good knowledge | 13(50.0) |
| Overall poor knowledge | 13(50.0) |

Note: n = frequency, (%) = percentage

Attitude of beef fattening house workers toward AMU and AMR

Overall 53.8 % of the respondents have good attitude toward AMU and AMR, While 46.2 % of the respondents have poor attitude toward AMU and AMR depending on mean as cut off point. 84.6 % of the respondents did not think usage of antimicrobial drugs for non-therapeutic reasons lead to antimicrobial resistance. Majority 69.2 % of the respondents do not agree there is relationship between antibiotic use in animals and development of resistance.

88.5 % of the respondents have not ever received training on proper drug use and antimicrobial resistance. Responses to attitude questions related to antimicrobial use and resistance summarized in (Table 6) below.

Table 6. Attitude of beef fattening farm workers toward AMU and AMR (N = 26)

| Questions | n (%) |
|---|--------------|
| Do you think usage of antimicrobials for non-therapeutic reasons lead to AMR? Yes | 4(15.4) |
| Do you agree restriction of antibiotic use in animals will lead more benefit than damage? Yes | 19(73.1) |
| Do you agree there is relationship between antibiotic use in animals and development of resistance? Yes | 8(30.8) |
| Have you ever received training on proper drug use and AMR? Yes | 3(11.5) |
| Do you agree purchasing of antimicrobials from a pharmacy with a legal permit is safe? Yes | 19(73.1) |
| Do you think there is enough awareness about AMU and AMR among cattle farmers? Yes | 22(84.6) |
| Overall good attitude | 14(53.8) |
| Overall poor attitude | 12(46.2) |

Note: n = frequency, (%) = percentage

Practice of beef fattening house workers toward AMU and AMR

Overall, 42.3 % of the respondents demonstrated a poor practice toward antimicrobial use and resistance, while the remaining 57.7 % exhibited a good practice depending on mean as cut off point. A considerable proportion 69.2% of respondents obtained antibiotics without a prescription from private pharmacies; this is because of previous experience. 42.3% of respondents reported did not observing withdrawal periods after administering antibiotics before selling animals to slaughterhouses. 73.1% of the respondents reported that they practice improper disposal of leftover or unused medications. Responses to practice questions related to Antimicrobial use and resistance summarized in (Table 7) below.

Table 7. Practice of beef fattening farm workers toward AMU and AMR (N = 26)

| Questions | Category | n (%) |
|---|--------------------------------|----------|
| Who administers antibiotics to cattle? | Veterinarian | 19(73.1) |
| | Farm worker | 7(26.9) |
| Have you given antibiotics for your animals in the last one month? | Yes | 6(23.1) |
| | No | 20(76.9) |
| Where did you obtain the antibiotics that you gave for your animals? | Without prescription | 18(69.2) |
| | From veterinarian prescription | 8(30.8) |
| How many times have you give antibiotics for your animals per a month? | 2-5 times | 10(38.5) |
| | Never | 16(61.5) |
| Do you use antibiotics when animals get sick without laboratory test? | Yes | 18(69.2) |
| | No | 8(30.8) |
| Do you stop giving the antibiotics, if animals feel better after the first day of treatment? | Yes | 11(42.3) |
| | No | 15(57.7) |
| Do you observe withdrawal periods after administering antibiotics before selling to slaughterhouse? | Yes | 15(57.7) |
| | No | 11(42.3) |
| How do you dispose leftover or unused medication? | Throw away | 19(73.1) |
| | Burn, bury | 7(26.9) |
| Overall good practice | | 15(57.7) |
| Overall poor practice | | 11(42.3) |

Note: n = frequency, (%) = percentage

Observations for hygiene and biosecurity practice at beef fattening farms

During observation of beef fattening farms, 84.62% of farms were properly fenced. Only 11.54% of farms displayed signs restricting unauthorized entry. The majority (92.31%) had no footbath at the entrance of farm. About 65.38% had separate areas for feed storage, animal housing, and waste disposal. Approximately 57.69% quarantined new animals before mixing them with others and kept records of animal origin. Only 46.15% of workers wore protective clothing.

Furthermore, only 11.54% of farms maintained treatment or vaccination records. The summary was in (Table 78).

Table 8. Observational checklist of beef fattening farms

| Variables | n (%) |
|--|--------------|
| Farm is properly fenced. Yes | 22(84.6) |
| Presence of sign limiting Yes unauthorized entry | 3(11.5) |
| Footbath present at entry. Yes | 2 (7.7) |
| Separate areas for feed storage, animal housing and waste disposal. Yes | 17(65.4) |
| Floors, roof and walls in good condition. Yes | 9(34.6) |
| Regular cleaning schedule available. Yes | 21(80.8) |
| Presence of disinfectant. Yes | 6(23.1) |
| New animal quarantined before mixing and record of animal origin kept. Yes | 15(57.7) |
| Manure is properly managed. Yes | 16(61.5) |
| Workers wear protective clothing. Yes | 12(46.2) |
| Hand washing facilities available. Yes | 13(50.0) |
| Vaccination and treatment record available. Yes | 3(11.5) |
| Water source (Tab water) | 17(65.4) |
| Presence of toilet. Yes | 24(92.3) |

Note: n = frequency, (%) = percentage

Association of farmers' socio-demographic characteristics with knowledge, attitude, and practice regarding antimicrobial use and antimicrobial resistance

The KAP assessment of beef fattening farmers revealed that educational level significantly affected knowledge, attitude and practice of farmers toward AMU and AMR. Medium farm size also associated with better knowledge. Longer farming experience associated with better KAP of farmers toward AMU and AMR. The association of sociodemographic characteristics and KAP of farmers were indicated in the (Table 79) below.

Table 9. Association between socio-demographic characteristics and knowledge, attitude and practice regarding AMU and AMR

| Variable | Good KAP n (%) | Fisher exact test |
|---|-----------------------|--------------------------|
| Association of sociodemographic data with good knowledge | | |
| Educational level | | 0.005 |
| Can't read and write | 0(0) | |
| Primary school | 6(46.2) | |
| Secondary school | 7(87.5) | |
| Farm size | | 0.041 |
| Small | 5(31.2) | |
| Medium | 8(80) | |
| Cattle fattening experience: | | 0.002 |
| 1-4 years | 0(0) | |
| 5-8 years | 13(72.2) | |
| Role of respondent in farm | | 1.000 |
| Owner | 10(47.6) | |
| Worker | 3(60) | |
| Age (in years) | | 0.163 |
| 19-35 | 6(46.2) | |
| 36-45 | 2(28.6) | |
| >45 | 5(83.3) | |
| Association of sociodemographic with good attitude | | |
| Educational level | | 0.009 |
| Can't read and write | 0(0) | |
| Primary school | 7(53.8) | |
| Secondary school | 7(87.5) | |
| Farm size | | 0.701 |
| Small Farm size | 8(50) | |
| Medium farm size | 6(60) | |
| Duration of working in cattle fattening | | 0.000 |
| 1-4 yrs. of experience | 0(0) | |
| 5-8 yrs. of experience | 14(77.8) | |
| Role in farm | | 0.635 |
| Owner | 12(57.1) | |
| Worker | 2(40) | |
| Age | | 0.204 |
| 19-35 age | 5(38.5) | |

| | | |
|--|----------|--------------|
| 36-45 age | 4(57.1) | |
| >45 age | 5(83.3) | |
| Association of sociodemographic data with good practice | | |
| Educational level | | 0.007 |
| Can't read and write | 0(0) | |
| Primary school | 8(61.5) | |
| Secondary school | 7(87.5) | |
| Farm size | | 0.109 |
| Small Farm size | 7(43.7) | |
| Medium farm size | 8(80) | |
| Duration of working in cattle fattening: | | 0.038 |
| 1-4 yrs. of experience | 2(25) | |
| 5-8 yrs. of experience | 13(72.2) | |
| Role in farm | | 0.128 |
| Owner | 14(66.7) | |
| Worker | 1(20) | |
| Age | | 0.767 |
| 19-35 age | 7(53.8) | |
| 36-45 age | 5(71.4) | |
| >45 age | 3(50) | |

Note: n = frequency, (%) = percentage

Significant at P value < 0.05 and fisher exact test was used due to some expected cell count was less than 5.

Sociodemographic characteristics of slaughterhouse and butcher shop workers

In this study, a total of 22 slaughter house workers and 60 butcher shop workers were participated. Majority of the participants from abattoir worker was male, between 21 and 35 years old (50%), and had a minimum of 1-10 years of experience (81.8%). Most of the respondents (72.7%) at least attended primary school; 22.7% completed college and above school. The findings on butcher shop workers indicated that the majority of them were male, between 26 and 30 years old 32(53.3%) and had a minimum of 1-4 years of experience (71.9%). Most of the respondents (70.0%) attended secondary school; 28.3% completed college and above school. The Sociodemographic profile of slaughterhouse and butcher shop workers was summarized in (table 10) below.

Table 10. Sociodemographic characteristics of slaughterhouse and butcher shop workers

| Source | Variable | | n (%) |
|----------------|-----------------------------|-------------------|----------------|
| Slaughterhouse | Gender | Female | 1(4.6) |
| | | Male | 21(95.5) |
| | Age | 21-35 | 11(50) |
| | | 36-45 | 8(36.4) |
| | | >46 | 3(13.6) |
| | | Educational level | Primary school |
| | | Secondary school | 1(4.6) |
| | | College and above | 5(22.7) |
| | Working experience in years | 1-10years | 18(81.8) |
| | | 11-20years | 4(18.2) |
| Male | | 60(100) | |
| Butcher shop | Gender | Female | 1(4.6) |
| | | Male | 21(95.5) |
| | Age | 20-25 | 12(20) |
| | | 26-30 | 32(53.3) |
| | | 31-36 | 15(25) |
| | | >36 | 1(1.7) |
| | | Educational level | Primary school |
| | | Secondary school | 42(70.0) |
| | | College and above | 1(1.7) |
| | Working experience in years | 1-4years | 43(71.9) |
| 5-8 years | | 17(28.3) | |
| Religion | | Orthodox | 38(63.3) |
| | Protestant | 22(36.7) | |

Note: n = frequency, (%) = percentage

Findings from observations of slaughterhouse and butcher shops

At the abattoir level, inspection before slaughter was conducted in more than half of the cases (55.6%), while on (50%) of slaughtered cattle there was waiting time before initiating dehiding after inspection and bleeding. All carcass dressing procedures were carried out using the vertical method (100%). In addition, there was sinks available for hand washing, but not inside of the slaughterhouse, and carcass washing after evisceration was universally practiced in all carcass.

Regarding the use of protective materials, the most commonly workers used only apron, boots, and white coat, head cover is used by a few workers of slaughter house. Workers in the slaughterhouse had no attention for their personal hygiene and were not interested in washing their hands, knife and axes during slaughtering process. Their clothes and hands were not clean all through the working days.

At the butcher shop level, none of the meat display cases had protective covers, and all butcher shop floors were made of concrete. Ventilation conditions of display cases and butcheries were fair across all butcher shop (ventilation allows air flow but do not sieve dust or other particles or allows very little air flow). A large majority of shops had clean walls and ceilings (81.7%), and almost all were painted with white color (96.7%). Despite these conditions, key hygienic facilities were lacking. Only 35% of butcher shops had meat cooling facilities, similarly, nearly all butcher shops (98.3%) lacked sinks for hand washing at the point of sale. In terms of cutting board materials, plastic boards were predominantly used (76.7%), compared to wooden boards (23.3%). All of the butcher shops have no hot water baths for dipping knives. Only 13(21.7%) of the butcher shop workers use white coat and head cover as protective materials.

Survey of hygienic practice of slaughterhouse and butcher shop workers

Only 3(13.6%) slaughterhouse workers used soap during hand washing. 14(63.6%) of workers reported using the same knife for both dehiding and evisceration processes. The majority 21(95.5%) did not wash their hands between different activities, and only 18.2% immersed their knives in hot water after each task. Overall, only 50% of the slaughterhouse workers demonstrated good hygienic practices. Most respondents 37(61.7%) of butcher shop workers did not wash their hands before touching meat, and nearly all 59(98.3%) did not use soap when washing their hands. Notably, none of the respondents 60(100%) used detergent or disinfectant for cleaning butchery utensils. Fly control practices were also inadequate, with 32(53.3%) of shops lacking routine fly control measures. Additionally, 37(61.7%) of respondents reported handling money while selling meat, which may increase contamination risk. Nearly all respondents 58(96.7%) had not received any training on hygienic meat handling and 32(70%) had not undergone medical checkups in the last six months.

25(58.3%) reported using the same equipment, for handling both meat and offal. Generally, 28(46.7%) of the butcher s workers have good hygienic practice toward meat handling over all summarized in (table 11) below.

Table 11. Hygienic practice of slaughterhouse and butcher shop workers

| Variables | n (%) |
|--|----------|
| slaughterhouse level hygienic practice | |
| Do you Wash your hands frequently during slaughter operation: Yes | 12(54.6) |
| Do you wash your hands with soap: Yes | 3(13.6) |
| Do you use the same knife for dehiding and evisceration: No | 8(36.4) |
| Do you wash your hands in between activities: Yes | 1(4.6) |
| Do you sink the knife in hot water after each activity: Yes | 4(18.2) |
| Have you Ever received any training on hygienic handling of meat: Yes | 15(68.2) |
| Have you gone for medical checkups to work at the slaughter house: Yes | 16(72.7) |
| If yes, how frequent you go for medical checkup: Every six months | 12(54.6) |
| Overall good practice | 11(50%) |
| Overall good practice | 11(50%) |
| Butcher shop level hygienic practice | |
| What is means of transporting meat from slaughterhouse to retail shops: closed vehicle | 60(100) |
| How frequent do you use washed the protective coat: Once per day in the evening | 40(66.7) |
| Do you have a refrigerator for storage of the meat that remains from daily sale: Yes | 49(81.7) |
| Do you wash your hands before touching meat: Yes | 23(38.3) |
| Do you wash your hands with soap? Yes | 1(1.7) |
| How often do you wash butchery equipment? More than twice per day | 22(18.4) |
| How often do you wash floors? Once per day | 43(71.7) |
| Do you use detergent/disinfectant for cleaning the butchery utensils: No | 60(100) |
| What is the way of cleaning butchery equipment: Cold water with soap | 36(60.0) |
| Is there routine control of flies at retail shop: Yes | 28(46.7) |
| Do you collect money while handling or selling meat No | 23(38.3) |
| Have you ever received any training on hygienic handling of meat Yes | 2(3.3) |
| Do you ever receive complaints from the consumers on the quality of the meat you sell: Yes | 3(5) |
| Have you gone for medical checkups in the last 6 months: Yes | 18(30) |
| How frequent you go for medical checkup: Every six month | 22(36.7) |
| Do you have different storage and display cabinets for offal's and meat Yes | 31(51.7) |
| Do you use the same equipment's such as knife while handling meat and the offal: No | 25(41.7) |
| Overall good practice | 28(46.7) |
| Overall good practice | 32(53.3) |

4.2. Prevalence and distribution of *E. coli* isolates

Out of 663 different samples examined at the Akaki Kality beef farm, abattoir and butchery shop, 176(26.5%) were positive for *E. coli*. Among the samples found positive, 31(18.7%) were isolated from beef farm, 88(34.2%) were from the abattoir and 57(23.8%) were isolated from butchery shops. Out of 176 *E. coli* isolates, 49 were non sorbitol fermenter *E. coli* which was considered as presumptive positive for *E. coli* O157:H7 isolates. Then out of 49, presumptive positive for *E. coli* O157:H7 39 randomly selected representative samples was taken, 19(2.9%) was confirmed as *E. coli* O157:H7 by Maldtoff technique. To support Maldtoff technique, latex agglutination test was done on the selected samples. Among the samples found positive, 7(4.2%) were isolated from beef farm, 9(3.5%) were isolated from abattoir and 3(1.3%) were from the butcher shops (table 12) below.

Table 12 . Occurrence of *E. coli* and *E. coli* O157:H7 isolated from different sample source (chi-square)

| Source | Sample type | Examined | <i>E. Coli</i> (%) | χ^2 | P-value | <i>E. coli</i> O157:H7 (%) | χ^2 | P-value |
|--------------|---------------|----------|--------------------|------------|---------|----------------------------|----------|---------|
| Farm | Fecal | 84 | 16(19%) | 4.045 | 0.245 | 2(2.4%) | 3.93 | 0.303 |
| | Hand swab | 29 | 2(6.9%) | | | 1(3.4%) | | |
| | Water | 26 | 7(26.9%) | | | 1(3.8%) | | |
| | Equipment | 27 | 6(22.2%) | | | 3(11.1%) | | |
| Total | | 166 | 31(18.7%) | | | 7(4.2%) | | |
| Abattoir | Fecal | 63 | 19(30.2%) | 25.55 4 | 0.001 | 2(3.2%) | 4.048 | 0.855 |
| | Carcass swab | 65 | 33(50.8%) | | | 2(3.1%) | | |
| | Hand swab | 40 | 11(27.5%) | | | 2(5%) | | |
| | Knife Swab | 40 | 8(20%) | | | 1(2.5%) | | |
| | T. vehicle | 8 | 5(62.5%) | | | 1(12.5%) | | |
| | Drainage | 6 | 1(16.7%) | | | 0 | | |
| | axe swap | 13 | 8(61.5%) | | | 1(7.7%) | | |
| | Water | 6 | 0 | | | 0 | | |
| hook swab | 16 | 3(18.8%) | 0 | | | | | |
| Total | | 257 | 88(34.2%) | | | 9(3.5%) | | |
| Butcher shop | Carcass swab | 60 | 15(25%) | 4.670 | 0.198 | 1(1.7%) | 0.713 | 0.294 |
| | Hand swab | 60 | 14(23.3%) | | | 0 | | |
| | Knife Swab | 60 | 9(15%) | | | 0 | | |
| | Cutting board | 60 | 19(31.7%) | | | 2(3.3%) | | |
| Total | | 240 | 57(23.8%) | | | 3(1.3%) | | |
| Over all | | 663 | 26.5% | | | 2.9% | | |

Key note; T. vehicle: transport vehicle

Accordingly, from beef farm samples, *E. coli* was detected in 16 (19%) of fecal samples whereas, *E. coli* O157: H7 was detected in 2(2.4%). From water sample *E. coli* was detected in 7(26.9%) and *E. coli* O157: H7 was detected in 1(3.8%). Additionally, in equipment *E. coli* was detected in 6(22.2%) whereas, *E. coli* O157: H7 was detected in 3(11.1%). From the abattoir samples, *E. coli* was detected in 19(30.2%) of fecal sample and of 33(50.8%) of carcass swab whereas, *E. coli* O157: H7 was detected in 2(3.2%) and 2(3.1%) of fecal and carcass swab

respectively. And also *E. coli* was detected in 11(27.5 %) of hand swab and 8(20 %) knife swab respectively, whereas, *E. coli* O157: H7 was detected in 2(5%) of hand and 1(2.5%) knife swab respectively. From butcher shop sample *E. coli* and *E. coli* O157: H7 was detected in 15(25%) and 1(1.7%) of carcass swab respectively. From cutting board *E. coli* was detected in 19(31.7%) and *E. coli* O157: H7 was detected in 2(3.3%) of cutting board.

The study revealed that, Carcass swab was highly contaminated with *E. coli* and *E. coli* O157:H7 by odd ratio of 1.995 (1.182 - 3.367) and 0.88 (0.19 - 4.00) times, transport vehicle also contaminated by 5.333(1.213 - 23.45) and 5.1(0.50 - 51.91) times and axe swab is 5.12(1.573 - 16.663) and 2.98(0.31 - 28.81) times than fecal sample and there was statistical significant difference in the occurrences of *E. coli* in the study site. But there was no statistically significant difference in distribution of *E. coli* O157:H7 across sample type and sampling site. Summary of logistic regression of *E. coli* isolate in (table 13 and table 14).

Table 13. Logistic regression of *E. coli* isolate

| Variables | Category | No. Positive | % | OR | 95%CI for OR | P value |
|------------------|--------------------|---------------------|----------|-----------|---------------------|----------------|
| Sample type | Fecal | 35 | - | Ref | - | - |
| | Carcass swab | 48 | 38.4% | 1.995 | 1.182 - 3.367 | 0.010 |
| | Hand swab | 27 | 20.9% | 0.847 | 0.479 - 1.497 | 0.568 |
| | Knife Swab | 17 | 17% | 0.655 | 0.344 - 1.249 | 0.199 |
| | T. vehicle swab | 5 | 62.5% | 5.333 | 1.213 - 23.45 | 0.027 |
| | Drainage swab | 1 | 16.7% | 0.64 | 0.0723 - 5.663 | 0.688 |
| | Cutting board swab | 19 | 31.7% | 1.483 | 0.764 - 2.878 | 0.244 |
| | axe swap | 8 | 61.5% | 5.12 | 1.573 - 16.663 | 0.007 |
| | Water | 17 | 21.9% | 0.896 | 0.357 - 2.248 | 0.815 |
| | Equipment swab | 6 | 22.2% | 0.914 | 0.342 - 2.444 | 0.858 |
| | hook swab | 3 | 18.7% | 0.738 | 0.199 - 2.74 | 0.650 |
| Study site | Abattoir | 88 | 34.2% | Ref | - | - |
| | Butcher shop | 57 | 23.8% | 0.598 | 0.402 - 0.886 | 0.010 |
| | Farm | 31 | 18.7% | 0.441 | 0.276 - 0.704 | 0.001 |

Key note; T. vehicle: transport vehicle

Table 14. Logistic regression of *E. coli* O157:H7

| Variables | Category | No. Positive | % <i>E. coli</i> O157:H7 | OR | 95% CI for OR | P value |
|--------------|--------------------|--------------|--------------------------|------|---------------|---------|
| Study sample | Fecal | 4 | 2.7 | Ref | - | - |
| | Carcass swab | 3 | 2.4 | 0.88 | 0.19 - 4.00 | 0.868 |
| | Hand swab | 3 | 2.3 | 0.85 | 0.19 - 3.88 | 0.835 |
| | Knife Swab | 1 | 1 | 0.36 | 0.04 - 3.28 | 0.366 |
| | T. vehicle swab | 1 | 12.5 | 5.10 | 0.50 - 51.91 | 0.168 |
| | Drainage swab | 0 | 0 | 1 | - | - |
| | Cutting board swab | 2 | 3.3 | 1.23 | 0.22 - 6.92 | 0.812 |
| | axe swap | 1 | 7.7 | 2.98 | 0.31 - 28.81 | 0.346 |
| | Water | 1 | 3.1 | 1.15 | 0.12 - 10.68 | 0.900 |
| | Equipment swab | 3 | 11.1 | 4.47 | 0.94 - 21.23 | 0.060 |
| | hook swab | 0 | 0 | 1 | - | - |
| Study site | Abattoir | 9 | 3.5 | Ref | - | - |
| | Butcher shop | 3 | 1.3 | 0.35 | 0.09 - 1.30 | 0.117 |
| | Farm | 7 | 4.2 | 1.21 | 0.44 - 3.32 | 0.707 |

Key note; T. vehicle: transport vehicle

The results of this study highlight that interconnected nature of *E. coli* O157:H7 transmission within a One Health framework. The detection of the pathogen in fecal samples confirms cattle as a primary reservoir, while its presence on carcasses, hands, and equipment indicates potential transmission along the beef value chain. Environmental sources such as transport vehicles and equipment showed relatively higher contamination levels, suggesting their role as critical points for cross-contamination. The study revealed that there is no statistically difference in the occurrence of *E. coli* O157:H7 in the study area. This means contamination is not specified to single point, but rather distributed across interconnected animal, human, and environmental interfaces within the beef value chain.

Even though, no statistically significant associations were observed between sample type or study site. The occurrence of *E. coli* O157:H7, across animal, human, and environmental interfaces emphasizes the importance of integrated control strategies. These findings highlight that effective mitigation requires coordinated interventions such as improving hygienic practices, environmental sanitation and strengthening livestock health practice.

4.3. Antimicrobial susceptibility profile of *E. coli* and *E. coli* O157:H7

From 23 selected *E. coli* isolated 15(65.2%), 14(60.9%), 11(47.8%) and 10(43.5%) were resistant to ampicillin, tetracycline, trimethoprim and amoxicillin-clavulanate. While 16(69.6%), 13(56.5%) and 13(56.5%) were susceptible to chloramphenicol, imipenem and ciprofloxacin (figure 4). While 13(68.4%), 11(57.9%), 10(52.6%) and 9(47.4%) *E. coli* O157:H7 isolate were resistant to tetracycline, trimethoprim, ampicillin and cotrimoxazole and 13(68.4%), 12(63.2%) and 11(57.9%) were susceptible to Imipenem, chloramphenicol and amoxicillin-clavulanate (figure 5).

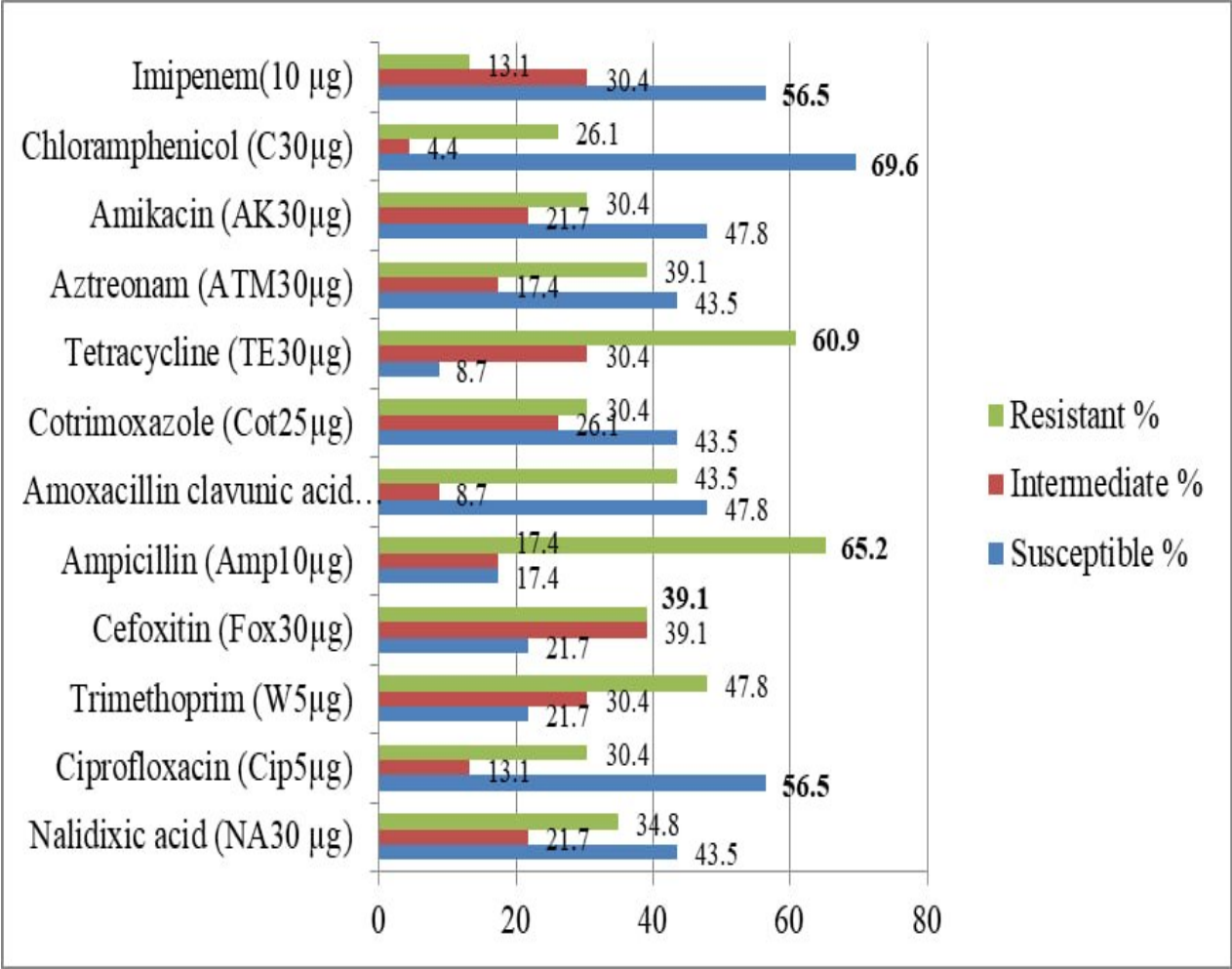


Figure 4. Resistance profile of *E. coli* summary (N=23)

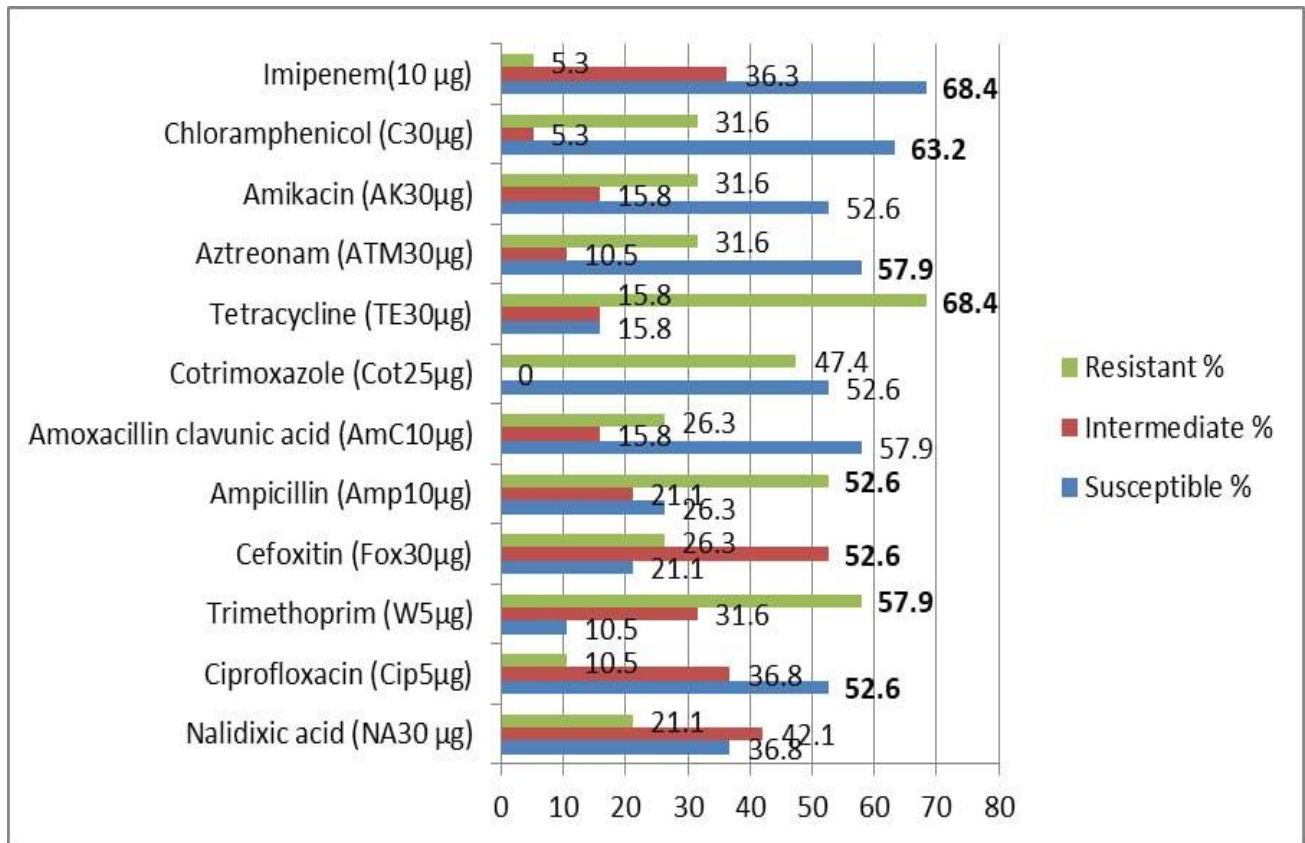


Figure 5. Resistance profile of *E. coli* O15:H7 summary (N=19)

Multi-drug resistance profiles

In this study, a total of 17(73.9%) *E. coli* and 16 (84.2%) *E. coli* O157: H7 isolates were resistant to three or more classes of antibiotics. . Multi-drug resistance profiles against three, four and greater than five antibiotic classes were 7(30.4%), 3(13.04%), 7(30.4%) and 6 (31.6%), 3(15.8%) and 7 (36.8%), *E. coli* and *E. coli* O157: H7 respectively. The frequency of resistant phenotype was more common for ampicillin, tetracycline trimethoprim, cotrimoxazole and amoxicillin-clavulanate.

5. DISCUSSION

This study assesses the Sociodemographic KAP of beef farmers and hygiene and biosecurity standards of slaughterhouse and butcher shop worker. Also prevalence and distribution of *E. coli* isolates, the antimicrobial susceptibility and multi-drug resistance profiles of *E. coli* isolate among beef farm, slaughterhouse, and butcher shops in Akaki Kality sub city, Addis Ababa, Ethiopia. The findings of the study revealed the critical gaps across the beef value chain, underscoring the persistent need for integrated interventions aimed at mitigating antimicrobial resistance through one health approach. The demographic profile indicates a predominance of young aged males was actively engaged in livestock and meat handling activities. This finding is in line with the study done in Bishoftu, Sheger and Addis Ababa, Ethiopia by (Gutema *et al.*, 2021; Leta, 2024 and Kallu *et al.*, 2024) respectively. This might be due to limited employment opportunities in other sectors. In current study most of the beef fattening farms were small-scale nature of farms this result is consistent with the study done in Jimma, Ethiopia by (Duguma, 2024 ; Wakaso *et al.*, 2025). The finding from this study shows majority of respondents had generally low level of education, with only 50% of them having primary education. This finding is comparable with the study done in Bishoftu, Sheger and East shoa zone, Ethiopia by (Gutema *et al.*, 2021; Leta, 2024; Yimana and Hassen, 2024).

This study reveals that only 46.15% of respondents know why antibiotics are given to cattle. This is comparable to the study done in Oromia speial zone, selected regions of Oromia and Southern Ethiopia by (Gebeyehu *et al.*, 2021; Tufa *et al.*, 2023; Endale *et al.*, 2026) revealing that farmers had low awerness level about the correct use of antibiotics. This awareness result is lower than that of study done in selected region of Oromia and Amhara, Ethiopia by (Gemeda *et al.*, 2020) which shows majority(84.2%) of participant know the use of antibiotics and the study done in Africa, by (Caudell *et al.*, 2020) which show farmers had higher understanding on the use of antimicrobials. The variation might be due to, difference in study population, training related to antimicrobial use. In this study, only 50% of the study participants heard about antimicrobial resistance.

This finding is lower than the study done in Amhara and Addis Ababa, Ethiopia by (Geta and Kibret, 2021; Kallu *et al.*, 2024) which showed 90.1 % and 70.8% participants heard about antimicrobial resistance, respectively. The difference might be due to awareness creation by doctors and veterinarian in the previous study area.

In this study 65.38% of the respondents did not know that improper antibiotic use in farm animals contributes emergence of resistant bacteria. Also 61.54% of farmers did not know that AMR transmission from animals to humans. This finding is in line with the research done in Oromia special zone and Yabello and Menz Gera, Ethiopia by (Gebeyehu *et al.*, 2021; Lemma *et al.*, 2025) found poor awareness of AMR transmission dynamics among livestock producers. These finding reflect limited (38.5) understanding of AMR transmission across animal, human and environment. These finding is lower than the study done in Tigray region, Ethiopia by (Romha, 2026; Woldemichael *et al.*, 2025) reported 71% of participants know transmission of AMR bacteria can be transmitted from animal to human and 52.9% of respondents know improper drug use in animal can lead AMR in humans. The difference might be due to study participants have high education level in the previous study and access to AMU and AMR related information. Overall 50 % of the study participants have good knowledge toward AMU and AMR; this is higher than study done in Oromia special zone, north eastern Ethiopia by (Gebeyehu *et al.*, 2021) who reported 19.8% of good knowledge and also higher than study done in Buno Bedelle and Illu Aba bora zones by (Tadese *et al.*, 2025) who reported (21.5%) respondents have good knowledge regarding the antimicrobials use, resistance, and residues. The variation might be due to study population and education level of participants.

This study result also shows 73.1% of participants agreed with idea on restriction of antibiotic use in animals will lead more benefit than damage, while 69.2 % did not agreed with, there is relationship between antibiotic use in animals and development of resistance. This study is consistent with study done in Ethiopia by (Kallu *et al.*, 2024; Endale *et al.*, 2026) and article review done by (Kimera *et al.*, 2020). This discrepancy might be due to the need for educational initiatives clarifying the connection between antimicrobial misuse and resistance.

This study revealed 84.6% of respondents were not think usage of antimicrobials for non-therapeutic reasons lead to AMR ; and this is in line with study done in selected regions of Oromia, Ethiopia by (Tufa *et al.*, 2023) who found many Ethiopian livestock producers lacked knowledge about the risks of non-therapeutic use of antibiotics. Similarly study done in Buno Bedelle and Illu Aba bora zones and Tigray region by (Tadese *et al.*, 2025; Woldemichael *et al.*, 2025) majority of respondents have misconception about antimicrobial use. And also, 88.5% of respondents had not ever received training on proper drug use and AMR; this agrees with study done in southern Ethiopia and Tigray region, Ethiopia by (Endale *et al.*, 2026; Romha, 2026) who reported lack of formal training for farmers regarding AMU and AMR. In general the overall 53.85 % of the respondents have good attitude about AMU and AMR, the finding is consistent with the study done in Amhara region, Ethiopia by (Geta and Kibret, 2021) which shows 52.5% of the farm owners/workers had positive attitudes on appropriate antibiotic use and resistance. This finding is lower than study done in Tigray region, Ethiopia by (Woldemichael *et al.*, 2025) who reported 68.2% of respondents have good attitude toward AMU and AMR. This study is higher than research done in Oromia special zone by (Gebeyehu *et al.*, 2021) who reported 14.7% livestock farmers had good attitude toward AMU and AMR.

In current study 69.2% of the farmers use antibiotics without prescription, this finding is consistent with the study done in Oromia, Amhara region and Addis Ababa, Ethiopia by (Gemedo *et al.*, 2020; Geta and Kibret, 2021; Kallu *et al.*, 2024) who found majority of farmers use antibiotics without prescription. And also this study shows that 42.3 % of farmers do not observe drug withdrawal periods. This finding in line with finding of the study done in Addis Ababa, Buno Bedelle and Illu Aba bora zones, Ethiopia by (Kallu *et al.*, 2024; Tadese *et al.*, 2025) where the farmers did not adhere to specified drug withdrawal periods before sending animals to the slaughterhouse. Similarly this study is agrees with study done in Tigray region, Ethiopia by (Romha, 2026) which indicates withdrawal period adherence were low. In addition improper disposal of leftover medications was observed and (73.1%) of respondents through away leftover medication.

This practice may facilitate dissemination of resistant bacteria and resistant genes; and this support one health concept that environmental contamination play an important role in AMR transmission between animals, humans and ecosystem (Despotovic *et al.*, 2023; Hossein and Ripanda, 2025). Overall, 42.3 % of the respondents demonstrated a poor practice toward antimicrobial use and resistance this is lower than (Gebeyehu *et al.*, 2021) who reported 78.5% livestock owners in Oromia special zone, were practice improperly toward AMU and AMR. This finding is comparable with (Romha, 2026),who reported 39.8% of farmers in Tigray region, had poor practice toward AMU, AMR and antimicrobial residue. And also similar with study of (Geta and Kibret, 2021) who found 52.75 % of animal farm owners or workers in selected regions of Amhara zone, Ethiopia had poor practice toward AMU and AMR. Farmers have high percentage of good practice than good knowledge toward AMU and AMR; this might be due to cultural practice and save cost.

Critical biosecurity measures such as absence of visitor restriction foot bath, absence of signs of unauthorized entry facilities and proper treatment record keeping were poorly implemented. Similar gabs in farm biosecurity practice have been reported in Ethiopian livestock keepers by (Duguma, 2020 ; Gemedo *et al.*, 2020 ; Nyokabi *et al.*, 2023) . 53.85% of farmers did not wear personal protective equipment, which can increase occupational exposure to zoonotic pathogens and resistant bacteria. Similar observations have been documented in Bishoftu, Ethiopia by (Gutema *et al.*, 2021) in slaughterhouse environment. Over one-third of farms lacked Separate areas for feed storage, animal housing and waste disposal; this can increase risks of cross-contamination and facilitate pathogen circulation within farms. Similar observation were described in Nigeria and Ethiopia by (Rekwot *et al.*, 2022; Wakaso *et al.*, 2025) reported that smallholder livestock systems frequently work with insufficient and limited infrastructure.

Educational status showed a statistically significant association with knowledge regarding AMU and AMR. 87.5% of farmers with secondary school education demonstrated good knowledge. This finding agrees with reports by (Gemedo *et al.*, 2020; Gebeyehu *et al.*, 2021; Geta and Kibret , 2021; Kallu *et al.*, 2024), which shows higher educational completion significantly improved awareness and understanding of AMU and AMR among livestock producers in Ethiopia.

Similarly, (Tufa *et al.*, 2023) reported that educated livestock producers were more likely to understand withdrawal periods, antimicrobial misuse, and the consequences of AMR. The present finding is also supported by (Woldemichael *et al.*, 2025; Endale *et al.*, 2026) who showed that illiteracy remains a major challenge for effective antimicrobial stewardship in Ethiopian livestock systems.

Farm size also had a significant association with knowledge, where 80% of farmers with medium farm size had good knowledge. This finding is in line with study done in selected regions of Oromia zone, Buno Bedelle and Illu Aba bora zones by (Tufa *et al.*, 2023; Tadese *et al.*, 2025), who observed that farmers involved in large size livestock farming have better awareness regarding proper antimicrobial use. This might be due to their regular interaction with veterinarians and health extension workers.

Duration of working in cattle fattening showed strong significant associations with knowledge, attitude, and practice. Farmers with 5 to 8 years of experience had significantly better KAP scores. Whereas 72.2%, 77.8% and 72.2% of experienced farmers had good knowledge, attitude and practice respectively. This result is consistent with studies conducted by (Gemeda *et al.*, 2020; Gebeyehu *et al.*, 2021; Romha, 2026), which indicated that longer involvement in livestock production increases farmers' understanding of AMU and AMR. Similarly, (Kallu *et al.*, 2024) reported that farming experience positively influenced proper antimicrobial usage practices among dairy farm workers in Addis Ababa.

Additionally, this study revealed that 45.45% and 86.36% of participants did not wash their hands frequently during slaughter operation and did not use soap while washing their hands respectively. The result is comparable with the research finding in Aweday and Harar by (Roba *et al.*, 2023) who observed inadequate hand hygiene identified a risk factor for contamination of meat with pathogenic bacteria. Most (63.64%) of slaughter house worker uses the same knife for dehiding and evisceration procedures without washing with hot water between activities. And majority (95.45%) did not wash their hands between different activities.

Related findings have been reported in Haramaya and Wolaita sodo, Ethiopia by (Amente *et al.*, 2022; Debebe *et al.*, 2022). Such like practices substantially rise risk of carcass contamination with food borne pathogens such as *E. coli* and *E. coli* O157:H7. And also, 81.82% of respondents did not sink the knife in hot water after each activity this finding is comparable with study done in Bishoftu and East shoa zone of the Oromia region, Ethiopia by (Abunna *et al.*, 2023; Yimana and Hassen, 2024). 31.82% of participants wear only apron and boot; this can be agrees with study done in Bishoftu and Haramaya by (Gutema *et al.*, 2021; Amente *et al.*, 2022; Mengistu and Hiko, 2022), reported that inadequate slaughterhouse sanitation and prolonged carcass handling were identified as important contributors to contamination of meat with *E. coli* O157:H7 and other foodborne pathogens.

All visited butcher shop have no any cover on meat display case and ventilation status allows air flow but do not sieve dust or other particles, similar finding is reported by (Beyi *et al.*, 2017; Haile *et al.*, 2022) where absence of cover on meat display associated with increased prevalence of *E. coli* O157:H7 in Addis Ababa and central Ethiopia. Only 1.67% of butcher shops have sink for washing hands. This result is comparable with studies conducted in Ambo, Aweday and Harar and Sheger city, Ethiopia by (Tadese *et al.*, 2021; Roba *et al.*, 2023; Leta *et al.*, 2024), who reported limited access to hand washing facilities in butcher shop. None of the visited butchers have no hot water baths for dipping knives. Similar study reported in Bishoftu by (Gutema *et al.*, 2021; Abunna *et al.*, 2023), explained lack of knife disinfection in butcher shops significantly contributed to contamination with *E. coli* O157:H7.

All butcher shops use closed vehicle for transporting meat from slaughterhouses, which represents a relatively good hygienic practice. This result is better than reports from some Ethiopian and African studies where open transportation systems were commonly practiced and were linked with increased contamination risks (Beyi *et al.*, 2017; Adzitey *et al.*, 2020). Most of butcher shop worker wear white coat, but only 21.67% used both white coats and head covers. Similar findings reported in Ambo and Wolaita Sodo, Ethiopia by (Tadese *et al.*, 2021; Debebe *et al.*, 2022).

While working in the butcherries, only (38.98%) of respondents wash their hands before touching meat, and only (1.7%) use soap when washing their hands this study is agreed with the study done in Aweday and Harar, and Sheger city, Ethiopia by (Roba *et al.*, 2023; Leta *et al.*, 2024). Even though in interviewed questionnaires 81.7% of respondents have refrigerator for storing of unsold meat, but during observation only 35% of butcher shops possessed refrigeration, while 65% lacked any refrigeration. Similar findings were reported in Jimma and Chelenko, Ethiopia by (Sebsibe and Asfaw, 2020; Mekonnen *et al.*, 2024) reported majority of the butcher shops in Ethiopia have no refrigerator for storing of unsold meat. All of respondents 100% of butcher shop worker did not sink the knife in hot water after each activity comparable with (Abunna *et al.*, 2023; Yimana and Hassen, 2024). 81.6% of respondents wash butchery equipment once per day and none of them did not used detergent for cleaning of utensils and 40% of them use cold water only or wiping with pieces of cloth. Similar finding have been reported from Bishoftu and East shoa zone, Ethiopia by (Gutema *et al.*, 2021; Yimana and Hassen, 2024) respectively, reported improper cleaning of butchery equipment was practiced.

53.3% of butcher shop lack routine fly control measures comparable with reports from Ambo, Ethiopia by (Tadese *et al.*, 2021), indicated that inadequate vector control contributed to contamination of meat in Ethiopian retail shops. 61.7% of respondents handled money while selling meat this is in line with the report from Wolaita sodo and Chelenko, Ethiopia by (Debebe *et al.*, 2022; Mekonnen *et al.*, 2024) which identified simultaneous handling of money and meat as a common but risky practice among butcher workers in Ethiopia. Only 96.7% of butcher workers had not received training on hygienic handling of meat this is in consistent with (Yimana and Hassen, 2024), who reported 82.6% of butcher shop worker in East Shoa zone of the Oromia region had not taken training on hygienic handling of meat. And also 58.3% use the same equipment such as knife while handling meat and offal similar result reported by (Gugsa *et al.*, 2022; Haile *et al.*, 2022; Abunna *et al.*, 2023), who recognized shared equipment and improper separation of meat products as major contributors to *E. coli* O157:H7 contamination in Ethiopian butcher shop.

Contamination of fecal, meat and meat contact surface with *E. coli* in beef value chain was 26.5%. this finding is in line with (Abey *et al.*, 2024) who reported 29.95% in Gondar. And this study finding is higher than study done in Jimma, Wolaita Sodo and Chelenko town by (Sebsibe and Asfaw, 2020; Debebe *et al.*, 2022; Mekonnen *et al.*, 2024) who reported 20.2%, 8.6% and 7.41% *E. coli*. the study is lower than (Abera *et al.*, 2025) who reported 81.0% of *E. coli* in Addis Ababa. The difference is due to variation in sample size and study period. Whereas, in this study *E. coli* O157:H7 was 2.9% this is in consistent with the result of (Atnafie *et al.*, 2017) who reported 2.4% of *E. coli* O157:H7 in cattle feces and meat contact surface in Hawasa town abattoir and butcher shop. This finding is lower than the study done by (Bekele *et al.*, 2014; Sebsibe and Asfaw, 2020; Amente *et al.*, 2022) who reported 10.4% *E. coli* O157:H7 in Addis Ababa, 5.4% *E. coli* O157:H7 at abattoir and butcher shops in Jimma town and 5.7% in Haramaya town respectively. And also the current finding is lower than (Abunna *et al.*, 2023) who reported 4% in Bishoftu town. This might be due to difference in geographic, study time and sample size.

Regarding source of sample, the prevalence of *E. coli* and *E. coli* O157:H7 at the beef farm level was 18.7% and 4.2%. This finding agrees with study done by (Gemedo *et al.*, 2023; Bedane *et al.*, 2024); Chekole *et al.*, 2025) reported occurrence of *E. coli* in livestock and environmental sources in Ethiopia. Occurrence of *E. coli* at farm level is expected due to cattle are the primary reservoir of it but presence in farm environmental sample is due to poor farm hygiene and biosecurity. In this study highest prevalence of *E. coli* was in abattoir 34.2% and butcher shop 23.8%, This finding is higher than (Sebsibe and Asfaw, 2020 ; Debebe *et al.*, 2022) who reported 19.3% and 21.4% and 7.41% and 10.12% of *E. coli* in slaughterhouse and butcher shop respectively. And also higher than finding of (Mekonnen *et al.*, 2024), who reported 16 (7.41%) and 17 (10.12%) in abattoir and butcher shop. Whereas, highest prevalence of *E. coli* O157:H7 was observed in beef farm 4.2% followed by abattoir 3.5%. This finding is comparable with study done by (Dejene *et al.*, 2022),who reported 4% *E. coli* O157:H7 along dairy farm in Akaki Kality sub city, Ethiopia. Similar findings were reported by (Gugsa *et al.*, 2022; Abebe *et al.*, 2023), who documented *E. coli* O157:H7 in abattoir samples in Ethiopia.

In this study there is no statistically significant variation among sample types in the occurrence of *E. coli* and *E. coli* O157:H7 except occurrence of *E. coli* in the abattoir this is comparable with study done by (Abebe *et al.*, 2023) in Dessie and Kombolcha towns. Highest occurrence of *E. coli* in beef value chain was found in carcass, fecal, cutting board, hand swab and water, the finding is comparable with study done in Hawasa, Jimma, Ambo and Bishoftu, Ethiopia by (Atnafie *et al.*, 2017; Sebsibe and Asfaw, 2020 ; Tadese *et al.*, 2021; Abunna *et al.*, 2023) which shows contamination of carcass and contact surface with *E. coli*. Similarly study done in South Africa and Nigeria by (Ateba *et al.*, 2008; Ajuwon *et al.*, 2021) indicate that feces is a major source of beef meat contamination. In contrast, *E. coli* O157:H7 was most frequently isolated from equipment samples (11.1%), followed by transport vehicle, water, hand swabs, feces and carcass swab. This finding supports study done in Jimma, Bishoftu, Haramaya and Gondar, Ethiopia by (Sebsibe and Asfaw, 2020; Gutema *et al.*, 2021; Amente *et al.*, 2022; Abey *et al.*, 2024), who reported contamination of meat and meat contact surface in abattoir and butcher shop with *E. coli* O157:H7. This might be due to inadequate sanitation practice and equipment handling.

At abattoir level, the overall prevalence of *E. coli* was reported as 34.2% with statistically significant differences among sample types. The result is higher than study done in Jimma by (Sebsibe and Asfaw, 2020) who reported 19.3% of *E. coli* contamination in abattoir. And also higher than study done Wolaita Sodo and Chelenko by (Debebe *et al.*, 2022; Mekonnen *et al.*, 2024). whereas prevalence of *E. coli* O157:H7 in slaughterhouse was 3.5% without significant difference and this is higher than with study done in Hawasa, Ethiopia by (Atnafie *et al.*, 2017) who reported 2.8% of *E. coli* O157:H7 in slaughterhouse without significant difference. This result is lower than (Sebsibe and Asfaw, 2020) who report 6.0% of *E. coli* O157:H7 in the abattoir sample. The prevalence of *E. coli* O157:H7 in butcher shop was (1.3%), which is comparable with study done in selected four cities of Central Ethiopia by (Beyi *et al.*, 2017) who reported 1.7% *E. coli* O157:H7. This finding is lower than study done in Addis Ababa by (Haile *et al.*, 2022; Abera *et al.*, 2025) who reported 3.64% of *E. coli* O157:H7 and 9.9% shiga toxin producing *E. coli* in butcher shop respectively.

The difference might be due to variation in sample size, study period and isolation procedure. Compared with abattoirs, butcher shops and farm showed significantly lower odds of contamination. Similar findings have been documented by (Beyi *et al.*, 2017; Sebsibe and Asfaw, 2020; Haile *et al.*, 2022). A lower odd of contamination was observed at butcher shops when compared with abattoir. Similar study has been reported in Mekele and Dessie and Kombolcha towns, Ethiopia by (Gugsa *et al.*, 2022; Abebe *et al.*, 2023). And also there was lower contamination at farms compared with abattoirs suggests that bacterial contamination becomes increased during slaughter and processing. This aligns with the One Health perspective described by (Fujita *et al.*, 2022 ; Parker *et al.*, 2024).

Occurrences of *E. coli* O157:H7 among sample type and sample source has not significant difference, this indicates contamination was broadly distributed throughout the beef value chain; this can be supports the study done in Nigeria by (Ajuwon *et al.*, 2021; Aliyu *et al.*, 2021), reports occurrence of *E. coli* O157:H7 in beef and animal products without significant difference. This is due to inadequate hygienic practice and poor sanitation in the farm, slaughter house and butcher shop facilitates circulation of the pathogen across the chain. As observed from antimicrobial susceptibility result, both isolate was highly resistant to ampicillin and tetracycline, Comparable high resistance to tetracycline and ampicillin has been reported in Addis Ababa and Burayu, Ethiopia by (Abera *et al.*, 2025), similarly (Abey *et al.*, 2024) reported high resistance to these antimicrobials among diarrhgenic isolates from Gondar and Bahir Dar cities, Ethiopian. Similar resistance patterns of *E. coli* O157:H7 isolate were also reported in Addis Ababa, Mekele, Ambo, Ethiopia by (Haile *et al.*, 2022; Gugsa *et al.*, 2022) and (Tadese *et al.*, 2021) from raw beef and slaughter environments. This is due misuse and over use these antimicrobials for therapeutics, prophylactics and growth promotion as survey question was indicates.

Trimethoprim resistance was 47.8% and 57.9% among the *E. coli* and *E. coli* O157:H7 isolate respectively. This result is lower than (Beyi *et al.*, 2017),who found 100% resistance of *E. coli* O157:H7 to Trimethoprim. Moderate resistance levels were observed against amoxicillin-clavulanate, cefoxitin and aztreonam.

These results were aligning with study done Ethiopia and Africa, that found resistant *E. coli* and *E. coli* O157:H7 isolates to β -lactam and folate pathway inhibitors (Sebsibe and Asfaw, 2020; Haile *et al.*, 2022; Abunna *et al.*, 2023; Adzitey *et al.*, 2020). Resistance to β -lactam antibiotics shows the presence of β -lactamase-producing strains, which are progressively reported among Enterobacteriaceae and became a growing public health concern (Dallenne *et al.*, 2010; Hasan *et al.*, 2022; Mustafai *et al.*, 2023).

Higher susceptibility was observed to ciprofloxacin, chloramphenicol, and imipenem in both *E. coli* and *E. coli* O157:H7 isolate groups. Ciprofloxacin susceptibility was 56.5% and 52.6%, chloramphenicol susceptibility reached 69.6% and 63.2%, and imipenem showed the highest effectiveness with susceptibility rates of 56.5% and 68.4% and minimal resistance (13.1% and 5.3%). Comparable result reported in studies done in some Ethiopian countries where carbapenems and fluoroquinolones taken relatively greater effectiveness against *E. coli* O157:H7 and generic *E. coli* isolates (Dejene *et al.*, 2022; Abebe *et al.*, 2023; Mekonnen *et al.*, 2024). The study revealed Nalidixic acid resistance was 21.1% to 34.8%, while cotrimoxazole resistance was 30.4% and 47.4% for *E. coli* and *E. coli* O157:H7 respectively. Similar resistance pattern among *E. coli* isolated from meat has been documented in eastern China, South Africa and Egypt, other countries by (Jaja *et al.*, 2020; Ma *et al.*, 2022; Ibrahim *et al.*, 2023).

The frequency of resistance phenotypes involving ampicillin, tetracycline, cotrimoxazole, and trimethoprim was observed; this is Comparable with reports of (Abey *et al.*, 2024; Mekonnen *et al.*, 2024; Abera *et al.*, 2025) in Gondar and Bahir Dar and Chelenko and Addis Ababa, among *E. coli* isolated from beef meat and meat contact surface. This might be due to antimicrobials are frequently used in livestock production due to they are inexpensive and broad spectrum antimicrobials. In the present study, the 17(73.9%) *E. coli* and 16 (84.2%) *E. coli* O157: H7 isolates show three or more drug resistance. This is comparable with earlier study done in Jimma, Mekele, Bishoftu, Addis Ababa and Amhara region, Ethiopia by (Sebsibe and Asfaw, 2020; Gugsa *et al.*, 2022; Abunna *et al.*, 2023; Abera *et al.*, 2025; Chekole *et al.*, 2025) who reported considerable multidrug resistance among *E. coli* and *E. coli* O157: H7 isolates obtained from cattle, beef meat and meat contact surface.

Also, (Haile *et al.*, 2022) documented a high multidrug resistant profile among *E. coli* O157:H7 isolated from retail beef in Addis Ababa. Similar findings have been reported by (Abebe *et al.*, 2023) in Dessie and Kombolcha, Ethiopia and in Ambo, Ethiopia by (Tadese *et al.*, 2021), where considerable proportion of *E. coli* O157:H7 isolated from beef meat were resistant to multiple antimicrobial classes. Similar MDR patterns among *E. coli* isolated from meat have also been reported in Ghana and South Africa (Adzitey *et al.*, 2020; Jaja *et al.*, 2020). This finding is lower than study done by (Segni *et al.*, 2018) in Bishoftu, Ethiopia, shows 92.5% of isolate were MDR to two or more antibiotics. This finding is higher than study done by (Messele *et al.*, 2022 ; Mumed *et al.*, 2022), in Australia and Haramaya and Harar town, Ethiopia who reported 35.5% and 20.1% of *E. coli* isolate was resistant to two or more drugs respectively. This difference is due to the variation in dose, route of administration, misuse of antimicrobials for therapeutic, prophylactic and growth promotion purpose, and geographic variation. The effectiveness of treatments and the ability to control infectious diseases in both animals and humans may be severely hampered due to rapid development of multidrug resistance (Debebe *et al.*, 2022; Mumed *et al.*, 2022).

Study limitations

This study plays a pivotal role in the understanding of AMU and AMR pattern across beef value chain in the study area. But some limitations of the study were listed below. Due to resource limitation antimicrobial susceptibility test was conducted on a limited number of generic *E. coli* isolates. Even though molecular characterization of antimicrobial resistance genes was initially included in the proposal phase it was not conducted due to resource limitation. This affects understanding of the genetic mechanisms and transmission pathways. The study was conducted in a single sub-city, which may limit the generalizability of findings to other regions with different management, hygiene, and antimicrobial use practices. In addition, some sample categories had small sample sizes, leading to potential instability in statistical estimates. Finally, the study did not assess seasonal variation because of time limitation.

6. CONCLUSION AND RECOMMENDATION

The current study revealed considerable challenges related to AMU, hygiene practices, and microbial contamination across the beef value chain. There was notable gap among beef fattening farmers' on KAP regarding AMU and resistance. Half of the farmers had poor knowledge of AMU and AMR, and many were unaware of the link between improper AMU and development of resistance. Educational level and farming experience significantly affected KAP of farmers toward AMU and AMR, suggesting that awareness and experience are important determinants of responsible AMU. And also there were biosecurity and sanitation gaps at farms, slaughterhouses, and butcher shops. Majority of farms lacked unauthorized entry, footbaths, disinfectants, treatment records and adequate hand washing facilities. Alike, assessment of hygienic practice of slaughterhouse and butcher workers indicates; limited hand washing with soap, inadequate equipment sanitation, lack of detergent or disinfectant use, and insufficient training and medical surveillance. Generally, the prevalence of *E. coli* was 26.5%, whereas *E. coli* O157:H7 prevalence was 2.9%. Highest contamination was observed in slaughterhouse (34.2%), followed by butcher shops and farms.

A coordinated One Health approach recommendation was provided as below.

- Targeted education and continuous training programs on AMU, AMR and hygienic handling of meat should be implemented for beef farmers, slaughterhouse and butcher shop workers.
- AMS in beef fattening farms through multisectoral collaboration among veterinary, medical, environmental, and food safety sectors is essential.
- Improving biosecurity at farm level and hygienic practice at farm-level, slaughterhouse and butcher shop following regular inspection and periodic follow up monitoring.
- Continuous surveillance including farms, meat processing facilities, environmental samples, and AST should be done for timely detection and implement intervention method.
- Studies involving broader geographical coverage should be done to improve national understanding of AMR and foodborne pathogens within Ethiopian beef production systems. Also, molecular characterization of resistant isolates and resistance genes should also be done for more understanding transmission pathway.

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8. ANNEXES

Annex 1. Ethical clearance letter

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Research Ethics Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/08/116/18/2026

Name of Applicant: **Chaltu Desalegn Muleta (BVSc, MSc Student)**

Address: Department of Microbiology, Parasitology and Poultry Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *A One health assessment of antimicrobial use and associated resistance in E. coli along the beef value chain in Akaki-Kality sub city, Addis Ababa, Ethiopia*


Date of application: **November, 2025**

Nature of the project: **Field investigation**
 Target animal species: **Cattle**
 Number of animals involved: **150**
 Study area: **Addis Ababa- Ethiopia**


Minutes No. and date of review: **VM/ERC/09/18/026, 27/02/2026**

The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of Student Chaltu Desalegn Muleta.

Professor Getachew Terefe (DVM, PhD)
Chairman



Signature



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Please quote Our Ref. No. When replying

ፋክስ } ስልክ } ፖ.ሣ.ቱ } ቢሾፍቱ፣ ኢትዮጵያ
Fax 251-11-4339933 } Tel. +251 114338450 } P.o.x. Box}34 } Bishoftu, Ethiopia

Annex 2. Participant consent form introduction

Greetings! My name is-----and I am currently conducting academic research on a one health assessment of AMU and associated resistance in *E. coli* along beef value chain in Akaki Kality sub city, Addis Ababa. The main objective of this study is to assess AMU and the prevalence of AMR in *E. coli* along the beef value chain in Akaki Kality sub city using one health approach in order to prevent inappropriate AMU, food contamination, which poses a risk to consumer health. I am going to collect from your animal, hand swab from you and I ask you some questions, the conversation explore extensively into the specifics of your job duties and your wellness practices. The interview employed a question and answer format, with an estimated duration of up to twenty minutes on average. To ensure the privacy of people and organizations, we manage all acquired data in compliance with accepted data protection guidelines. There are no negative repercussions if you decide not to participate in the survey interview; your participation is entirely voluntary. If you decide to participate, you are free to skip any questions you prefer not to answer. Should you have any further inquiries about the survey, please feel free to ask.

Certificate of Consent

It is important to note that I am participating in this interview voluntarily and without payment. I have the right to withdraw from study at any point without facing any consequences. If I feel uneasy during the interview, I can choose not to answer certain questions or terminate. Therefore, I hereby confirm my voluntary agreement and participation in this study. I have received a copy of this consent form for my records.

Print Name of Participant _____ Signature of Participant _____ Date / / Month Year

Statement of Researcher

Through joint examination of the information page, I ensured that the potential participant understood it all. I ensured that the participant inquire about the research. I verify that the person provided consent voluntarily and without any pressure. I have provided the participant with a copy of this consent form a Print

Name of Researcher _____ Signature of Researcher _____ Date / / / Month, Year

Annex 3. Data collection tools (Questionnaires)

Annex 3.1 Questionnaire on KAP of cattle fattening workers regarding antimicrobial use and resistance

A. Basic information

1. Date _____

2. Code _____

B. Sociodemographic characteristics of the respondents

1. Role in farm: owner worker veterinarian

2. Gender: Male Female

3. Age: _____years

4. Educational Background or Level:

can't read and write Primary School Secondary School College and above

5. Duration of working in cattle fattening: _____years

6. Type of fattening farm: small scale medium large scale

Section 1: Knowledge and Awareness

1. Do you know why antibiotics are given to cattle? Yes No

2. If yes to question number one, for what purpose: (Check all that apply) Treatment Prevention Growth promotion other purpose specify _____

3. Do antibiotics can be used for all types of diseases in animals? Yes No

4. Have you heard about antimicrobial resistance (AMR)? Yes No

5. Do you believe that the use of antibiotics in farm animal without proper guidance can cause AMR? Yes No

6. Do you know AMR in animals have associated health risks in humans?

Yes No

Section 2: Attitudes toward antimicrobial use

1. Do you think usage of antimicrobials for non-therapeutic reasons lead to AMR?

Yes No

2. Do you agree restriction of antibiotic use in animals will lead more benefit than damage?
 Yes No
3. Do you agree there is relationship between antibiotic use in animals and development of resistance?
 Yes No
4. Have you ever received training on proper drug use and AMR?
 Yes No
5. Do you agree purchasing of antimicrobials from a drug company or cooperative with a legal permit is safe?
 Yes No
6. Do you think there is enough awareness about AMU and AMR among cattle farmers?
 Yes No
7. If no to question 5 above please list the reason of lack of awareness? (Check all that apply)
 - Lack of access to information on AMU and AMR
 - Limited training to farmers
 - Lack of exposure to awareness campaigns on AMU and AMR
 - Limited interaction with veterinary professionals
 - Others specify _____

Section 3: Practices towards Antimicrobials use and Antimicrobials resistance

1. Have you given antibiotics for your animals in the past month?
 Yes No
2. Where did you obtain the antibiotics that you gave for your animals?
 From veterinarian prescription Without prescription from private pharmacy Leftover from previous course none
3. If answer for question number two is without prescription, why you used antibiotics for your

animal without prescription?

Minimize cost previous experience Quick relief Lack of time

4. How many times have you give antibiotics for your animals per a month?

Never Once 2-5 times More than 5 times

5. Do you use antibiotics when animals get sick without laboratory test?

Yes No

6. Do you stop giving the antibiotics, if animals feel better after the first day of treatment?

Yes No

7. Do you observe withdrawal periods after administering antibiotics before selling to slaughterhouse?

Yes No Not sure

8. How do you dispose leftover or unused medication?

Burn Bury Through away

Section 4: Observations check list for hygiene and biosecurity practice at beef fattening farms

1. Farm is properly fenced Yes No

2. Presence of sign limiting unauthorized entry Yes No

3. Footbath present at entry Yes No

4. Separate areas for feed storage, animal housing and waste disposal Yes No

5. Floors, roof and walls in good condition Yes No

6. Regular cleaning schedule available Yes No

7. Presence of disinfectant Yes No

8. New animal quarantined before mixing and record of animal origin kept Yes No

9. Manure are properly managed Yes No

10. Workers wear protective clothing Yes No

11. Hand washing facilities available Yes No

12. Vaccination and treatment record available Yes No

13. Water source Tap well River other

14. Presence of toilet Yes No

Annex 3.2. Questions and check list to assess hygienic handling practices at slaughterhouses

A. Basic information

1. Date _____

2. Code _____

B. Sociodemographic characteristics of the respondents

1. Job role: Slaughter man Cleaner Inspector Manager

2. Gender: Male Female

3. Age _____

4. Educational background or level: can't read and write Primary School Secondary School College and above

5. Duration of working in slaughterhouse: _____yrs.

C: Hygienic handling practices at slaughterhouses

C-1. Check list for observations

1. Inspection before slaughter: Yes [] No []

2. If yes to the above question type of inspection method used:

Visual Inspection

Palpation (touch and feel)

Auscultation (listening to internal sounds)

Laboratory Testing (e.g., microbiological, chemical tests)

Physical Examination (checking for abnormalities)

3. Method of carcass dressing: Vertical (hanging) Horizontal (on floor):

4. Presence of sink for washing hands in the slaughterhouse: Yes [] No []

5. Carcass washing after evisceration: Yes [] No []

6. Use of the following protective materials while working in the slaughterhouse: Yes No

| Protective materials | Response |
|----------------------|--|
| Apron | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| white coat | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| Head cover | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| Gloves | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| Boot | <input type="checkbox"/> Yes <input type="checkbox"/> No |

C-2. Questions for face to face interviews

1. Do you wash your hands frequently during slaughter operation: Yes [] No []
2. Do you wash your hands with soap: Yes [] No []
3. Do you use the same knife for dehiding and evisceration: Yes [] No []
4. Do you wash your hands in between activities: Yes [] No []
5. Do you sink the knife in hot water after each activity: Yes [] No []
6. What do you think is the possible sources for carcass contamination: [] Feces during evisceration [] hides during dehiding [] handlers hand [] knife [] floor [] hanging hook [] Others (specify)_____
7. What is the source of water used in the slaughterhouse: City/Municipal tap water [] borehole [] collected rain water [] River [] others (specify) _____
8. Have you ever received any training on hygienic handling of meat: Yes [] No []
9. Have you gone for medical checkups to work at the slaughterhouse: Yes [] No []
10. If yes, how frequent you go for medical checkup: every three months [] every six months [] once per year []
11. Do you think improvements are needed to avoid contamination of carcass at the slaughterhouse: Yes [] No []
12. If yes, what kind of improvement:
 - Enhanced Hygiene Practices
 - Improved Cleaning and Sanitation Procedures
 - Staff Training on Hygiene Protocols

- Implementation of Hazard Analysis and Critical Control Points
- Better Equipment Maintenance
- Segregation of Clean and Dirty Areas
- Use of Personal Protective Equipment (PPE)
- Control of Cross-Contamination During Processing
- Regular Monitoring and Inspection
- Improved Waste Management
- Facility Design Improvements for easy cleaning and reduced contamination risk

Thank You Very Much for Your Time and Answer!

Annex 3.3. Questions and check list to assess hygienic handling practices at butcher shops

A. Basic information

1. Date_____

2. Code_____

B. Sociodemographic of characteristics Basic Information about the Participant

1. Gender: MaleFemale

2. Age_____

3. Educational background or level: can't read and write Primary School Secondary School College and above

4. Duration of working in butcher shop: _____yrs.

5. Religion . Orthodox Muslim Protestant Waqefata

C. Hygienic handling practices at butcher shops

C-1. Check list for observations

1. Presence of any cover on meat display case: Yes [] No []

2. Retail shop floor is made of : Concrete [] Tile [] Wood material [] others(specify)_____

3. Wall and ceiling are clean or free of dust: Yes [] No []

4. Wall painted with white color: Yes [] No []

5. Ventilation status of display case and butchery: Good [] Fair [] Poor []

Good-ventilation allows air flow into the butchery but sieves off dust and other particles []

Fair-ventilation allows air flow but do not sieve dust or other particles or allows very little air flow []

Poor-ventilation does not allow air flow at all []

- 6. Presence and use of bulbs at the display case: Yes [] No []
- 7. Meat cooling facility (refrigerator) at the display cabinet: Yes [] No []
- 8. Presence of sink for washing hands at beef sale point: Yes [] No []
- 9. Type/kind of cutting board used: Wood [] plastic [] Metal [] concrete [] Marble []
- 10. Presence of hot water baths for dipping knives: Yes [] No []
- 11. Material used to pack or wrap meat for sale: Newspaper [] Plastic [] Used paper []
- 12. Use the following protective materials while selling or handling meat:

| Protective materials | Response |
|----------------------|--|
| Apron/white coat | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| Head cover | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| Gloves | <input type="checkbox"/> Yes <input type="checkbox"/> No |

C-2. Questions for face to face interviews

- 1. What is the means of transporting meat from slaughterhouse to the retail shop: Open vehicle [] Closed vehicle [] Animal transport (Cart horse) []
- 2. How frequent do you use washed the protective coat (white coat and apron): Once per day in the evening [] Twice per day, morning and evening [] Once after every two days [] Once per week [] others [] _____
- 3. Do you have a refrigerator for storage of the meat that remains from daily sale: Yes [] No []
- 4. Do you wash your hands before touching meat: Yes [] No []
- 5. Do you wash your hands with soap: Yes [] No []
- 6. What is the source of water for use in the butchery: City/Municipal tap water [] borehole [] rain collected water [] river [] others (specify) []
- 7. How often do you wash the following butchery surfaces and equipment?

| | Equipment's /surfaces | | | | | |
|-----------------------------|-----------------------|----------------|----------|-----------------|-------|--------|
| Frequency of washing | Knife | Cutting boards | Saw/Axes | Display cabinet | Hooks | Floors |
| Once per day in the morning | | | | | | |
| Once per day in the evening | | | | | | |
| Twice per day | | | | | | |
| More than twice | | | | | | |
| Once in every two days | | | | | | |
| Others (specify) | | | | | | |

8. Do you use detergent/disinfectant for cleaning the butchery utensils: Yes [] No []

9. If “Yes” what types of detergent/disinfectant:

- Mild Detergents (for general cleaning)
- Alkaline Detergents (effective against fats and proteins)
- Acidic Detergents (for removing mineral deposits and scale)
- Chlorine-based Disinfectants (e.g., bleach solutions)
- Quaternary Ammonium Compounds (broad-spectrum disinfectants)
- Phenolic Disinfectants
- Iodophors (Iodine-based disinfectants)
- Peracetic Acid
- Hydrogen Peroxide Solutions
- Enzymatic Cleaners (for organic matter breakdown).

10. What is the way of cleaning butchery equipment: Using cold water only [], cold water with soap [] hot water only [] hot water with soap [] wiping with pieces of cloths [] others (specify)_____

11. Is there routine control of flies at the retail shop: Yes [] No []

12. If “Yes”, what is the method used to control flies:

- Use of Insecticide Sprays or Aerosols
- Fly Traps (Glue Boards or Electric Fly Catchers)
- Ultraviolet (UV) Light Traps
- Screens and Fly Nets on Windows and Doors
- Proper Waste Management and Regular Disposal
- Sanitation and Cleaning to Remove Breeding Sites
- Environmental Controls (e.g., Fans or Air Curtains)
- Biological Control Methods (e.g., beneficial insects)
- Use of Fly Repellents or Natural Deterrents
- Regular Monitoring and Inspection

13. How long does the meat stay in your butchery before it is sold? Less than

12 hours [] One day [] Two days []

14. Do you collect money while handling or selling meat: Yes [] No []

15. Have you ever received any training on hygienic handling of meat: Yes [] No []

16. Do you ever receive complaints from the consumers on the quality of the meat you sell: Yes [] No []

17. If yes, what kind of complaint: Abdominal upsets[]Hard meat []Dirty meat []others

18. Have you gone for medical checkups in the last 6 months: Yes [] No []

19. How frequent you go for medical checkup: Every three months [] very six months [] Once per year []

20. Do you have different storage and display cabinets for offal's and meat: Yes [] No []

21. Do you use the same equipment's such as knife while handling meat and the offals: Yes [] No []

22. Do you believe that the butchery where are you working requires some improvement for better handling of meat: Yes [] No []

23. If yes, what kind of improvement:

- Enhanced Hygiene and Sanitation Practices
- Proper Storage Facilities and Temperature Control
- Improved Meat Handling and Cutting Techniques
- Staff Training on Meat Hygiene and Safety
- Better Waste Management and Disposal Systems
- Upgraded Equipment and Tools
- Implementation of Hazard Analysis and Critical Control Points
- Segregation of Raw and Processed Meat Areas
- Regular Cleaning and Maintenance Schedule
- Improved Ventilation and Lighting_____

Thank You Very Much for Your Time and Answer

Annex 4. Sample plating and media record sheet

| No | Date of sampling | Sample type | Sample code | Sampling site | Culture media | | | | | Biochemical test: +/- | | | | | MAL DFOB | Serological test |
|----|------------------|-------------|-------------|---------------|---------------|------|-----|-------|----|-----------------------|----|----|---------|-----|-------------|--------------------------|
| | | | | | BPW | MCKA | EMB | SMCKA | NA | Indole | MR | VP | Citrate | TSI | Result | Latex agglutination test |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
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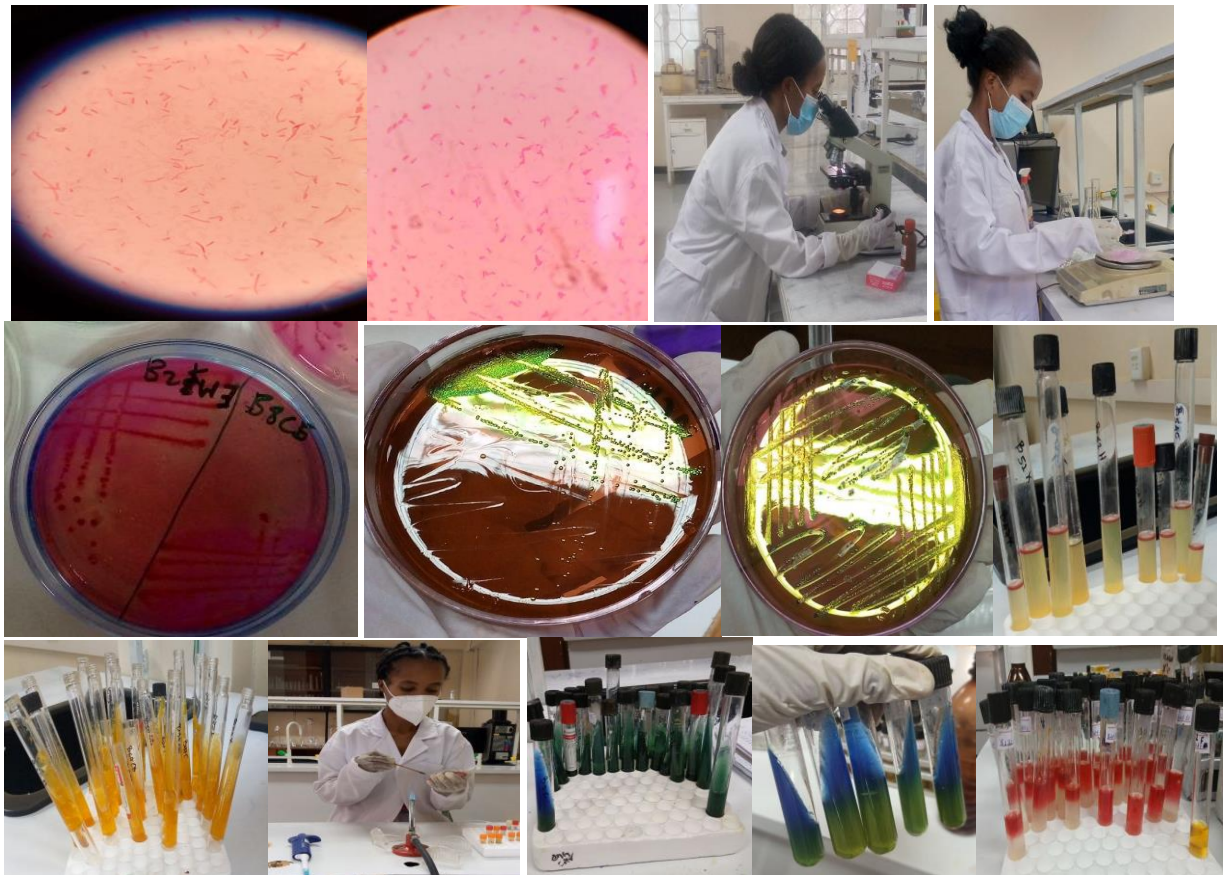
N.B.: BPW=Buffered peptone water EMBA= Eosin methylene blue agar, VP = Voges-Proskauer, MR= Methyl red, MCKA = MacConkey, SMCKA =Sorbitol MacConkey, NA= Nutrient agar

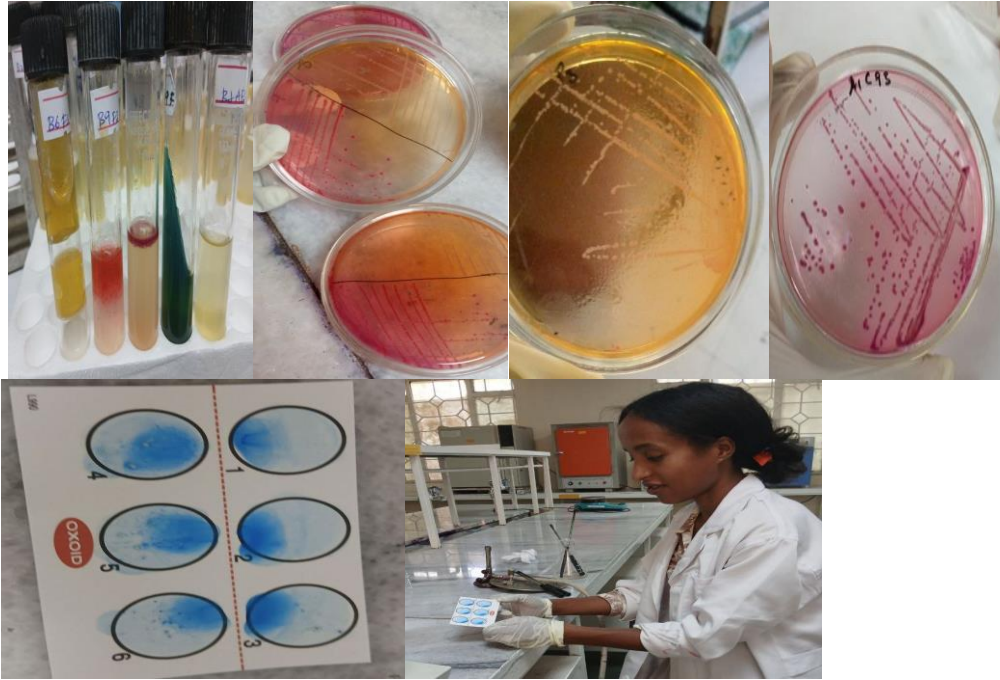
Annex 5. Photo taken during sample collection and laboratory analysis

Picture taken during field sample data collection from beef fattening farms, abattoir, and butcher shops

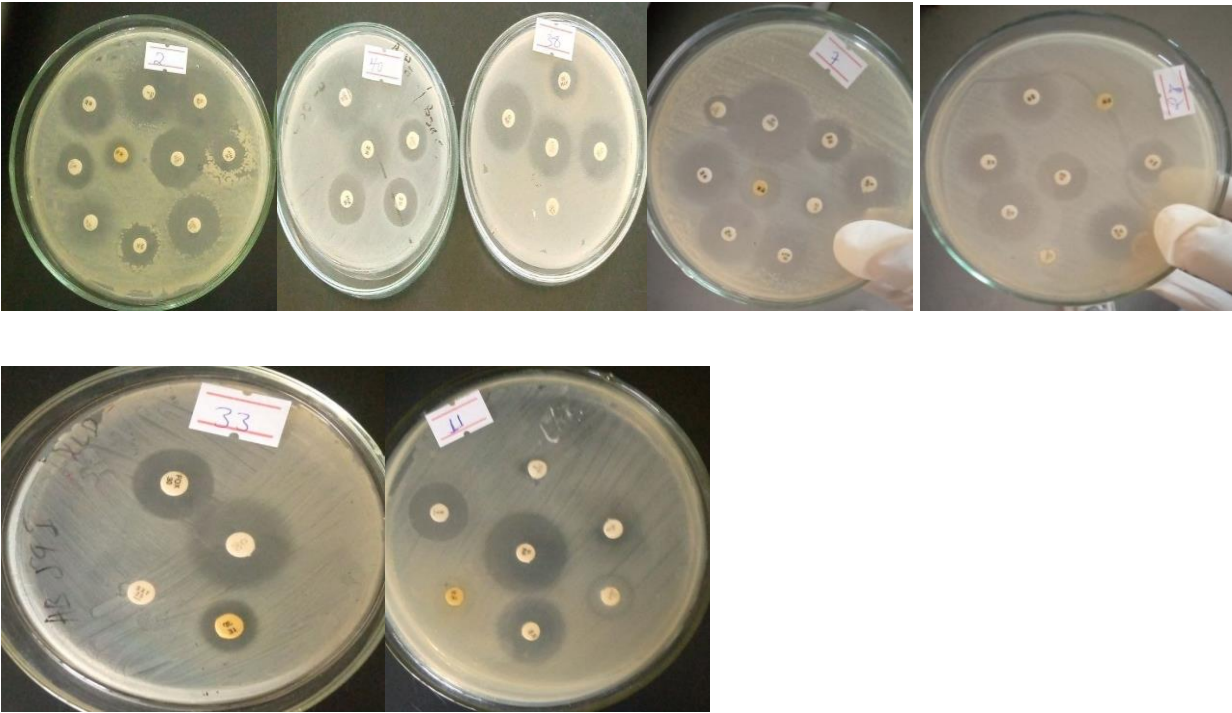


Photo taken, during isolation and characterization





Picture taken during antimicrobial susceptibility test of *E.coli* and *E.coli* O157:H7



Annex 6. Figure showing test result report on MALD TOF



AHI
TEST RESULT REPORT FORM



Customer name: Chaltu Desalegn
 Contact: phone : 0901152563, email :
 Sampling Place: Region: Oromiya, District: Bishoftu
 Kebele: Bishoftu

Reference No. SR/Ref/123/2026
 Test Report No. 123/2026

Date reported: 22-04-2026

| Lab Code | Sample Type | Species | Sampling Date | Date Received | Date Analyzed |
|-----------|-------------------|---------|---------------|---------------|---------------|
| 9025-9063 | Bacterial Isolate | Bovine | 12/11/2025 | 24/03/2026 | 24/03/2026 |
| | | | | | 24/03/2026 |

| Testing Lab | No. | Positive | Negative | Doubtful | Test | Method | TM |
|-------------|-----|----------|----------|----------|-------------------------------------|-----------|------------------|
| AHI | 39 | 19 | 20 | 0 | E.coli isolation and identification | MALDI-TOF | AHI-TM-BACL-003* |

*Accredited Test (AHI is accredited for tests marked *)

Verified by:
Olani Abebe

Approved by:

Dr. Getnet Abie Mekonnen
Deputy Director General

NOTE: the results shown on this test report only refers the number of samples tested and the respective customer not for other purpose. is responsible for all the information provided on this report, except for the information provided by the customer.

| | | |
|------------------------------|--|-----------------------------------|
| Prepared by: Aseggedch Sirak | Reviewed by: Ayelech Muluneh, Abera Kebede | Approved By: |
| Effective Date: 2025-08-07 | Document Type: FRM | Document No.: AHI-FRM-GEN-025-10A |

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