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COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF MICROBIOLOGY, PARASITOLOGY AND POULTRY HEALTH

MASTER OF SCIENCE PROGRAM IN ONE HEALTH

MOLECULAR DETECTION AND ANTIMICROBIAL RESISTANCE PATTERNS OF *ESCHERICHIA COLI* O157:H7 AT THE HUMAN-ANIMAL-ENVIRONMENT INTERFACE IN ADDIS ABABA: A ONE HEALTH PERSPECTIVE

MSC THESIS

BY:

TIGIST NIGATU

JUNE, 2025

BISHOFTU, ETHIOPIA

MOLECULAR CHARACTERIZATION AND ANTIMICROBIAL SUSCEPTIBILITY OF *E. COLI* O157:H7 STRAIN ACROSS DAIRY ANIMALS, HUMANS AND FARM ENVIRONMENTAL INTERFACE IN SELECTED SUB-CITIES OF ADDIS ABABA

A Thesis Submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in Partial Fulfillment of the Requirement for the Degree of Master of Science In One Health

By:

Tigist Nigatu

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As MSc research advisors, we certify that we have read and evaluated the thesis prepared under our guidance by Tigist Nigatu entitled: "**Molecular Characterization and Antimicrobial Susceptibility of *E. coli* O157:H7 Strain Across Dairy Animals, Humans and Farm Environmental Interface in Selected Sub-Cities of Addis Ababa**." We recommend that it be accepted as fulfilling the thesis requirement for the degree of Master of Science in One Health.

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First , I certify that this thesis is my original work and that all references used have been properly cited. This thesis has been submitted to the College of Veterinary Medicine and Agriculture, Addis Ababa University, in partial fulfillment of the requirements for the Master of Science degree. The thesis is deposited in the university library and made accessible to borrowers in accordance with the library's regulations. I also affirm that this thesis has not been submitted to any other institution for the award of any academic degree, diploma, or certificate.

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DEDICATION

To my family and friends, I dedicate this thesis manuscript in appreciation of their unwavering support and affectionate care. They are the key to my success in my entire life.

BIOGRAPHICAL SKETCH

Tigist Nigatu was born in 1998 in Bishoftu Town, Oromia Region, to her mother, Saba Habeteab and her father, Nigatu Mekuriya. She completed her elementary and primary education at Guardian Angels Catholic School between 2003 and 2015. She then pursued her secondary education at Beza Primary and Secondary School from 2016 to 2017. In 2018, she enrolled at Addis Ababa University, College of Veterinary Medicine and Agriculture, where she earned her Doctor of Veterinary Medicine (DVM) degree in July 2023. In November 2023, she joined the postgraduate programme at the same institution to pursue a Master of Science (MSc) degree in One Health.

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LIST OF ABBREVIATIONS

AMR	Antimicrobial Resistance
CFUS	Colony Forming Units
DAEC	Diffusely Adherent *E. coli*
DEC	Diarrheagenic *E. coli*
E. COLI	*Escherichia coli*
EHEC	Enterohemorrhagic Subtype
EIEC	Enteroinvasive *E. coli*
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
EXPEC	Extraintestinal Pathogenic *E. coli*
HACCP	Hazard Analysis Critical Control Points
HC	Hemorrhagic Colitis
HUS	Hemolytic Uremic Syndrome
IPEC	Intestinal Pathogenic *E. coli*
LEE	Locus Of Enterocyte Effacement
MDR	Multidrug Resistance
ORFS	Open Reading Frames
SPSS	Statistics For Social Science
STEC	Shiga Toxin-Producing *E. coli*
STX	Shiga Toxin
TSI	Triple Sugar Iron
TTP	Thrombocytopenic Purpura
UK	United Kingdom
UPA	Urban and Peri-Urban Agriculture.
USA	United States of America
VTEC	Vero Cytotoxin-Producing *E. coli*

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ABSTRACT

Escherichia coli O157:H7 is a zoonotic pathogen of major public health concern due to its high virulence, ability to survive in the environment, and increasing resistance to antibiotics. This study adopted a One Health approach to investigate the prevalence, molecular characterization, and antimicrobial susceptibility patterns of *E. coli* O157:H7 isolated from dairy animals, humans, and farm environment in Addis Ababa, Ethiopia. A cross-sectional study was conducted between November 2024 and April 2025 across 60 dairy farms, with 470 samples samples collected from raw milk, human hand swabs, water, and manure (from both bedding and waste areas). A structured questionnaire was administered to 60 farms to identify potential risk factors. Isolation and identification was done using selective culture media and biochemical tests, while confirmation of the *E. coli* O157:H7 serotype was done via PCR. Antimicrobial susceptibility was determined using the Kirby-Bauer disk diffusion method, and data were analyzed using Descriptive statistics and chi-square.Out of 470 total samples, 32.7% (154/470) tested positive for *E. coli*, and 0.6% (3/470) were confirmed as *E. coli* O157:H7. Based on analysis, sample types had a significant effect on the occurrence of *E. coli* and *E. coli* O157:H7. The prevalence of *E. coli* O157:H7 was 1.7% in both manure and hand swabs, and 0.4% in milk samples. Overall *E. coli* detection was significantly highest in waste-area manure (66.7%) compared to bedding manure (56.7%), hand swabs (53.3%), water (23.3%), and milk (14.8%) ($p < 0.05$). All *E. coli* O157:H7 isolates exhibited resistance to ampicillin(100%) and tetracycline (90%), while high susceptibility was observed for norfloxacin (90%), cefotaxime (85%), and cefoxitin (85%). Multidrug resistance was observed in isolates showing resistance to at least three antibiotics. The questionnaire survey revealed that lack of handwashing before milking, absence of disinfectant use, and poor farm hygiene were significantly associated with *E. coli* O157:H7 presence ($p < 0.05$).The findings highlight the importance of promoting rational antibiotic use and improving hygienic practices at the farm level to reduce the growth of drug-resistant *E. coli* O157:H7.

Keywords: Addis Ababa, Antimicrobial Susceptibility Test, Dairy Farms, *E. coli* O157:H7, OneHealth,Prevalence.

1. INTRODUCTION

Escherichia coli is gram- negative commensal micro flora that is found in the intestinal tract of animals and humans (Foster and Pallen, 2022). However, most strains of *E. coli* are harmless,some strains such as the enterohemorrhagic subtype (EHEC), Shiga toxin-producing *E. coli* (STEC) cause severe and life-threatening illness in humans such as haemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Cho *et al*., 2018; Li and Wang, 2017). *E. coli* O157:H7 is a foodborne pathogen that is characterized by the presence of somatic (O) antigen 157 and flagella (H7) antigens 7 and cause disease in human (Balakrishnan *et al*.,2016; Chase *et al*., 2023).This shiga toxin (stx) producing strain has major virulence factor that are encoded by the stx1 and stx2 genes which result in damage to the intestinal lining leading to diarrhoea. Stx2 is frequently causes severe human diseases and has two most clinically important variants, the Stx2c and Stx2d while stx1 cause less serious human diseases (Rivas *et al*., 2016). Other important virulence factors include intimin (eae gene) and enterohemolysin (hlyA gene) (Hizlisoy *et al*.,2017).Because of its high virulence potential, low infectious dosage (10–100 CFUs), environmental persistence, and resistance to treatment alternatives, *E. coli* O157:H7 represents a serious threat to human health (Lupindua, 2018).

Cattles are considered the major reservoirs of *E. coli* O157:H7 (Munns *et al*., 2015). Infected cattles are typically asymptomatic but intermittently shed the pathogen through faeces (Kim *et al*., 2016). From the environment, the pathogens can extend further to the udders, milking utensils, water sources, bedding and bulk storage vessels (Sapountzis *et al*., 2020), especially when hygiene practices are suboptimal contamination of milk can occur. (Otero *et al*., 2017). Environmental persistence of *E. coli* O157:H7, particularly in manure, soil, and water, further amplifies risk of transmission (Persad and LeJeune, 2015). Furthermore, dairy farms with no discernible farm treatment had a high incidence of it (Radostits *et al*., 2016).A rising frequency of *E. coli* O157:H7 has been linked to a number of host and farm practices (Widgren *et al*., 2015; Segura *et al*., 2018). Human infections are frequently linked to the ingestion of tainted foods originating from animals, including raw milk and dairy products (Rangel *et al.,* 2005). The interconnection between animal, human, and environmental health is central to the One Health approach, which promotes multidisciplinary collaboration to manage zoonotic threats like *E. coli* O157:H7 (Abdissa *et al*., 2017; Atnafie *et al*.,2017). Another emerging challenge is *E. coli* O157:H7

development of antimicrobial resistance (AMR). Because antibiotics have the potential to promote the development and secretion of Shiga toxins, which could hasten the start of HUS in humans, their use in *E. coli* O157:H7 infections is controversial (Helke *et al*., 2017). Resistance to commonly used antibiotics such as ampicillin, amoxicillin, ceftriaxone, chloramphenicol, ciprofoxacin, cotrimoxazole and tetracycline has been documented and complicates clinical management (Berhe *et al*., 2021).Unfortunately, inappropriate antimicrobial use has significantly contributed to the growth and spread of resistance (Wong *et al*.,2000). Antibiotic resistance in *E. coli* O157: H7 has been increasingly noted over the last 20 years (Tadesse *et al*.,2015). According to a recent study, different regions of Ethiopia had higher incidence rates of *E. coli* O157:H7 antibiotic resistance (Bedasa *et al*., 2018; Sebsibe and Asfaw, 2020). In order to lessen the occurrence of their increasing threat, a one-health approach to researching microbes, their transmission, resistance behaviour, and genetic relationships with other organisms will be helpful.

Despite the increased likelihood of contracting an *E. coli* O157:H7 infection and the growing prevalence of antibiotic resistance, there is a limited information about the prevalence and antibiotic resistance profile of *E. coli* O157:H7 isolated from cattle faeces, raw milk, milker hand swabs, manure and water in the dairy farms using a one health approach in Addis Ababa. Understanding the prevalence and resistance patterns, across animal, human, and environmental sources is crucial for designing effective interventions. Applying a One Health framework to this issue will enhance surveillance, reduce cross-species transmission, and inform public health strategies. This study was therefore carried out to address these gaps. Therefore the objectives of this study were:

General objective

- The general objective of the current study was to isolate, molecular characterize and determine antimicrobial susceptibility profiles of *Escherichia coli* O157:H7 strain isolated from dairy animals, humans, and the farm environmental interface in Addis Ababa City through the One Health approach.

Specific Objectives

- To assess the prevalence of *E. coli* O157:H7 in dairy animals, humans, and the farm environmental interface.
- To assess the antimicrobial susceptibility of *E. coli* O157:H7 isolates.
- To assess the Sanitation and hygienic practices associated with the prevalence of *E. coli* O157:H7 isolates.

2. LITERATURE REVIEW

2.1. General Overview of *Escherichia coli*

Escherichia coli (E. coli) is a Gram-negative, facultative anaerobe, non-spore-forming rod-shaped bacterium that belongs to the family Enterobacteriaceae which is found as commensal bacteria in the gastrointestinal tract of food-producing animals and humans (Abdeltawab *et al*., 2015). Most *E. coli* strains are harmless and live in their hosts with mutually beneficial associations. But some strains of *E. coli* are classified as pathogenic strains depending on their virulence type and the host's clinical symptoms. These pathogenic strains are classified as intestinal pathogenic *E. coli* (IPEC) and extraintestinal pathogenic *E. coli* (EXPEC) (Lindstedt *et al*., 2018).

Intestinal pathogenic *E. coli* includes enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), enterohemorrhagic *E. coli* (EHEC) and Vero cytotoxin-producing *E. coli* (VTEC) or Shiga toxin-producing *E. coli* (STEC) (Alizade *et al*.,2019). Some intestinal pathogenic *E. coli* strains, such as EIEC and EAEC, are found exclusively in humans (Sora *et al*.,2021). Among the STEC group, EHEC strains are known to cause severe disease in humans. Although over 200 STEC serotypes have been isolated from food-producing animals and food products, not all of them are pathogenic. Among these, *E. coli* O157:H7 is the most significant serotype, capable of causing bloody diarrhoea, which may progress to the potentially fatal hemolytic uremic syndrome (HUS). This serotype is among the STEC strains responsible for global infections (Balakrishnan *et al*., 2016).

2.2. Characteristics of *E. coli* O157:H7

The standard method for serotyping *Escherichia coli* is based on identifying three types of antigens: somatic lipopolysaccharide (O), capsular (K), and flagellar (H) antigens. Based on the standard method, 700 *E. coli* serovars have been identified. Among these, more than 200 *E. coli* serovars produce Shiga toxins (Stxs) (Murinda *et al*., 2019). 187 and 53 of *E. coli* species have been identified as carrying somatic lipopolysaccharide (O) and flagellar (H) antigens (Dolye *et al*., 2016). Serogroups such as O157:H7, O91, O26, O55, O103,

O111, O121, and O145 are recognized as enterohemorrhagic *E. coli* (EHEC), which are associated with severe human diseases (Heredia and Garcia, 2018). Almost all *E. coli* O157:H7 strains that cause HC and HUS possess related virulence factors. But this is not true for other serogroups (CDC, 2016).

In addition to serotyping, distinct metabolic traits are used to identify *E. coli* O157:H7. Most *E. coli* O157:H7 isolated strains lack β-glucuronidase enzyme due to this they cannot hydrolyze 4-methylumbrelliferyl-D-glucuronide and are not capable to fermenting sorbitol within 24 hours. *E. coli* O157:H7 unable to grow above 44.5 °C, with the addition of the above mentioned two characteristics are vital in identifying from other *E. coli* strains (Assefa, 2019). The biochemical characteristics are oxidase negative, catalase positive, indole positive, urease negative, Voges- Proskauer negative, and citrate negative. The characteristics and the outer cell membrane's antigenic composition also help in identifying *E. coli* O157:H7 (Fan *et al*., 2019; Eichhorn *et al*., 2015).

E. coli O157:H7 has a lipopolysaccharide component within its outer membrane which is different from the cytoplasmic membrane and thus this cell envelope structure is typical of Gram negative cells (Yang *et al*., 2023). The carbohydrate composition and structure of the lipopolysaccharide help define the O157 antigen. The unique polypeptide structure of the flagella is used to determine H7 antigen (Sun *et al*., 2022). *E. coli* O157:H7 the ability to survive in foods that have adverse pH(close to 2.5) helps overcome the acidity barrier of the stomach allowing the entrance and colonization of the intestinal tract (Castro *et al*., 2017). Compared to commensal *E. coli* strains, O157:H7 is significantly more acid-tolerant and may become further adapted to acidic conditions after exposure to weak acids in the rumen. These phenotypic, biochemical and antigenic characteristics make *E. coli* O157:H7 a highly distinguishable and clinically significant serotype among STEC strains. (Ashang *et al*., 2015).

2.3. Epidemiology of *E. coli* O157:H7

2.3.1. Geographic Distribution

E. coli O157:H7 lineages differ between regions, but the infections arise globally. This possibly induces the occurrence and disease severity in humans (CFSPH, 2016). *E. coli*

O157:H7 has been causing numerous outbreaks and diseases globally related to gastrointestinal diseases since its first outbreak was recorded in the USA (Parsons *et al*., 2016). In North America, Europe, South Africa, Japan, South America and Australia. *E. coli* O157:H7 has become a main concern, ever since its recognition in 1982. Predominantly, *E. coli* O157:H7 is usually related to clinical disease in people found in North America, Japan and the UK. HUS endemic and high rates of occurrence of *E. coli* O157:H7 are present in South America, particularly in Argentina (Constable *et al*., 2016). 390 *E. coli* O157:H7 outbreaks were recorded during 2003–2012; 928 diseases, 1,272 (26% of illnesses) hospitalizations, 299 (6%) physician-diagnosed HUS cases, and 33 (0.7%) deaths were recorded due to these outbreaks. foodborne (255 outbreaks, 65%), animal contact (39, 10%), person-to-person (39, 10%), waterborne (15, 4%), and different or unknown (42, 11%) were the main transmission pathways. Diseases (3,667, 74%), hospitalizations (1,035, 81%), physician-diagnosed HUS cases (209, 70%), and deaths (25, 70%) were caused by food-borne outbreaks. (Heiman *et al*., 2015).

Around the African continent, reports of the isolation of the *E. coli* O157:H7 strain that generates Shiga toxin in humans, animals, food, and the environment have been documented. The first human infection was discovered and recorded in Johannesburg, South Africa, as early as 1990.(Beyi *et al*., 2017). But in 1996, in the Central African Republic, harmful bacteria were recovered from patients suffering from haemorrhagic colitis, which led to death. In 1998, *E. coli* O157:H7 isolation in humans was reported following the emergence of bloody diarrhoea in Cameroon (Havelaar *et al*., 2015). There have been reports of pathogen isolation in Tanzania, Kenya, and Ethiopia in East Africa. In terms of zoonotic disease outbreaks on the African continent, Ethiopia is second only to Nigeria. In Ethiopia, the epidemiology of foodborne infections, particularly *E. coli* O157: H7, has not been thoroughly investigated or documented in recent years. However, reports of the organism's incidence level in dairy and beef products have been rising recently (Ayalew Assefa.,2019).

2.3.2. Status of E. coli O157:H7 In Ethiopia

Few research studies have documented the prevalence of *E. coli* O157:H7 using a one health approach in Ethiopia. While reports from someparts of Ethiopia are encompassed, most of the studies were carried in central part of the country(Mohammed, 2023).

Table 1: Status of *E. coli* O157:H7 in the Ethiopia

Study area	Sample type	Sample size	No of positive	Percentage(%)	*E. coli* strain	References
Central Ethiopia	water, milker handswab and raw milk	450	27	6.0%	O157:H7	Dejene *et al*.,2022
Adami Tulu Jido Kombolcha District	water, milk, manure, and feces	408	19	4.7%	O157:H7	Mesele *et al*., 2023
Wolaita Sodo Town	fecal, environmental swabs, and milk	300	10	3.3%	O157:H7	Kassahun,Yordans,2024
Sebeta	Milk	142	0	0%	O157:H7	Dadi *et al*., 2020
Asosa Town	Milk	380	11	2.9%	O157:H7	Disassa *etal*., 2017

2.3.3. Reservoirs and Susceptibility of Escherichia coli O157:H7

Cattle are the primary natural sources of *E. coli* O157:H7, and livestock are the most significant reservoir (Bekele *et al*., 2014). Although sub-adult cattle from weaning to 24 months of age exhibit the highest shedding, EHEC can affects all ages of cattle (Joris *et al*., 2012). cattle and sheep are the principal reservoirs of *E. coli* O157:H7 but the bacterium is also found in the guts of healthy cattle, deer, goats, and sheep (Balcha *et al*., 2014). The outbreaks of this bacteria are associated with eating foods of bovine origin for instance, beef and dairy products (Perelle *et al*., 2007). STEC infections can occur in people of any age. The elderly and young children are more vulnerable and likely to experience more severe symptoms (Azanza *et al*., 2019). Certain businesses, including slaughterhouses, farms, hospitals, nursing homes, nursery schools, and food processing facilities, have a higher risk of infection for their employees (Walker *et al*., 2012). The potential for *E. coli* O157:H7 to persist through passive shedding by in-contact animals and to replicate in the environment without the need for a specific host is supported by its ability to survive and

replicate in faeces, the environment, and a variety of host species, including cattle (Ongeng *et al*., 2015).

2.3.4. Major Virulence Factors and Pathogenesis of E. coli O157:H7

The ability of the microorganism to produce the stx1 and stx2; the presence of the intimin (eae gene), which is necessary for the organism's adherence to the intestinal epithelium (attaching and effacing mechanism) (Hessain *et al*., 2015) And a number of bacterial virulence factors, including enterohemolysin, in addition to host factors, are the main causes of *E. coli* O157:H7 pathogenicity (Ferdous, 2017).Toxin production, plasmid O157, and locus of enterocyte effacement (LEE) are the main virulence factors thought to be required for the pathogenicity of *E. coli* O157:H7. (Smith *et al*., 2015).Forty-three open reading frames (ORFs) in the whole sequence of pO157 were found to be comparable to known proteins. The pathophysiology of *E. coli* O157:H7 infections was found to involve only 19 genes, including haemolysin (ehxA), serine protease (espP), zinc metalloprotease (stcE), type II secretion system apparatus (etp), putative adhesion (toxB), catalase peroxidase (kat), and an eae conserved fragment (ecf) (Ayaz *et al*., 2019). Lesions on host intestinal epithelial cells are caused by the LEE (Sapountzis *et al*., 2020). At least in certain STEC serotypes, the presence of LEE is associated with HUS (Alharbi *et al*., 2022). Noteworthy is the fact that STEC harboring-LEE is known as EHEC (Pakbin *et al*., 2020).

Shiga and intimin toxins are crucial to the pathophysiology of STEC infection. During colonisation, the intimin helps the bacteria form a close bond with the intestinal epithelium. Shiga toxins are produced by STEC after adhesion and reach the bloodstream. Shiga toxins attach to cells such as endothelial cells, which include globotriaosyl-ceramide (Gb3) in their cell membrane, after they have translocated to the blood circulation. The target host cells are then killed when the Shiga toxins enter the cells by endocytosis and then cleave ribosomes to prevent protein synthesis (Hauser *et al*., 2020). It is generally believed that STEC becomes more virulent when the stx2 and eae genes are present (Lupindu, 2018). Systemic sequelae are more likely to occur in strains encoding the subtype Stx2a (Fitzgerald *et al*., 2019).

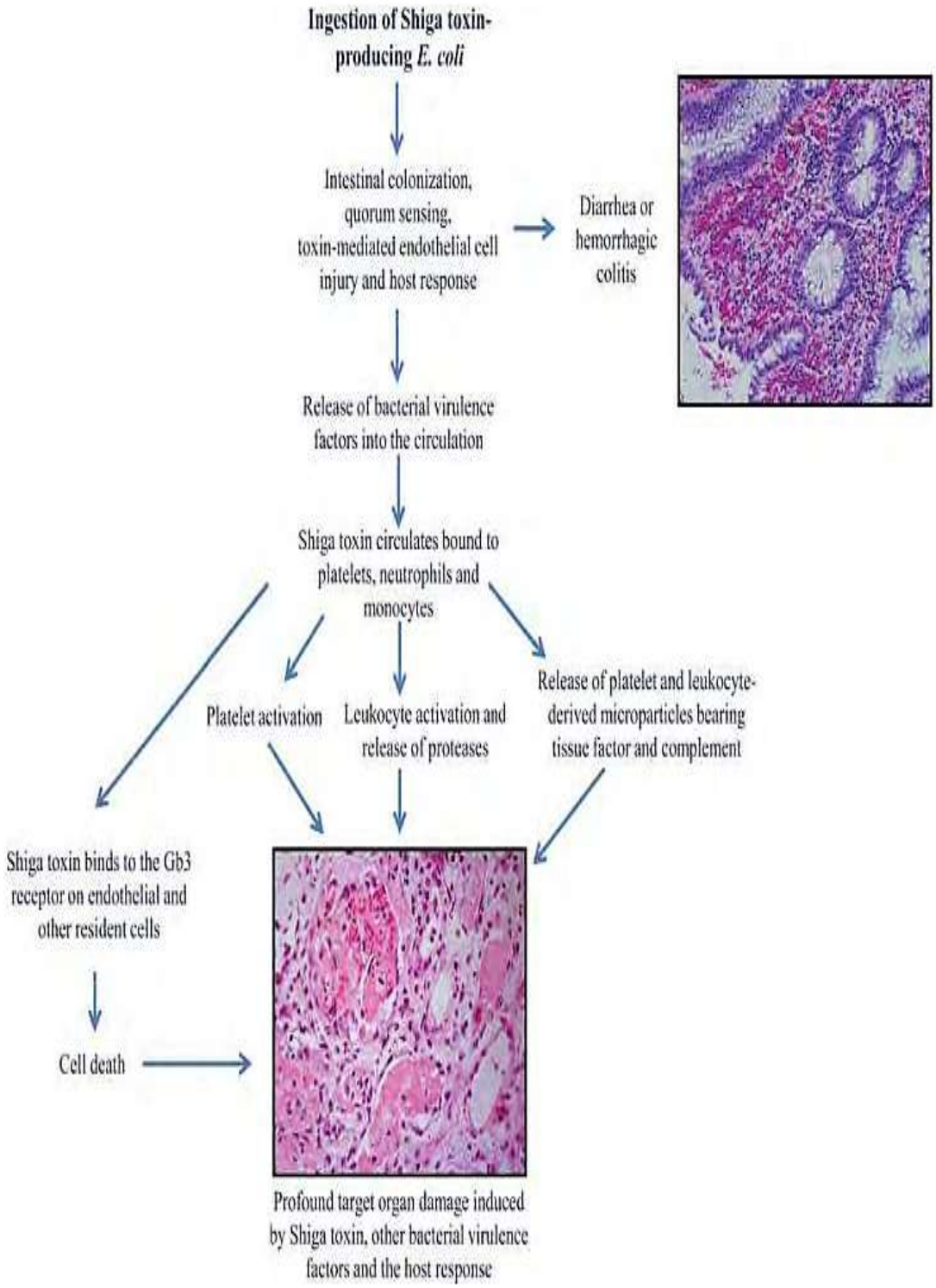

Figure 1: schematic presentation of pathogenesis of shiga toxin-induced disease.

Source: Karpman, 2012

2.3.5 Source of Infection and Transmission Routes of Escherichia coli

The fecal-oral pathway is the main way that enterohemorrhagic *Escherichia coli* (EHEC), particularly *E. coli* O157:H7, are spread. Animals can spread the disease to one another directly or indirectly through polluted pastures, shared water supplies, feed, and environmental surfaces. Consuming contaminated animal-based foods, such as undercooked beef, unpasteurised milk, and dairy products, is the most frequent way for people to become infected. Furthermore, faecal contamination during food handling, raw vegetables, and tainted water are known causes of illness (Ghasemian *et al*., 2017). There have also been reports of direct person-to-person transmission, particularly in settings like childcare facilities. Furthermore, there is increasing worry about zoonotic transmission through close contact with diseased animals or their surroundings. Visits to petting zoos, dairy farms, and campgrounds where cattle had previously grazed have been connected to a number of outbreaks (Lim *et al*., 2010; Constable *et al*., 2016).

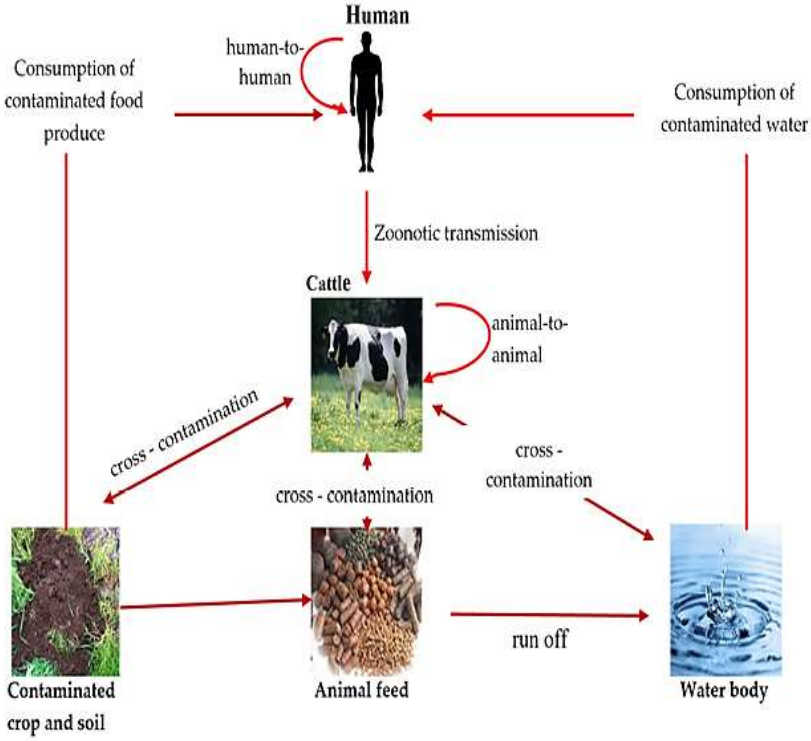

Figure 2: Transmission route of pathogenic *E. coli* among, animal, human and Environment.
Source: Oluwarinde *et al*., 2023

2.3.6 Environmental Survival of Escherichia coli O157:H7

Escherichia coli O157:H7 can live and persist in a variety of environments, such as soil, water, food, and animal reservoirs. It has been shown to survive for 21 months in raw, uncomposted manure and for a year in manure-treated soil. Composting manure efficiently eliminates *E. coli* O157:H7 if the temperature is maintained above 50°C for six days. It has a long lifespan in water, particularly in colder climates. Water trough sediments contaminated by bovine faeces can operate as a long-term (>8 months) reservoir for *E. coli* O157:H7, and the bacteria that survive in contaminated troughs can infect others (Noviyanti *et al*., 2018). It thrives in Acanthamoeba polyphaga and reproduces there. A widespread environmental protozoan found in soil, water, and faecal slurry is called Polyphaga. Therefore, under these conditions, it may be an effective *E. coli* O157:H7 gearbox vehicle (Mohammed *et al*., 2014).To flourish in a range of environments, it needs the ability to adapt to variances or abrupt changes in the temperature, pH, and osmolarity conditions normally seen in nature. For example, when subjected to heat and acid, *E. coli* O157:H7 generates exopolysaccharides, and heat stress alters the lipid content of the membrane (Yuk and Marshall, 2004). these environmental adaptations have a major impact on *E. coli* O157:H7 survival and expansion on farms as well as the increased transmission from cattle to cattle. Furthermore, there is a higher likelihood that the disease will contaminate crops and food through contact with diseased animals, irrigation with contaminated water, or direct contact with their manure because it may remain beyond the host reservoir (Maule, 2000).

2.4. Public Health Importance of *E. coli* O157:H7

E. coli is one of numerous kinds of bacteria that typically live as commensal organisms in both human and animal intestines (Bekele *et al*., 2014). Certain *E. coli* strains, such *E. coli* O157:H7, can cause illness when the immune system is weakened or when an individual is exposed to certain environmental factors. Additionally, it is a newly discovered foodborne pathogen that has drawn more attention recently. this bacteria is often referred to as an enterhemorrhagic *E. coli* (Abdissa *et al*., 2017). Over the past 20 years, *E. coli* has been linked to an increasing number of food-borne diseases. The most significant organism that causes life-threatening infections, including haemolytic uremic syndrome, kidney failure, bloody diarrhoea, abdominal pain, and HC, especially in humans globally, is *E. coli*

O157:H7(Pal et al., 2016). The pathogenic strains of *Escherichia coli* are referred to as enterocytotoxin-producing, enterotoxin-producing, or Shiga-toxin-producing organisms. In North America, Europe, and other parts of the world, *E. coli* O157:H7, which produces Shiga toxin, poses a serious risk to public health and causes severe, occasionally fatal illnesses in humans. *E. coli* O157:H7 infections in humans can range widely in clinical presentation, from asymptomatic to fatal. The majority of cases start with non-bloody diarrhoea and go away on their own without any more problems. But in one to three days, some patients get HC or bloody diarrhoea. Five to ten percent of HC patients may develop thrombocytopenic purpura (TTP) or HUS, two potentially fatal consequences (Kiranmayi *et al*., 2010).

2.5. Antimicrobial Resistance Patterns

Antimicrobial resistance is a growing phenomenon globally and represents a serious danger to human health and the economy (Pulingam *et al*., 2022). In 2019, it was projected that 4.95 million deaths were attributable to bacterial AMR and its consequences, and 1.27 million of these were affected directly by bacterial AMR. These fatalities occurred predominantly in sub-Saharan Africa and Southern Asia (Murray *et al*., 2022). By 2050, it is estimated that AMR will cause the loss of both lives and capital, affecting around 10 million people and causing a loss of $300 billion to $1 trillion annually (Burki, 2018; O'Neill, 2016). The discovery of the synthetic chemical arsphenamine's antisypohilitic activity in 1909 led to the use of therapeutic antimicrobial drugs in human health care for more than a century. Later, a variety of medicinal substances were found, such as cephalosporins, penicillins, and other naturally occurring substances (Chaisatit *et al*., 2012).

The prevalence of antibacterial resistance in dairy farms can be attributed to the widespread use of antibiotics in animal production (Dehkordi *et al*., 2014). The acquisition of antibiotic resistance genes, such as plasmids, and mutations brought on by the selective pressure of bacterial populations exposed to antimicrobials are the causes of antimicrobial resistance (Vidovic and Vidovic, 2020). Most drivers of AMR arise from inappropriate antimicrobial use in human ,animal, agriculutre or enviromental contamination (Holmes *et al*.,2016). A worldwide problem, the frequency of pathogenic multi-drug resistant *E. coli* is

rising quickly (Ali *et al*., 2016). Antimicrobial medications are utilised in animal production for growth promotion, prevention, and therapy. Drug use puts bacterial populations under selective pressure, which leads to the selection of antimicrobial resistances and the propagation of the resistance gene pool in the environment (Bruno and Mackay, 2012). There have been reports of *E. coli* strains becoming resistant to antibiotics all across the world, and both developed and developing nations are seeing an increase in these resistance rates. Antibiotic-resistant microorganisms make treating illnesses more difficult. Furthermore, the rise of antibiotic resistance in bacteria like *E. coli* O157:H7 is a serious public health issue.The efficacy of treatments and the capacity to manage infectious diseases in both humans and animals can be impacted by the pathogen's resistance to antibiotics (Thaker *et al*., 2012).Globally, antibiotic-resistant microorganisms are becoming a bigger issue. Due to evidence that suggest antibiotics may promote the production of Shiga toxin, the use of antibiotics to treat STEC infections has long been controversial. Rehydration therapy and, in certain situations, dialysis are examples of the supportive therapies that are advised (Mühlen and Dersch, 2020). Antimicrobial resistance to *E. coli* O157:H7 has been reported more frequently, despite the fact that antimicrobials are used less frequently to treat human diarrhoeal illness. Resistance in *E. coli* O157:H7 strains found in humans, animals, and food in Ethiopia (Rosa Abdissa *et al*., 2017; Mir and Kudva, 2019; Mukherjee *et al*., 2017).

According to a recent review by Mir and Kudva (2019), *E. coli* O157:H7 exhibits varying resistance to antimicrobials such ampicillin, tetracycline, streptomycin, and sulfonamides.the presence of antibiotic resistance in pathogenic bacteria can lead to the treatment failure, high morbidity and mortality. Antimicrobial resistant bacteria which live in the gut of carrier animals can be the source of environmental contamination and spread of antibiotic resistant bacterial strain in the environment and in animal products. Even if *E. coli* O157:H7 infections were generally not treated with antibiotics, its can share resistance-encoding genetic materials among themselves and with other pathogenic and nonpathogenic members of family enterobacteriaceae. The transmission of multidrug resistant *E. coli* O157:H7 from animals to -humans can cause food born infection which was difficult to control with antibiotic therapy, lead to protracted illness and death (Van *et al*., 2007).

2.6. Detection Methods For *E. coli* O157: H7

Detection of *E. coli* in the clinical laboratory depends on distinguishing the pathogenic serotypes from normal faecal flora containing commensal strains of *E. coli*. Fortunately, *E. coli* O157: H7 has two unusual biochemical markers; delayed fermentation of D-sorbitol and lack of D-glucuronidase activity, which help to phenotypically separate *E. coli* O157:H7 isolates from nonpathogenic *E. coli* strains (Assefa, 2019; Kargar and Homayoon, 2015). MacConkey agar containing 1% D-sorbitol instead of lactose is a useful and inexpensive medium on which non-sorbitol-fermenting *E. coli* grow as small, round greyish-white colonies. Selectivity is improved by the addition of 0.5% rhamnose, and addition of 0.05 mg/liter cefixime inhibits overgrowth by Proteus spp. Aeromonas, Pleisomonas, Morganella and Providencia (Kiranmayi and Krishnaiah, 2010). While fewer presumptive colonies require testing on this medium (OIE, 2018). Laboratory diagnosis of *E. coli* O157:H7, HC and HUS cases is accomplished by isolation of *E. coli* O157:H7 from specimens by bacteriologic culture followed by O157 antigen detection, toxin detection, or biochemical characterization.

Latex agglutination test is often used for the rapid identification of *E. coli* O157:H7. The test is best used in conjunction with Sorbitol MacConkey Agar. A positive result is indicated by agglutination with the test reagent, whilst the control reagent should appear milky and smooth (Al-Dragy and Baqer, 2014). The PCR technique has enabled the detection of genetic markers rather than the use of biochemical properties to identify bacteria in cultures and clinical specimens. Molecular-based techniques are distinctly advantageous because of their sensitivity, selectivity, and their rapid results.However, molecular-based techniques are appreciably more expensive than traditional plating techniques (Parsons *et al*., 2016).The selective methods used to detect *E. coli* O157:H7, but do not identify *E. coli* O157: H 7 or non-O157 EHEC, which are biochemically similar to other *E. coli* and do ferment sorbitol (Heredia and Garcia, 2018). Non-O157 enterohemorrhagic *E. coli* (EHEC) are typically identified based on their production of verotoxins. For O157 detection, the gold standard involves an initial enrichment of samples in buffered peptone water, followed by selective plating on cefixime tellurite sorbitol MacConkey (CT-SMAC) agar and incubation at 37°C for 24 hours. Suspected colonies are then confirmed using latex agglutination and PCR assays directing to identify the rfb gene

(specific for the O-antigen), the Shiga toxin genes (stx1 and stx2), and the eae gene (Williams and Dhungyel, 2014).

2.7. Treatment and Management

The effectiveness of using antibiotics to treat STEC infections is up for debate. Following the treatment of antibiotics for STEC infection, there have been reports of a rise in the production of Shiga toxins and a higher risk of fatal consequences (Zhang *et al*., 2000). Nonetheless, others contend that certain antibiotics may stop the development of HUS if given early in the course of illness (Schroeder *et al*., 2002). According to in vitro research, the majority of strains are susceptible to a variety of antibiotics; nevertheless, at sub-lethal concentrations, some antibiotics may enhance the production of a toxin that is similar to Shiga and has been linked to the development of HUS (Collins and Green, 2010). Antimicrobials have the ability to either boost the expression of stx genes in vivo or lyse bacterial cell walls, which releases stx (Schroeder *et al*., 2002). Therefore, replacing fluids and electrolytes is the primary method of treating infections caused by EHEC strains, such as *E. coli* O157:H7 (Rahal *et al*., 2012). Antimicrobial agents can disrupt bacterial cell walls, potentially leading to the release of Shiga toxins (Stx) or triggering enhanced expression of stx genes within the host (Schroeder et al., 2002). Consequently, managing infections caused by EHEC, including *E. coli* O157:H7, primarily focuses on supportive care through fluid and electrolyte replacement rather than antibiotic therapy (Rahal *et al*., 2012).

2.8. Prevention and Control

E. coli O157:H7 levels on farms can be reduced by following good sanitation and hygiene procedures; feed and water should be designed to keep waste out. The possibility for bacterial transmission would be reduced by cleaning pens and beddings with adequate water drainage (Desta *et al*., 2016).Washing hands with soap and running water for 20 seconds before eating or drinking, especially after working on the farm or handling products that can be contaminated with manure, could limit the spread of *E. coli* O157: H7 from animal to human (Zelalem *et al*., 2003). Strict hygiene measures should be put in place to reduce the risk of human infection from direct contact with farm animals. These measures include limiting access to farm animals, managing visitor movement, providing

easy access to washing facilities, offering a disinfection method in case visitors come into contact with the animals, and separating dining areas from animal housing areas (Fairbrother and Nadeau, 2006).In general, *E. coli* O157: H7 transmission from animals to humans could be prevented by implementing hazard analysis critical control points (HACCP). The widely recognised food safety management system is HACCP (Pennington, 2010). Implementing control methods that lessen the various effects of *E. coli* O157:H7 in human and animal populations is one of One Health's strategies. More efficient and financially feasible approaches to disease management than those that just target the human population may be provided by interventions that reduce infection in animal populations or stop disease transmission from animals to humans (Halliday *et al*., 2015). Recently, a vaccine for cattle was created to help reduce shedding in cattle. Cattle are immunised against the proteins produced on cell surfaces in order for the vaccine to function. It has been demonstrated that the vaccination lowers shedding because these proteins function as a receptor in intestinal walls, enabling the bacteria to colonise (Smith, 2015).

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in Addis Ababa, the capital city of Ethiopia. Addis Ababa is situated at latitude of 8º55' and 9°3'North and 38°43' and 38º50'East, longitude and it has an area of 51,000 hectare in the central highlands with an average altitude of 2000-2560 meters above sea level. The area is characterized by a relative humidity varying from 70% to 80% during the rainy season and 40% to 50% during the dry season, and bimodal rainfall with an average of 1800mm, the highest percentage of rain falls is during the long rainy season from June to September. The short rainy season is from February to April. Its annual average maximum and minimum temperature are 26 °C and 11° C respectively; with an overall average of 18.7 °C. The city has about 5, 200 dairy farms with a total of 38,572 dairy cows. AddisAbaba has 11sub-cities and the majority of UPA farms are found in five sub-cities (Akaki-Kaliti, Bole, Kirkos, Kolfe-Keranio and NifasilkLafto).The farms are categorized into small, medium, and large-scale operations. Small scale farms, numbering between 3,640 and 4,160, Medium-scale farms, estimated between 780 and 1,040 and Large-scale farms, ranging from 260 to 520. (Tadesse *et al*. 2019).

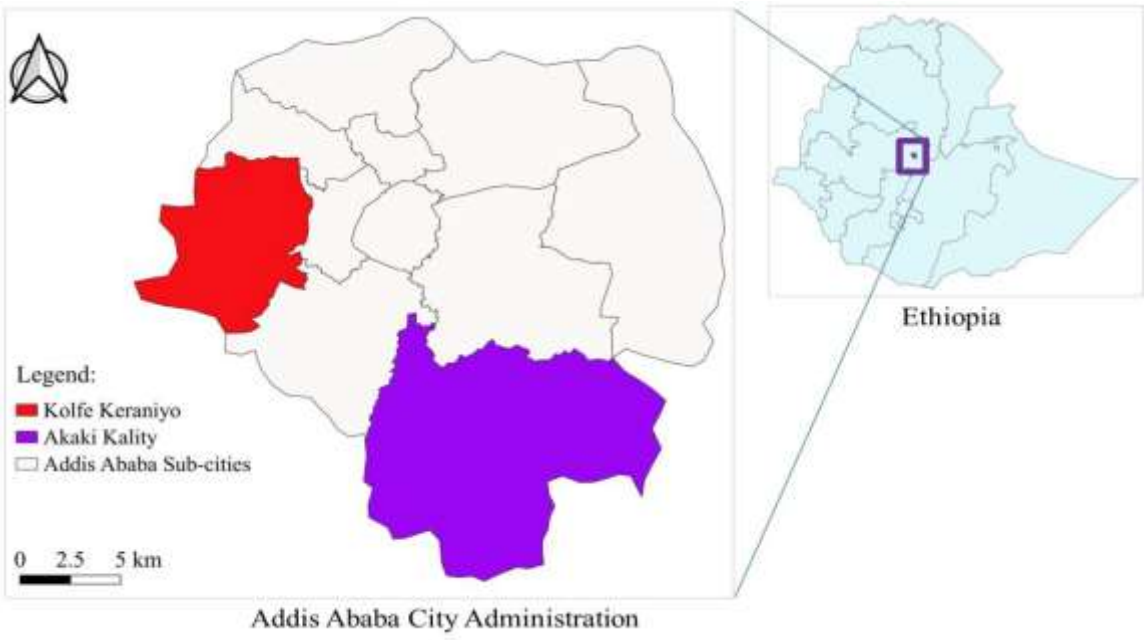

Figure 3: Map of study area.

Source: made using ARC GIS

3.2 Study Design and Population

A cross-sectional study was conducted from November 2024 to April 2025 to assess the antimicrobial susceptibility and molecular characterization of *E. coli* O157: H7 isolated from milk from dairy animals, samples collected from the farm environment (water and manure) and swabs from farm workers. The study involved 60 dairy farms selected from two sub-cities of Addis Ababa: Akaki Kality and Kolfe Keranyo. A semi-structured questionnaire was used to collect information on risk factors associated with the occurrence of *E. coli* O157:H7 in the dairy farms. The study involved two types of dairy farms: small-scale and medium-scale. A sampling frame was prepared, and the sampling units were stratified based on herd size into three categories: small (5–10 animals), medium (11–20 animals), and large (>20 animals), following the classification by Naess and Bårdsen (2013). The reference population in this study consisted of dairy animals and farm workers on the selected farms in the study areas. The study population included exotic dairy cattle breeds kept under intensive management systems in small and medium-scale farms. It also encompassed individuals involved in dairy farm activities, such as farm workers who carried out routine tasks like milking, feeding, and cleaning.

3.3. Sample Size Determination

The Sample size was determined based on the expected prevalence of *E. coli* O157:H7 and the desired absolute precision by using the standard formula described by Thrusfield (Thrusfield, 2018).

$$N = \frac{(1.96)^2 * Pexp\ (1\text{-}Pexp)}{d^2}$$

Where: N= required sample size, P = expected prevalence, d = desired absolute precision

The required sample size was calculated using a previously reported prevalence of 6.09% for *E. coli* O157:H7 in raw milk samples (Dejene *et al*., 2022), with a 95% confidence level and 5% desired absolute precision. The sample size obtained was 88. However, to improve the precision of the study, the total sample size for raw milk was increased to 230. The list of dairy farms available in study area was obtained from the Addis Ababa city administration farmers and urban agriculture development commission. Based on the data obtained from the each sub-cities has 50 medium and 167 small -scale dairy farms. From the total of 217 dairy farms identified in each sub-city of Addis Ababa, a total of 60 farms

were selected using stratified random sampling. The sample size was determined based on considerations of feasibility, resource availability, and the need for balanced representation of farm types across sub-cities. While proportional stratified sampling would suggest selecting approximately 46 small-scale and 14 medium-scale farms, the actual distribution of 36 small-scale and 24 medium-scale farms was intentionally used to ensure comparative analytical balance between farm types. Given that small-scale farms greatly outnumber medium-scale ones, including them in exact proportion would have made statistical comparisons less reliable due to the smaller medium-scale sample. 3 animals were sampled from each small-scale farm, resulting in 108 animals and 5 animals were sampled from each medium-scale farm, yielding 186 animals. The number of humans in contact was determined based on the expected prevalence of *E. coli* O157:H7 and the desired absolute precision according to a previously described formula. Accordingly, the sample size of hand swab from farm workers was 60, as determined from a previous prevalence report of *Escherichia coli* O157:H7 in humans in contact in dairy farm at in Bishoftu (4.1%) (Dejene *et al*., 2022). The expected prevalence was set at 13.4% based on a previous study conducted by (Abebe *et al*., 2014) resulting in 180.Thus, 60 water and 120 manure samples(60 waste and 60 bedding area) were collected.

3.4. Sampling Techniques

Two sub-cities, namely, Akaki-Kaliti and Kolfe-Keranio, were purposively selected based on the high density and the availability of dairy farms. Based on baseline data from the selected sub-cities, a total of 60 dairy farms, among them 36 farms small-scale and 24 farms medium-scale, were selected by using the simple random sampling technique. The number of animals and farm attendants were selected by a simple random sampling method from each selected dairy farm.

3.5. Sample Collection and Transportation

Milk samples were aseptically collected directly from the teats after thorough cleaning and drying of the udder and teats. Each teat was disinfected using cotton swabs soaked in 70% ethyl alcohol. The initial 3–4 streams of milk were discarded, and approximately 5 mL of milk was collected into a sterile, screw-capped universal bottle .Human hand swab samples were obtained from any humans in contact with dairy animals using sterile cotton swabs.

Water samples (5ml) were collected using sterile capped universal bottles. Pooled manure (1 g) samples were also collected from the selected dairy farms using sterile stomacher bags from bed, and dung storage area. The samples were shipped carefully on the day of collection using an ice box containing ice packs and processed within 24 hrs in Microbiology Laboratory of the Addis Ababa University college of veterinary medicine and agriculture.

3.6. Laboratory Isolation and Identification of *E. coli* O157:H7

3.6.1. Bacterial Isolation Process

The isolation and identification of *E. coli* O157:H7 were carried out using standard bacteriological techniques. Approximately 1 gram of feces, 1 mL of water or milk, and hand swab samples were each enriched in 9 mL of buffered peptone water and incubated at 37°C for 24 hours. Following enrichment, the samples were streaked onto MacConkey Agar and incubated at 37°C for another 24 hours. Pink-colored colonies were then sub-cultured onto Eosin Methylene Blue (EMB) agar and incubated aerobically at 37°C for 24 hours. Colonies with a characteristic green metallic sheen indicative of presumptive *E. coli* were transferred onto nutrient agar and incubated at 37°C for 24 hours for biochemical tests. Confirmatory identification was performed using a series of standardized biochemical tests, including Indole, Methyl Red, Voges-Proskauer, Citrate utilization, and Triple Sugar Iron (TSI) tests (Annex 3). Isolates showing positive results for Indole and Methyl Red, negative for Voges-Proskauer and Citrate, and TSI reactions indicating acid and gas production without hydrogen sulfide (H_2S) were identified as *E. coli* (Leber, 2020) (Annex 2).

Identified *E. coli* colonies were further sub-cultured onto Sorbitol MacConkey (SMAC) agar and incubated at 37°C for 24 hours to distinguish *E. coli* O157:H7 from other strains. Colonies that appeared colorless or pale, indicating non-sorbitol fermentation, were presumed to be *E. coli* O157:H7, while pinkish colonies (sorbitol fermenters) were considered non-O157:H7 *E. coli* strains.The pale colonies/colourles were encountered on SMAC which was taken for further confirmatory testing by polymerase chain reaction.

3.6.2. Molecular characterization of E. coli O157:H7

The PCR assay targeted the eae and stx2 genes specific to *E. coli* O157:H7, using gene-specific primers to amplify the corresponding toxigenic sequences. The reaction was carried out in a total volume of 20 µL, which included 10 µL of 2× GoTaq Green Master Mix. 2 µL each of forward and reverse primers, 3 µL of nuclease-free water (Promega Corporation, USA), and 3 µL of DNA template extracted from *E. coli* O157:H7 isolates. The mixture was transferred into a 200 µL PCR tube for amplification.PCR cycling conditions consisted of an initial denaturation at 94°C, followed by 35 cycles of denaturation at 94°C, annealing at 55°C, and extension at 72°C, with a total reaction time of approximately 2 hours and 30 minutes. The amplified products were analyzed using gel electrophoresis on a 2% agarose gel. A 450 bp DNA ladder was used as a molecular size marker to identify the expected amplicon sizes. The positive and negative controls were used in this study as *Escherichia coli* O157:H7 (ATCC- 43894). (Annex 4)

3.6.3. Antimicrobial susceptibility test

The Clinical and Laboratory Standards Institute's Kirby Bauer disc diffusion method on Mueller Hinton agar was used to determine the antimicrobial susceptibility pattern of *E. coli* O157:H7 isolates. The isolated of *E. coli* O157:H7 was evaluated for sensitivity to the most commonly used antimicrobials both in humans and animals, including Ciprofloxacin (CIP) (5 µg), Sulphonamides (S3) (300 µg), Norfloxacin (NOR) (10 µg), Ampicilin (AM) (10 µg), Meropenem (MEM)(10 µg), Amoxycilin Claulanic Acid (AMC) (30 µg), Streptomycin (S) (10 µg), Tetracycline (TE) (30 µg), Trimethoprim sulphamethaxazole(SXT) (25 µg), Cefoxitin (FOX) (30 µg), Cefotaxime (CTX) (30 µg) and Gentamicin (CN) (10 µg), Furthermore, isolates that showed resistance to two or more antimicrobials were labeled as multidrug-resistant. The results were classified as sensitive, intermediately resistant, and resistant according to the standardized table supplied by the CLSI (CLSI, 2024) (Annex 5).

3.7. Questionnaire Survey

A Semi-structured questionnaire was used to collect data from 60 dairy farms on hygienic practices at the dairy farms. Accordingly, information regarding washing before milking, washing udder before milking, provision of protective gear to workers, use of disinfectants,

hygienic condition of the farms, knowledge of antibiotic resistance and using them as feed additives. These factors were investigated to evaluate their possible association with the presence of *E. coli* O157:H7 within the dairy farms (annex 6).

3.8. Data Management and Analysis

The data and result obtained from the study were recorded in the format developed for this purpose and entered in Microsoft Excel 2013© and filtered for completeness. Then the data was exported to SPSS Version 20 software for analysis. Descriptive statistics such as frequency and percentages were used to express the proportion of prevalence of *E. coli* O157:H7 strain and the antimicrobial susceptibility profiles of the isolates. The proportions of prevalence of *E. coli* O157:H7 strain in dairy cattle, farm workers and farm environment samples were calculated by dividing the number of positive samples by the total number of samples tested from each sample source multiplied by 100. Chi square (χ^2) were used to assess the difference in the proportion of *E. coli* O157:H7 strains among the different risk factors. A P-value less than 0.05 was considered statistically significant.To examine the relationship between demographic variables andhygienic practicescores,we used multivarable logistic regression.The odds ratio was used to measure the impact of demographic variables.

3.9. Ethical Consideration

After having the study plans ethically reviewed by the animal health ethical committee of the College of Veterinary Medicine and Agriculture, Addis Ababa University and Aklilu Lemma Institute of Pathobiology Institutional research ethics review committee (ALIPB-IRERC) approved with reference number VM/ERC/04/67/17/2025and ALIPB IRERC 163/2017/25, respectively (annex 8).

4. RESULTS

4.1 The overall prevalence of *E. coli* and *E. coli* O157:H7

Among the total sampled taken during the study period, 32.7% (154/470) were positive for *E. coli* on EMB and biochemical tests. Bacteriologically, out of the 470 samples tested, 20 were initially suspected to be presumptive colonies of *E. coli* O157:H7 based on Sorbitol MacConkey agar (SMAC). Among the 20 isolates 0.6% (3/470) were *E. coli* O157:H7 positive by using polymerase chain reaction confirmatory test (Table 2 and Figure 4).

Table 2: The overall *E. coli* O157:H7 isolates in different tests 450bp

No of sample examined	Test	No of positive animals	Prevalence
470	SMAC	20	4.2%
470	PCR	3	0.6%

Note: SMAC – Sorbitol MacConkey Agar; PCR-Polymerase Chain Reaction

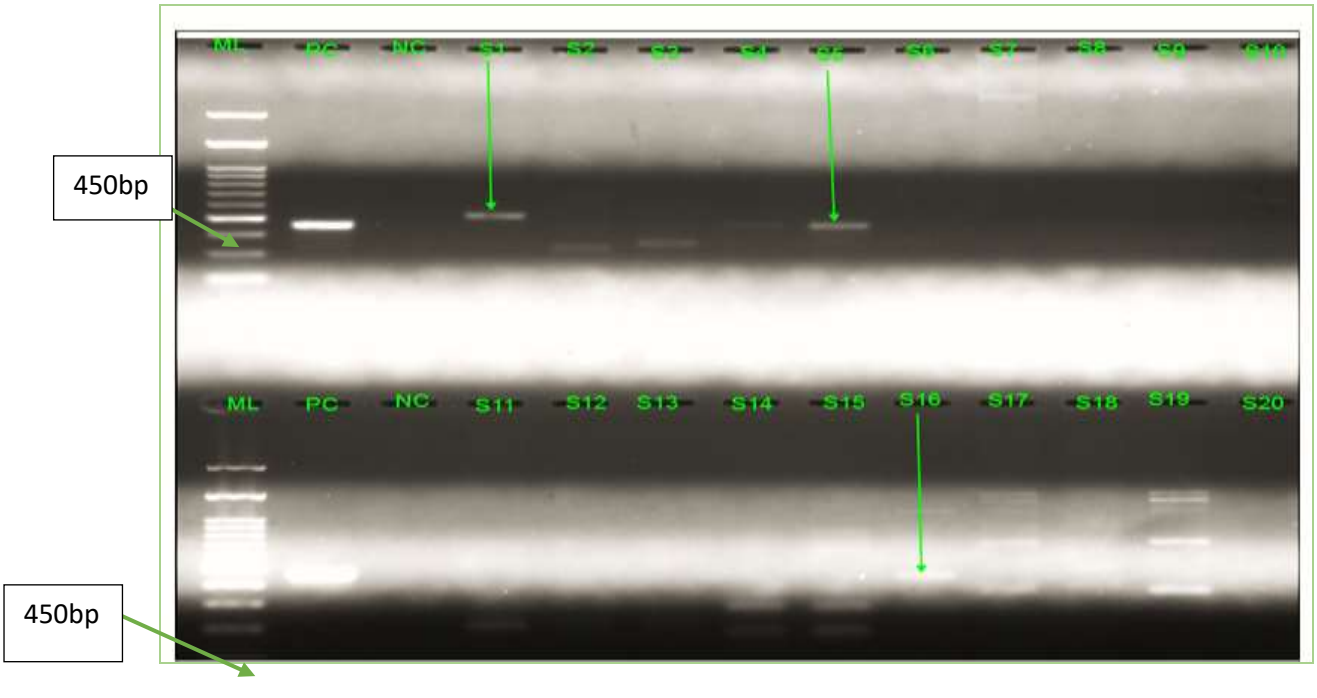

Figure 4: Agarose gel electrophoresis for detection of the *eae* and *stx2* genes in *E. coli* O157:H7 isolates.

Note: ML-Ladder ; PC-Positive control; NC-Negative control; BP: Base Pair; S 1 - S 20: samples.

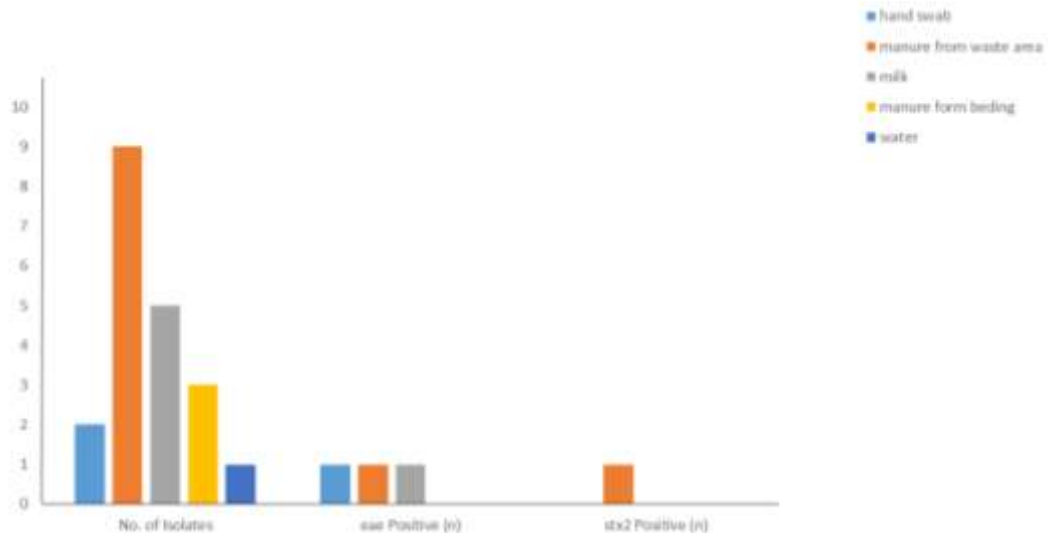

Figure 5: virulence gene distribution by sample type.

The study revealed that the sample type was significantly correlated with the isolation of *E. coli* from samples (p =0.001). Out of positive isolate high occurrence of *E. coli* observed in manure from waste area sampled (66.7%) followed by manure from beding (56.7%), hand swab (53.3%), water(23.3%) and milk(14.8%) as indicated in the Table 3.

Table 3: The occurrence of *E. coli* isolates in different sample type

Sample type	**Total sample examined**	***E. coli* isolates n(%)**	**X^2**	**P- value**
Milk	230	34(14.8)	94.568	**0.001**
Hand Swab	60	32(53.3)		
Water	60	14(23.3)		
Manure (waste)	60	40(66.7)		
Manure (beding)	60	34(56.7)		

The this study revealed that the sample type was not significantly correlated with the isolated *E. coli* O157:H7 (p =0.571). out of positive isolate high occurrence of *E. coli* O157:H7 observed in manure from waste area and hand swab sampled (1.7%) compared to 0.4% in milk (Table 4).

Table 4: The occurrence of *E. coli* O157:H7 isolates in different sample type

Sample type	Total sample examined	*E. coli* O157:H7 Strains n(%)	X^2	P- value
Milk	230	1(0.4)	2.922	0.571
Hand Swab	60	1(1.7)		
Water	60	0(0)		
Manure (waste)	60	1(1.7)		
Manure(beding)	60	0(0)		

This study revealed that the isolated of *E. coli* and *E. coli* O157:H7 was not significantly correlated with farm size (p=0.125 and p=0.060) and study area with (p=0.789 and p=0.089). however medium sized dairy farms were infected with *E. coli* (29.2%) and *E. coli* O157:H7 (1.4%) compared with small scale farms 35.8% and 0% respectively. 33.3% and 1.2% in kolfe keranyo; 32.2 % and 0% in Akaki Kality (Table 5).

Table 5: Prevalence of *E. coli* and *E. coli* O157:H7 strains based on farm scale and study area

Risk factors	Total sample examined	*E. coli strains n(%)*	*E. coli* O157:H7 strains n(%)
Farm scale			
Small	254	91(35.8)	0(0)
Meduim	216	63(29.2)	3(1.4)
X2(P- value)		2.350(0.125)	3.550(0.060)
Study area			
Akaki kality	230	74(32.2)	0(0)
Kolfe keraniyo	240	80(33.3)	3(1.2)
X^2(P- value)		0.072(0.789)	2.893(0.089)

4.2 Antimicrobial susceptibility pattern of *E. coli* O157:H7 isolates

Among the total isolates presumed to be *E. coli* O157:H7(n=20) was tested against twelve commonly used antimicrobial drugs. All (100%) isolates of *E. coli* O157:H7 were resistant to Ampicillin, 90% resistant to Tetracycline and 65% resistant to amoxicillin clavulanic acid. On the contrary, 90% of the isolates were sensitive to Norfloxacin and equal level of sensitive (85%) to cefotaxime and cefoxitin ;(80%) to streptomycin and trimethoprim sulphsmethaxazole respectively. 75% of isolates were sensitive to ciprofloxacin. 65%, 55% and 45% of isolated sensitive to Gentamicin, sulphonamides and Meropenem respectively (Table 6).

Table 6: Antimicrobials susceptibility patterns of *E. coli* O157:H7 isolates

Antimicrobial drugs	Resistance level n(%)	Intermediate level n(%)	Susceptibility level n(%)
Ciprofloxacin(5 µg)	1(5)	4(20)	15(75)
Streptomycin(10 µg)	3(15)	1(5)	16(80)
Norfloxacin(10 µg)	2(10)	0(0)	18(90)
Amoxicillin clavulanic acid (30 µg)	13(65)	3(15)	4(20)
Ampicillin(10 µg)	20(100)	0(0)	0(0)
Meropenem(10 µg)	5(25)	6(30)	9(45)
Gentamicin(10 µg)	5(25)	2(10)	13(65)
Cefotaxime(30 µg)	3(15)	0(0)	17(85)
Sulphonamides(300 µg)	5(25)	4(20)	11(55)
Cefoxitin(30 µg)	3(15)	0(0)	17(85)
Tetracycline(30 µg)	18(90)	0(0)	2(10)
Trimethoprim sulphsmethaxazole(25 µg)	4(20)	0(0)	16(80)

4.3 Multidrug Resistance Patterns

A total of 20 *E. coli* O157:h7 isolates multidrug resistance (MDR) was exhibited 30% (6/20) Resistance to three antimicrobial classes. Resistance to four antimicrobial classes was observed in four isolates (20%). Resistance to five antimicrobial classes was found in one isolates (5%). Furthermore, two isolate (10%) exhibited resistance to eight antimicrobial classes (Table 7).

Table 7: Multidrug resistance patterns of *E. coli* O157:H7

No of AM Class	Resistance pattern	No of isoate	percent (%)
3	AM,AMC,TE,MEM(1) AM,CN,TE(1) AM,TE,MEM(1) AM,AMC,STX,TE(1) AM,AMC,S3,SXT,TE(1) AM,AMC,CTX,FOX,TE(1)	6	30
4	AM,AMC,S,S3,TE(1) AM,AMC,CIP,STX,TE(1) AM,AMC,CN,S3,TE(2)	4	20
5	AM,AMC,FOX,CN,TE,MEM(1)	1	5
8	AM,CTX,S,CN,NOR,S3,TE,MEM(1) AM,AMC,CTX,FOX,S,NOR,SXT,TE,MEM(1)	2	10

Note:AM:antimicrobial;MDR:multidrugresistance;CIP:Ciprofloxacin; S3: Sulphonamides; NOR:Norfloxacin; AM:Ampicilin; MEM:Meropenem; AMC:Amoxycilin Claulanic Acid; S:Streptomycin; TE:Tetracycline; SXT:Trimethoprim sulphamethaxazole;FOX :Cefoxitin; CTX: Cefotaxime and CN:Gentamicin

4.3 Questionnaire Data

4.3.1. Demographic and Education status of the respondents

During the study period, 80% of the respondents were male, while 20% were female. Regarding age distribution, 20% were aged 20–30 years, 30% were 31–40 years, another

30% were 41–50 years, and 20% were above 50 years. In terms of educational status, 10% had completed primary school, 40% had finished high school, 20% had attained university-level education, and 30% were illiterate. These findings suggest that males, individuals aged between 31 and 50 years, and those with a high school education were the most represented groups in the questionnaire survey (Table 8).

Table 8: Demographic Characteristics of Respondents

Variable		**Frequency(%)**
Sex	Male	48(80)
	Female	12(20)
Age	20-30	12(20)
	31-40	18(30)
	41-50	18(30)
	>50	12(20)
Educational status	Illiterate	18(30)
	primary school	6(10)
	high school	24(40)
	university level	12(20)

4.3.2. Dairy Farm Hygiene and Management Practices

To assess the Sanitation and hygienic practices associated with the prevalence of *E. coli* O157:H7 icontamination in 60 dairy farms. The most significant risk factor identified is the lack of Hand Washing Before Milking ($p = 0.000$). Only 35% (21/60) of farms practiced this. Farms rated as having "Poor" Hygienic Conditions (55%, 33/60) showed a very strong association with contamination ($p = 0.000$).Not Using Disinfectants (85%, 51/60) was significantly associated with higher contamination risk ($p = 0.008$). Awareness of Antibiotic Resistance was critically low (only 10% or 6/60 farms aware) among respondents. While the association approached significance ($p = 0.072$). The study found that certain practices were not significantly associated with the risk of *E. coli* O157:H7 contamination. Infrastructure factors such as the type of floor, which was predominantly concrete (98.4%), and the use of stainless steel milk containers (86.7%) showed no meaningful correlation with the presence of the bacteria ($p=0.311$, $p=0.633$). Similarly,

having a specific type of water supply, reported by 95% of respondents, did not show a significant association (p=0.548). Despite being widely practiced by 90% of participants, washing the udder before milking was not found to be significantly protective (p=0.292). The study also examined manure management methods, including composting (15%), field application (20%), and dumping (65%), but found no notable link to bacterial risk (p=0.606). Providing workers with protective gear, adopted by 35% of participants, did not demonstrate any association with contamination risk (p=1.000). Additionally, the use of antibiotics as additives, practiced by 75%, showed no significant impact on the presence of *E. coli* O157:H7 (p=1.000).

Table 9: Sanitation and hygienic practices associated with the prevalence of *E. coli* O157:H7 isolates

Variable		**n(%)**	**X^2**	**P-Value**
Type of floor	Concrete	59(98.4)	1.026	0.311
	Mud	1(1.6)		
Method of manure disposal	Composting	9(15)	1.002	0.606
	ApplicationTo Fields	12(20)		
	Dumping	39(65)		
Type of Water source	Pipe	57(95)	0.360	0.548
	Well	3(5)		
Type of milk containers	Plastic	8(13.3)	0.229	0.633
	Stainless Steel	52(86.7)		
Washing udder before milking	Yes	54(90)	1.111	0.292
	No	6(10)		
Hand washing before milking	Yes	21(35)	15.824	**0.000**
	No	39(65)		
Workers provided with protective gear	Yes	21(35)	0.000	1.000
	No	39(65)		

Use antibiotics as additives	Yes	45(75)	0.000	1.000
	No	15(25)		
Are you aware of antibiotic resistance?	Yes	6|(10)	3.243	0.072
	No	54(90)		
Hygienic condition of the farms	Good	27(45)	25.859	**0.000**
	Poor	33(55)		
Do you use disinfectants	Yes	9(15)	7.059	**0.008**
	No	51(85)		

4.3.3 Association of demographic characteristics and hygiene practice score of respondents

The study assessed associations between demographic characteristics and hygienic practices among participants. Age was the only demographic variable significantly associated with hygiene. Specifically, respondents aged 31–40 years had significantly lower odds of practicing good hygiene compared to the 20–30 age group (OR = 0.086; p = 0.026), indicating that younger individuals may be more attentive to hygiene-related practices. Although not statistically significant, educational level showed a tendency toward better hygienic practices with higher education. Participants with university-level education had 3.46 times higher odds of good hygienic practices compared to illiterate individuals, while those with primary education had lower odds (OR = 0.32) of maintaining hygiene standards. Gender was not significantly associated with hygiene outcomes; however, females showed lower odds of good hygiene than males (OR = 0.64; p = 0.625), a finding that may be influenced by differing roles or access to hygiene resources on farms.These findings highlight the need for targeted hygiene training and awareness campaigns, particularly for middle-aged groups and those with lower educational attainment.(Table10)

Table 10: The association of demographic characteristics with hygienic practice scores

Demographic variable		**Hygienic practice**	
		OR(95% CI)	P-vaule
Gender	Male	Ref	
	Female	0.64(0.105 – 3.876)	0.625
Educational status	illiterate	Ref	
	primary school	0.32(0.049 – 2.076)	0.233
	high school	1.05(0.091 – 12.026)	0.971
	university level	3.46(0.443 – 26.999)	0.237
Age	20-30	Ref	
	31-40	0.086(0.010 – 0.741)	**0.026**
	41-50	0.259(0.037 – 1.812)	0.173
	>50	2.78(0.203 – 38.160)	0.444

Note: OR: odds ratio; CI: confidence interval; Ref: Referent.

5. DISCUSSION

In Ethiopia, *E. coli* is regarded as a major threat to dairy farm development and public health (Mohanty *et al*.,2013). This study also suggests that *E. coli* is the most significant dairy farm development obstacle in the study area. In the current study, out of 470 samples collected, the isolation of *Escherichia coli* was 32.7%.This result was comparable with previous research findings from AmboTown (36.8%) Tadese (2021) and Asosa town (33.9%) Disassa *et al*.,(2017). This prevalence was lower when compared to reports from Bangladesh (100%) Salauddin *et al*., (2020), urban and peri-urban dairy farms of Hawass town (69.1%) Fesseha *et al*., (2022), Bishoftu 53/75 (70.7%) Yeshiwas and Fentahun, (2017). This finding was higher than reports from Jimma town (5.4%) Sebsibe and Asfaw,(2020), Ambo town (9.1%) Tadese etal.,(2021), Bishoftu town (6.3%) Gutema *et al*.,(2021) and Kerala twon in India (8.8%) Vanitha *et al*.,(2018). The variation of prevalence of *E. coli* in the different study area might be due to differences in sample size, farming system, farmsize, methodological approaches, diagnostic techniques and geographical locations.

Regarding the risk factors associated with the prevalence of *E. coli* ,estimates of the prevalence of *E. coli* among farm size and study area showed no significat association. but the prevalence of *E. coli* among sample type showed significant difference (P=0.001) in which *E. coli* was recovered at higher rate in manure from waste area sampled (66.7%) followed by manure from beding (56.7%), hand swab (53.3%), water(23.3%) and milk(14.8%). This can be due to the fact that cattle are a major reservoir of *E. coli* and infected cattle could shade 101 to 107 cfu of *E. coli* per gram of feces. Given that typical cattle excrete 20 to 50 kg of feces per day, this provides a large inoculum of *E. coli* for the farm environment and could contaminate dairy products in the presence of poor hygiene and sanitary practices during milking and subsequent handling (Mathhews *et al*., 2014).

The present study revealed an overall 0.6%. Prevalence of *E. coli* O157: H7. The result is in line with the result reported from Greece (0.74%) Solomakos *et al*., (2009), Egypt (0.5%) Ahmed and Shimamoto, (2014), Addis Ababa and Debre Berhan cities (0.8%) Abdissa *et al*.,(2017). In contrast, the prevalence observed in the current study was lower than those reported by other researchers, including findings from Bishoftu (12%) Bedasa *et al*., (2018), Jimma (9.33%) Akililu *et al*., (2017), Holeta (5.2%) Ashenafi *et al*., (2020)

and Libya (3.5%) Garbaj *et al*.,(2016).This study showed a slightly higher prevalence of *E. coli* O157:H7 compared with the reports from Turkey (0.0%) Sancak *et al*., (2015).The prevalence of *E. coli* O157:H7 among farm size,sample type and study area were showed no significant association. The lack of statistically significant associations between the prevalence of *E. coli* O157:H7 and risk factors such as farm size, sample type, and study area may be attributed to the low overall prevalence observed in the current study which could reduce the power to detect meaningful differences.

In the present study, the prevalence of *E. coli* O157:H7 was found to be 4.2% using Sorbitol MacConkey Agar (SMAC) and 0.6% using polymerase chain reaction (PCR). The lower detection rate by PCR compared to SMAC may appear unexpected, as PCR is generally regarded as a more sensitive method for identifying *E. coli* O157:H7. Similar findings were reported by Fikadu *et al.* (2023), who recorded a 2.7% prevalence at the Bedele Municipal Abattoir in Southwest Ethiopia, and by Robi and Gelalcha (2020), who reported a 2.4% prevalence in Hawassa using PCR.The discrepancy observed in this study may be attributed to several factors influencing PCR performance, including the quality of DNA extraction, storage conditions, and handling of samples. Although PCR can detect low levels of the target organism by amplifying specific DNA sequences, its effectiveness depends heavily on the integrity and purity of the extracted DNA (Elbastawisy *et al*., 2023).

Antibiotic use plays a crucial role in both human and animal health. However, the emergence of antimicrobial resistance (AMR) has become a serious global public health threat, particularly within the One Health framework, where resistant bacteria readily circulate between humans, animals, and the environment (McEwen & Collignon, 2018; Fesseha *et al*., 2022). The indiscriminate use of antimicrobial agents in human medicine, veterinary care, and agriculture is a key driver of AMR (Mude Shecho *et al*., 2017). In the present study, *E. coli* O157:H7 isolates exhibited 100% resistance to ampicillin. This is consistent with previous findings by Tadese *et al*., (2021) and Eregena *et al*., (2023),who reported similarly high resistance rates in Ethiopia. However, this contrasts with Abebe *et al*., (2023), who observed complete susceptibility to ampicillin among *E. coli* O157:H7 isolates from bovine-origin foods in Dessie and Kombolcha towns. The variation may be attributed to differences in antibiotic usage patterns across regions. The high resistance rate observed in our study could reflect the frequent and potentially unregulated use for

therapeutic and preventive purposes in the study area. A high prevalence of resistance was also noted for tetracycline (90%) and amoxicillin-clavulanic acid (65%). These findings are consistent with reports from various parts of Ethiopia, including studies by Geletu *et al*., (2022), Welde *et al*., (2020), and Shecho *et al*., (2017). In contrast, Ahmed and Van Velkinburgh (2014) reported tetracycline susceptibility in *E. coli* isolates from Dire Dawa and Shumi *et al*.,(2021) from jimma indicating possible geographic based differences in antimicrobial use. The widespread use of tetracycline as a first-line drug for disease prevention and growth promotion in food-producing animals in Ethiopia and easily available in local markets by non-professionals may have resulted to the emergence of tetracycline-resistant bacterial species observed in this study.On the other hand, all isolates were highly susceptible to norfloxacin (90%), cefotaxime (85%), cefoxitin (85%), streptomycin (80%), and trimethoprim-sulfamethoxazole (80%). These results align with findings by Beyi *et al*., (2017) in Ethiopia, suggesting these antimicrobials may still be effective against *E. coli* O157:H7 in the region. On the contrary the findings of this study were inconsistent with the study by Hiko *et al*., (2008) and Bekele *et al*., (2014) from Ethiopia and Magwira *et al*., (2005) from Botswana revealed the resistance of *E. coli* O157:H7 mainly to streptomycin. Meanwhile, isolates were sensitive to cefotaxime, cefoxitin and trimethoprim sulphsmethaxazole.

The high susceptibility observed for these drugs might be due to their infrequent veterinary use, implying that such antibiotics can potentially be engaged to treat *E. coli* O157:H7 infections in humans in the study area. Ciprofloxacin sensitivity was observed in 75% of isolates, which is consistent with findings from Addis Ababa (76.5%) Bekele *et al*., (2014), Nigeria (78.9%) Reuben & Owuna (2013) and Dessie and Kombolcha (72%) Abebe *et al*., (2023). Despite being a commonly used Fluoroquinolone, its relatively high efficacy may reflect more controlled use or lower resistance development in the study area. Sensitivity rates to gentamicin (65%), sulphonamides (55%), and meropenem (45%) were moderate to low. The result of present study for gentamicin closely matches Alam *et al*.,(2017) from Bangladesh finding of 66.7%, and Natvig *et al*.(2002) from Norway report of 81%. In contrast, studies by Mesele *et al*.(2023) from Adami Tulu Jido Kombolcha and Ahmed *et al*.(2006) in Italy reported gentamicin sensitivities of 100% and 36.6%, respectively. These differences may result from varying levels of drug usage and regulation across countries. The main concern is the reduced sensitivity to meropenem, a last-resort carbapenem, which may indicate emerging carbapenem resistance and warrants further monitoring in the study

area. The resistance of an isolate to more than three tested antimicrobials is known as multidrug resistance (MDR) (Hill *et al*., 2005). In the current study, all 13 *E. coli* O157:H7 isolates exhibited multidrug resistance (MDR), with 30% (6/20) resistance to three antimicrobial classes. Resistance to four antimicrobial classes was observed in four isolates (20%). Resistance to five antimicrobial classes was found in one isolates (5%). Furthermore, two isolate (10%) exhibited resistance to eight antimicrobial classes.This level of MDR is comparable to findings by Asmaretal, (2024), who reported 100% MDR among *E. coli* O157:H7 isolates from dairy farms in Ethiopia, with the majority resistant to three or more antimicrobial classes, highlighting the widespread presence of resistant strains in similar settings. However, our findings show a higher degree of resistance compared to the report by Osman *et al*. (2018), where only 60% of *E. coli* isolates were resistant to three or more classes, possibly due to differences in sample sources, antibiotic usage patterns, or geographical variations in antimicrobial exposure.Overall, the findings of this study reflect the local challenge of AMR and emphasize the need for targeted antimicrobial stewardship, continuous surveillance, and integrated One Health interventions to prevent the further spread of resistant *E. coli* O157:H7 strains.

The questionnaire survey analysis revealed significant risk factors associated with the detection of *E. coli* O157:H7 on dairy farms in Addis Ababa. Notably, lack of handwashing before milking, absence of disinfectant use, and poor farm hygiene were strongly associated with higher detection rates ($P < 0.05$). Among these, hand hygiene before milking showed the strongest association ($P = 0.000$). This suggests that farms where milkers did not wash their hands were more likely to test positive for *E. coli* O157:H7 compared to those where handwashing was practiced. Similarly, failure to use disinfectants was associated with an increased likelihood of contamination ($P = 0.008$), and poor farm hygiene showed an even more substantial impact, with ($P = 0.000$), highlighting the critical importance of maintaining cleanliness in the dairy environment. Conversely, variables such as type of floor, method of manure disposal, type of milk containers used, washing udder before milking,workers provided with protective gear, use antibiotics as additives for growth gromotion, aware of antibiotic resistance and type of water source did not show significant associations in this study. This suggests that direct contact and hygienic practices during milking may play a more crucial role than infrastructure alone.

The study assessed associations between demographic characteristics and hygienic practices among participants. Age was the only demographic variable significantly associated with hygiene. Specifically, respondents aged 31–40 years had significantly lower odds of practicing good hygiene compared to the 20–30 age group (OR = 0.086; p = 0.026), indicating that younger individuals may be more attentive to hygiene-related practices. Although not statistically significant, educational level showed a tendency toward better hygienic practices with higher education. Participants with university-level education had 3.46 times higher odds of good hygienic practices compared to illiterate individuals, while those with primary education had lower odds (OR = 0.32) of maintaining hygiene standards. Gender was not significantly associated with hygiene outcomes; however, females showed lower odds of good hygiene than males (OR = 0.64; p = 0.625), a finding that may be influenced by differing roles or access to hygiene resources on farms.These findings highlight the need for targeted hygiene training and awareness campaigns, particularly for middle-aged groups and those with lower educational attainment.

These findings are consistent with reports by Mesele *etal*.,(2023) who explained inadequate handwashing during milking and poor farm hygiene were significantly associated with the presence of the *E. coli* O157:H7 in the Adami Tulu. Similarly, the findings of this study are in line with those reported by Hamiroune *et al.* (2016) on dairy farms in Algeria and Mogotu *et al.* (2022) in selected farms in Kenya. The significant influence of farm management practices on the presence of *E. coli* O157:H7 in milk contamination may be linked to inadequate hand hygiene prior to milking. Therefore, proper hygienic practices, particularly handwashing, may serve as a crucial control point in minimizing the risk of *E. coli* O157:H7 transmission within dairy farm environment

6. CONCLUSION AND RECOMMENDATIONS

This study demonstrated the presence of *E. coli* and *E. coli* O157:H7 across dairy animals, farm workers, and environmental sources in selected sub-cities of Addis Ababa, with the overall prevalence of *E. coli* was 32.7%, while *E. coli* O157:H7 was found in 0.6% of samples. Manure, hand swabs, and milk samples were found to harbour the pathogen, with the highest occurrence in manure, indicating poor waste management and hygiene practices as major risk factors. Antimicrobial susceptibility testing revealed alarming resistance patterns, with all isolates of *E. coli* O157:H7 showed resistance to ampicillin 100% and 90% resistance to tetracycline, suggesting the spread of multidrug-resistant strains within dairy farms. Meanwhile, higher sensitivity to norfloxacin, cefotaxime, and cefoxitin indicates these drugs remain relatively effective. The questionnaire surveys further highlighted significant gaps in hygiene practice, with only 35% of farm workers washing hands before milking, 15% using disinfectants and 7.5% having awareness about AMR. These behavioural and infrastructural deficiencies contribute to both the distribution and development of antimicrobial resistance *E. coli* O157:H7.

Based on the above findings and conclusions of this study, the following recommendations are forwarded:

- Regular training for dairy farm workers should provide on hygiene practices and antibiotic use.
- Promotion of good hygienic practice, Sanitation Standard Operating Procedures should be needed.
- Establish routine surveillance to monitor the emergence and spread of antibiotic-resistant pathogens and ensure rational antibiotic use.
- Develop and enforce One Health-based policies to control AMR.
- Strengthen hygiene protocols at both animal and environmental levels to break persistent transmission cycles within farms.
- Additional studies to elaborate molecular detection of antimicrobial resistance genes should be done.

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8. ANNEXES

Annex 1: Sample collection and laboratory activities work sheet for the isolation of *E. coli* and *E. coli* O157:H7

No	Date	Sample Type	Sample code	Enrichment	EMB Agar	MacConkey Agar	CTS MAC	Biochemical test				
								Indole	MR	VP	Citrate	TSI

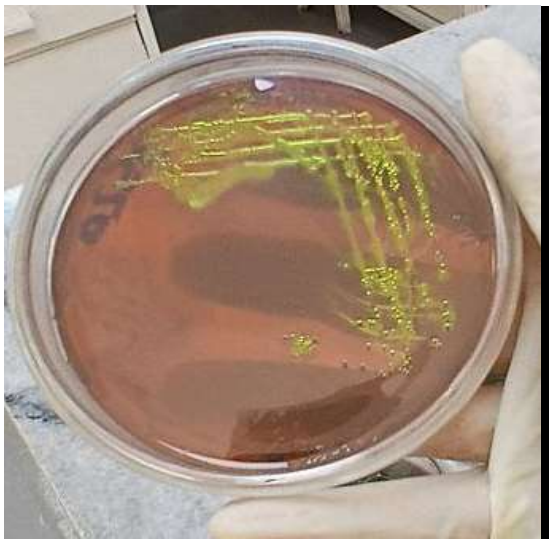
Annex 2: Isolation of *E. coli* by Culture and Biochemical Tests

1. Isolation and Identification

For the primary isolation of *E. coli,* typical pink colonies indicating lactose fermentation were observed on MacConkey agar. A single isolated colony was then sub-cultured onto Eosin Methylene Blue (EMB) agar. Colonies displaying a characteristic green metallic sheen on EMB agar, indicative of *E. coli*, were subsequently transferred to nutrient agar for further processing. From these, a single isolated colony was selected and subjected to a series of primary biochemical tests to confirm identification, following the procedures described by (Lagier, *et al*., 2015).

MacConkey agar

SMAC agar



EMB agar

A. Indole Test

The test organisms were inoculated into 5mL of peptone water containing tryptophan and incubated at 37°C for 24 hours. After incubation, 5 drops of Kovac's reagent was carefully added along the side of the test tube. The appearance of a red-colored ring at the interface indicated a positive indole test result.

B. Citrate Utilization Test (Simmon's Citrate Slant) Procedure

A loopful of the bacterial colony was streaked onto a Simmons citrate agar slant and incubated at 37°C for 24 to 48 hours. A color change of the medium to blue indicated a positive citrate utilization test, while no color change (remaining green) indicated a negative result.

C. Methyl-Red Test Procedure

A single colony from the pure culture of the test organism was inoculated into 5 mL of sterile MR-VP broth and incubated at 37ºC for 24 hours. After incubation, 5 drops of methyl red solution were added to the broth, and the color change was observed. A red color indicated a positive result, while a yellow color indicated a negative result.

D. Vogas-Proskauer Test Procedure

The test organisms were cultured in 3 mL of sterile MR-VP broth at 37ºC for 48 hours. After incubation, 0.6 mL of 5% alpha-naphthol and 0.2 mL of 40% potassium hydroxide solution containing 0.3% creatine were added per milliliter of broth culture. The mixture was thoroughly shaken and then allowed to stand for 10–15 minutes. The appearance of a red color indicated a positive Voges-Proskauer test result.

E. Triple Sugar Iron

Isolated colonies were picked using a sterile wire loop. The surface of the slant was streaked, and the butt of the tube was stabbed with the loop. The inoculated tubes were then incubated at 37°C for 18 to 24 hours.

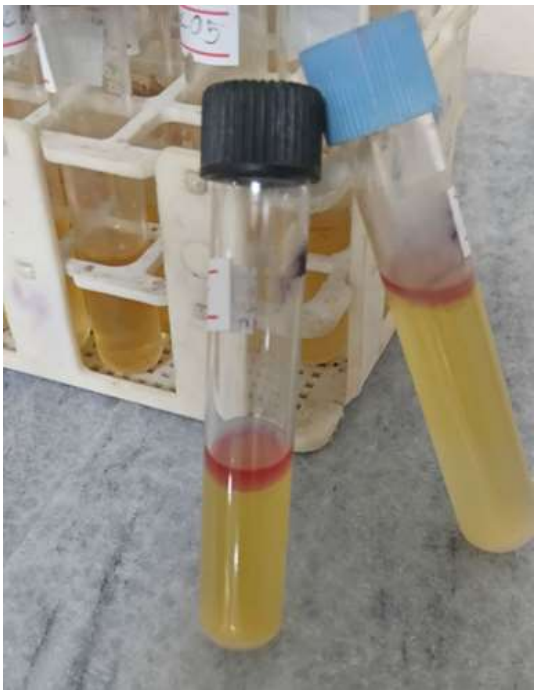
A: Butt of the tube — turns yellow if glucose is fermented; remains red or unchanged if glucose is not utilized; turns black if hydrogen sulfide (H_2S) is produced; and may show bubbles if gas is formed.

B: Slant surface — turns yellow if lactose and/or sucrose are fermented; remains red or unchanged if lactose and/or sucrose are not utilized.

MR positive

Citrate negative

TSI test positive



Indole test positive

Annex 3: Biochemical characterization of *E. coli*

Table 10: Biochemical characterization of *E. coli*.

Biochemical tests	Reaction
Indole	Positive
Methyl Red	Positive
Voges Proskauer	Negative
Simmons Citrate	Negative

Source: (Rai *et al*., 2017)

Annex 4: Polymerase chain reaction

DNA extraction procedures

1. Add 200ul cell suspension into 2ml centrifuge tube then add the single bacterial colony.
2. Add 20ul proteinase-k and 200ul buffer, vortex for homogenous mixing, and incubate in 70^{o}c water bath for 20 minutes, briefly centrifuge at 8000rpm (15 seconds) to remove drops from inside the lid.
3. Add 200ul ethanol (96-100) and vortex to homogenize the sample, briefly centrifuge at 8000 rpm (15 seconds) to remove drops from inside the lid.
4. Place mini-spin column tube on to 2ml collection tube, pipette the sample mix in to the mini-spin column tube, centrifuge at 8000rpm for 1 minute, place the mini-spin column tube on to new 2ml collection tube, and discard the filtrate.
5. Add 500ul buffer AW1 into the spin column tube and centrifuge at 8000rpm for 1 minute, place the mini-spin column tube on to new 2ml collection tube and discard the filtrate.
6. Add 500ul buffer AW2 and centrifuge at 14000rpm for 3 minutes, to dry the membrane.
7. Place the mini-spin column tube on to the new 2ml collection tube and centrifuge at 14000rpm for 1 minute (without adding any buffer).
8. Place the mini-spin column tube on to new 1.5ml collection tube, pipette 100ul buffer AE directly into the membrane, incubate at room temperature for 5 minutes, then centrifuge at 8000rpm for 1 minute to elute.

Annex 5: Antibiotic resistance tests

Antimicrobial susceptibility testing (AST) was performed on 20 *E. coli* O157:H7 isolates using the disk diffusion method. The bacterial suspension was standardized to 0.5 McFarland turbidity. After preparing the suspension, it was evenly inoculated onto Mueller-Hinton Agar (MHA) plates. Using sterile forceps, antibiotic discs were placed carefully on the surface of the agar to ensure proper contact. The inoculated plates were incubated at 37°C for 18–24 hours. After incubation, the diameters of the inhibition zones around each antibiotic disk were measured using a digital caliper. These measurements were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines using the Kirby-Bauer chart. Based on the measured zone diameters, isolates were classified as Susceptible (S), Intermediate (I), or Resistant (R) to each antibiotic tested. The findings were systematically recorded as per the predefined data collection format.

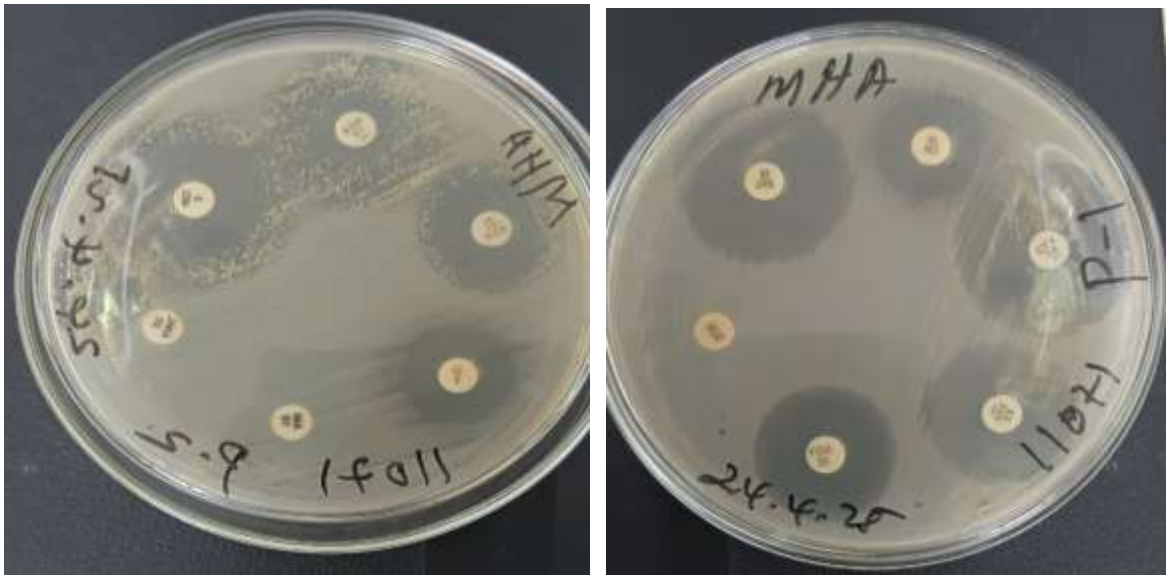


Table 11: Antimicrobial disks utilized, their respective concentrations and cut-off points

No	Antimicrobials	Disc code (concentration)	Resistance	Intermediate	Susceptible
1	Ciprofloxacin	CIP(5mcg)	≥21	16-20	≤15
2	Sulphonamides	S3(300ug)	≥16	13-15	≤12
3	Norfloxacin	NOR(10ug)	≥17	13-16	≤12
4	Ampicilin	AMP(10ug)	≥17	14-16	≤13
5	Meropenem	MEM(10ug)	≥23	20-22	≤19
6	Amoxycilin Claulanic Acid	AMC(30ug)	≥18	14-17	≤13
7	Streptomycin	S(10mcg)	≥15	12-14	≤11
8	Tetracycline	TE(30mcg)	≥15	12-14	≤11
9	Trimethoprim sulphamethaxazole	STX(25mcg)	≥16	11-15	≤10
10	Cefoxitin	FOX(30ug)	≥18	15-17	≤14
11	Cefotaxime	CTX(30u g)	≥23	13-22	≤14
12	Gentamicin	CN(10mcg)	≥15	13-14	≤12

Source: (CLSI, 2024)

Annex 6: Questionnaire format

Demographic Information

Respondent Name: _____ Address: _____

Educational Status:

☐ Illiterate ☐ Grade 1-6

☐ Grade 7-12 ☐ Grade >12

Gender:

☐ Male ☐ Female

Age of Respondent:

☐ <20 ☐ 20-30 ☐ 31-40

☐ 41-50 ☐ >50

1 .Do you clean the udder before milking? A. Yes B. No

2. Do you wash your hands before milking your cows? A. Yes B. No

3. Are workers provided with protective gear (e.g., gloves, boots)? A. Yes B. No

4. Do you use disinfectants for cleaning the farm? A. Yes B. No

5. What types of milk containers do you use? A. Plastic B. stainless steel

6. What type of Water source do you use? A. Pipe B. Well

7. What type of flooring is used in animal sheds? A. Concrete B. Mud

8. How is manure disposed of?

A. Composting

B. Direct application to fields

C. Dumping

D. Others (Specify): _____

9. What is the hygienic condition of the farm? A. Good B. Poor

10. Do you use antibiotics as additives for growth promotion in feed? A. Yes B. No

11. Are you aware of antibiotic resistance? A. Yes B. No

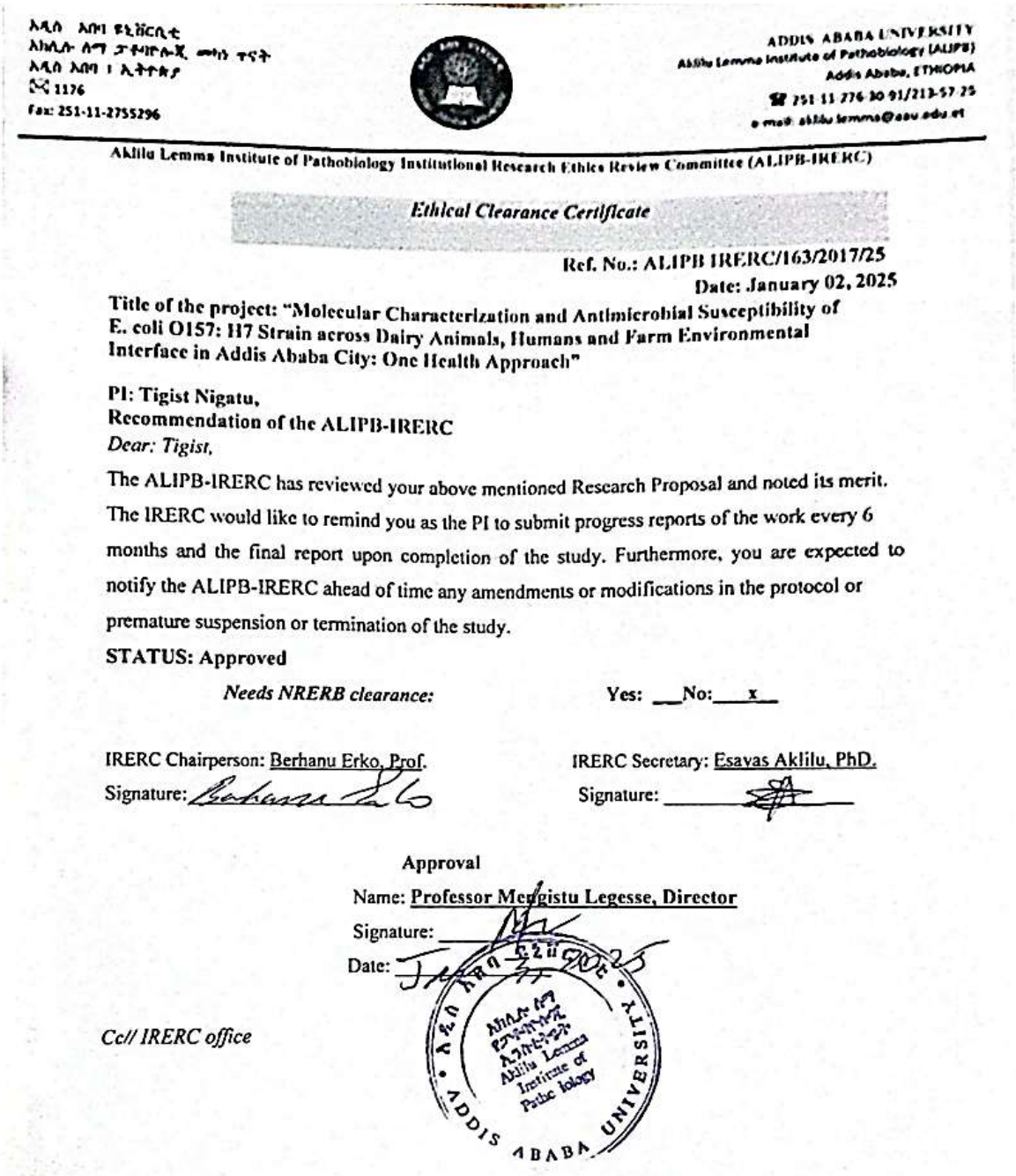
Annex 7: Declaration of informed voluntary consent

Verbal Consent Form

Hello, my name is Tigist Nigatu. I am an MSc student at Addis Ababa University. I would like to ask you a few questions about the sanitary conditions of your farm. Some questions require physical observation and taking milk, manure and swab samples from your hands. The objective of this study is to assess practices related to milk hygiene, which is important for improving the sanitary status and safeguarding the safety of milk reaching consumers. Your cooperation is voluntary, and all information you provide will be kept strictly confidential. You are free to skip any questions or stop the interview at any time. Do I have your permission to continue?

1. ☐ Yes (continue to the next page)
2. ☐ No (thank the participant and move to the next participant)

Annex 8: Ethical consideration approval letter



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Fax: 251-11-2755296

ADDIS ABABA UNIVERSITY
Aklilu Lemma Institute of Pathobiology (ALIPB)
Addis Ababa, ETHIOPIA
251 11 276 30 91/213-57 25
e mail: aklilu.lemma@aau.edu.et

Aklilu Lemma Institute of Pathobiology Institutional Research Ethics Review Committee (ALIPB-IRERC)

Ethical Clearance Certificate

Ref. No.: ALIPB IRERC/163/2017/25
Date: January 02, 2025

Title of the project: "Molecular Characterization and Antimicrobial Susceptibility of E. coli O157: H7 Strain across Dairy Animals, Humans and Farm Environmental Interface in Addis Ababa City: One Health Approach"

PI: Tigist Nigatu,
Recommendation of the ALIPB-IRERC
Dear: Tigist,

The ALIPB-IRERC has reviewed your above mentioned Research Proposal and noted its merit. The IRERC would like to remind you as the PI to submit progress reports of the work every 6 months and the final report upon completion of the study. Furthermore, you are expected to notify the ALIPB-IRERC ahead of time any amendments or modifications in the protocol or premature suspension or termination of the study.

STATUS: Approved

Needs NRERB clearance: Yes: __ No: x

IRERC Chairperson: Berhanu Erko, Prof.
Signature:

IRERC Secretary: Esayas Aklilu, PhD.
Signature:

Approval
Name: Professor Mengistu Legesse, Director
Signature:
Date:

ADDIS ABABA UNIVERSITY
Aklilu Lemma Institute of Pathobiology

Cc// IRERC office

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/04/67/17/2025

Name of Applicant: **Tigist Nigatu** (DVM, MSc student)

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

Title of the project: *Molecular characterization and antimicrobial susceptibility of E. coli O157 H7 strain across dairy animals, humans and farm environmental interface in selected sub-cities of Addis Ababa*

Date of application:	**December, 2024**
Nature of the project:	Field investigation
Target animal species:	**dairy cattle**
Number of animals involved:	**294**
Study area:	**Addis Ababa, Ethiopia**

Minutes No. and date of review: **VM/ERC/04/17/025, 25/02/2025**

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 Tigist Nigatu .

Additional clearance from concerned body is required for samples to be collected from human subjects

The Federal Democratic Republic of Ethiopia

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature


applied biosystems
Cefixime
Qty: 1 mg
78843264
2026-04-30
For testing of Food and Environmental
Thermo Fisher Scientific Baltics UAB
V.A. Graiciuno 8, LT-02241 Vilnius, Lithuania
Made in United Kingdom
applied biosystems
Potassium Tellurite
Qty: 50 mg
Store at 2 °C to 8 °C
78843250
2026-04-30
For testing of Food and Environmental samples only
Thermo Fisher Scientific Baltics UAB
Vilnius, LT-02241
Made in United Kingdom