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**ISOLATION, IDENTIFICATION, AND ANTIMICROBIAL RESISTANCE PROFILE  
OF *E. COLI* O157:H7 IN SELECTED DAIRY FARMS IN WOLAITA SODO TOWN**

**MSC THESIS**

**BY**

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**JUNE, 2024  
BISHOFTU, ETHIOPIA**

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**A Thesis Submitted to College of Veterinary Medicine and Agriculture of Addis Ababa  
University in Partial Fulfillment of the Requirement for Degree of Masters of Science in  
veterinary public health**

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**JUNE, 2024  
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I Yordanos Kassahun hereby declare that the MSc thesis entitled “**Isolation, Identification, and Antimicrobial Resistance Profile Of *E. Coli* O157:H7 in Selected Dairy Farms in Wolaita Sodo Town, South Ethiopia**” submitted by me for the award to study her master of veterinary science in Veterinary public 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. This thesis was submitted for examination with my approval as a university advisor.

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## **DEDICATION**

This thesis manuscript is dedicated to my family for their unreserved encouragement, nursing me with affection and love. They are the key of my success in my entire life.

## **BIBLOGRAPHY**

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## LIST OF ABBREVIATIONS

A/EEC	Attaching and effacing <i>Escherichia coli</i>
AMR	Antimicrobial Resistance
EAEC	Enterogaagregative <i>Escherichia coli</i>
<i>E. COLI</i>	<i>Escherichia coli</i>
EHEC	Enterohaemorrhagic <i>Escherichia coli</i>
EIEC	Enteroinvasive <i>Escherichia coli</i>
EPEC	Enteropathogenic <i>Escherichia coli</i>
ETEC	Enterotoxigenic <i>Escherichia coli</i>
FBD	Food Borne Disease
HC	Hemorrhagic Colitis
HUS	Hemolytic Uremic Syndrome
LPS	Lipopolysaccharide
PCR	Polymerase Chain Reaction
SMAC	Sorbitol Macckoney Agar
STEC	Shiga-toxigenic <i>Escherichia coli</i>
TTP	Thrombotic Thrombocytopenic Purpura
VTEC	Vero Toxin-producing <i>Escherichia coli</i>

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## ABSTRACT

*Escherichia coli* is one of the microorganisms that are frequently connected to foodborne disease. It is the common cause of bloody diarrhea. A cross-sectional study design with simple random sampling was employed to isolate and identify *E. coli* O157:H7 in milk, feces, and environmental samples collected from selected dairy farms in Wolaita Sodo Town during the study period. A fecal sample, an environmental swab, and a milk sample were collected from 300 dairy cows. A questionnaire survey was conducted on 100 respondents from the selected farm workers. Bacteriological methods were applied. A polymerase chain reaction was applied for the confirmation of *E. coli* O157:H7. Antimicrobial susceptibility testing was done using the Kirby-Bauer disc diffusion technique for confirmation of *E. coli* O157:H7 isolates. Chi-square was used to analyze the data. Based on the analyzed data, 66% (198/300) of dairy cows were positive for *E. coli*, and 3.3% (10/300) were *E. coli* O157:H7 isolates. Based on analysis, age, sample type and farm size were significant effect on the occurrence of *E. coli*. Medium-sized farms (74.19%) were significantly more affected by *E. coli* than small-scale farms (62.32%). Among the total samples, fecal, environmental swabs, and milk were 4.62% (6/130), 2.5% (1/40), and 2.31% (3/130) positive for *E. coli* O157:H7, respectively. The occurrence of *E. coli* was significantly higher in old-aged dairy cows (73.86%) than in adult animals (58.93%) and young dairy cows (51.47%) ( $p < 0.05$ ). The antibiotic susceptibility profiles of the isolates indicated that *E. coli* were 57.14% more sensitive to ciprofloxacin than other antibiotics. On the other hand, *Escherichia coli* O157: H7 isolates were 85.71%, 78.57%, and 71.43% resistant to ampicillin, tetracycline, amoxicillin, doxycycline, streptomycin, and ciprofloxacin, respectively. The questionnaire survey analysis showed that hygienic practices during and after milking, as well as the hygienic conditions of the farm and knowledge of the withdrawal period of the drug, were significant potential factors for milk contamination ( $P < 0.05$ ). The drug-resistant *E. coli* O157:H7 and unhygienic practices were important issues in the current study. It is highly recommended that rational use of antibiotics and proper hygienic practices on the farms should be applied.

**Key words:** *Antimicrobial Resistance Profile, E. coli O157:H7, Dairy Farms Wolaita Sodo Town*

# 1. INTRODUCTION

## 1.1. Background

Foodborne diseases (FBD) are worldwide problems. Consuming tainted foods of animal origin is linked to potential threats to food safety and is a major cause of foodborne illnesses (FBD). The severity of FBD is worse in developing nations, including Ethiopia. (Abdisa *et al.*, 2017). This could be attributed to dietary changes, mass catering, complicated and drawn-out food supply processes with increased international travel, poor food handling and sanitation procedures, insufficient food safety laws, weak regulatory systems, a lack of funding, and awareness of proper food handling, which fosters the spread of foodborne and food poisoning etiologic agents (Bevilacqua *et al.*, 2017).

A huge diversity of pathogens contribute to food-borne illness, the majority of which have zoonotic origins and carried in healthy food animals from which they move to an expanding spectrum of foods derived from animals and are regarded as primary carriers of foodborne diseases. (Sanchez *et al.*, 2011). Globally, developing countries carry the majority of the burden associated with foodborne infections, which are a serious public health concern. (Soomro *et al.*, 2002).

There is widespread worry about the safety of dairy products in terms of food borne illness, but this is particularly true in underdeveloped nations where milk and other dairy products are produced in unhygienic circumstances using poor production techniques. The consumption of raw milk and its derivatives is common in Ethiopia, which is not safe for consumers in a health point of view as it may lead to the transmission of various zoonotic disease-causing organisms (Umer *et al.*, 2022).

One of these microorganisms that are frequently connected to foodborne illness is *Escherichia coli* (*E. coli*). *E. coli* is a Gram-negative, non-spore-forming, flagellate, rod-shaped, and facultative anaerobic bacterium belonging to the family Enterobacteriaceae. (Edilu *et al.*, 2023). *Escherichia coli* is a common resident of both animal and human

intestines, but because it may contain enteropathogenic and/or toxigenic strains, its recovery from food raises questions about public health. *E. coli* has several types of strains that are divided into six groups of pathotypes based on the mechanism of disease cause. Enteropathogenic *E. coli* (EPEC), Attaching and effacing *E. coli* (A/EEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), EHEC and Enteroaggregative *E. coli* (EAEC). *E. coli* strains that produce the Stx toxins have been referred to as Vero Toxin-producing *E. coli* (VTEC), Shiga-toxigenic *E. coli* (STEC) and enterohaemorrhagic *E. coli* (EHEC) (Karmali, 1989; Nataro and Kaper, 1998). Most *E. coli* strains are harmless, however, the *E. coli* O157 strain is a significant foodborne pathogen with a low infective threshold and high resistance to treatment (Edilu et al., 2023) Escherichia coli O157:H7, often known as *E. coli* O157 that produces verocytotoxins or shiga toxins, is the most significant foodborne pathogen to have been identified globally. *E. coli* O157 originally raised public health concerns when its first outbreak was recorded in the USA in 1982.

Human diseases ranging from gastrointestinal disorders characterized by diarrhea and vomiting to other, more widespread, and even fatal foodborne illnesses are brought on by milk-borne bacteria. They are significant for both economic and public health reasons. They not only result in significant financial losses for the dairy cow industry, but they also offer a significant barrier to the trade of animals and animal products, which could substantially impede socioeconomic development, particularly in emerging nations like Ethiopia. (Munyeme *et al.*, 2010).

Pathogens found in milk are important for both public health and business. They not only result in significant financial losses for the dairy cow industry, but they also offer a significant barrier to the trade of animals and animal products, which could substantially impede socioeconomic development, particularly in emerging nations like Africa. Additionally, farmers in most of these nations are not aware of the economic and public health significance of zoonotic illnesses, which further hinders efforts to control these infections. (Mosalagae *et al.*, 2010).

Cattle, sheep, horses, pigs, turkeys, dogs, and a number of different wild animal species have all had *E. coli* O157:H7 isolated from their faeces or gastrointestinal tracts. It has also been isolated from water sources including wells and water troughs and has been discovered to live for months in dung and sediments from water troughs. (Sargeant *et al.*, 2000).

The main source of *E. coli* O157-H7 is cattle, although it's also possible that sheep and goats are to blame. *E. coli* O157-H 7 related human illness has been associated with direct contact with bovine faeces, raw milk, dairy products, vegetables, unpasteurized fruit juices, and water. Its natural reservoir is cattle, which serves as a significant source of *E. coli* O157 for human infections. Despite the fact that the organism does not cause any clinical illnesses in its natural habitat, it can cause a wide range of clinical abnormalities in people, including mild diarrhoea, hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), bloody diarrhoea, and thrombotic thrombocytopenic purpura (TTP) (Hajian *et al.*, 2011).

Antimicrobial medication overuse in food processing has been associated with an increase in antimicrobial resistance in both animals and people, although little focus has been placed on. The ability of a bacterium to live and multiply in the presence of antibiotic doses that were previously believed to be effective against them is known as antimicrobial resistance (AMR), and it is a growing issue on a worldwide scale. AMR has currently reached alarming levels in both human and veterinary medicine and is now recognised as a serious growing danger to global public health and food security. (Chuanchien *et al.*, 2014)

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

Similar to other nations, Ethiopia lacks a foodborne pathogen surveillance programme and regularly underreports cases of foodborne illness. Food safety is also hampered by the difficulty of determining the degree of contamination in milk and milk products. Due to Ethiopia's chronically poor sanitary conditions, hunger, and lack of proper medical care, foodborne diseases continue to be a significant public health concern. (Haile, 2015)

A number of researches have reported contamination of raw milk with *E. coli* O157 (Munyeme *et al.*, 2010). According to Arafa and Soliman (2013), of the raw milk and fresh cream tested in Egypt, 2.6% and 1%, respectively, were contaminated with *E. coli* O157:H7. Allerberger *et al.* (2001) reported 3% of the milk samples tested in Austria to be positive for *E. coli* O157:H7 and Klie *et al.* (1997) found that 3.9% of the raw milk analyzed in Germany was contaminated with *E. coli* O157:H7. There are not many studies on this topic, especially ones from developing nations; despite the fact that research indicates that multi-drug resistance in *E. coli* is a big concern worldwide (Sarba *et al.*, 2019).

Little research on the prevalence, distribution, and related virulent genes of *E. coli* O157:H7 in humans, animals, or foods of animal origin have been conducted in Ethiopia. According to a study on the frequency and antimicrobial susceptibility profile of *E. coli* O157:H7 isolated from traditionally marketed raw cow milk in and around Asosa town, 380 samples of raw milk were examined, and 129 (33.9%) and 11 (2.9%), respectively, were found to be contaminated with *E. coli* and *E. coli* O157:H7 (Disassa *et al.*, 2017). Diriba, (2018) reported 4.6 % prevalence of *E. coli* O157 from milk and fecal samples collected from apparently healthy lactating cows managed under extensive husbandry systems in Borana. The same study reported 3.29% prevalence of *E. coli* O157 in camels' feces.

Despite the fact that the majority of Ethiopia, notably Wolaita Sodo Town, regularly consumes raw milk and milk products, studies on the presence of foodborne pathogens like *E. coli* O157 in milk and milk products and faeces and antimicrobial susceptibility of the isolates has not been reported in the area.

Therefore the objectives of this study were:

#### General

- To isolate and identify *E. coli* O157:H7 strains from raw milk, faeces and environmental samples using bacteriological and molecular methods.

#### Specific

- To determine the antimicrobial susceptibility profile of *E. coli* O157:H7 isolates.

- To assess potential risk factors associated with the occurrence of *E. coli* O157:H7 within the dairy farms.

## 2. LITERATURE REVIEW

### 2.1. General Overview of *E. coli*

*Escherichia coli* is a rod-shaped, gram-negative, facultatively anaerobic, motile, nonspore-forming bacteria. They are members of the family Enterobacteriaceae, which are typically found in the gastrointestinal tracts of animals, people, and birds. However, some of its strains have evolved to cause diarrhoea and a variety of extra-intestinal illnesses, mostly in immunocompromised hosts. (Guabiraba and Schouler, 2015). Theodore Esherichin, a German paediatrician, was the first to isolate it from the faeces of newborn humans in 1884. Since then, there have been numerous documented outbreaks. There are hundreds of serotypes for the genus *E. coli*, which are categorized according to different surface antigens called Somatic (O), Capsular (K), Flagellar (H), and Fimbrial (F) (Stenutz *et al.*, 2006).

*E. coli* comes in both pathogenic and non-pathogenic varieties. The benign forms of *E. coli* prevent the growth of hazardous bacteria, create vitamins, and are not contagious. Additionally, they play a number of significant roles in humans, including carrying out certain metabolic processes that humans lack, modifying the morphology and physiology of the gut, and helping the immune system grow. (Shah *et al.*, 2018). On the other hand, pathogenic *E. coli* can cause three clinical syndromes, including sepsis/meningitis, urinary tract infections, and diarrhoea. Additionally, *E. coli* that causes diarrhoea is categorised into various "pathotypes" according to the illness they induce. *i.e.* Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Attaching and effacing *E. coli* (A/EEC), Enteroinvasive *E. coli* (EIEC) and Enterohaemorrhagic *E. coli* (EHEC) strains and Enteroaggregative *E. coli* (EAEC) has been found to be diarrhoeagenic strains (Goomes *et al.*, 2016).

Some *E. coli* strain like STEC O157 have acquired Virulence factors that have allowed them to adapt to new niches and in some cases to cause serious disease (Diriba, 2018). *E. coli* O157:H7 is distinguished by antigenic components such O, H, and LPS on its surface. The O-antigen (Ohne), a polysaccharide domain in the lipopolysaccharide (LPS)

of the bacterium, and the H-antigen (Hauch), a flagella protein, are classified as these structures. (Ratnam *et al.*, 1988).

*E. coli* O157:H7 is one of the EHEC strains that have been the subject of the most research due to its global distribution, unusual tolerance to several physical and chemical therapies, severity of sickness, and low dosage infectiousness. It was originally recognized in 1982 as a human enteric pathogen, and since then, it has been linked to both HUS and hemorrhagic colitis (HC). (Beneduce *et al.*, 2003).

## **2.2. Bacteriology of *Escherichia coli* O157:H7**

In contrast to many other strains, *Escherichia coli* O157:H7 does not ferment sorbitol, which enables clinical laboratories to distinguish the strain from others. Strains of *E. coli* that express Shiga and Shiga like toxins acquired this ability through infection with a prophage containing the structural gene coding for the toxin, and nonproducing strains may become infected and produce shiga like toxins after incubation with shiga (Zhang *et al.*, 2000). The prophage responsible seems to have infected the strain's ancestors fairly recently, as viral particles have been observed to replicate in the host if it is stressed in antibiotics (Berger *et al.*, 2019).

## **2.3. Clinical Manifestations of *Escherichia coli* O157:H7 Infections**

Hemorrhagic colitis, HUS, and TTP are the three main symptoms of sickness that are brought on by *E. coli* O157:H7 infection in humans. Usually 3 to 5 days after intake, hemorrhagic colitis's earliest signs and symptoms (abdominal pain, bloody diarrhoea, and dehydration) start to manifest. While most situations are self-limiting and terminate without any negative consequences HUS or TTP-related complications happen in roughly 5% of cases. (Zangari *et al.*, 2014). Hemolytic anaemia (intravascular red blood cell destruction), thrombocytopenia (low platelet counts), and severe renal failure are common signs of HUS. Children under 5 years of age, the elderly and immune compromised individuals are most susceptible to HUS with a mortality rate of 3–5%. By

comparison, TTP causes less renal damage and has more neurological involvement (seizures, strokes and coma) than HUS (Puligundla and Doyle, 2022).

#### **2.4. Public Health and Economic Significance of *E. coli* O157: H7**

*E. coli* O157: H7 and other serotypes of enterohemorrhagic *E. coli* are zoonotic pathogens associated with serious human diseases. Numerous outbreaks and isolated cases of disease linked to *E. coli* O157:H7 have been recorded from Argentina, Australia, Belgium, Canada, China, and many other nations since it was determined to be the cause of human illness (Bach *et al.*, 2002). During July–October 2014, an outbreak of 119 cases of *E. coli* O157:H7 infections associated with exposure to contaminated pork products occurred in Alberta, Canada (Honish *et al.*, 2017). Transmission typically happens when an infected animal comes into close touch with another one, consumes undercooked or contaminated meals of bovine origin, or faeces contaminate other food products. (Kiranmayi *et al.*, 2010).

Costs are significant due to the severity and long-term effects of infections with *E. coli* O157:H7 and other verocytotoxin-producing *E. coli*. Abe *et al.* (2002) evaluated the direct and indirect economic costs of *E. coli* O157: H7 outbreak in Japan that was linked to the lunches at primary schools. The economic impact of the outbreak was estimated to be about 82,686,000 yen. The hazard of *E. coli* O157:H7 infection has direct consequences for humans, but there are also indirect costs for cattle and dairy producers, meat packers and dairy processors, meat and milk distributors, etc. The cost of *E. coli* O157:H7 to the food sector in the United States alone is estimated to be in the billions of dollars as a result of recalls, destroyed food, control measures, and lost demand due to loss of customer trust (Roberts *et al.*, 2000).

## **2.5. Environmental Survival of *Escherichia coli* O157:H7**

*Escherichia coli* O157:H7 is capable of surviving and persisting in a wide range of habitats, including food, water, soil, and animal reservoirs. It has been demonstrated to live for a year in soil treated with manure and for 21 months in raw, uncomposted manure. If the temperature is kept above 50°C for 6 days, composting manure effectively kills *E. coli* O157:H7. It can endure in water for a long time, especially in cold climates. Bovine feces-infected water trough sediments can behave as a long-term (>8 months) *E. coli* O157:H7 reservoir, and the surviving bacteria in contaminated troughs can cause infection. (Noviyanti *et al.*, 2018). It survives and replicates in *Acanthamoeba polyphaga*. Polyphaga is a common environmental protozoan that is widely distributed in soil, water and fecal slurry. Thus, it can be an efficient transmission vehicle of *E. coli* O157:H7 in these environments (Mohammed *et al.*, 2014).

It requires the capacity to adjust to variations or dramatic changes in temperature, pH, and osmolarity conditions typically experienced in nature in order to thrive in a variety of habitats. For instance, *E. coli* O157:H7 produces exopolysaccharides when it is exposed to heat and acid, and heat stress causes membrane lipid composition to change. (Yuk and Marshall, 2004). The survival and spread of *E. coli* O157:H7 on farms as well as the rising transmission from cattle to cattle are both significantly influenced by these environmental adaptations of *E. coli* O157:H7. Additionally, since the disease may persist beyond the host reservoir, there is a greater chance that it will contaminate crops and produce through interaction with infected animals, irrigation with contaminated water, or direct contact with their dung. (Maule, 2000).

## **2.6. Treatment and Management**

It is debatable whether or not antibiotics should be used to treat STEC infection. There have been reports of increase in the level of shiga toxin production and a greater risk of fatal complications following administration of antibiotics in STEC infection (Zhang *et*

*al.*, 2000). However, some suggest that some antimicrobials, if administered early in the course of infection, may prevent disease progression to HUS (Schroeder *et al.*, 2002).

In vitro studies show that most strains are susceptible to various antibiotics, although certain antibiotics, at sub-lethal concentrations may increase the release of shiga-like toxin which has been associated with the development of HUS (Collins and Green, 2010). Antimicrobials may lyse bacterial cell walls, thereby liberating stx, and/or cause increased expression of stx genes in vivo (Schroeder *et al.*, 2002). As a result, treatment of infection with EHEC strains, including *E. coli* O157:H7 is mainly based on fluid and electrolyte replacement (Rahal *et al.*, 2012)

## **2.7. Control and Prevention of *E. coli* O157:H7 Infection**

The three main strategies for preventing and controlling *E. coli* infections are management interventions, infection control, and vaccine programmes. (Kabir, 2010). In cattle, control of *E. coli* O157:H7 is possible through vaccination. Either reducing the vulnerability of cattle to *E. coli* O157:H7 colonisation or shortening the duration of such colonisation is the aim of vaccination. In order to attach to the large intestine wall, *E. coli* O157:H7 secretes virulence factors into host cells. A vaccine that stimulates the generation of antibodies against these virulence factors and causes the organism's expulsion from the digestive tract could prevent the organism's adhesion. (Bach *et al.*, 2002).

Prevention and control measures for *E. coli* O157:H7 infection need to be applied at all stages of the food chain, from farm to fork. So, effective control of the agent requires a multi-faced approach. (Kiranmayi *et al.*, 2010). Control of *E. coli* in environmental sources, such as water troughs, feed, and manure, is essential in stopping the cycle of infection and re infection of livestock. This includes minimising the frequency and intensity of *E. coli* faecal shedding by animals exposed to the organism and reducing the amount of manure they produce. (Bach *et al.*, 2002).

People who are affected during STEC outbreaks in the public or hospitals should be isolated to stop the outbreak. Person-to-person contact can spread infections that could

become worsened by HUS. Therefore, the most successful intervention is hand washing. (Dagne *et al.*,2019). Food should not be consumed in areas where animals are maintained, areas for eating should be isolated from areas for keeping animals healthy, and visitors should be told about precautions to take to avoid contracting diseases from animals. (National Associate of State Public Health Veterinarians, 2011).

Strict hygiene procedures should be put in place to reduce the risk of human infection from coming into contact with farm animals directly. These procedures should include restricting access to farm animals, controlling visitor flow to farms, providing easy access to washing facilities, and offering a means of disinfection in case visitors come into contact with the animals. ( Fratamico *et al.*, 2016).

## **2.8. Antimicrobial Resistance**

One of the most significant risks to global public health this century is antimicrobial resistance (AMR). The abuse of antibiotics in both medicine and agriculture is one cause of AMR, particularly of dangerous bacteria. There is growing concern about the establishment and spread of antibiotic-resistant strains of *E. coli* O157:H7, a major zoonotic foodborne pathogen that could cause sporadic cases to serious outbreaks over the world. (Tadesse *et al.*, 2012). According to CLSI (2018), the disc diffusion method can be used to assess the isolates' antimicrobial susceptibility, employing varying quantities of antibiotic discs. The majority of *E. coli* O157:H7 isolates (50%) are resistant to tetracycline (81.8%), streptomycin (81.8%), kanamycin (63.6%), cefoxitin (54.5%), and norfloxacin (54.5%), according to a study by Disassa *et al.* (2017). Another study by Minda and Shimelis (2021) on food of animal origin at different catering establishments in the selected study settings of Arsi Zone indicated that, of the four *E. coli* O157 : H7 isolates subjected to 10 panels of antimicrobial discs, 3 (75%) were highly resistant to kanamycin, streptomycin, and nitrofurantoin. An important public health problem is the emergence of antibiotic resistance in bacteria like *E. coli*. Treatment effectiveness and the capacity to manage infectious diseases in both humans and animals could be seriously affected. (Thaker *et al.*, 2012).

## 2.11. Status of *E. coli* O157:H7 in the Ethiopia

Numerous studies have documented the presence of *E. Coli O157:H7* in feces and milk, in Ethiopia. While reports from some parts of Ethiopia are included, the majority of the researches were conducted in central part of the country (Mohammed, 2023).

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

Location	Sample type	Sample size	No of positive	Percent	<i>E.Coli</i> strain isolated	References
Assosa	Milk	380	11	2.9%	O157:H7	Disassa <i>et al.</i> , 2017
Bishoftu	Milk	200	24	12%	O157:H7	Bedasa <i>et al.</i> , 2018
Adami tulu	Feces	154	6	3.7%	O157:H7	Mesele <i>et al.</i> , 2023
Adami tulu	Milk	154	7	4.5%	O157:H7	Mesele <i>et al.</i> , 2023
Central Ethiopia	Milk	450	27	6.0%	O157:H7	Dejene <i>et al.</i> , 2022
Hawassa	Feces	47	22	46.8%	O157:H7	Fesseha <i>et al.</i> , 2022
Hawassa,	Feces	150	7	4.7%	O157:H7	Atnafie <i>et al.</i> , 2017

### 3. MATERIAL AND METHODS

#### 3.1. Study area

The study was conducted from December, 2023 to May, 2024 in Wolaita sodo town, Wolaita Zone, South Ethiopia. It is located 390 kilometers south of Addis following the tarmac road that passes through Shashamane to Arbaminch. Alternatively, it is located 330 km southwest of Addis Ababa following the tarmac road that passes through Hosanna to Arbaminch. It has a total area of 4,541km<sup>2</sup> and is composed of 12 districts and 3 registered towns (WZAO, 2011). It is at an altitude of 2025 meters above sea level, 8°50' N latitude, and 37° 45' E longitude. The maximum rainfall in the study area between July and September is 801–1600 mm, with an annual average of 15.1-31 °C. With a population density of approximately 385 people per square kilometer (ppkm<sup>2</sup>), the region is the most populous in the country. It is estimated that there are 886,242 cattle, 117,274 sheep, 99,817 goats, 41,603 horses, and 442,428 chickens in the Wolaita area (WZFEDD, 2022).

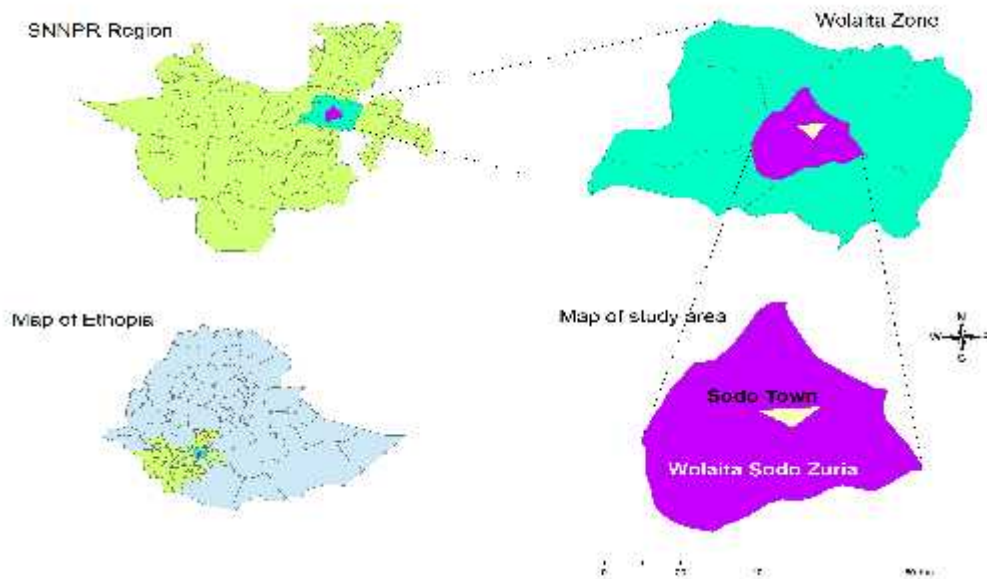


Figure 1: Map of the Study Area

### 3.2. Study design

A cross-sectional study was carried out from December 2023 to May 2024 to isolate and identify *E. coli* O157 in milk, feces, and environmental swab samples collected from selected dairy farms in Wolaita Sodo Town. A total of 300 samples were collected from the selected dairy farms. Data regarding the different host risk factors, such as age, herd size, and sample type, were collected from apparently healthy lactating dairy cows. The samples were transported to the Wolaita Sodo Regional Veterinary Laboratory in an icebox with ice packs for bacteriological analysis.

### 3.3. Sample size determination and sampling technique

The sample size of the study animals were determined by using the formula given for census sampling methods (Thrusfield, 2005) by using an expected prevalence of 2.4 % were *E. coli* O157:H7 isolates in the study area (Atnafie *et al.*, 2017).

$$n = \frac{1.96^2 P_{\text{exp}} (1 - P_{\text{exp}})}{d^2}$$

Where,

n = required sample size;

p = expected prevalence (p =2.4% =0.024);

d = 5% = 0.05, 1.96 (CI = 95%)

n = 36.

Hence, by using this formula, the sample size was calculated to be 36. However, total sample size of the study was increased to 300 in order to increase the precision of study. Simple random sampling technique was employed to estimate the to isolate and identify *E. coli* O157:H7 in milk, feces, and environmental swab samples collected from lactating dairy cows in selected dairy farms in Wolaita Sodo Town.

### **3.3. Sample Collection**

From the selected dairy farms found in Wolayta sodo town, all lactating dairy cows in the farms were included. 93 and 207 dairy cows were selected from medium and small scale farms. Fecal sample, milk and environmental swab samples were collected from only lactating animals found during study period. Twenty five grams of the fecal samples were collected from the selected dairy cows. From each farm, environmental pooled swabs were collected. After discarding three streams of milk, a 25 ml sample of milk was taken from each quarter of each of the chosen individual milking cows using sterile labelled screw capped glass bottles. After the milk was well mixed, sterile screw-capped glass bottles with labels were used to collect approximately 25 milliliters of tank milk samples from dairy farms. All pertinent information about the samples, including the research site, sample source, sample type, date of collection, and condition of the sample sources, was recorded in a pre-designed manner during the sample collecting process. The samples were shipped carefully on the day of collection using an ice box containing ice packs and processed within 24 hrs in Sodo regional veterinary laboratory, Ethiopia.

### **3.4. Isolation and Identification of *Escherichia coli* O157:H7**

#### **3.4.1. Bacterial Isolation Process**

Isolation and identification of *E. coli* O157: H7 was done by standardized bacteriological methods. About 1 g of feces and 1 ml of milk was enriched into an Erlenmeyer flask containing 9 mL of modified tryptone soya broth and incubate at 37°C for 24 hours. The pre-enriched samples were further inoculated into MacConkey broth and incubated at 37°C for 24 hrs for selective enrichment. The enrichments were then streaked on MacConkey Agar plates and incubated at 37°C for 24 hrs. Pink-colored colonies were aseptically streaked on nutrient agar plates and incubated at 37°C for 24 hrs (Kalin *et al.*, 2012).

A single isolated colony was picked and streaked on Eosin Methylene Blue Agar (EMB) medium and incubated aerobically at 37°C for 24 hrs. The presumptive *E. coli* colonies

that showed greenish metallic sheen were picked up with a sterile inoculating loop and allowed to grow on nutrient agar plates at 37°C for 24 hrs for biochemical examination. Furthermore, confirmatory identification of presumptive *E. coli* isolates was performed by standardized biochemical tests. Indole test was conducted according to Cheesbrough; Methyl Red and Voges Proskauer tests were done according to Cheesbrough (1985); Citrate utilization test was applied. TSI test was performed according to Vanderzant and Splitt stresser. All the biochemical tests were interpreted and isolates which were indole positive, methyl red positive, Voges-Proskauer negative, citrate negative, and TSI positive were confirmed to be *E. coli* due to producing acid with gas and without hydrogen sulfide production (Leber, 2020).

The identified *E. coli* colonies were further subcultured onto SMAC agar plates at 37°C for 24 hrs to differentiate *E. coli* O157:H7 strain from other *E. coli* strains. The colorless or pale colonies were confirmed as *E. coli* O157: H7 strain where Sorbitol-fermenters (pinkish colonies) were considered as non-O157:H7 *E. coli* strains. The pale colonies/colourless were encountered on SMAC which taken for further confirmatory test polymerase chain reaction.

#### 3.4.2. Molecular characterization of *E. coli* O157:H7

The PCR assay was based on the sequence of the uidA, Stx1, and Stx2 genes of *E. coli* O157:H7. Specific primers targeting each of the toxigenic genes were used to amplify the gene products. A 25 µL of total volume of PCR reaction (12.5 µL of 2×Go Taq Green Mix Master (Promega Corporation, USA), including 2 µL of Primer-F and Primer-R, 6.5 µL of DNeasy-free water (Promega Corporation, USA), and (v) 4 µL DNA template of *E. coli* O157:H7) was used. The whole mixture was placed in the Eppendorf tube of 200 µL (Biozym, Oldenhof, Germany). Finally, by using gel electrophoresis, DNA ladder 100 bp, and 2% agarose gel (Peqlab, Erlangen, Germany), the amplicons of the specific sequence was determined (Jinneman *et al.*, 2003).

#### 3.4.3. Antimicrobial susceptibility testing of *E. coli* O157:H7

Antimicrobial susceptibility testing was done for 16 confirmed *E. coli* O157:H7 isolates by using Kirby-Bauer disc diffusion technique for the following 6 selected antimicrobial agents included Amoxicillin, ciprofloxacin, tetracycline, doxycycline, ampicillin and streptomycin. The selection of these antibiotics was based on the availability and frequent use of these antimicrobials in the study area both in veterinary and human medicine. First, the suspension was adjusted to 0.5 McFarland's standard. This suspension was inoculated onto Mueller Hinton agar (MHA) and the above antimicrobial agents was placed using sterile forceps and pressed gently to ensure the contact of a medium. After overnight incubation of the plate at 37°C, the zones of inhibition around each of the antibiotic disks were observed. The diameters of inhibition zones were measured using a digital caliper comparing the zone of inhibition with the Kirby–Bauer chart as recommended by CLSI guidelines (CLSI, 2014) and the findings were recorded in a pre-designed format. The inhibition zone results around individual antibiotic disks were interpreted and the isolates were classified as Sensitive (S), Intermediate (I), and Resistant.

### **3.5. Questionnaire Survey**

Semi-structured questionnaire survey was used as a tool to gather information on the hygienic practices at the dairy farms. Accordingly, information regarding washing before milking, washing udder before milking, head cover during milking, using of disinfectants and antiseptics, hygienic conditions for drainage system, hygienic condition of the farms, knowledge on the withdrawal period of the drug were factors for milk contamination with *E. coli*.

### **3.6. Data Management and Analysis**

Data generated from laboratory investigations was recorded and coded using Microsoft Excel spreadsheet (Microsoft Corporation) and was analyzed using STATA version 16.0 for Windows (Stata Corp. College Station, TX, USA). The prevalence of *E. coli* O157:H7 isolated from the selected farms in Wolaita sodo was calculated as the number of

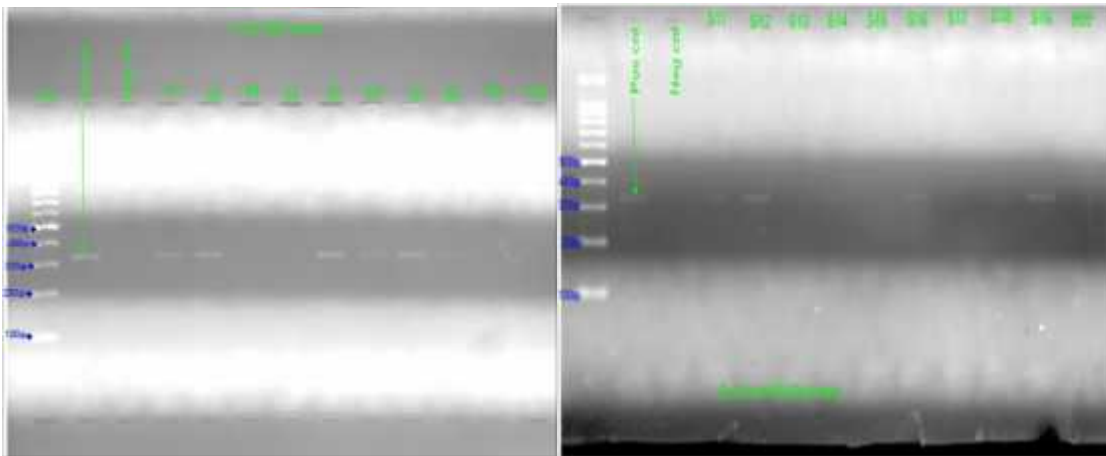
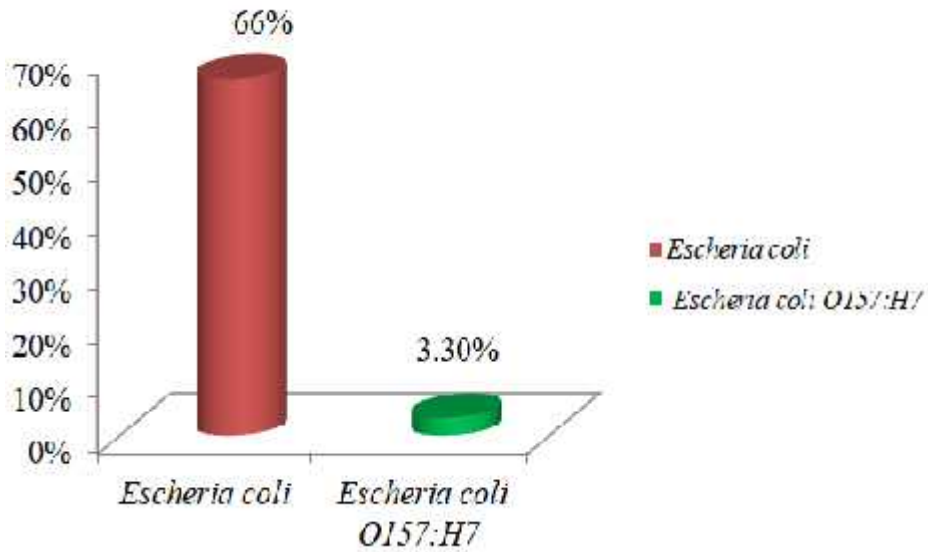
positive (confirmed) samples divided by the total number of samples investigated (processed) in the laboratory. Chi-square test was used to check the association of risk factors with the occurrence of *E. coli* O157: H7. The association was taken as significant when p-value is less than 0.05 and not significant when P-value is greater than 0.05.

### **3.7. Ethical considerations**

After having the study plans ethically reviewed by the animal health ethical committee of the College of Veterinary Medicine and Agriculture, Addis Ababa University, the study objectives were communicated to farm owners, managers and other study participants. Before the start of the research work, verbal consent was obtained from all farm owners, managers and participants. Furthermore, the information obtained was kept private and anonymous.

#### 4. RESULTS

Among the total sampled taken during the study period, 66% (198/300) were positive for *E. coli* on EMB and 3.3% (10/300) were *E. coli O157:H7* isolates since polymerase chain reaction confirmatory test was performed ( Table 1 and Figure 2).



**Figure 2:** The overall prevalence of *E. coli* and *E. coli O157:H7* based on EMB and PCR

According to the table 2, the prevalence of *E. coli* O157:H7 were 6.67% and 3.33% in SMAC and PCR respectively.

**Table 2:** The overall prevalence of *E. coli* O157:H7 in the different test

No of sample examined	Test	No of positive animals	Prevalence
300	SMAC	20	6.67%
300	PCR	10	3.33%

The current study revealed that the sample type was significantly correlated with the isolation of *E. coli* from samples ( $p = 0.000$ ,  $\chi^2 = 31.7376$ ). Fecal sample in the *E. coli* (82.31%) were isolated than the milk in the *E. coli* (67.50%) and environmental pooled swab (49.23%) as indicated in the Table 3.

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

Sample type	Total sample examined	<i>E. coli</i> isolates	$\chi^2$	P value
<b>Fecal sample</b>	130	82.31	31.7376	0.000
<b>Milk</b>	130	67.50		
<b>Environmental pooled swab</b>	40	49.23		

The distribution of *E. coli* O157:H7 isolates among the test sample types is illustrated on Table 3. *E. coli* O157:H7 strains were isolated in 6 out of 130 fecal samples (4.6 %), one out of 40 environmental pooled samples (2.5 %), and 3 out of 130 milk samples (2.31 %). ( $\chi^2 = 1.1737$ ,  $p = 0.005$ ).

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

Sample type	Total sample examined	<i>E. coli</i> O157:H7 strains	<sup>2</sup>	P value
Fecal sample	130	4.62% (6/130)	1.1737	0.005
Milk	130	2.31% (3/130)		
Environmental pooled swab	40	2.5% (1/40)		

The current study revealed that the farm size was significantly correlated with the isolation of *E. coli* from samples ( $p = 0.045$ , 4.0323). Medium sized dairy farm cows were infected with *E. coli* (74.19) compared with small scale (62.32%) as indicated in the Table 5

**Table 5:** *Escherichia coli* isolate association with farm size risk factors.

Farm scale	No of animals examined	No of positive animals	Prevalence	<sup>2</sup>	P value
Medium sized	93	69	74.19	4.0323	0.045
Small scale	207	129	62.32		

According to the current study, about 73.86% of the *E. coli* isolates were recovered from old aged animals, 58.93% of the *E. coli* were found in the adult animals and 51.47% of the *E. coli* were isolated from young dairy cows. Based on this study finding, isolation of *E. coli* was statistically correlated ( $p < 0.05$ ,  $\chi^2 = 12.49$ ) with age of the dairy cows (Table 6).

**Table 6:** *Escherichia coli* isolate association with age of the animals

Age	No examined animals	No of positive animals	Prevalence	<sup>2</sup>	P value
Adult	56	33	58.93	12.49	0.002
Old	176	130	73.86		
Young	68	35	51.47		

The antibiotic susceptibility profiles of the isolates showed that *Escherichia coli* O157:H7 were 57.14% sensitive to ciprofloxacin. On the other hand, *Escherichia coli* O157:H7 isolates were 85.71%, 78.57%, 71.43% resistant to ampicillin, tetracycline, and amoxicillin, respectively.

**Table 7:** Antimicrobial susceptibility pattern of *E. coli* O157:H7 isolates

Antimicrobial drugs	Resistance level	Susceptibility level	Intermediate
Ampicillin	85.71% (12/14)	0	14.29% (2/14)
Amoxicillin	71.43% (10/14)	21.43%(3/14)	7.14%(1/14)
Ciprofloxacin	7.14%(1/14)	57.14% (8/14)	35.71% (5/14)
Tetracycline	78.57%(11/14)	21.43%(3/14)	0
Doxycycline	35.71% (5/14)	21.43%(3/14)	42.86% (6/14)
Streptomycin	21.43% (3/14)	21.43%(3/14)	57.14%(8/14)

Among respondents during study period, 19% and 81% were male and female, respectively. 22%, 61%, 4%, 5%, and 8 % were below 18 years, 18-30 years, 31-40 years, 40-60 years and above 60 years, respectively. The educational status in the study period include primary school, high school and university level and illiterate were 23%, 46%, 20% and 11% respectively. This indicated female, age between 18-30 years and high school educating people were more participated during questionnaire survey (Table 8).

**Table 8:** Demographic Characteristics of Respondents

Variable		Frequency	Percentage
Sex	Male	19	19.00%
	Female	81	81.00%
Age	Below 18 years	22	22.00%
	18-30 years	61	61.00%
	31-40years	4	4.00%
	40-60 years	5	5.00%
	Above 60 years	8	8.00%
Educational status	High school	46	46.00%
	Illiteracy	11	11.00%
	Primary school	23	23.00%
	University level	20	20.00%

According to Table 9, hand washing before milking, washing udder before milking, head cover during milking, using of disinfectants and antiseptics, hygienic conditions for drainage system, hygienic condition of the farms, knowledge on the withdrawal period of the drug were significant potential factors for milk contamination ( $P < 0.05$ ). However, water supply and type of milk containers were not potential risk factors for the milk contamination ( $p > 0.05$ ).

**Table 9:** KAPS on risk factors associated with contamination of milk on Dairy farm and handling practices

Variable		Frequency	Percentage	<sup>2</sup>	P value
Hand washing before milking	Yes	1	1.49%(1/67)	31.7940	0.000
	No	15	45.45%(15/33)		
Type of milk containers used	Plastic	6	17.65 % (6/34)	0.1040	0.747
	Stainless	10	15.15 %(10/66)		
Washing udder before milking	Yes	16	16.33%(16/98)	9.2971	0.002
	No	2	100.00% (2/2)		
Do you use head cover during milking	Yes	5	6.02% (5/83)	47.4416	0.000
	No	13	76.47% (13/17)		
Is there water supply	Yes	16	16.84%(16/95)	1.7259	0.189
	No	2	40.00% (2/5)		
Do you use disinfectants and antiseptics	Yes	3	3.53% (3/85)	80.3922	0.000
	No	15	100% (15/15)		
How did hygienic conditions for drainage system	Good	3	5.88%(3/51)	11.6372	0.001
	Poor	16	32.65% (16/49)		
Hygienic condition of the farms	Good	5	9.62% (5/52)	6.1995	0.013
	Poor	14	29.17% (14/48)		
Do you know the withdrawal period of the drug	Yes	4	4.88% (4/82)	59.0326	0.000
	No	15	83.33 (15/18)		

## 5. DISCUSSION

In the current study, Out of 300 samples collected, the isolation of *Escherichia coli* was 66% (198/300). This result was comparable with other previous research findings of Majeed *et al.* (2011) 64% in Kuwait, Dawit (2012) 64% in Addis Ababa and Debre Zeit, Ethiopia, Fesseha *et al.* (2022) 69.1% in urban and peri urban dairy farms of Hawassa town, Nazir and Hussain (2007), who reported 60% and Yeshiwas and Fentahun (2017) who reported 53/75 (70.7%) in the Debre-Zeit.

On the other hand, the finding in the current study was higher than previous reports of Sebsibe and Asfaw (2020) (20.2%), Dereje (2012) 43.1%, Masud *et al.* (2012), who reported 44% and Gebregiorgis and Tessema (2016) 36.8%, Tadese *et al.* (2021) (23.4%). On the other hand, the current research was higher than the report of Darsema (2008) 13.5%, Razzaque *et al.* (2006) who reported 24%, Megersa *et al.* (2009), who reported 37%, Lanz Uhde *et al.* (2008) 5.5%, Aggernesh (2010) 38% in Debre Zeit and Demissie (2007) 43.1% from Addis Ababa dairy farms. The variation of prevalence of *E.coli* in the different study area might be due to differences in sample types, methodological approaches, diagnostic techniques, geographical locations, hygienic conditions, handling and transportation of samples. Generally, this high prevalence of the *E.coli* in the study area may be attributed to poor sanitation practices, such as inadequate sewage treatment and disposal, can contribute to the spread of *E. coli* in the study area. When sewage is not properly treated, it can release harmful bacteria, including *E. coli*, into the environment.

The present study revealed an overall 3.3%. Prevalence of *E. coli* O157: H7. This finding of *E. coli* O157:H7 is comparable with that of Klie *et al.* (1997) (3.9%) in Germany, Disasa *et al.* (2017) (2.9%) in Asossa, and Allerberger *et al.* (2001) (3%) in Austria. On the contrary the present prevalence was lower than reports from other researchers, from Jimma Ethiopia by 9.33% Akililu *et al.*, (2017), Mekuria and Beyene (2014) (10.4%) in Tigray, from Ethiopia 8% by Hiko *et al.* (2008), from Iran 8.3% by Hashemi *et al.* (2010) and 9.6% by Shekarforoush *et al.* (2006), Bedasa *et al.* (2018) (12%) in Bishoftu were positive for *E. coli* O157:H7. On the other hand, the result of

present study was lower than the reports of 2 % by Adem Hiko *et al.* (2008) in DebreZeit. The reasons for this low isolation of *E. coli* O157:H7 in the study area could be due current study result was confirmed by polymerase chain reaction which can reduce end up result than research performed by screening test.

In the current study, the prevalence of *E. coli* O157:H7 were 6.67% and 3.33% in sorbitol mackoney agar (SMAC) and polymerase chain reaction (PCR). Thus, prevalence of *E. coli* O157:H7 in PCR is lower than SMAC. This might be to PCR is generally considered to be more sensitive than SMAC agar for detecting *E. coli* O157:H7. This finding was comparable with reports of Fikadu *et al.* (2023) who explained that *E. coli* O157:H7 was 2.7% in the Bedele Municipal Abattoir, South West Ethiopia and finding of Robi and Gelalcha (2020) who reported 2.4% overall prevalence of *E. coli* O157: H7 from Hawassa by using PCR. This means that PCR is more likely to detect *E. coli* O157:H7 even when it is present in low numbers. PCR requires DNA extraction from the sample, which can be affected by factors such as sample storage, handling, and DNA extraction efficiency. SMAC agar, on the other hand, requires proper sample enrichment and incubation conditions to allow for the growth and identification of *E. coli* O157:H7 (Elbastawisy *et al.*, 2023).

Prevalence of *E. coli* O157:H7 in the fecal sample 4.62% (6/130) was significantly higher than environmental swab 2.5% (1/40) and milk samples 2.31% (3/130) ( $2=1.1737$ ,  $p=0.005$ ). This finding was not comparable with reports of Abebe *et al.* (2023) who confirmed that *E. coli* O157:H7 was not detected in milk products from foods of Bovine origin in Dessie and Kombolcha towns, Ethiopia. This high prevalence of *E. coli* O157:H7 in the fecal sample than environmental swab and milk samples because might be because of fecal contamination of milk and environmental samples.

Antibiotic use is an important factor in maintaining human and animal health worldwide. Recently, the development of antibiotic resistance in most bacterial species has become a serious threat to global public health (Fesseha *et al.*, 2022). The results of this investigation demonstrated the significant ciprofloxacin sensitivity of the *E. coli* isolates were found. Ampicillin, tetracycline, and amoxicillin resistance was present in the

majority of the isolates. Similar findings were made by Hiko *et al.* (2008), Bekele (2012), and Magwira *et al.* (2005) from Ethiopia, Botswana, and Ethiopia, respectively. This finding is consistent with reports (Mude *et al.* 2017) showing *E. coli* is resistant to tetracycline. Moreover, the study reported that tetracycline resistance was frequently detected among *E. coli* in Ethiopia (Bekele *et al.*, 2014) and (Taye *et al.*, 2013). However, Mohammed *et al.* (2014) reported in Dire Dawa that *E. coli* was sensitive to tetracycline, which runs counter to the results of the current investigation.

This *E. coli* sensitivity to ciprofloxacin might be attributed to the fact ciprofloxacin is a broad-spectrum antibiotic that is effective against a wide range of Gram-negative bacteria, including *E. coli*. Ciprofloxacin works by inhibiting the bacterial enzyme DNA gyrase, which is essential for DNA replication by inhibiting DNA gyrase, ciprofloxacin prevents the bacteria from replicating its DNA and dividing. Moreover, the high sensitivity of *E. coli* to ciprofloxacin is due to infrequently usage of ciprofloxacin in the study area.

The increased resistance of Ampicillin, tetracycline, and amoxicillin to *E. coli* O157:H7 in the present study may be due to the following reasons. Tetracycline can be pumped out of *E. coli* cells via efflux pumps, which lowers the intracellular concentration of the drug and increases bacterial resistance. Amoxicillin's beta-lactam ring can be broken down by beta-lactamase enzymes produced by *E. coli*, rendering the antibiotic useless. Penicillin-binding proteins (PBPs) affinity for ampicillin can be changed by mutations in the genes that encode them, which lowers the antibiotic's capacity to bind and prevent the formation of cell walls. The variation in resistance for a single drug may be due to the expression of resistant gene coded by the pathogen which is associated with emerging and reemerging aspects of the isolates with regard to different agroecology (Reuben, and Owuna, 2013). This might be due to the inappropriate use of antibiotics for the treatment of diseases or irrational use of antimicrobials for therapeutic and prophylactic treatment (Majaliya, *et al.*, 2010). As a result, the emergence of antibiotic resistance in bacteria such as *E. coli* O157:H7 is a major public health concern. The variation in multidrug resistance development for bacteria may be due to variations in dose, route of administration, regimen, continuous and indiscriminate use of antimicrobials for treatment and feed

additive in various study areas, as well as level of awareness and geographic location for studies abroad. Because of the rapid development of multidrug resistance, the efficiency of treatments and the ability to manage infectious diseases in both animals and people may be significantly limited (Thaker *et al.*, 2012)

The current investigation indicates that around 73.86% of the *E. coli* isolates were detected in old animals, 58.93% were in adult animals, and 51.47% were isolated from young dairy cows. The results of this investigation showed a statistically significant correlation ( $p < 0.05$ ,  $\chi^2 = 12.49$ ) between the age of the dairy cows and the isolation of *E. coli*. This study finding is not lined with previous reports of different studies such as Gebregiorgis and Tessema, 2016, Muktar, 2014; Muktar *et al.*, 2015, Villarroel, 2009 who confirmed younger animals were mostly clinically affected. This highly prevalence of the *E. coli* in old animals than other age group of animals may be attributed to as animals age, their immune system becomes weaker. This makes them more susceptible to infections, including *E. coli*\* infections. In addition, as animal's age, their gastrointestinal tract undergoes changes that can make them more susceptible to *E. coli* infections. For example, the stomach acid production decreases with age, which can allow *E. coli* to survive in the stomach and reach the intestines (Peng *et al.*, 2022).

According to the current investigation, there was a significant correlation ( $p = 0.045$ ,  $\chi^2 = 4.0323$ ) between the size of the farm and the isolation of *E. coli* from samples. Compared to small-scale dairy farms (62.32%), cows on medium-sized farms had higher rates of *E. coli* infection (74.19). This finding was the corroborates with the finding of Tadesse *et al.* (2024) who reported higher rate isolation of *E. coli* was encountered in sample of the medium sized farms than small holder farms. Medium-sized farms typically have a higher density of animals compared to smallholder farms. This increased animal density can lead to higher levels of *E. coli* in the environment, as the bacteria are shed in animal feces. The combination of higher animal density, less stringent management practices, and potential gaps in hygiene and knowledge can contribute to a higher prevalence of *E. coli* in medium-sized farms compared to smallholder farms.

The questionnaire survey analysis revealed that significant associations were observed for milk contamination ( $P < 0.05$ ) including hand washing before milking, washing the udder before milking, covering the head during milking, using disinfectants and antiseptics, maintaining a clean drainage system, keeping farms clean, and being aware of the drug's withdrawal period. Water supply and milk container type, however, did not appear to be significant risk factors for the contamination of milk. This survey study was allied with previous reports of Deddefo *et al.*, (2023) who explained hygienic practices in the dairy farms can be significant factors for milk contamination in dairy farms in Asella, Ethiopia. Moreover, this survey result was comparable with reports of Hamiroune *et al.* (2016) on farms in Algeria; Mogotu *et al.* (2022) in selected counties in Kenya. This significant effect of dairy farm practices on the milk contamination may be attributed due to improper hand washing before milking: This increases the risk of bacteria from the milker's hands contaminating the milk. Absence of proper washing of the udder before milking allows dirt and bacteria on the udder to get into the milk. A dirty environment increases the risk of disease and contamination of the milk. Not being aware of the drug's withdrawal period can lead to residual medication ending up in the milk, which can pose health risks to consumers.

## 6. CONCLUSION AND RECOMMENDATIONS

The current study revealed high magnitude of *E. coli* contamination and drug-resistant *E. coli* O157:H7 H7 is prevalent in the study area. The high prevalence of *E. coli* contamination might be due to unhygienic practices. The multidrug resistance pattern of all *E. coli* O157:H7 isolates might be due to the injudicious and extensive use of antibiotics in both veterinary medicine and public health. Factors such as the age, farm size and sample type were found to be significantly ( $p < 0.05$ ) correlated with the occurrence of *E. coli* in the dairy cows. Due to toxin gene profiles, *E. coli* O157:H7 isolates obtained from the fecal, environmental swab and milk samples in this study might be hazardous for public health. Drug like ciprofloxacin was effective in inhibiting the growth of the isolates while ampicillin, tetracycline, amoxicillin performed poorly. Questionnaire surveys revealed that washing before milking, washing udder before milking, head cover during milking, using of disinfectants and antiseptics, hygienic conditions for drainage system, hygienic condition of the farms, knowledge on the withdrawal period of the drug were significant potential factors for milk contamination which can be lead to highly occurrence of *E. coli*.

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

- ❖ Regular training for dairy farm workers should provide on proper hygiene practices and antibiotic use.
- ❖ Promotion of good hygienic practice, Sanitation Standard Operating Procedures should be needed.
- ❖ The emergence and spread of antibiotic-resistant pathogens should be assessed regularly and rational use of antibiotics should be practiced.
- ❖ Conduct surveys and interviews with dairy farm workers to better understand the factors contributing to contamination and identify potential solutions.
- ❖ Conduct further studies on the prevalence of *E. coli* O157:H7 in dairy farms and explore the impact of antibiotic use on the development of resistance.

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## 8. ANNEXES

### Annex I: Age Determination in dairy cattle

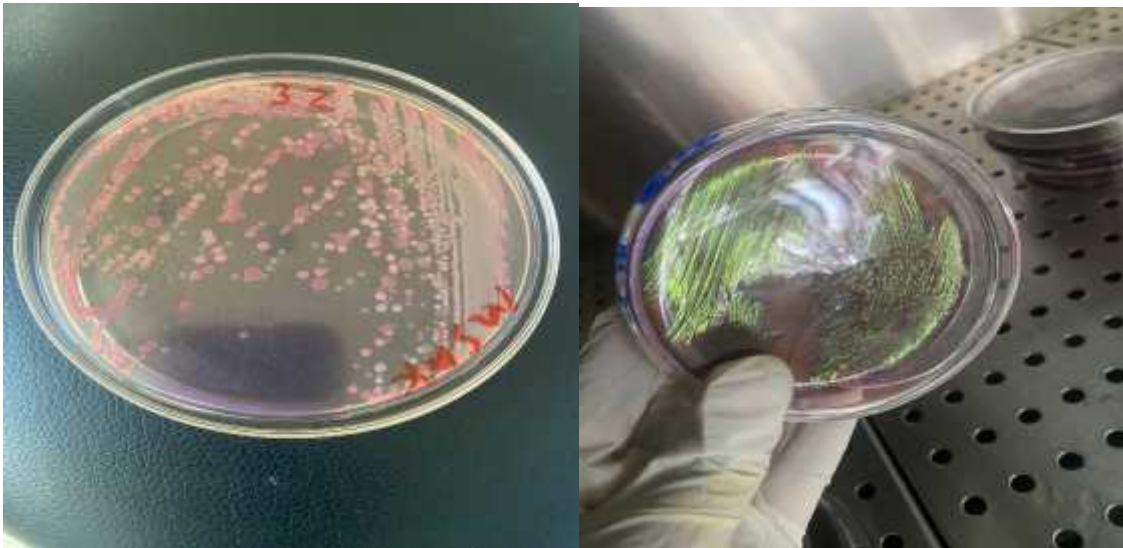
Eruption	Age in year
I1 erupted	1.5-2Years
I2 erupted	2-2.5Years
I3 erupted	3Years
C erupted	3.5-4Years
All I in wear	5Years
I1 is leveled and neck is emerged from gum	6 Years
I2 is leveled and neck is emerged from gum	7Years
I3 is leveled and neck is visible, I4 may also leveled	8Years
C is leveled and neck is visible	9 Years
Dental stars are squared in I2 and	10 Years
Dental stars are squared in all teeth	12 Years

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

### 1. Isolation and Identification

Typical colonies on Mac Conkey agar for primary isolation of *E. coli* (Difco laboratories, USA)

(Pink, due to their ability to ferment lactose). A single, isolated colony was pick and Sub-culture to Eosin Methylene- Blue (EMB) agar. The colonies with green metallic sheen on EMB agar which was typical feature of *E. coli* was transferred to nutrient agar then used for primary biochemical tests, a single isolated colony was picked. The suspected *E. coli* isolates were subjected to the biochemical tests (Lagier, *et al.*, 2015).



SMAC test result

EMB test result

#### A. Indole Test

The test organisms were cultured in 3 ml of peptone water containing tryptophan at 37°C for 24 hrs. One ml of diethyl ether was added, shake well and allowed to stand until the ether rises to the top. Then 0.5 ml Kovac's reagent was gently run down the side of the test tube to form pink colored ring indicates a positive result.

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

A loopful of colony was streaked onto a Simmons-citrate agar slant, and then incubated for 24 to 48 hours. At 37°C in incubator. Change in medium color to blue color indicates positive result but if it remains green it is citrate negative.

#### C. Methyl-Red Test Procedure

Single colony from the pure culture of the test organism was inoculated in 5 ml of sterile MR-VP broth. After 24 hrs. Incubation at 37°C, 5 drops of methyl red solution was added and observed for color formation. Development of red or yellow color indicated positive or negative result, respectively

#### D. Vogas-Proskauer Test Procedure

The test organisms were grown in 3 ml of sterile MR-VP broth at 37°C for 48 h and then 0.6 ml of 5% alpha-naphthol and 0.2 ml of 40% potassium hydroxide containing 0.3% creatine was added per ml of broth culture. Following well shaking, the broth was allowed to stand of 5-10 minutes to observe the color formation. Development of pink-red color was indicated positive result.

#### E. Triple Sugar Iron

Composition (g/l): meat extract 3.0; yeast extract 3.0; peptone 20.0; sodium chloride 5.0; Lactose 10.0; sucrose 10.0; glucose 1.0; ferric citrate 0.3; sodium thiosulphate 0.3; phenol red 0.024; agar 12.0. Preparation: suspend 65 g in 1 liter of distilled water and bring to boiling Dissolve completely. Mix well and distribute into container then sterilize by autoclaving at 121°C for 15 minutes and dispense into test tubes. Allow the medium to set in sloped form with butt 1 inch deep. Well isolated colonies are picked with a sterile wire. The slant is streaked and the butt is stubbed. Incubate inoculated tubes at 37°C for 18 to 24 hours.

A: butt: yellow if glucose used, red/unchanged if glucose is not used/black if H<sub>2</sub>S is formed and bubble if gas is formed.

B: Slant surface: yellow if lactose and /or sucrose used and red /unchanged if lactose and/or sucrose is not used



Indole test positive result



Citrate negative result



TSI test result

**Annex III:** Biochemical characterization of *E. coli*.

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

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

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

#### **Annex IV:** Polymerase chain reaction

The PCR assay was based on the sequence of the *uidA*, *Stx1*, and *Stx2* genes of *E. coli* O157:H7. Specific primers targeting each of the toxigenic genes were used to amplify the gene products. A 25  $\mu\text{L}$  of total volume of PCR reaction (12.5  $\mu\text{L}$  of 2 $\times$ Go Taq Green Mix Master (Promega Corporation, USA), including 2  $\mu\text{L}$  of Primer-F and Primer-R, 6.5  $\mu\text{L}$  of DNeasy-free water (Promega Corporation, USA), and (v) 4  $\mu\text{L}$  DNA template of *E. coli* O157:H7) was used. The whole mixture was placed in the Eppendorf tube of 200  $\mu\text{L}$  (Biozym, Oldenhof, Germany). Finally, by using gel electrophoresis, DNA ladder 100 bp, and 2% agarose gel (Peqlab, Erlangen, Germany), the amplicons of the specific sequence was determined.

Source: (Jinneman *et al.*, 2003).

## **Annex V: Antibiotic resistance tests**

Antibiotic resistance tests of 14 *E. coli* O157:H7 isolates were screened for susceptibility. McFarland's standard of 0.5 was applied to the suspension. Using sterile forceps, the antimicrobial agents used in the study period were carefully deposited onto Mueller-Hinton agar (MHA) after this suspension was infected there. This was done to guarantee that the medium was in touch. Following a 37°C overnight incubation period, the zones of inhibition surrounding each antibiotic disk were examined. The diameters of the inhibition zones were measured using a digital caliper by comparing the zone of inhibition with the Kirby-Bauer chart. The results were then documented in a manner that had been pre-planned. The isolates were categorized as s Susceptible (S), intermediate (I), and resistant (R) after the inhibition zone results surrounding specific antibiotic disks were analyzed.



Result of AST by disk diffusion test

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

No	Antimicrobials	Resistance	Intermediate	Susceptible
1.	Ciprofloxacin	$\leq 11$	22-25	$\geq 26$
2.	Tetracycline	$\leq 11$	12-14	$\geq 15$
3.	Ampillicin	$\leq 13$	14-16	$\geq 17$
4.	Amoxicillin	$\leq 13$	14-16	$\geq 17$
5.	Doxycycline	$\leq 10$	11-13	$\geq 14$
6.	Streptomycin	$\leq 11$	12-14	$\geq 15$

Source: (CLSI, 2014)

**ADDIS ABABA UNIVERSITY**  
**POSTGRADUATE PROGRAM DIRECTORATE**  
**COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE**  
**DEPARTMENT OF VETERINARY PUBLIC HEALTH**

Here by the following Questionnaire is set for the Dairy milk producers/farmers to carry out MSc thesis research on the title of Isolation, Identification, and Antimicrobial Resistance Profile of *E. Coli* O157:H7 in Selected Dairy Farms in Wolaita Sodo Town, South Ethiopia. Background information concerning hand washing before milking, washing the udder before milking, covering the head during milking, using disinfectants and antiseptics, maintaining a clean drainage system, keeping farms clean, and being aware of the drug's withdrawal period, water supply and milk container type in the dairy farms found in the Wolaita Sodo Town was targeted. The personal profile obtained from the respondents with regard to the subject matter is kept confidential and would not have any consequence on the respondent in any way. Thus, please give your genuine response for contributions of your foot print to validate the information available at your area. Your cooperation in completing the questionnaire was highly appreciated!

**Socio Demographic Information**

Name of the respondent: \_\_\_\_\_ Sex: \_\_\_\_\_ Kebele name:  
\_\_\_\_\_ Date: \_\_\_\_\_ Age: \_\_\_\_\_

- 1 .Level of education a) Illiterate b) Primary school c,) Secondary School d) high school e) Collage and above
- 2 .Do you clean the udder before milking? A. Yes B. No
3. Do you wash your hands before milking your lactating cows? A. Yes B. No
- 4 What types of milk containers do you use? A. Plastic B. stainless steel
- 5 Do you use apron/white coat and or head cover during Milking process? A. Yes B. No
6. Do you use detergent /disinfectants for cleaning the farm? A. Yes B. No .....
- 7 What is the hygienic condition of drainage systems? A. Good B.Poor .....

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

9. What is the status of water supply? A. Good B .poor

10.Do you know about drug withdrawal period? A. Yes B. No

**Annex VI: Declaration of informed voluntary consent: (English Version)**

I have read or had read to me the participant information sheet. I have clearly understood the Purpose of the research, the procedures, the risks and benefits, issues of confidentiality, the right of participation and the contact address for any queries. I have been given the opportunity to ask any questions about things that may have been unclear. I was informed that I have the right to withdraw from the study at any time or not answer any question that I do not want. Therefore, I declare my voluntary consent to participate in this study with my signature as indicated below.

Name of lactating cow owners: \_\_\_\_\_ Signature \_\_\_\_\_

Data collector: Name: \_\_\_\_\_ Signature \_\_\_\_\_

N.B.: This is to be signed face-to-face in the presence of the data principal, researcher, or collector.

Please provide a copy of this signed consent to the participant.

