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PREVALENCE OF *ESCHERICHIA COLI* O157:H7 AND ANTIMICROBIAL RESISTANCE PROFILE IN DAIRY FARMS AND IN CONTACT HUMANS IN BISHOFTU AND MODJO TOWNs

MSc THESIS

BY

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DEPARTMENT OF MICROBIOLOGY, PARASITOLOGY AND POULTRY HEALTH

MASTERS PROGRAM IN ONE HEALTH

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JUNE, 2025

BISHOFTU, ETHIOPIA

ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

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A thesis submitted to the College of Veterinary Medicine and Agriculture, Addis Ababa University in partial fulfillment of the requirements for the degree of Masters of Science in one health

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As members of the examining Board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by **Taye Dinku** entitled **Prevalence of *Escherichia Coli* O157:H7 and Antimicrobial Resistance Profile in Dairy Farms and in Contact Humans in Bishoftu and Modjo Towns** and recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters of science in One Health.

Chairperson	Signature	Date
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STATEMENT OF THE AUTHOR

I Taye Dinku hereby declare that MSc entitled "**Prevalence of *Escherichia Coli* O157:H7 and Antimicrobial Resistance Profile in Dairy Farms and in Contact Humans in Bishoftu and Modjo Towns"** submitted by me for in partial fulfillment of the requirements of master of science in one health, Addis Ababa University College of Veterinary Medicine and Agriculture which is my original work and it has not been presented for the award of any degree, diploma, fellowship or other similar titles of any other university or institution and that all sources of materials I have used or quoted for this thesis have been dully indicated and acknowledged by a complete reference.

BIBLOGRAPHY

Taye was born in 1991 from his father Dinku Regasa and his mother Tayitu Kumbi, in Lume woreda, Oromia, Ethiopia. He attended his primary school in Haro Gumbu primary school and his high school at Bishoftu secondary and preparatory school until June 2008. After he completed his high school, he joined Jimma University and attended Veterinary medicine and graduated in 2014. After graduation, he worked in government veterinary clinic as a veterinarian. He joined Addis Ababa University College of Veterinary Medicine and Agriculture to study his degree in Master of Science in One Health from October, 2023 to date.

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LIST OF ABBREVIATIONS

A/E	Attaching and Effacing
AMR	Antimicrobial Resistance
BPW	Buffered Peptone Water
E. coli	*Escherichia coli*
CFU	Colony Forming Units
CLSI	Clinical Laboratory Standards Institute
EMB	Eosin Methylene Blue
FAO	Food and Agriculture Organization
HC	Hemorrhagic Colitis
HUS	Hemolytic Uremic Syndrome
LEE	Locus of enterocyte effacement
MDR	Multidrug Resistance
PAIs	Pathogenicity Islands
p H	Potential of Hydrogen
STEC	Shiga toxin-producing *Escherichia coli*
Stx	Shiga toxins
T3SS	Type III secretion system
Tir	Trans located intimin receptor
TSI	Triple sugar Iron
USA	United States of America
VPH	Veterinary Public Health
WHO	World Health Organization

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ABSTRACT

E. coli O157:H7 poses a serious challenge to public health worldwide with ongoing acquisition of resistant to different antimicrobials. The cross-sectional study with stratified and simple random sampling was conducted to isolate and assess antimicrobial resistance profile of *E. coli* O157:H7 from dairy cattle, milk, in contact humans, soil and different water sources used in dairy farms as well as assess the associated risk factors of *E. coli* O157:H7 infection in dairy farms and in contact humans in Bishoftu and Modjo towns. Three hundred eighty two (382) containing cows' feces, milk, stool and environmental samples were collected and processed using bacteriological isolation, biochemical identification and confirmed by serological assay. Descriptive analyses were used to compute the prevalence of *E. coli* O157:H7 and the association between the outcomes and independent variables was analyzed using chi-square test at p value <0.05 and 95% confidence interval. Overall prevalence of *E. coli* O157:H7 was found to be 3.9%. The occurrence in soil and floor swab, fecal, stool 6.7%, 5.7%, 4.6%, respectively while it was 3.3% in milk, water and udder swab samples. There was no occurrence of *E. coli* O157:H7 detected on equipment and hand swabs. The occurrence of *E. coli* O157:H7 in fecal sample was significantly associated with floor type, cleaning frequency of the pen, pen cleanness and manure management sites. The prevalence in milk samples showed a statistically significant association ($p<0.05$) with factors like floor type, ways of udder cleaning, hand washing practice and manure management sites. Occurrence in soil samples was significantly associated ($p<0.05$) with floor type and manure disposal site while only manure management sites had statistically significant association ($p<0.05$) with occurrence in water samples. Risk factors such as age, drinking of raw milk, history of diarrhea and hand washing practice were found to be statistically significantly associated with *E. coli* O157:H7 occurrence in human. Antimicrobial sensitivity test to selected drugs revealed that all of the isolates (100%) were susceptible to nalidixic acid while all of the isolates (100%) were resistance to ceftazidime with multidrug resistance observed in 86.7% of the isolates. Therefore, there should be strict dairy farm hygienic practices such as manure management and rational uses of drug were recommended.

Key words: Antimicrobial resistance, Children, Dairy farm, *Escherichia coli* O157:H7,

1. INTRODUCTION

Dairy production is one of the major backbones for economy of Ethiopia and dairy farming is rapidly developing with the involvement of a large number of small and large scale farms. Ethiopia has vast potential for dairy development due to its extensive livestock population and diverse topographic and climatic conditions suitable for sustainable dairy farming. It has approximately about 15 million dairy cows producing approximately 7.1 billion liters of milk on average annually (MOA, 2022). However, several constraints, notably inadequate implementation of biosecurity measures on dairy farms and close cohabitation of human and livestock, a common practice in Ethiopia, facilitates a cross species transmission of pathogens, including foodborne pathogens (Getabalew *et al*., 2019; Moje *et al*., 2023).

Food-borne illnesses are severe global health dilemmas posing a significant obstacle to the socio-economic development of countries and have been a priority concern due to the rise in food-borne pathogens. It is estimated that around 600 million people became sick with foodborne illness from contaminated food, leading to approximately 420,000 deaths worldwide (Madilo *et al.,* 2023) which account for about 30% of deaths in children less than five years of age (WHO, 2022).

E. coli O157:H7 is among emerging food-borne pathogens and it is more significant than other foodborne pathogens due to several factors such as the severe consequences of infection that affect people of all age groups, its low infective dose, and its unusual tolerance to acidic environments (Haile *et al.*, 2017). Globally, Shiga toxin producing *E. coli* is estimated to cause around 2.8 million acute illnesses each year (Lupindu, 2018).

E. coli O157:H7 is a Shiga toxin producing serotype of *E. coli* strains which is highly virulent foodborne bacterial pathogens. It is a leading cause of acute life-threatening infections including hemolytic uremic syndrome and hemorrhagic colitis in humans (Kwan *et al*., 2019; Al-Ajmi *et al*., 2020). Human infections are typically due to ingestion

of contaminated food of animal origins, via animal contacts, waterborne (Heiman *et al*., 2015; Faraj & Wirtu, 2021; Altaie *et al*., 2025).

Among domestic animals, cattle and small ruminants, like sheep and goat, are the major animal reservoirs of *E. coli* O157:H7. Cattle, in particular, are a major reservoirs for *E. coli* O157:H7 and are significant sources of human *E. coli* O157:H7 infection cases (Gambushe *et al.,* 2022). Cattle primarily acquire the pathogen through consumption of contaminated feed or water, direct contact with infected animals or environmental exposure (Akomoneh *et al.,* 2020) and adult cattle often carrying it asymptomatically in their guts while newborn calves exhibiting clinical sign such as watery diarrhea (Antaki-Zukoski *et al.,* 2018).

The farm environment, including pen surfaces, bedding and water and manure plays a crucial role in the survival of *E. coli* O157:H7. Additionally, contaminated drinking water and soil which contaminated primarily by use of untreated animal manure serves as secondary habitat for *E. coli* O157:H7, contributing its survival and spread on the farms (Iwu *et al.,* 2021; Mesele *et al*., 2023).

Antimicrobial resistance has become is a major global health challenges, with a growing number of antibiotic resistant bacteria isolated from humans, food animals and environmental reservoirs over the last two decades, largely due to the misuse of antimicrobials (Hailu *et al.,* 2021; Murray *et al*., 2022). Studies conducted in different parts of the world revealed high rates of resistance of *E. coli* O157:H7 isolates to commonly used antibiotics. Similarly, multidrug resistance was also documented consistently, as 70-100% of the isolates were resistance to two or more classes of antimicrobial in cattle feces, humans and milk samples (Dejene *et al*., 2022; Tadesse *et al*., 2024; *Hunduma et al*., 2024).

Environmental elements, such as soil and water, can serve as reservoirs for antimicrobial resistance genes and further aggravate antimicrobial resistance dissemination. Study reported that 73% of *E. coli* O157 isolates from soil of manure-amended farms in the

USA exhibited multidrug resistance, with complete resistance to aminoglycosides and sulfa drugs (Hailu *et al*., 2021).

Previous studies conducted on *E. coli* O157:H7 in Ethiopia showed a prevalence ranging from 2.8%-38.2% in humans (Gutema *et al.,* 2021; Zelelie *et al.,* 2023), 4.7%-7.1% in food animals (Atnafie *et al.,* 2017; Gutema *et al.,* 2021), 0.2%-59.3% in raw milk (Sarba *et al*., 2023;Yihunie *et al*., 2024). Dejene and coauthors reported the prevalence of *E. coli* O157:H7 in water samples from dairy farm in Central Ethiopia was as high as 8% (Dejene *et al.,* 2022). Similarly, 9.89% prevalence of *E. coli* O157:H7 in water from Adami Tulu Jido Kombolcha district (Mesele *et al*., 2023).

1.1.Statement of the problem

Studies conducted in different parts of Ethiopia, showed that *E. coli* O157:H7 is prevalent in dairy cattle, milk, humans and dairy farm environment like water and manure. Although few studies are conducted on the dairy cattle, milk and farm environment (Mesele *et al*., 2023) they fail to incorporate in contact humans and environmental domains such as soil that serve as reservoir. They primarily focused on humans, food animals, and animal-derived foods separately. On the other hand those studies showed that dairy farming in the country lack good biosecurity practice(Moje *et al*., 2023) and farmers keep their cattle in open house attached to residence, backyard enclosure or separate house in the residential compounds (Gizaw *et al*., 2016). This close cohabitation may attribute to the easy transmission of *E. coli* O157:H7 from animal to humans through direct contact to animals and exposure to the environment.

The presence of potential risk factors like poor sanitation practices, unimproved drinking water sources, consumption of undercooked food like raw milk and beef puts Ethiopia at risk of *E. coli* O157:H7, with greatest burden being among children under the age of five (Zelelie *et al.,* 2023). In Ethiopia, consumption of raw milk is common and consumption of various food items, primarily foods of animal origin like milk, which can be contaminated by feces, manure, and polluted water on dairy farms, could be a significant

source of *E. coli* O157:H7 infection for humans. However, there is limited information on the contribution of variety of environmental factors, such as soil, water sources, and the interactions between humans, animals, and their environment as potential sources of *E. coli* O157:H7 to both humans and animals. However, there is a need for understanding of the epidemiological connections between human, dairy cattle, milk, soil and water in dairy farms for effective prevention and control.

General objective of the study:-

To determine the prevalence and risk factors of *E. coli* O157: H7 in Dairy cows, milk, soil, water and in contact humans in Bishoftu and Modjo towns.

Specific objectives of the study:-

- To isolate and identify *E. coli* O157:H7 from dairy cattle, milk, in contact humans, soil and different water sources used in dairy farms in Bishoftu and modjo towns.
- To assess the risk factors of *E. coli* O157:H7 infection in dairy farms and in contact humans
- To assess antimicrobial resistance profile of *E. coli* O157:H7 isolated from different samples

The research hypothesis is as follows:

- There is an association between factors such as cleaning of pen, milking place, herd size, hand washing and ways of udder cleaning during milking, floor type and prevalence of *E. coli* O157:H7 in dairy cows, farm environment and milk
- There is an association between contacts with dairy cattle, consumption of raw milk and prevalence of *E. coli* O157:H7in humans
- There are an antimicrobial resistant *E. coli* O157:H7 in samples from dairy cattle, milk, humans, soil and water sources

2. LITERATURE REVIEW

2.1. Morphology and growth characteristics of *E. coli* O157:H7

E. coliO157:H7 is Gram-negative, facultative anaerobe, flagellated, non-spore forming which belongs to the genus *Escherichia* and Enterobacteriaceae family. It was isolated and recognized as of enteric diseases in human in 1982 (Lim *et al.,* 2010). It is typically motile by peritrichous flagella. They are typically rod-shaped, with 1–3 μm long and 0.4–0.7 μm wide in size. The growth range for *E. coli* O157:H7 has been found to be 30°C to 45°C, with an optimum growth temperature of about 37°C. This bacterium is more heat sensitive and can easily be killed by standard pasteurization processes that are more than 60°C in nature. *E. coli* O157:H7 optimally grows at near neutral pH levels but is still capable of growth at pH as low as 4.4. It is notably acid-tolerant, allowing it to survive in foods with low pH values ranging from 3.6 to 4.0, and environments. Gastric acidity as well as volatile fatty acid is unable to eliminate *E. coli* O157:H7. A minimum water activity required for the growth of *E. coli* O157:H7 is 0.95. These characteristics highlight the adaptability of *E. coli* O157:H7, particularly in acidic environments and at lower temperatures, which is crucial for food safety management (Samelis *et al.,* 2005; Lupindu, 2017).

2.2. Pathogenicity

Escherichia coli O157:H7 is a highly virulent pathogen that can cause illness in humans and cattle with very few bacteria needed to start an infection as few as 4 to 24 CFU in humans and even fewer, around 100 CFU, in cattle. Its ability to cause disease is mainly due to certain factors like Shiga toxins (Stx), intimin, and a special system called the type III secretion system (T3SS) (Lupindu, 2018; Lange *et al*., 2022). Shiga toxins are potent toxins produced by bacteria and are classified into two types Stx1 and Stx2 which look different from each other. They stick to receptors on host cells, block protein production, and can lead to cell death (Lupindu, 2018). Among the two, Stx2 is much more toxic and is linked to more serious health problems in humans, like hemorrhagic colitis (HC) and

hemolytic uremic syndrome (HUS) (Gambushe *et al*., 2022). Another important factor is intimin, a protein that helps the bacteria attach tightly to host intestinal cells, and it's made by a gene called eae. The eae gene, found within specific regions called pathogenicity islands (PAIs) known as the locus of enterocyte effacement (LEE), produces a protein called intimin, which plays a key role in how the bacteria stick to the cells lining the intestines. This attachment leads to the formation of attaching and effacing (A/E) lesions, which damage the intestinal lining and can cause diarrhea and tissue injury (Lupindu, 2018; Lange *et al*., 2022). The Type III secretion system is a complex needle-like structure that spans the bacterial membranes. It injects special proteins called effectors into host cells, including the translocated intimin receptor (Tir). Tir then acts as a docking point for intimin, helping the bacteria firmly attach and establish themselves in the gut. These different virulence factors Stx toxins are causing cell death, intimin helping bacteria stick, and T3SS delivering effectors work together, allowing *E. coli* O157:H7 to colonize the host, evade the immune system, and cause serious gastrointestinal and widespread illnesses (Lange *et al*., 2022; Gambushe *et al*., 2022).

2.3. *E. coli* O157:H7 in dairy cattle

Dairy cows are the primary sources of *E. coli* O157:H7 strain, which contaminate milk through direct contact with the animals and their farming environments (Ariyanti *et al.*, 2022). The *E. coli* O157:H7 strain is found in the feces of various animals, including sheep, birds, cattle, bats, goats, pigs and dogs. Cattle and small ruminants, like sheep and goat, serves as the major animal reservoirs for human pathogenic strains of *E. coli* O157:H7. Cattle and sheep, in particular, are a significant reservoirs for this serotype and are significant sources of human infection with *E. coli* O157:H7 (Gambushe *et al.*, 2022). *E. coli* O157:H7 infects animals intermittently and transiently, for varying periods. Cattle are typically carrying it asymptomatically in their intestines and intermittently shedding it in their feces. Shedding is not continuous, shed at high density for short periods, followed by long intervals with no fecal shedding or only low concentration shedding of the bacteria (CFU) per gram of feces (Larzábal *et al.,* 2020; Lange *et al.,* 2022).

The survival of *E. coli* O157:H7 in herds of cattle depends on a small group termed "supersheders", individuals shedding in excess of 10^4 CFU/g of feces. These animals can excrete 100 to 1000 times more *E. coli* O157:H7 than typical shedders and they accounts for the majority of the *E. coli* O157:H7 released to the farm environment. The epidemiological studies show that >96% of *E. coli* O157:H7 isolates can originate from 10% of a small subset of colonized animals on the farm and supershedder cattle increase the number of *E. coli* O157:H7 in barns that enhance herd prevalence on the farm (Mir *et al.,* 2020; Katani *et al.,* 2021). Super shedding occurs when biofilms of *E. coli* O157:H7 within the rectum lumen are shed from the intestinal epithelium and enter and elevated levels of bacterium in the feces (FAO & WHO, 2022).

Cattle primarily acquire the pathogen through the feacal-oral route, which occur via ingestion of contaminated feed or water, direct environmental exposure or contact with infected animals (Akomoneh *et al.,* 2020). Younger animals are more susceptible to *E. coli* O157:H7 colonization, with higher fecal presence observed in young milk fed calves compared to mature adult dairy cows (Stenkamp-Strahm *et al.,* 2018). Adult cattle are asymptomatic carriers of *E. coli* O157:H7. Nevertheless, newborn calves can display clinical symptoms and tissue damage due to *E. coli* O157:H7 infections, including watery diarrhea, neutrophilic invasion of the intestinal mucosa, necrosis and sloughing of the epithelial cells of large and small intestines (Larzábal *et al.,* 2020; Lange *et al.,* 2022).

A farm environment is a key factor in supporting a population of viable *E. coli* O157:H7 which can survive in feces, manure, barn surfaces, bedding and water. In particular, cattle manure can enable the prolonged survival of the bacterium outside the host. Additionally, contaminated drinking water may play a role in the spread and persistence of *E. coli* O157:H7 on farms (Mesele *et al.,* 2023).

The prevalence of *E. coli* O157:H7 in cattle feces has been investigated across multiple geographical regions and production systems, with study reporting variable prevalence rate. In Ethiopia, Fikadu *et al.,* (2023) identified overall prevalence of 4.7% in cattle

feces at Bedele, while Hunduma *et al*., (2024) documented a slightly lower rate 3.9% in dairy cattle under pastoral production systems. Similarly, in Nigeria Mohammed (2023) reported a 2.3% prevalence of *E. coli* O157:H7 in fecal samples from dairy and beef cattle. Studies in Tunisia Tayh *et al.,* (2022) and Iraq Altaie *et al.,* (2025)found comparable prevalence of 4.6% and 3%, respectively, though the Iraq study noted the lower prevalence in animal samples compared to the human and food samples. In Turkiye, Babacan (2024) observed a 3% prevalence of *E. coli* O157:H7 in ruminant feces in Balikeser province. A meta-analysis study conducted on prevalence and concentration of *E. coli* O157:H7 in cattle, products and the environment in United States of America reported a pooled prevalence of 1.5% in cattle feces with herd level prevalence of 31.7%, aligning with Obaidat & Stringer (2019) finding of 2.3% in Jordan dairy cattle. However, a notable outlier was observed by Fesseha *et al.,* (2022), where they recorded 46.8% prevalence in diarrheic calves from peri-urban and urban dairy farms in Hawassa, Ethiopia. This exceptionally high rate give rise to the potential of high infection risks in clinically affected populations compared to healthy cattle groups. The data collectively indicate that *E. coli* O157:H7 has low but consistent prevalence in healthy cattle populations across diverse geographical regions. This consistency suggests that *E. coli* O157:H7 is endemic in cattle herds around the world.

2.4. Milk as a source of *E. coli* O157:H7

Theoretically, raw milk from healthy animal is considered as safe for human consumption at time of milking. However, contamination can occur either by endogenous contamination when the pathogens are transferred directly from an infected animal's blood or udder into the milk or exogenous contamination during or after milking due to contact with animal feces, soil, air, water, feed, equipment, the exterior of the udder and teats, and peoples (Altaie *et al.*, 2025). Common factors contributing to high prevalence of *E. coli* O157:H7 in dairy products can be attributed to poor milking hygienic practices, poor facility sanitation, absence of post milking teat disinfectant practices, and failure to segregate cows by age during milking operations. Failure to comply with biosecurity and herd management practices can increase the fecal-oral spread of the bacteria, as *E. coli*

O157:H7 is predominantly associated with fecal contamination of milk, equipment, or udders. A case in point: not dipping the teat at the end of milking elevates the risk of bacterial colonization, or milking older and younger cows together increases the risk of cross-contamination due to age-related variation in the shedding rate of the pathogen (Ababu *et al.,* 2020; Ariyanti *et al.,* 2022). Its presence in the milk products show recent fecal matter contact whose origin would most likely be unhygienic handling, contaminated equipment or storage. Such milk contamination has severe public health concerns as consumption of *E. coli* O157:H7 may cause milk born infections among humans(Alam e*t al.,* 2017; Akinjogunla *et al.,* 2022). The other main risk factors for milk contamination by *E. coli* is in the handling of bulk tank milk, which has amplified contamination due to extended contact periods and pooling from multiple cows (Disassa *et al.,* 2017). Warmer and more humid climate support *E. coli* growth which leads to increased contamination rate(Abebe *et al.,* 2023) .

The type of utensils used also affects contamination level, with plastic containers showing higher contamination than stainless steel, likely due to differences in cleaning characteristics. Water quality is another critical factors; the type of water used for hand washing and milking utensils significantly affect microbial contamination (Sarba *et al.,* 2023). Aseffa and Behon's research indicated a 26% pooled prevalence of *E. coli* O157:H7 contamination in milk, based on combined data of different studies conducted in Ethiopia. This high prevalence is due to fecal contamination from cattle during milking and unhygienic handling practice by the handlers (Assefa & Bihon, 2018).

2.5. Environment as reservoir of *E. coli* O157:H7

Environments, such as soil and water are secondary habitat of *E. coli*. The survival capability of *E. coli* O157:H7 in soil is highly dependent on the inherent heterogeneity of the ecosystems, which produces high variability in its persistence. Abiotic factors such as near neutral PH and moderate moisture content, and the availability of water soluble organic carbon and soluble organic nitrogen enhance *E. coli* O157 survival, while elevated salinity and extreme temperatures reduce its viability and biotic factors such as

competition with native microbial population interact synergistically to shape the survival of the pathogen in the soil (Han *et al.,* 2020). *E. coli* O157:H7 contamination of soil is driven primarily by use of untreated animal manure that introduces the pathogen into the soil. Once introduced, the pathogen's survival is prolonged by organic matter in manure enriched soils, which provides nutrients and stabilizes environmental conditions, enabling persistence for 21-45 days in the soil (Iwu *et al.,* 2021).

The occurrence of *E. coli* O157:H7 in agricultural soils varies extensively between regions based on environmental as well as anthropogenic factors. In South Africa, *Iwu et al*., (2021) reported 38% prevalence of *E. coli* O157:H7 in agricultural soils which is in line with Dusek *et al*., (2018), who reported a 41% prevalence of *E. coli* from different soil samples with highest prevalence(9%) in pasture soils, attributing this to frequent fecal deposition from livestock. Comparatively, Mohammed & Mustafa, (2015) documented a markedly higher prevalence of 68% of *E. coli* O157:H7 in Iraq dairy farm soils, likely reflecting intensive livestock activity and limited sanitation practices. In contrast, lower rates were observed in Ethiopia and India. Gemeda *et al*.,(2023) indicated 6.8% prevalence of *E. coli* O157:H7 from soil sample taken from areas in small holder livestock production systems, while Sekhar & Hirbaye, (2024) showed 4% prevalence in agricultural soils in Ethiopia. Vanitha *et al*., (2018) also indicated 8.33% prevalence of *E. coli* O157:H7 in soil samples from dairy farm environments in India. On the other hand, Hailu *et al*., (2021) reported a low prevalence of 0.9% of O157 in soil samples from dairy cattle and poultry manure amended farms in USA, likely reflecting strict biosecurity measures and waste management practices.

Soil acts as a significant extra host habitat for *E. coli* O157:H7 and soil contaminated due to direct shedding of *E. coli* O157:H7 onto the land by animals. Study indicated that compounds with unrestricted animal movement exhibit higher *E. coli* levels compared to those without animals (Ercumen *et al*., 2017). From contaminated soil, *E. coli* O157:H7 can transfer to surface runoff, infiltrate deeper soil layers, and colonize plant roots which creates the pathway for human infection through the food chain (Williams *et al*., 2015).

Water is a significant route of infection of *E. coli* O157:H7 in humans and animals. The contamination of water sources with *E. coli* is driven by factors such as inadequate wastewater management and urbanization. In densely populated urban areas, untreated wastewater discharge from households, septic systems and storm drains introduce fecal bacteria into rivers (Banu *et al.,* 2021).

Multiple studies across diverse geographical regions highlight the persistent presence of *E. coli* O157:H7 in water sources associated with animal husbandry, highlighting its role as critical transmission vector. In South Africa, irrigation water sampled from agricultural setting showed a 37% confirmation rate of *E. coli* O157:H7(Iwu *et al*., 2021), while dairy farm water sources, including from animal drinking troughs, yielded a prevalence of 18.7% (Myataza *et al*., 2017).

Studies in Ethiopia reported 8% prevalence of *E. coli* O157:H7 in animal drinking water (Mesele *et al.,* 2023). Similarly, Dejene *et al.,* (2022) identified 5.2% prevalence of *E. coli* O157:H7 in dairy farm water samples. In contrast, higher prevalence was observed in abattoir wastewater documenting 33.3% prevalence of *E. coli* O157:H7 in Addis Ababa which may due to concentrated fecal loads and inadequate sanitation practices in slaughterhouse effluents (Hayet *et al*., 2021).

2.6. Public health implication

E. coli O157:H7, a shiga toxin-producing *E. coli* (STEC) strains is considered a public health threat due to its potential to cause life threatening infections, including hemolytic uremic syndrome and kidney failure. Globally, STEC infections causes over 2.8 million cases of acute illness each year, with an estimated incidence rate of 43.1 cases per 100000 person –years which results in 3890 HUS cases and 230 deaths per year. STEC infections account for 10200 cases per year in Africa, 10% of which is caused by STEC O157:H7 (Lupindu, 2018; Gambushe *et al.,* 2022) . *E. coli* O157:H7 is a dominant serotype responsible annually for greater than 15000 and 1000 human infection in North America and United Kingdom respectively(Fitzgerald *et al*., 2019). STEC is the third

most frequent bacterial agent detected in foodborne outbreaks in the European Union with 42 outbreaks, 273 cases, 50 hospitalizations and 1 death in 2019 on which bovine meat and milk were the main sources of the diseases (Boelaert *et al.*, 2021) and *E. coli* O157:H7 is accountable for 20% food-borne outbreaks worldwide (Rani *et al*., 2021).

Human infection with *E. coli* O157:H7 are often attributed to the consumption of contaminated food of animal origins, including raw or undercooked meat, raw milk and unpasteurized dairy products (Boelaert *et al*., 2021). It also transmitted through direct contact with animals or their environment as well as person to person. Reports of outbreak of *E. coli* O157:H7 in United States identified 65% of the outbreak were due to consumption of contaminated food of animal origins, 10% through animal contacts, 10% person to person and 4% waterborne (Heiman *et al*., 2015;Altaie *et al*., 2025).

Several studies were conducted on prevalence of *E. coli* O157:H7 from different parts of the world and reported a consistence occurrence. Study conducted in Egypt on detection of *E. coli* O157:H7 from patients with gastroenteritis reported 11% prevalence with high rates in patients that have direct contact with animals(Abdel-Aziz & Eid, 2024) which is in line with study conducted in Ethiopia on *E. coli* O157:H7 among diarrheic patients and their cattle that reported 11.1% in diarrheic patient with high prevalence among under 5 children and over 64 years of age and those patients who had cattle in their house were three times more likely to have *E. coli* O157:H7 associated diarrhea than patients who did not have(Engda *et al*., 2023). Study in Nigeria on isolation of *E. coli* O157:H7 revealed 19.07% and 4.17% prevalence in diarrheic and non-diarrheic children respectively (Joseph Fuh, 2018). Similarly, study conducted in Iraq on prevalence across human, animal, and animal product samples found 21.3% high prevalence of *E. coli* O157:H7 in human samples, particularly from diarrheal patients (Altaie *et al*., 2025). Hospital based study on prevalence of *E. coli* O157:H7 and associated factors in under-five children with diarrhea in Eastern Ethiopia reported 15.3% prevalence and they revealed that children from households with livestock were four times more likely to contract *E. coli* O157:H7 than households without livestock (Getaneh *et al*., 2021).

2.7.Transmission of *E. coli* O157:H7

E. coli O157:H7 is transmitted to humans through ingestion of contaminated food and/or person-to-person contact (Lupindu, 2018). Cattle acquire the pathogen via the faecal-oral route through consumption of contaminated feed, water, or by direct contact with the environment or other animals (figure 1) (Gambushe *et al*., 2022)

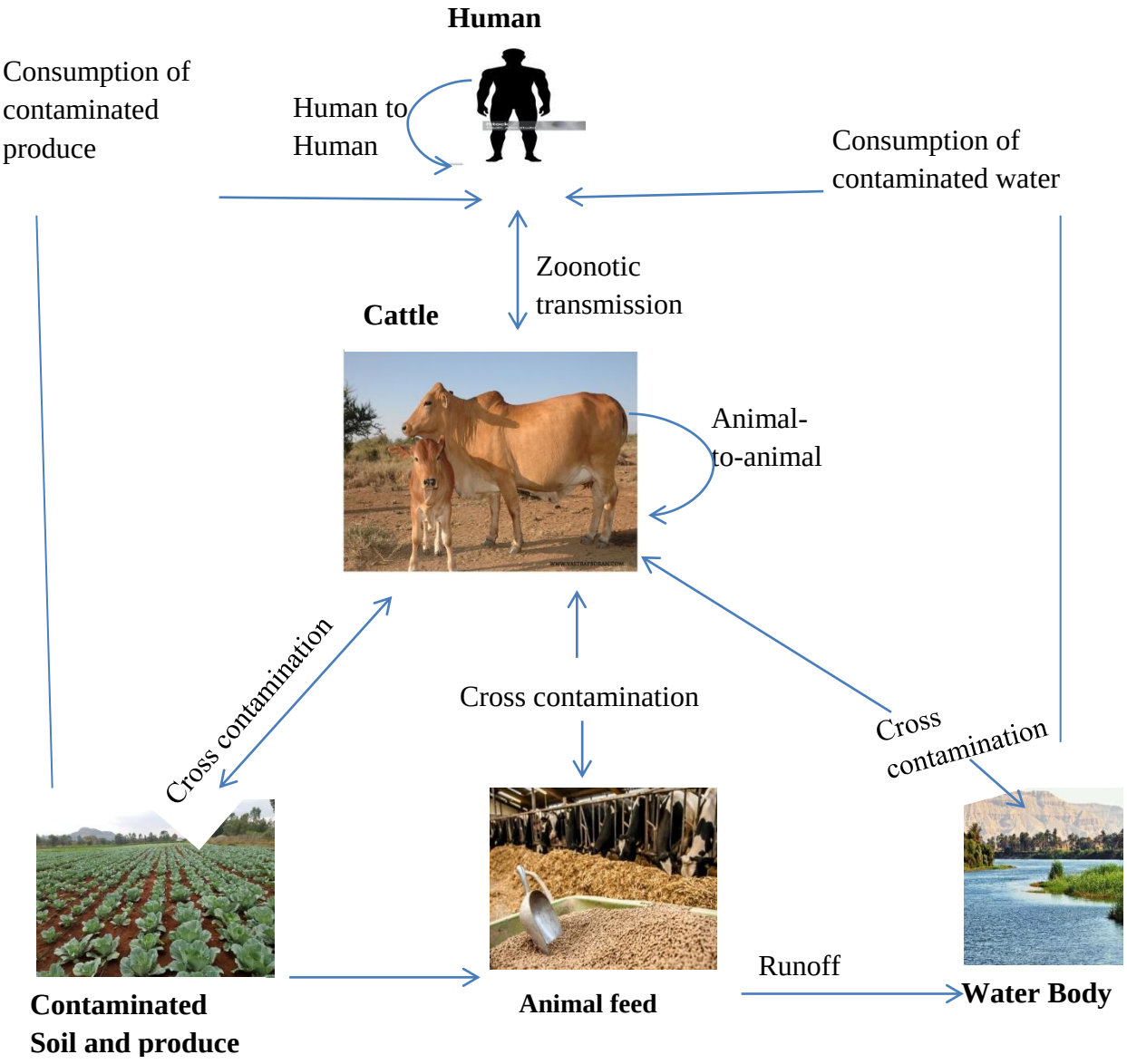

Figure 1: transmission route among human, animals and environment

Sources: (Oluwarinde *et al*., 2023)

2.8.Antimicrobial susceptibility of *E. coli* O157:H7

Antimicrobial resistance (AMR) represents a critical global health challenges, posing substantial risks to human populations, food producing animals and environments. The number of antimicrobial resistant bacteria isolated from humans, animals, and the environment has increased globally over the last two decades due to the overuse and misuse of antibiotic (Hailu *et al*., 2021). Recent study estimated that AMR causes 4.95 million deaths out of which bacterial AMR directly caused 1.27 million deaths globally in 2019 (Murray *et al*., 2022). By 2050, if no effective measures not taken to reduce the rise of drug resistance, the estimated number of deaths will increase up to 10million with more than USA $100 trillion economic output (Garg, 2024).

AMR in humans is inter-linked with AMR in farm animals and wider environment. Cross-species transmission of resistant bacteria between humans, animals and environments from which farm animals are important segment of this complex systems as they exposed to large quantities of antibiotics so act as reservoir of resistance gene (Woolhouse *et al*., 2015).

E. coli is one of the most important vectors of Antimicrobial resistance among various microbiota due to its persistent in the environment and participate in a reservoir of resistance genes that could be easily exchanged between Enterobacteriaceae in the host and the environment(Barrera *et al*., 2019). There is increasing AMR amongst *E. coli* O157:H7 isolates from various sources such as humans, milk, cattle feces, soil and water (Myataza e*t al*., 2017). There are a range of research works on the antimicrobial resistance of *E. coli* O157:H7 isolated from animals and humans have indicated that bacteria that are resistant to antimicrobial used in animals would also be resistant to antimicrobial used in humans (Gugsa *et al*., 2022; Engda *et al*., 2023).

Studies conducted in Jordan on AMR in *E. coli* O157:H7 isolates from dairy cattle revealed 85.7% of the isolates are resistant to three or more antimicrobial classes with high resistance to streptomycin(92.9%), cefalothin(85.7%) and tetracycline(78.6%)

(Obaidat & Stringer, 2019). Similarly, studies conducted in Tunisia, Ethiopia and South Africa revealed high resistance rates in cattle derived *E. coli* O157:H7 isolates to commonly used antibiotics such as cefuroxime (50%), streptomycin (30-81.8%) and tetracycline(30-81%), kanamycin (63.6%), cefoxitin (54.5%), and norfloxacin (54.5%) (Msolo *et al*., 2016;Disassa *et al*., 2017;Tayh *et al*., 2022). Multidrug resistance was also documented consistently, as 70-100% of the isolates were resistance to two or more classes of antimicrobial in cattle feces, humans and milk samples (Dejene *et al*., 2022; Tadesse *et al*., 2024; Hunduma *et al*., 2024). Tadesse *et al*., (2024) reported 93% multidrug resistant E. coli isolates in humans in contact with dairy cattle in central Ethiopia. Similarly, Engda *et al*., (2023) found 54.7% resistance to tetracycline and 43.8% to sulfamethoxazole/trimethoprim in diarrheic patients with multidrug resistance observed among 43.8% and 33.3% isolates of *E. coli* O157:H7 in diarrheic patients and their cattle respectively.

Farm environmental such as soil and water, can serve as reservoirs for antimicrobial resistance genes and further aggravate antimicrobial resistance dissemination. Hailu *et al*., (2021) reported that 73% of *E. coli* O157 isolates from manure-amended farms in the USA exhibited MDR, with complete resistance to aminoglycosides and folate antagonists. On the other hand, Veloo *et al*.,(2025) reported 12.5% of isolates from the soil samples were show MDR on study conducted on prevalence and antimicrobial resistance patterns of E. coli in the environment, cow dung, and milk of selangor dairy farms, Malesia. Study conducted in Ethiopia on antimicrobial susceptibility patterns of *E. coli* O157:H7 revealed 100% MDR from animal drinking water isolates (Mesele *et al*., 2023).

3. MATERIAL AND METHODS

3.1. Study area

The study was carried out on dairy farms in Bishoftu and Modjo towns, central Ethiopia. Bishoftu and Modjo town are located in southeast of Addis Ababa at distance of 47km and 73km, respectively in East shewa zone of Oromia region of Ethiopia **(Figure 2)**. They are recognized to have high potential for dairy production due to their strategic advantages in accessing dairy related technologies and institutional support such as improved breeding practices, advanced husbandry and management systems, animal health interventions and dairy product processing technologies, which enhances their capacity for dairy production. Dairy farms in these towns primarily supports livelihood of the inhabitants, with production systems concentrated in peri urban and urban areas. While small scale farms dominate the sector, there is a noticeable emergence of the medium scale commercial dairy farms in these areas (Mellese & Jemal, 2013;Shanko *et al*, 2020; Moje *et al.*, 2023). According to Bishoftu and Modjo towns administration bureau of urban agriculture, there are 100, 34, and 7 small, medium and large scales dairy farm respectively in Bishoftu town while 60 small scale, 20 medium and 3 large scale dairy farm in Modjo town with cross breed dominated the dairy farms in both towns. People and domestic animals share space and live in close contact, which may favor cross- species transmission of many diseases, including the foodborne pathogens.

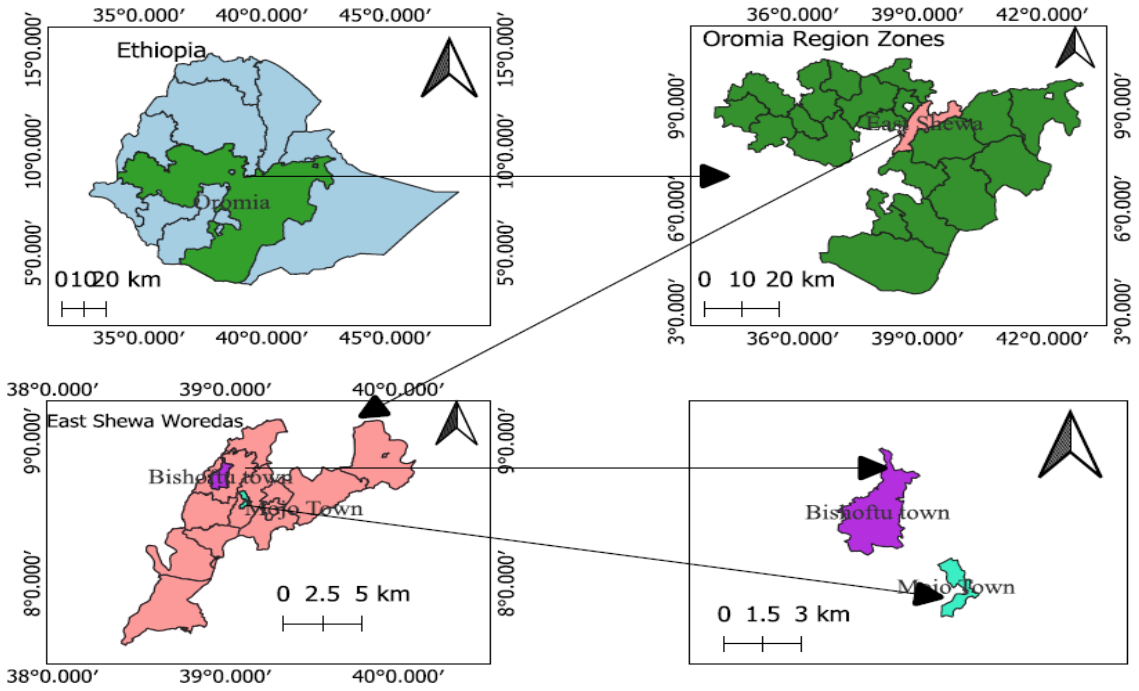

Figure 2: Map of Ethiopia and Oromia Regional State depicting the location of study areas

3.2. Study design

The study was cross-sectional that was conducted from October 2024 to May 2025 to determine the prevalence and antimicrobial resistance profile of *E. coli* O157:H7 from dairy cattle, milk, farm environment and humans in direct contact with dairy farms.

3.3. Study population and study animals

The study population consists of dairy cattle and their products, farm environment and in contact humans in Bishoftu and Mojo farms. The study animals comprise those lactating dairy cattle of local, cross and exotic breed, milk, and environment and in contact humans in randomly selected small and medium scale farm in Bishoftu and Modjo towns and humans that have direct contact with dairy farms.

3.4. Sample size estimation

The required sample size was estimated considering previous prevalence of *E. coli* O157:H7 4.7% in dairy cattle by Mesele *et al*., (2023), 2.8% from humans stool by Gutema *et al*., (2021), 4.08% in milk samples by Dejene *et al*., (2022) with consideration of 95% confidence interval and 5% precision, and the calculation was carried out using the formula given by Thrustfield (2005);

$$n = \frac{1.96^2 \, Pexp \, (1-Pexp)}{d^2}$$

Where, n = required sample size, Pexp = expected prevalence, d = desired absolute precision

A total of 69, 43 and 60 samples were collected from dairy cattle, humans and milk respectively. The sample size for water, soil, floor, milking equipment and milker hand swab conveniently determined based on the number of dairy farms sampled. Overall 382 samples (69 feces samples, 43 stool samples, 60 milk samples, 60 udder swab, 30 water samples, 30 soil samples, 30 floor swab samples, 30 milking equipment swab samples and 30 milker hand swab samples) were collected.

3.5. Sampling technique and sample collection

3.5.1. Dairy cattle

Dairy farms were identified by consulting Bishoftu and Modjo towns administration bureau of urban agriculture. The farms were categorized into three strata, small scale (<10 animals), medium scale (10 to 50 animals) and large scale (>50 animals) using the classification by Mesele *et al*., (2023) . Then, the farms were selected by simple random sampling using lottery system after providing each farm specific number. Individual cattle were selected at random and fecal samples were collected directly from rectum using disposable gloves and transferred to sterile 50 mL capacity falcon tube. Milk samples were collected directly from teats after the udder and teat were cleaned and

dried. Approximately 10ml of milk of each cow from all quarters was collected and pooled into a single sterile bottle.

3.5.1. Environmental samples

The farm floor, milking equipment and personnel hand swab were collected by sterile cotton tipped swab and put in universal bottle containing Buffered Peptone Water (BPW) (microgen, India). Pooled floor surface swab samples were collected from different parts of the pen including resting area and outlet of the pen. Similarly, pooled milking equipment swab was collected from those farms with two or more milking equipment's. Water samples were collected from animal drinking through and source of water using sterile capped universal bottle. Approximately 10g of soil free of fecal contaminations was collected into sterile vials from the barn areas of dairy farms from 2-5cm beneath the surface.

3.5.3. Stool samples

After securing the consent and collection of samples from dairy cattle and farm environment, the owners were asked for stool samples from children who had direct contact or use milk from that dairy farms and stool specimens were collected from children. Collection of stool samples was carried out by medical personnel. Approximately 10 grams of fresh stool specimen were collected into leak proof and clean stool screw cupped bottles using sterile applicator stick immediately after direct defecation.

All samples collected from dairy cattle, children and farm environments were labeled and transported in icebox to veterinary public health laboratory of College of Veterinary Medicine and Agriculture of Addis Ababa University for bacteriological analysis.

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

After arrival at the laboratory, a nonselective enrichment was initially performed on all samples to resuscitate the bacteria exposed to the stress and enable the recovery of the isolates when present at low concentration in the samples as described by OIE (2018). One gram/ml of feces, stool, soil, raw milk and water samples were diluted in 9ml of Buffered Peptone Water (microgen, India) and homogenized manually using a vortex mixer then incubated aerobically at 37 ^{O}C for 24hrs. Similarly, floor, milking equipment, and milker hand swabs samples in BPW were incubated for 24hrs at 37 ^{O}C. After carefully mixing the enrichment, the suspension was serially diluted and a loop full sample was streaked on MacConkey Agar (HIMEDIA, India) and incubated at 37 ^{O}C for 24hrs. Presumptive *E. coli* colonies smooth, circular and pink colonies were selected subcultured on Eosin Methylene Blue (EMB) Agar (HIMEDIA, India) and grown at 37 ^{O}C for 24hrs. Isolates with a green metallic sheen appearance on EMB plate were considered *E. coli*. Following that, typical colonies on EMB Agar were further subcultured on Sorbitol MacConkey Agar (HIMEDIA, India) and incubated at 37 ^{O}C for 24hrs. Non-Sorbitol fermenting colonies which were circular and colorless were considered presumptive *E. coli* O157:H7 strains whereas pinkish colored colonies (Sorbitol Fermenter) were considered non- O157:H7 *E. coli* strains (Akomoneh *et al*., 2020). Then colonies presumptively identified as *E. coli* O157:H7 were transferred to nutrient agar to be used for additional confirmatory biochemical tests.

3.7. Biochemical test identification

The suspected on Sorbitol MacConkey Agar colonies of *E. coli* isolates were further identified using biochemical tests including Indole test, Triple sugar Iron Agar test (TSI) and citrate utilization test as described by Lupindu, (2017) (Annex 4) . Isolates that were Indole positive with red ring on the top of the culture broth, Simon's citrate test negative (with no color change from green to blue) and isolates with yellow slant, yellow butt and presences of gas bubbles without hydrogen sulfide (H2S) on TSI test were considered *E. coli* isolates. Similarly, sorbitol fermentation test was conducted and isolates with no

color change from red to yellow were considered as sorbitol fermentation negative (non-sorbitol fermenter). Then, isolates were stored in deep freeze in brain heart infusion broth containing 16% glycerol. Finally, confirmation of *E. coli* O157:H7 was performed using latex agglutination test.

3.8. Serological identification of *E. coli* O157:H7

Colonies were serologically confirmed using *E. coli* O157:H7 latex test (Oxoid, England). *E. coli* O157:H7 latex contains 3 reagents. The particles in each reagent are coated with a different antibody: one against *E. coli* serotype O157, another against *E. coli* serotype H7, and the third with normal rabbit globulin to serve as control latex. A drop of Latex was dispensed into the circle of the reaction card. Using a loop, colonies were taken and added into the circle which contained latex reagent. The test latex particles were mixed with fresh colonies of *E. coli* O157:H7, and in immunochemical reactions, those undergoing agglutination within a minute were registered as positive for *E. coli* O157:H7. The absence of agglutination indicates that the test isolates were not *E. coli* O157:H7.

3.9. Antimicrobial susceptibility test

All confirmed *E. coli* O157:H7 isolates were subjected to antimicrobial susceptibility test using the standard kirby-Baeur disc diffusion assay on Muller-Hinton agar according to Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2015). Nine antimicrobial discs belonging to six antimicrobial classes commonly prescribed in veterinary medicine and public health including, streptomycin (25µg), sulphonamides (300µg), gentamycin (10µg), tetracycline (10µg), ceftazidime (30µg), oxytetracycline (30µg), sulphamethoxazole (100µg), vancomycin (30µg) and nalidixic acid (30µg) were used in this study. Around 2-3 colonies were picked from culture and suspended in 5 mL of 0.85% normal saline and the turbidity of broth culture was adjusted to 0.5 McFarland standards. Then, sterile cotton swab was dipped into the suspension and streaked on the entire surface of Muller-Hinton agar. The inoculated plates were dried at room

temperature for 3-5 minutes and antibiotic discs were placed on the inoculated Muller-Hinton agar and gently pressed using sterile thumb forceps. After 24 hours of incubation, the diameter of clear zone of inhibition was measured in mm using ruler and interpreted and determined as susceptible, intermediate, and resistant.

3.10. Multi-drug resistance

Multi-drug resistance is the ability of microorganisms, particularly bacteria, to resist the effects of multiple drugs that are typically effective against them. It defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos *et al*., 2012) and isolates that showed resistance to at least one drug in three or more antimicrobial class were considered as multi-drug resistant isolates.

3.11. Data collection

After securing the consent, information such as age, contact with animals, water sources, hand washing practice, eating raw meat, drinking raw milk, eating raw vegetables and use of latrine were collected using structured checklist by asking take giver or family. During sampling, the farm husbandry practices evaluated including identification number, sampling date, age, sex, type of sample, farm scale, breed, floor type, milking place, calf rearing place, cleaning of pens, hand washing and udder cleaning practice during milking, manure disposal areas were documented on recording format with a structured checklist and by direct observations.

3.12. Data management and analysis

All data were recorded on data collection sheet prepared for this purpose and entered to excel spread sheet and checked for completeness and analyzed using STATA version 14 (college station, Texas, 77845, USA). Descriptive analyses were used to compute frequency and proportion. The overall prevalence of *E. coli* O157:H7 in dairy cattle, milk, dairy farm environments and in contact children was determined using descriptive

analysis. The association between the outcomes and independent variables such as herd size, floor type, pen cleanness, manure disposal, age and others was analyzed using chi-square (X^2) test at p value <0.05 and 95% confidence interval.

3.13. Ethical consideration

The study received ethical approval from College of Veterinary Medicine and Agriculture and Aklilu Lemma Institute of Pathobiology, Addis Ababa University of research ethics and review committee (Ref. No: VM/ERC/04/69/17/2025) (Annex 8). Before collecting the samples, the objectives of the study were briefed to the farmers were and asked for verbal agreement to collect samples from cows, farm environments and in contact human. Further information obtained were kept private and confidential

4. RESULTS

4.1. Overall prevalence of *E. coli* O157:H7

In this study, out of 382 samples collected and processed from dairy cattle, milk, farm environments and children the overall prevalence of *E. coli* O157:H7 was found to be 3.9% (15/382) based on Latex Agglutination assay. As presented in Table 1, the highest prevalence was observed in soil samples 2(6.7%) and floor swab 2(6.7%) followed by fecal samples 4(5.7%) and children stool samples 2(4.6%) but the difference in *E. coli* O157:H7 prevalence among different samples was not statistically significant ($p>0.05$) ($X^2=4.4610$, $p=0.725$). There was no occurrence of *E. coli* O157:H7 observed in hand swab and material swab in the current study.

Table 1: Prevalence of *E. coli* O157:H7 in different sample types

Sample sources	**Categories**	**N^0 tested**	**N^0 (%)positive**	**X^2**	**p-value**	**(95% CI)**
Dairy cattle	Feces	69	4(5.7)	4.4610	0.725	.0213818- .1477213
	Milk	60	2(3.3)			.0080138 -.1283032
	Udder swab	60	2(3.3)			.0080138 -.1283032
Humans	Stool	43	2(4.6)			.0109966 -.1762825
	Hand swab	30	0(0.0)			-
Environment	Water	30	1(3.3)			.0041394-.2224335
	Soil	30	2(6.7)			.0153435 -.2466589
	Floor swab	30	2(6.7)			.0153435 -.2466589
	Equipment swab	30	0(0.0)			-
Overall		**382**	**15(3.9)**			

4.2. Association of risk factors for occurrence of *E. coli* O157:H7 in cow feces and milk samples

The analysis of risk factors related to the occurrence of *E. coli* O157:H7 in cattle feces showed that there was statistically significant association ($p<0.05$) with floor type, cleaning frequency of the pen, pen cleanness and manure management sites. However, no statistically significant association ($p>0.05$) was found between occurrence of *E. coli* O157:H7 and factors such as sample collection area, age, farm scale, breed and rearing place. The prevalence of *E. coli* O157:H7 in milk samples showed a statistically significant association ($p<0.05$) with factors like floor type, ways of udder cleaning, hand washing practice and manure management sites while there was no statistically significant association with sample collection area, age, farm scale, breed, milking and rearing place (Table 2).

Table 2: Factors affecting occurrence of *E. coli* O157:H7 in fecal and milk samples collected from dairy farms

Risk factors	**Categories**	**Feces samples**			**Milk samples**		
		N^0 (%) positive	**X^2**	**p-value**	**N^0 (%) positive**	**X^2**	**p-value**
Site	Bishoftu	2(5.6)	0.0080	0.929	1(4.3)	0.1191	0.730
	Modjo	2(6.1)			1(2.7)		
Age	3-6 years	3(7.0)	0.3826	0.826	0(0.0)	4.8276	0.089
	6-9 years	1(4.3)			2(12.5)		
	>9 years	0(0.0)			0(0.0)		
Farm scale	Small	4(8.3)	1.3882	0.239	1(2.0)	1.1638	0.281
	Medium	0(0.0)			1(8.3)		
Breed	Cross	3(7.1)	1.9667	0.374	1(2.9)	0.7835	0.676
	Local	1(12.5)			0(0.0)		
	Exotic	0(0.0)			1(6.25)		
Floor type	Concrete	1(1.8)	6.3996	0.011	0(0.0)	5.6897	0.017

	Soil	3(18.7)			2(12.5)		
Milking	In barn	3(6.1)	0.0328	0.856	2(4.8)	0.8867	0.346
place	Out of barn	1(5.0)			0(0.0)		
Rearing	In barn	3(7.3)	0.4274	0.513	1(2.4)	0.3214	0.571
place	Out of barn	1(3.5)			1(5.3)		
Udder	Using water	3(7.5)	0.5054	0.477	2(11.7)	6.7980	0.009
cleaning	Using towel and detergents	1(3.4)			0(0.0)		
Hand	Before and	2(3.9)	1.2593	0.262	0(0.0)	5.2333	0.022
washing	after milking						
practice	Only rarely	2(11.1)			2(16.7)		
Cleaning of	Once a per	3(27.2)	20.093	0.000	0(0.0)	5.2333	0.073
pen	day		4				
	Twice per day	1(5.0)			2(11.7)		
	Frequently	0(0.0)			0(0.0)		
Pen	Poor	4(16.6)	7.9615	0.019	2(11.1)	4.8276	0.089
cleanness	Moderate	0(0.0)			0(0.0)		
	Good	0(0.0)			0(0.0)		
Manure	In barn	3(42.8)	20.093	0.000	2(18.2)	13.448	0.001
disposal	In compound	1(4.3)	4		0(0.0)	3	
	Out of compound	0(0.0)			0(0.0)		

4.3. Association of risk factors with *E. coli* O157:H7 occurrence in udder of milking cows

The occurrence of *E. coli* O157:H7 in udder swab indicated that there was statistically significant association ($p<0.05$) with floor type, pen cleaning frequency, ways of udder cleaning and manure management sites (Table 3)

Table 3: Results of descriptive analysis of association of risk factors with occurrence of *E. coli* O157:H7 in udder of milking cows

Risk factors	Categories	N^0 positive (%)	X^2	p-value
Area	Bishoftu	1(3.4)	0.0023	0.962
	Modjo	1(3.2)		
Farm scale	Small scale	1(20.0)	1.1638	0.281
	Medium scale	1(8.3)		
Floor type	Concrete	0(0.0)	6.2069	0.013
	Earthen	2(13.3)		
Pen cleaning frequency	Once a day	2(13.3)	6.2069	0.045
	Twice a day	0(0.0)		
	Frequently	0(0.0)		
Manure disposal	In barn	2(25.0)	13.448	0.001
	In compound	0(0.0)		
	Out of compound	0(0.0)		
Cleaning of udder	Using water	2(10.5)	4.4646	0.035
	Using towel & detergents	0(0.0)		

4.4. Association of risk factors with *E. coli* O157:H7 occurrence in soil and water

Out of 30 soil samples examined 6.7% of them were found to be contaminated with *E. coli* O157:H7.The prevalence of *E. coli* O157:H7 in soil samples was significantly associated with floor type and manure disposal site of the dairy farms. There was more likelihood of occurrence 2(25%) of earthen floor type as compared to those dairy farms with concrete floor type (0.0%) (X^2=5.8929, p=0.015). Conversely, there was no statistically significant association with sample collection area, farm scale and pen cleanness. There was no statistically significant association with risk factors like sample collection area, farm scale, floor type except manure management which had a significant association ($p<0.05$) (X^2=9.3103, p=0.010) with occurrence of *E. coli* O157:H7 in water samples from animal drinking through (Table 4).

Table 4: Association of risk factors with occurrence of *E. coli* O157:H7 in soil and water samples

Risk factors	Categories	Soil samples			Water samples		
		N^0 (%) positive	**X^2**	**p-value**	**N^0 (%) positive**	**X^2**	**p-value**
Area	Bishoftu	0(0.0)	1.8750	0.171	1(7.1)	1.1823	0.277
	Modjo	2(12.5)			0(0.0)		
Farm scale	Small scale	1(4.2)	1.2054	0.272	1(4.2)	0.2586	0.611
	Medium scale	1(16.7)			0(0.0)		
Floor type	Concrete	0(0.0)	5.8929	0.015	0(0.0)	1.5517	0.213
	Earthen	2(25.0)			1((8.3)		
Pen cleanness	Poor	2(18.2)	3.7013	0.157	1(10.0)	2.0690	0.355
	Moderate	0(0.0)			0(0.0)		
	Good	0(0.0)			0(0.0)		
Manure disposal	In barn	2(50.0)	13.9286	0.001	1(33.3)	9.3103	0.010
	In compound	0(0.0)			0(0.0)		
	Out of compound	0(0.0)			0(0.0)		

4.5. Association of risk factors with *E. coli* O157:H7 occurrence in stool samples

In this study, different variables were considered as risk factors for *E. coli* O157:H7 infection in humans. Risk factors such as age, drinking of raw milk, history of diarrhea and hand washing practice were found to be statistically significantly associated with *E. coli* O157:H7 occurrence in children. However, risk factors like contact with animals, water sources, eating raw meat and vegetables had no statistically significant association with *E. coli* O157:H7 infection in humans (Table 5).

Table 5: Associated risk factors of occurrence *E. coli* O157:H7 among children

Risk factors	Categories	N^0 tested	N^0 (%) positive	X^2	p-value
Age	<2 years	10	0(0.0)	12.9350	0.002
	2-5years	27	0(0.0)		
	>5-10years	6	2(33.3)		
Hand washing	Not always	6	2(33.3)	12.9350	0.000
	Frequently	37	0(0.0)		
History of eating raw meat	Yes	9	1(11.1)	1.0711	0.301
	No	34	1(3.0)		
Drinking raw milk	Yes	10	2(20.0)	6.9220	0.009
	No	33	0(0.0)		
Eating raw vegetables	Yes	13	1(3.3)	0.3886	0.533
	No	30	1(7.6)		
History of diarrhea	Yes	8	2(25.0)	9.1768	0.002
	No	35	0(0.0)		
Contact with animals	Yes	24	2(10.5%)	2.6496	0.104
	No	19	0(0.0)		

4.6. Antimicrobial susceptibility profiles of *E. coli* O157:H7 isolates

The result of antimicrobial sensitivity test to selected drugs revealed that all of the 15 *E. coli* O157:H7 isolates identified in this study 15(100%) were susceptible to nalidixic acid while all of the isolates 15(100%) were resistance to ceftazidime. In addition, higher percentages of the isolates were resistant to Vancomycin 12(80%), gentamycin 9(60%), tetracycline 8(53.3%) and sulfonamides 7(46.7%) as presented in Table 6.

Table 6: The results of antimicrobial susceptibility profiles of *E. coli* O157:H7 isolated from different samples

Antimicrobial agents	**Status of antimicrobial agents to the isolates**		
	SusceptibleN^0 (%)	IntermediateN^0 (%)	ResistanceN^0 (%)
Streptomycin	8(53.3)	0(0.0)	7(46.7)
Gentamycin	5(33.3)	1(6.7)	9(60.0)
Tetracycline	5(33.3)	2(13.3)	8(53.3)
Nalidixic acid	15(100.0)	0(0.0)	0(0.0%)
Vancomycin	0(0.0%)	3(20.0)	12(80.0)
Sulphamethoxazole	7(46.7)	2(13.3)	6(40.0)
Ceftazidime	0(0.0%)	0(0.0%)	15(100.0)
Sulfonamides	5(33.3)	3(20.0)	7(46.7)
Oxytetracycline	7(46.7)	0(0.0)	8(53.3)

This study revealed that multi-drug resistance or resistance to more than three different classes of drugs was observed among 86.7% *E. coli* O157:H7 isolated from fecal, milk, human, floor swab, soil, udder swab and water samples. Among the *E. coli* O157:H7 isolates tested 5(33.3%) showed resistance to eight drugs belonging to five antimicrobial classes (sulfonamides, tetracycline, glycopeptide, aminoglycoside, cephalosporin) and 4(26.7%) isolates resistant to three drugs belong to three antimicrobial classes (glycopeptide, cephalosporin, aminoglycoside). None of the isolates were resistant to seven drugs composition (Table 7).

Table 7: MDR profiles of *E. coli* O157:H7 isolates from different samples

Drug class	**Multi-drug resistance profile**	**Sample sources of MDR**	**N^0 (%)**
Glycopeptide, cephalosporin, aminoglycoside	VAN∗CAZ∗GEN	Soil, children, floor swab	4(26.7)
Sulfonamides, glycopeptide, cephalosporin	S3∗ VAN∗CAZ	Fecal	1(6.7)
Tetracycline, aminoglycoside, cephalosporin	TE∗S∗OT∗CAZ	Soil	1(6.7)
Tetracycline, glycopeptide aminoglycoside, cephalosporin	TE∗VAN∗OT∗S∗CAZ	Water	1(6.7)
Sulfonamides, tetracycline, glycopeptide, cephalosporin	S3∗TE∗VAN∗OT∗CAZ∗RL	Fecal	1(6.7)
Sulfonamides, tetracycline, glycopeptide, aminoglycoside cephalosporin	S3∗TE∗VAN∗OT∗GEN∗S∗CAZ∗RL	Children, milk fecal, udder swab	5 (33.3)
	Overall MDR	-	13(86.7)

S3-sulfonamides, CAZ- ceftazidime, VAN- vancomycin, GEN-gentamycin, TE-tetracycline, S-streptomycin, OT-oxytetracycline, RL-sulfamethoxazole

5. DISCUSSION

The Ethiopian government has prioritized dairy production to achieve food security through its so called "*Ye Lemat Tirufat*" initiative. Hence, expansion of dairy farms of all scales is ongoing. However, such activities are not without drawbacks. In the absence of stringent biosecurity and understanding of contamination of dairy products, increased production and supply of dairy products could contribute to spread of infection to consumers and personnel involved along the value chain. Understanding of the sources of contamination by foodborne pathogens such *E. coli* O157:H7 is crucial for devising effective preventive measures. This study provides information on the occurrence of *E. coli* O157:H7 in dairy cattle, milk, the environment, personnel and water sources in Bishoftu and Mojo towns, two of the major dairy sheds in the country.

The current study revealed the overall *E. coli* O157:H7 prevalence of 3.9% from different dairy cattle, in contact humans and dairy farm environment and water samples. The finding was in agreement with previous studies reported by Hunduma *et al*., (2024) and Mesele *et al*.,(2023) who reported overall prevalence of 3.9% and 4.7%, respectively from dairy cattle feces, milk and farm environment in Ethiopia. The current finding showed lower prevalence of *E. coli* O157:H7 compared to reports of Dejene *et al*., (2022) who reported overall prevalence of 6% from milk, milker hand swab and water in dairy farm in central Ethiopia and reports of Altaie *et al*., (2025) who reported 7.9% detection rate of *E. coli* O157:H7 in sample from humans, animal feces and animal products from Kirkuk province, Iraq. The difference observed in the prevalence between the current study and the previous ones could be due to the difference in the management systems and agro-ecology, which can affect the survival and multiplication of the bacterium in the environment and its spread along the value chain.

Upon this study, from different sample types examined the prevalence of *E. coli* O157:H7 was higher in soil samples (6.7%) and floor swab (6.7%) followed by fecal samples (5.7%) and children stool (4.6%). The study result showed the 6.7% prevalence of *E. coli* O157:H7 in soil samples was in line with the reports of Gemeda *et al*., (2023)

and Vanitha *et al*., (2018) who reported 6.8% in soil from homestead and animal barn in Ethiopia and 8.3% in soil samples from dairy farm environment in India respectively. The result of the study were not in accordance with Mohammed & Mustafa, (2015) who recorded higher prevalence of 68% in soil samples from different direction of dairy farms in Iran. On the other hand, the current result were slightly higher than the findings of Sekhar & Hirbaye, (2024) who reported 4% prevalence of *E. coli* O157:H7 in soil samples fertilized with bovine manures in Negele, Ethiopia. Soil could be contaminated due to direct shedding of *E. coli* O157:H7 onto the land by animals. Previous study indicated that compounds with unrestricted animal movement exhibit higher *E. coli* O157:H7 levels compared to those without animals (Ercumen *et al.*, 2017). The potential sources of *E. coli* O157:H7 in soils may include the use of poorly decomposed organic fertilizers, feces from livestock and wild animals, runoff from animal waste piles, and pathogen-laden dust deposited on the land (Huang *et al*., 2020). Manure can serve as the main sources of pathogen dissemination on dairy farms and broader environments (FAO & WHO, 2022) and sewage from cattle barn could result in contamination of surrounding land(Mesele *et al*., 2023) so soil in nearby dairy farm has high chance for *E. coli* O157:H7 contamination in farms with poor manure management practices.

The survival of *E. coli* O157:H7 on pen floors and in the immediate environment of animals is crucial for infection, as contaminated pen floors are significant sources that affect the dynamics of *E. coli* O157:H7 population (Faraj & Wirtu, 2021). In this study, the prevalence of *E. coli* O157:H7 from floor swab was found to be 6.7%, which is slightly higher than the 2.5% occurrence rate reported by Yordanos *et al*., (2024) for environmental swabs taken from dairy farms in Wolaita Sodo town, Ethiopia. Contamination of pen floor with *E. coli* O157:H7 starts when infected cattle execrate the bacterium that adhere to the floor surfaces and spread across the floor through footwear, equipment, rodents and water runoff during cleaning practices (Beauvais *et al*., 2018).

Drinking water for animals also identified as a potential source of *E. coli* O157:H7 contamination on dairy farms (Mesele *et al*., 2023) and contaminated drinking water was identified as the primary transmission route of *E. coli* O157:H7 to cattle, playing a critical

role in disseminating the pathogen within herds (Faraj & Wirtu, 2021). Result of the present study also revealed that 3.3% water samples from animal drinking through were contaminated with *E. coli* O157:H7 in study area which is in line with the 1.5% and 2.4% prevalence documented by Benjamin *et al*., (2015) and (Faraj & Wirtu, 2021) in water from drinking through cattle ranches in California and USA, respectively. However, the result of this study is much lower than the reports of Vanitha *et al*., (2018) who documented 11.11% of *E. coli* O157:H7 contamination of water from dairy farm environment from India and Mesele *et al*., (2023) who reported 8% contamination rate of animal drinking water in Ethiopia. Contamination of drinking trough with fecal matter from dairy cows may cause frequent detection of *E. coli* O157:H7 in cattle water troughs, indicates that sediments and biofilms within these troughs may act as environmental reservoirs for the bacteria (Beauvais *et al*., 2018). The above mentioned studies and the current result highlight the proper handling of animal drinking water and cleaning of drinking trough has been suggested to reduce load of *E. coli* O157:H7 in water trough.

Cattle are significant reservoirs of *E. coli* O157:H7, typically carrying it asymptomatically in their intestines and intermittently shedding it in their feces (Lange *et al.*, 2022). In the present study, a 5.7% occurrence rate of *E. coli* O157:H7 was recorded in fecal samples from dairy cows. This is in line with findings of Hayet *et al*., (2021) who reported 6.4% prevalence of *E. coli* O157:H7 were reported from cattle fecal samples in Addis Ababa, Ethiopia. Comparable prevalence were also reported by Atnafie *et al*., (2017) 4.7% prevalence of *E. coli* O157:H7 in cattle feces from Hawasa, Ethiopia, by Tayh *et al*., (2022) 4.2% from Tunisia, by Hunduma *et al*., (2024) and Mesele *et al*., (2023) reported similar prevalence of 3.9% in dairy cattle feces under pastoral production systems and lactating dairy cows in Ethiopia. On the other hand, the result of current study showed higher occurrence of *E. coli* O157:H7 in dairy cows when compared with reports by Altaie *et al*., (2025) 2.85% from Iraq, Mohammed, (2023) 2.5% from Nigeria, Birdal and Ak (2018) 1.04% from Turkey, Al-Ajmi *et al*., (2020) 1.46% from United Arab Emirates and Faraj & Wirtu (2021) 1.5% from USA. Conversely, compared with present study, higher prevalence of *E. coli* O157:H7 have been reported 11.4% from Ethiopia (Gemeda *et al*., 2023), 19.8% from India (Vanitha *et al*., 2018) and 75% from

Iraq (Mohammed & Mustafa, 2015). These data indicated that dairy cows can serve as a reservoir for *E. coli* O157:H7 even though the occurrence rate of *E. coli* O157:H7 vary in different studies which may due to various factors such as climate and environmental conditions, farm management practices, farming systems, study population dynamics (breed, age group, health status) and detection methods.

Raw milk is a vehicle for *E. coli* O157:H7 transmission and consumption of unpasteurized milk is important transmission route to humans (Obaid *et al*., 2022). The isolation rate of *E. coli* O157:H7 in milk samples in current study was 3.3% and the finding was slightly in agreement with different reports from the country such as that of Disassa *et al*., (2017) 2.9% from Asosa, Hunduma *et al*., (2024) 2.6% from Borana Zone, Geresu, (2021) 3.1% from Arsi, Dejene *et al*., (2022) 4.08% from Bishoftu and Mesele *et al*., (2023) 4.5% from Adami Tulu district. But, the isolation rate was far lower when compared to reports of Abebe *et al*., (2023) 16.7% in Dessie, Gugsa *et al*., (2022) 12.4% in Mekele, Bedasa *et al*., (2018) 12% in Bishoftu, Ariyanti *et al*., (2022) 15.6% in Indonesia, Vanitha *et al*., (2018) 8.8% in India, and Msolo *et al*., (2016) 11% from South Africa. On the other hand, the current result was higher than the prevalence recorded by Sarba *et al*., (2023) 0.2% from Bako Ethiopia and Ghali-Mohammed *et al*., (2023) 2.3% from Nigeria. These mentioned studies and the present result collectively highlights the role of raw milk as a potential vehicle for transmission of *E. coli* O157:H7. Contamination of milk with *E. coli* O157:H7 may occur during or after the milking process as a result of contact with contaminants such as animal feces, soil, air, water, feed, equipment, the outer surfaces of the udder and teats, animal hides and humans (Ababu *et al*., 2020; Altaie *et al*., 2025). The variation in *E. coli* O157:H7 contamination proportion of current finding from the mentioned study could be attributed to difference in sample size, hygiene practices, milking procedure and diagnostic method used.

The present study found that the prevalence of *E. coli* O157:H7 from udder swab sample was 3.3% which is lower than the reports of Msolo *et al*., (2016) who found the 55% prevalence of *E. coli* O157:H7 from cattle udder swab. The variation could be due to difference in detection methods, farm management practices and sample sizes. On the

other hand studies indicated that dirty udder contributes to favorable condition for multiplication of the pathogen (Ababu *et al*., 2020) this result in contamination of milk during milking process.

The prevalence of *E. coli* O157:H7 in children stool obtained in the current study is 4.6%. This is comparable with previous study conducted in Bahir Dar city and Bishoftu town, Ethiopia which recorded a prevalence of 6.1% and 2.8% from diarrheic patients(Gutema *et al*., 2021; Balew & Kibret, 2023). Contrarily, the prevalence of *E. coli* O157:H7 in current study was lower compared to reports from Iraq 21.33% (Altaie *et al.*, 2025), from Ethiopia 15.3% (Getaneh *et al*., 2021), and reports of 11% prevalence in Egypt (Abdel-Aziz & Eid, 2024). On the other hand, the result is higher when compared to 1.39% prevalence recorded by Joseph Fuh, (2018) from non-diarrheal children in Nigeria and 0% in human stool from Turkiye (Apan *et al*., 2018). The difference observed in the prevalence between the current study and the previous ones could be due to the difference in sample size, study population (diarrheic versus non- diarrheic), detection methods, dietary habits and hygienic practices in the study area.

The analysis of risk factors related to the occurrence of *E. coli* O157:H7 in cattle feces indicated that there was statistically significant association ($p<0.05$) with floor type, cleaning frequency of the pen, pen cleanness and manure management sites. These findings were in agreement with finding of Mesele *et al*., (2023) who reported floor type and cleaning of pens as significantly associated factors to occurrence of *E. coli* O157:H7 in dairy farm. Pen floor can influence the occurrence of *E. coli* O157:H7 in dairy cattle in which muddy or soil environments may promote fecal- oral transmission of enteric agents (FAO & WHO, 2022). Manure can serve as the source of *E. coli* O157:H7 so improper disposal of manure increase the risk of infection. In present study, manure disposal site shows significant association with occurrence of *E. coli* O157:H7 in which high prevalence occurred in farm disposing manure in the cattle barn. This may occur pathogen can be transmitted back to the cattle through contamination of water and feed. Similarly, pen cleanness and cleaning frequency shows significant association with occurrence of *E. coli* O157:H7 in dairy cattle feces with high prevalence in farms with

poor cleanness. Factors related to poor hygienic practices were found to influence the occurrence of bacteria (Mesele *et al*., 2023). In current study, the prevalence of *E. coli* O157:H7 in milk samples showed a statistically significant association ($p<0.05$) with factors like floor type, ways of udder cleaning, hand washing practice and manure management sites. In previous study it was indicated that milking practices such as washing and drying of udder have a significant role in the highest prevalence of *E. coli* O157: H7 (Ababu *et al*., 2020) might support the findings.

The prevalence of *E. coli* O157:H7 in soil sample was significantly associated with floor type and manure disposal site of the dairy farms. There was more likelihood occurrence (25%) of earthen floor type as compared to those dairy farms with concrete floor type (0%). Earthen floor tends to retain moisture content more than concrete floor which can lead to higher survival rate, proliferation and may create favorable environment for persistence of the pathogen from which *E. coli* O157:H7 contaminate the soil around the farms with workers footwear, equipment and water runoff. Concrete surfaces are generally, easier to clean and removal of waste and contaminants (Islam *et al*., 2020). Manure disposal in barn more likelihood for occurrence of *E. coli* O157:H7 in soil may due to farms dispose the dung in the barn may allow the pathogen to remain concentrated in the area, encouraging their survival and potential transmission to soil through workers footwear and equipment.

Out of risk factors observed on occurrence of *E. coli* O157:H7 in children, risk factors such as age, drinking of raw milk, history of diarrhea and hand washing practice were found statistically significant association with *E. coli* O157:H7 occurrence in children. The current result found that the prevalence in children with history of diarrhea (25%) were higher prevalence as compared to children with no history of diarrhea (0%) and the findings was agree with reports of Joseph Fuh, (2018) from Nigeria, Zelelie *et al*., (2023) from Ethiopia, Altaie *et al*., (2025) from Iraq who reported higher occurrence rate of *E. coli* O157:H7 in diarrheic than non-diarrheic individuals. High prevalence in diarrheic than non-diarrheic individuals could indicate the clinical importance of *E. coli* O157:H7. In present study occurrence of *E. coli* O157:H7 was higher (33.3%) in age groups

between 5-10 years compared to age groups <2 and 2-5 years which could be these age groups more likely consume a wide variety of food including raw dairy products, common vehicles for *E. coli* O157:H7 transmission and as age increases the exposure to various infection sources increases (Altaie *et al*., 2025). Drinking of raw milk also has an association with *E. coli* O157:H7 occurrence in children drinking raw milk (20%) than children not consume raw milk (0%). The finding agree with Kirchner *et al*., (2013) from Germany who reported significant association of consumption of raw milk with confirmed infection in school children. Hand washing practice also found to be significant risk factors for *E. coli* O157:H7 occurrence in children with high prevalence (33.3%) in children not frequently washing their hands than those who wash their hands frequently. Reports indicated that proper hand washing before meals and after defecation can lower exposure of children to enteric pathogens (Wolde *et al*., 2021).

AMR represents a critical global health challenges, posing substantial risks to human populations, food producing animals and environments (Hailu *et al*., 2021). There is increasing AMR amongst *E. coli* O157:H7 isolates from various sources such as humans, milk, cattle feces, soil and water (Myataza e*t al*., 2017). In present study, isolates from different samples like children stool, milk, udder swab, cattle feces and environmental samples such as water and soil showed drug resistance to at least to two antimicrobials. All of 15 *E. coli* O157:H7 isolates were susceptible to nalidix acid which is in line with findings of Sarba *et al*., (2023), Gemeda *et al*., (2023) and Mesele *et al*., (2023) who documented 100% susceptibility of *E. coli* O157:H7 isolates to nalidixic acid. Contrarily, all *E. coli* O157:H7 isolates (100%) were resistance to ceftazidime which in line with studies conducted in Uganda and Nigeria who reported 100% of resistance to ceftazidime by Odongo *et al*., (2020) and Ajuwon *et al*., (2021), respectively but disagree with study conducted in Tunisia on prevalence and antimicrobial resistance profile of *E. coli* O157:H7 from health animals that recorded more than 80% of isolates susceptible to Ceftazidime (Tayh *et al*., 2022). On present study 80%, 60%, 53.3%, 46.7%, 46.7% and 40% of the isolates were resistant to Vancomycin, Gentamycin, tetracycline, sulfonamides, streptomycin and sulphamethoxazole respectively. The finding was in agreement with reports of Ababu *et al.*, (2020) who revealed 45.45% and 54.54% of

isolates resistant to streptomycin and sulphamethoxazole respectively, Yihunie *et al*., (2024) who documented 57.9% of the isolates resistant to tetracycline and gentamycin. The finding slightly disagree with resistance rate reported by Disassa *et al*., (2017) 81.8% resistance to streptomycin and tetracycline.

MDR, defined as resistance to at least one agent in ≥3 antimicrobial classes, is known to be one of the most serious global health challenges with MDR pathogens emerging across the globe (Magiorakos *et al.*, 2012; Catalano *et al*., 2022). The emergence of MDR *E. coli* O157:H7 become a thoughtful public health problem (Su *et al*., 2021). This study revealed that MDR to three and more than three classes was observed among 86.7% of *E. coli* O157:H7 isolated from fecal, milk, human, floor swab, soil, udder swab and water samples. This aligns with findings from Jordan where Obaidat & Stringer, (2019), reported 91.7% of *E. coli* O157:H7 isolated from dairy farm were resistance to three or more antimicrobial classes. Dejene *et al.*, (2022) and Mesele *et al*., (2023) revealed 100% MDR of *E. coli* O157:H7 isolates from raw milk, water, manure and milker hand swab in Ethiopia. Similarly, Hunduma *et al*., (2024) observed MDR in 70% of the *E. coli* O157:H7 isolates from fecal and milk samples of cows on study conducted in pastoral area of Ethiopia. Hailu *et al*., (2021) reported that 73% of *E. coli* O157 isolates from manure-amended farms in the USA exhibited MDR. Contrarily, Engda *et al.*, (2023) found multidrug resistance among 43.8% and 33.3% isolates of *E. coli* O157:H7 in diarrheic patients and their cattle respectively and Tayh *et al*., (2022) reported no multi-drug-resistance on study conducted in healthy cattle in Tunisia which are much lower than current finding.

In present study, the higher rate of multi drug resistance was observed among *E. coli* O157:H7 isolates in which 33.3% isolates showed resistance to eight drugs belong to five antimicrobial classes followed by 26.6% isolates resistant to three drugs belong to three antimicrobial classes and 6.7% isolates resistant to four, five and six drugs each. This was not in consistent with findings of Dejene *et al*., (2022) who documented the higher rate of multidrug-resistance for four drugs (29.63%) followed by six (18.52%), three (18.52%) and two (14.81%) drugs.

Current study observed high occurrence of AMR and MDR in *E. coli* O157:H7 isolates from different sample sources in the study area and detection of resistance isolates in children, dairy cattle, milk, soil and water indicates interconnected transmission path ways of resistant strains. Resistance originate in human, animal and farm environments will lead to dissemination of infection with resistance bacteria in wider environments (Woolhouse *et al*., 2015) The high resistance rate to important antibiotics highlights the emerging threat of AMR in human as well as dairy environments. These observed findings correlate with global trends of increasing AMR in *E. coli* O157:H7 (Myataza e*t al*., 2017).

6. CONCLUSSION AND RECOMMENDATIONS

Escherichia coli O157:H7 is detected in cattle, farm environment such as soil, pen floor, water and milk samples, implying that these can serve as source of contamination to milk and infection to humans. The current study also revealed the occurrence of *E. coli* O157:H7 in, in contact children at Bishoftu and Modjo town. Factors such as floor type, pen cleanness, manure management sites, ways of udder cleaning and hand washing practices are significantly associated with the occurrence of *E. coli* O157:H7 in dairy cows, soil and farm water in dairy farms. On the other hand, drinking of raw milk, history of diarrhea and hand washing practices were the factors that are significantly associated with its occurrence in children. In addition, *E. coli* O157:H7 isolates exhibited resistance to commonly used drugs including multi- drug resistance.

Based on the above conclusion the following recommendations are forwarded:-

- Training should be given to farm owners and workers on the dairy farm hygienic practices including on manure management, pen cleanness, udder hygiene, good milking practices to prevent *E. coli* O157:H7 survival and transmission in dairy farms
- There should be awareness creation on the risk of raw milk of consumption for public health to prevent *E. coli* O157:H7 transmission through contaminated raw milk
- Rational use of drug should be encouraged to reduce the risk of antimicrobial resistance through strict regulation, creating awareness on rational drug usage and health professionals should take antimicrobial resistance into account in their practices

Study limitation

Study has limitation in characterizing and identifying antimicrobial resistance genes due to lack of primer. The study also lack to gene sequencing to identify the strains that rotate between human, animal and environment due to lack of resources.

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8. ANNEXES

Annex 1: Data collection format for dairy farm

No	Date	Type of samples	Herd size	Breed	Age	Floor type	Milking place	Rearing place	Hand washing practice	Cleaning of pen	Udder cleaning	Pen cleannness	Manure disposal

Annex 2: Data collection format from humans

No	Date	Age	Contact with animals	Water sources	Hand washing practice	Eating raw meat	Drinking raw milk	Eating raw vegetables	History of diarrhea	Use of latrine

Annex 3: Preparation media used for isolation, biochemical and antibiotic sensitivity tests

1. Buffered peptone water (DM1614-500g, microgen, India)

Preparation: The medium was prepared according to the manufacturer's instructions in which 20mg powdered medium were dissolved in 1Liter of distilled water and well mixed and sterilized by autoclaving at 121 ^{O}C for 15 minutes.

2. MacConkey agar (MH081-500g, HIMEDIA, India)

The medium was prepared according to the manufacturer's instructions whereby 49.53 g of the medium dissolved in 1Liter of distilled water boiled to dissolve completely and sterilized by autoclaving at 121 ^{O}C for 15 minutes then cooled to 45 ^{O}C and poured to sterilize petri dishes after which incubated for 24 hrs at 37 ^{O}C to check sterility.

3. Eosin Methylene Blue (M317-500g, HIMEDIA, India)

The medium was prepared according to the manufacturer's instructions whereby 35.96g of the medium dissolved in 1Liter of distilled water heated to dissolve completely and sterilized by autoclaving at 115 ^{O}C for 15 minutes then cooled to 45 ^{O}C and dispense to sterilize petri dishes after which incubated for 24 hrs at 37 ^{O}C to check sterility.

4. Sorbitol macConkey agar (M298-500G, HIMEDIA, India)

The medium was prepared according to the manufacturer's instructions whereby 50.03 g of the medium dissolved in 1Liter of distilled water heated to dissolve completely and sterilized by autoclaving at 121 ^{O}C for 15 minutes then cooled to 45 ^{O}C and dispense to sterilize petri dishes after which incubated for 24 hrs at 37 ^{O}C to check sterility

5. Mueller Hinton Agar (M173-500G, HIMEDIA, India)

The medium was prepared according to the manufacturer's instructions whereby 38.0 g of the medium dissolved in 1Liter of distilled water heated to boiling to dissolve completely and sterilized by autoclaving at 121 ^{O}C for 15 minutes then cooled to 45 ^{O}C and dispense to sterilize petri dishes after which incubated for 24 hrs at 37 ^{O}C to check sterility

6. Simmons Citrate Agar (M099-500G, HIMEDIA, India)

The medium was prepared according to the manufacturer's instructions whereby 24.28 g of the medium dissolved in 1Liter of distilled water heated to boiling to dissolve completely and distribute in to test tubes of suitable capacity to obtain the portion necessary for the test. Finally, the solution sterilized by autoclaving at 121 ^{O}C for 15 minutes and used for test

7. Triple Sugar Iron Agar (M021-500G, HIMEDIA, India)

The medium was prepared according to the manufacturer's instructions whereby 64.52 g of the medium dissolved in 1Liter of distilled water heated to boiling to dissolve completely and dispended in to test tubes of suitable capacity to obtain the portion necessary for the test. Finally, the solution sterilized by autoclaving at 121 ^{O}C for 15 minutes and used for test.

Annex 4: Biochemical test used for identification of *E. coli*

1. Indole test

Indole positive bacteria possess an enzyme, tryptophanase, which converts tryptophan to indole. When indole reacts with para-dimethyl aminobenzaldehye (Kovac's reagent) a red or pink colored complex is produced.

Indole reacts with the aldehyde in the kovac's reagent to give red or pink color ring in the top of the tube. The isolates to be tested were inoculated to trypto soya broth prepared according to manufacturer's instructions and the mixture was incubated at 37 ^{O}C for 24 hrs after which five drops of kovac's reagent added to the broth and formation of red or pink colored ring on the top is a positive reaction and *E. coli* are indole positive.

2. Citrate utilization test

Citrate utilization test indicate detects the ability of bacteria to utilize citrate as a sole sources of carbon and energy. Citrate agar media was prepared according to manufacturer's instruction and allow forming slant. Then the isolates were streaked to the slant without streaking the butt and incubated at 37 ^{O}C for 24 hours. Positive reaction is blue color and *E. coli* is citrate negative.

3. Triple Sugar Iron Test

Triple sugar iron slant agar is used to determine whether an organism ferments glucose, lactose and sucrose. When bacteria ferment sugar, they produce acid turning the medium yellow and if there is only glucose fermentation, the limited acid quickly oxidizes at the slant, reverting it to red while the butt stays yellow due to low oxygen. Fermentation of lactose or sucrose produces enough acid to keep the entire tube yellow.

TSI agar was prepared according to manufacturer's instruction and allows forming slant and the butts of the slant were stabbed with the tip of the inoculating wire loop and carefully with draw it up and streaked of the slant and incubated overnight at 37 ºC. During interpretation red slant and yellow butt indicate glucose fermentation only, yellow slant and yellow butt indicate lactose and / or sucrose attacked as well as glucose and blackening of the medium indicate hydrogen sulphide production

4. Sorbitol fermentation test

The sorbitol test in identifies if a microbe can ferment the sugar sorbitol, producing acid as a byproduct that the pH of the medium, which is indicated by a color change in a pH indicator. The medium color changed typically from red to yellow to indicate sorbitol fermentation.

Sorbitol phenol red broth was prepared by adding 1g sorbitol to 1Liter buffered peptone water that prepared according to the manufacturer's instructions in which 20mg powdered medium were dissolved in 1Liter of distilled water and sterilized by autoclaving at 121 ^{O}C for 15 minutes and phenol red indicator was added to the medium.

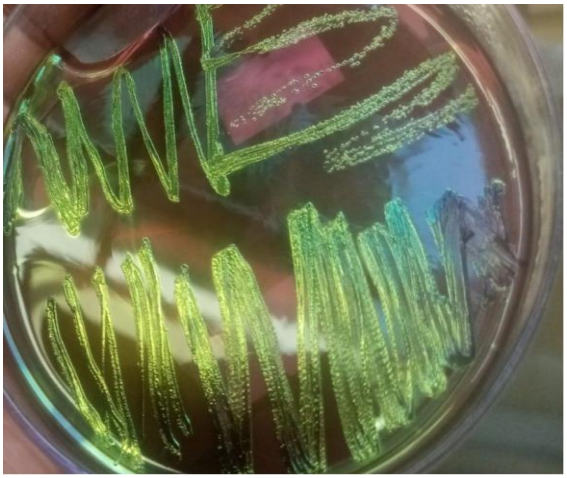
Then pure colonies were inoculated into the broth and incubated for 24hrs at 37^{O}C after which change in color was observed and color change from red to yellow can shows sorbitol fermentation and E. coli O157:H7 is non-sorbitol fermenter.

Annex 5: Zone diameter interpreting criteria for Enterobacteriaceae

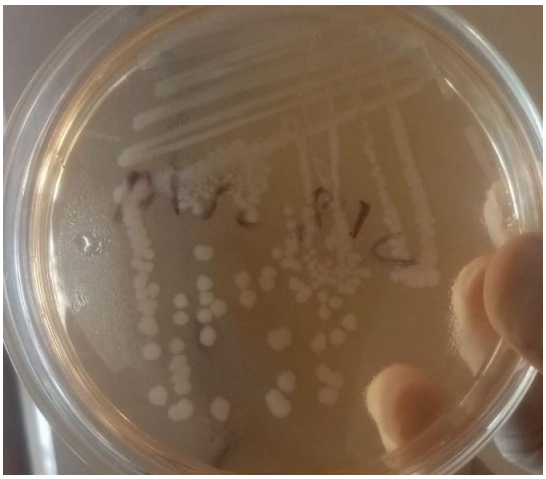
Antimicrobial Agent	**Concentration (µg/disk)**	**Susceptible (mm)**	**Intermediate (mm)**	**Resistance (mm)**
Ceftacidime	30	≥21	18-20	≤17
Gentamycin	10	≥15	13-14	≤12
Streptomycin	15	≥15	12-14	≤11
Tetracycline	30	≥15	12-14	≤11
Nalidixic acid	30	≥19	14-18	≤13
Sulfonamides	300	≥17	13-16	≤12
Sulphamethoxazole	100	≥16	11-15	≤10
Vancomycin	30	≥17	14-16	≤13
Oxytetracycline	30	>15	12-14	<11

Annex 6: Picture taken during sample collection and laboratory work

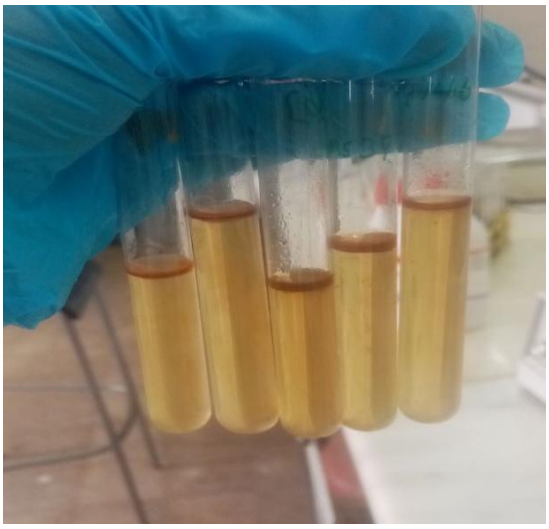
Sample collection



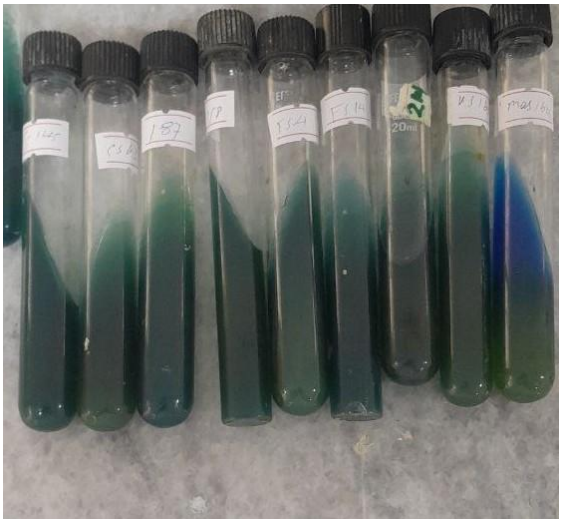
Green metallic sheen on EMB agar



Colorless colonies on SMAC



Indole test positive



Citrate utilization negative

TSI test

Sorbitol test with no color change and color change

Antimicrobial sensitivity test

Annex 7: Informed Consent Form (English version)

Tittle of the study: Occurrence of *Escherichia coli* O157:H7 and antimicrobial resistance profile in dairy farms: Dairy cattle, Milk, Soil, Water and in contact human in Bishoftu and Modjo town

Principal Investigator: Taye Dinku

Department: Microbiology, Immunology and Veterinary public Health

Phone: 0910016569

Email: tayedinku@gmail.com

Purpose of Study

You are being asked to take part in a research study. Before you decide to participate in this study, it is important that you understand why this research is being done and what it will involve. Please read the following information carefully. The purpose of this study is To isolate *E. coli* O157:H7 from dairy cattle, milk, diarrheic patients attending government health facilities and different water sources used in dairy farms and human health facilities, to assess the associated risk factors of *E. coli* O157:H7 infection in dairy farms and human patients and assess antimicrobial resistance profile of *E. coli* O157:H7 isolated from different samples

If you agree to participate in this study, you will be asked to complete a questionnaire that will take approximately 10 minutes to complete and provide a stool sample using the provided collection kit. There are minimal risks associated with participation, including, discomfort while completing the questionnaire and discomfort related to providing a stool sample. There will be no direct benefit to you for your participation in this study. However, I hope that the information obtained from this study may help to identify the sources, route of transmission and ways of control and prevention of *E. coli* O157:H7 the study area.

All information collected will be kept confidential. Your responses will be coded, and only the researchers will have access to the data. No identifying information will be published. Participation in this study is entirely voluntary. You may choose not to participate or withdraw at any time without any consequences. If you have any questions about the study or your rights as a participant, please contact Taye Dinku at 0910016569

I have read the information above, and I understand the purpose and procedures of the

study. I have had the opportunity to ask questions and have received satisfactory answers. I consent to participate in this study.

Participant's signature _____ Date _____

Investigator's signature _____ Date _____

Annex 8: Animal research ethical clearance

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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/04/69/17/2025

Name of Applicant: **Taye Dinku** (DVM, MSc student)

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

Title of the project: *Prevalence of E. coli O157: H7 and antimicrobial resistance profile in dairy farm: dairy cows, milk, soil, water and in contact humans in Bishoftu and Modjo towns*

Date of application:	**December, 2024**
Nature of the project:	Field investigation
Target animal species:	**dairy cattle**
Number of animals involved:	**69**
Study area:	**Central Oromia, 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 Taye Dinku.

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

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

The Federal Democratic Republic of Ethiopia

Addis Ababa University College of Veterinary Medicine and Agriculture

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Please quote Our Ref. No. When replying

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