

**ADDIS ABABA UNIVERSITY**  
**COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE**  
**DEPARTEMENT OF CLINICAL STUDIES**



**EPIDEMIOLOGY AND ASSESSMENT OF CRITICAL CONTROL POINTS OF *E. COLI***  
**O157:H7 ALONG DAIRY SUPPLY CHAINS IN CENTRAL ETHIOPIA**  
**MVSc THESIS**

**By**  
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**JUNE 2018**  
**BISHOFTU, ETHIOPIA**

**EPIDEMIOLOGY AND ASSESSMENT OF CRITICAL CONTROL POINTS OF *E. COLI*  
O157:H7 ALONG DAIRY SUPPLY CHAINS IN CENTRAL ETHIOPIA**



**A Thesis Submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Veterinary Science in Veterinary Epidemiology**

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**JUNE 2018  
BISHOFTU, ETHIOPIA**

**Addis Ababa University**  
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## LIST OF ABBREVIATIONS

AAU	Addis Ababa University
AU	Ambo University
BPW	Buffered Peptone Water
CAVS	College of Agriculture and Veterinary Science
CCP	Critical Control Points
CLSI	Clinical and Laboratory Standards Institute
CVMA	College of Veterinary Medicine and Agriculture
EAggEC	Enteroaggregative <i>Escherichia coli</i>
EHEC	Enterohemorrhagic <i>Escherichia coli</i>
EIEC	Enteroinvasive <i>Escherichia coli</i>
EPEC	Enteropathogenic <i>Escherichia coli</i>
ETEC	Enterotoxigenic <i>Escherichia coli</i>
Gb-3	Globotriaosylceramide-3
HACCP	Hazard Analysis Critical Control Point
HC	Hemorrhagic Colitis
HUS	Haemolytic Uraemic Syndrome
MDR	Multidrug-Resistant
NSF	Non-sorbitol Fermenting
PCR	Polymerase Chain Reaction
SMAC	Sorbitol MacConkey
STEC	Shiga Toxin <i>Escherichia coli</i>
Stx	Shiga Toxins
TTP	Thrombotic Thrombocytopenic Purpura

## ABSTRACT

A cross-sectional study was carried out from November 2017 to May 2018 to assess the handling and hygienic practices of milk and to determine the epidemiology, antimicrobial resistance pattern and critical control points of *E. coli* O157:H7 along the dairy supply chains in Akaki Kality sub-city of Addis Ababa, Bishoftu and Sululta towns of central Ethiopia. Accordingly, a total of 91 owners of dairy farms, milk collection centers and cafeterias were participated in survey study and interviewed using structured questionnaire. The result indicated that, 13.8% of the producers in the study areas did not manage hygienic practices of udder and hand washing before and after milking. Also, 57.7% of cafeterias in the study areas used well water sources for routine sanitation practices. However, a total of 450 raw cow milk (294), milker hand swab (65) and water (91) samples were collected from dairy farms, milk collection centers and cafeterias and processed according to the standards in order to isolate and identify *E. coli* O157:H7. Out of the total 450 samples examined, 6.0% were contaminated by *E. coli* O157:H7. Accordingly, 9.89% of water, 9.23% of milker hand swab and 4.08% of raw milk samples were contaminated by the pathogen. Furthermore, the prevalence of *E. coli* O157:H7 was 7.79%, 6.21% and 3.97% in Akaki kality sub-city, Sululta and Bishoftu towns, respectively. The result of multivariable logistic regression analysis revealed that there was statistically significant difference observed ( $p < 0.05$ ) between the occurrence of the pathogen in the milk and water samples, and the sources of water used. The study also revealed that, varying level of resistance of *E. coli* O157:H7 isolates against nine antimicrobial discs tested and 100% ( $n = 27$ ) of the isolates showed multidrug-resistance comprising from two up to seven antimicrobial drugs. The assessment of critical control point result also revealed that, the pathogen was also isolated from dairy farm and cafeterias in the study areas. In conclusion, this study has clearly indicated that, there is poor milk handling practices, the occurrence of *E. coli* O157:H7 and its multiple drug resistant profiles in milk samples along the dairy supply chains and its risk for public health and food safety. Therefore, proper hygienic practices from dairy farms to fork and rational drug usage are recommended.

**Keywords:** *Antimicrobial Resistance, Critical Control Points, Escherichia coli O157:H7, Ethiopia, Milk*

## 1. INTRODUCTION

Dairying is an important enterprise for many countries of the world and is an especially important source of income generation for rural families in the developing agricultural nations like Ethiopia (CSA, 2008). Milk is one of the dairy products and demand in the market. Since, milk is the most perfect single balanced food of high biological value and food of outstanding interest which has been taken by mammals since the earliest pre-historic time which still forms the basis of most nations' economies (Melese and Beyene, 2009; Mesfine *et al.*, 2015). It provides the primary source of nutrition for young mammals before they can digest other types of food (Olatunji *et al.*, 2012). Thus, it contains almost all ingredients of food in the right proportion in an easily digestible form (Mesfine *et al.*, 2015). However, apart from its nutritional value, milk can be easily contaminated by many food-borne pathogens if not appropriately managed and handled.

Despite the extensive scientific progress and technological developments achieved in the past years in developed countries, food-borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and medical and social costs (Ali *et al.*, 2010). Moreover, sporadic cases and outbreaks of human diseases caused by food-borne pathogens have been linked to ground beef, raw milk, meat and dairy products, vegetables, unpasteurized fruit juices and water. From those, contaminated raw milk is one of the primary sources of food-borne illnesses (Rahimi *et al.*, 2012). Furthermore, changes in eating habits, mass catering, complicated and long supply chains with increased international movement and poor hygiene practices are major contributing factors. Thus, it is essential to keep the health and hygiene of the cow, the environment in which the cow is housed and milked, hygiene during milking and storage equipment influence microbial contamination of milk (Javaid *et al.*, 2009).

Raw milk being as nutritious food; it serves as an ideal medium for the growth of various bacteria including pathogenic organisms which have a significant impact on public health (Popescu and Angel, 2009). Many microorganisms can get access to milk and milk byproducts among which species, *Escherichia coli* is recognized to be of primary concern (Thaker *et al.*, 2012). Moreover, many *E. coli* strains are harmless or even beneficial to the host. However, some strains of *E. coli*

can be pathogenic to humans and are harbored in food animals. Among them *E. coli* O157:H7 is the best known pathogenic strain (Riemann and Cliver, 2006; Kozub-Witkowski *et al.*, 2008).

Enterohemorrhagic *Escherichia coli* (O157:H7) is recognized as the primary cause of bloody diarrhea or hemorrhagic colitis. Thus, the characteristic feature of this group is the production of verocytotoxins (Bandyopadhyay *et al.*, 2012), also known as Shiga toxins (Stx). This Shiga toxin is produced in the colon and damages the tissue resulting in bloody diarrhea (Gordillo *et al.*, 2011). It then travels to kidney through the bloodstream damaging renal endothelial cells resulting in renal inflammation (Bandyopadhyay *et al.*, 2012). Moreover, cattle are the primary reservoir of *E. coli* O157:H7 and an important source of human infection (Varela-Hernández *et al.*, 2007), undercooked ground beef and unpasteurized milk being common vehicles of the pathogen transmission (Su *et al.*, 2012). Worldwide the food-borne *E. coli* O157:H7 estimated to cause 2.8 million acute illnesses each year (Majowicz *et al.*, 2014). Also, an estimated 73,480 illnesses due to *E. coli* O157:H7 infection occur each year in the United States, leading to an estimated 2,168 hospitalizations and 61 deaths annually (Rangel *et al.*, 2005; Scallan *et al.*, 2011) resulting in an economic burden of 607 million US dollars (Scharff, 2012). It has emerged as an important global zoonotic food and water-borne pathogen, which produces serious illness in humans such as haemorrhagic colitis, haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) and an important cause of acute renal failure in children (Chekabab *et al.*, 2013; Pal *et al.*, 2016).

In order to mitigate the risk of the transmission of zoonotic infections associated with contaminated food, international food management agencies, especially the World Health Organization (WHO), the Food and Agriculture Organization (FAO) and the International Hazard Analysis Critical Control Point (HACCP) alliance have already provided guidelines to member countries about safe handling procedures such as HACCP and Good Manufacturing Practices (GMP) (Ali *et al.*, 2010; Sikora *et al.*, 2013). HACCP is not well known in the context of our country and not implement as quality management systems in food industries.

There are scanty information about the prevalence, distribution and associated virulent genes of *E. coli* O157:H7 in humans, animals or in foods of animal origin in Ethiopia (Hiko *et al.*, 2008;

Mersha *et al.*, 2010; Taye *et al.*, 2013). Furthermore, it has not been determined well to what extent milk producers and retailers house environment serve as a source of *E. coli* O157:H7 contamination for dairy products. Thus, the investigation of this pathogenic organism contributes to the understanding of its epidemiology by identifying the transmission routes, vehicles and source of milk contamination. Knowledge of the transmission routes and vehicles also allows consumers to be aware and facilitate so as to decrease the risk for infection. Therefore, this study was designed with the following objectives.

### **General objective**

- To assess the handling and hygienic practices of milk and to determine the epidemiology, antimicrobial resistance pattern and assessment of critical control points of *E. coli* O157:H7 along the dairy supply chains in Akaki Kality sub-city of Addis Ababa, Bishoftu and Sululta towns of central Ethiopia.

### **Specific objectives**

- ❖ To assess the hygienic milking and milk handling practices along the dairy supply chains.
- ❖ To estimate the prevalence of *E. coli* O157:H7 from raw cow milk and environmental samples.
- ❖ To determine the antimicrobial resistance pattern of isolates by disc diffusion method.
- ❖ To assess the critical control points of *E. coli* O157:H7 along the dairy supply chains.

## 2. LITERATURE REVIEW

### 2.1. A general overview of *Escherichia coli*

*Escherichia coli* are gram negative, facultative anaerobic, rod shaped, highly mobile and non-sporulating bacteria. They are often classified under *Enterobacteriaceae*, known to be normal inhabitants of the gastrointestinal tract of both animals and human beings (Oliver *et al.*, 2009), but only some strains of *E. coli* have become highly adapted to cause diarrhea and a range of extra-intestinal diseases. Moreover, the cells are about 2  $\mu\text{m}$  long and 0.5  $\mu\text{m}$  in diameter with a cell volume of 0.6 to 0.7  $\mu\text{m}^3$  (Markey *et al.*, 2013). Furthermore, *Escherichia coli* were first isolated by a German paediatrician, Theodore Esherich, in 1884 from faeces of human neonates (Khan and Steiner, 2002). Up till now in the genus *E. coli*, there are hundreds of serotypes of *E. coli* which are classified on the bases of various surface antigens referred to as Somatic (O), Capsular (K), Flagellar (H) and Fimbrial (F) (Zhang *et al.*, 2006).

Thus, there are approximately 174 O antigens, 56 H antigens, and 103 K antigens that have been identified. Additionally, there are several strains of the pathogen that have been isolated (Frenzen *et al.*, 2005). Moreover, the pathogenic groups of *E. coli* are divided into five groups on the basis of their virulence properties such as enterohemorrhagic (EHEC, found in human, cattle and goats), enteroinvasive (EIEC, found only in humans), enteropathogenic (EPEC, causative agent of diarrhea in humans, rabbits, dogs, cats and horses), enterotoxigenic (ETEC, causative agent of diarrhea in humans, pigs, sheeps, goats, cattle, dogs and horses) and enteroaggregative (EAggEC, which found only in human) *E. coli* (Biswas *et al.*, 2006; Xia *et al.*, 2010). Hence to that, regarding on the zoonoses, the most important category is enterohemorrhagic (EHEC), which is also the most severe (Nguyen and Sperandio, 2012).

*Escherichia coli* O157:H7 (EHEC) is considered as a worldwide threat to public health (Chinen *et al.*, 2009; Signorini and Tarabla, 2009; Fedio *et al.*, 2011) and it has several virulence factors such as Shiga toxins (Stx1 and Stx2), intimin (*eaeA*) and enterohemolysin (*hlyA*). Thus, Shiga toxins (Stx) are capable of inhibiting protein synthesis and can cause eukaryotic cell injury and

death (Rey *et al.*, 2006; Bentancor *et al.*, 2012). On the other hand, intimin encoded by the *eaeA* gene, is responsible for adherence on the intestinal mucosa and is important for causing intestinal lesions (Rey *et al.*, 2006; Mora *et al.*, 2007; Bentancor *et al.*, 2012). Also, enterohemolysin is encoded by *hlyA* and may contribute to virulence by lysing of erythrocytes and therefore, providing a source of iron to the bacterium to survive in the intestine (Fu *et al.*, 2005; Rey *et al.*, 2006). Therefore, *Escherichia coli* O157:H7 is not only a major cause of haemorrhagic colitis (HC) but also haemolytic uraemic syndrome (HUS), which is characterized by thrombocytopenia, haemolytic anaemia and nephropathy (Gordillo *et al.*, 2011; Bandyopadhyay *et al.*, 2012). Thus, the pathogen is non-sorbitol fermenting (NSF) and biochemically, oxidase negative, catalase positive, indole positive, urease negative, Voges-Proskauer negative, and citrate negative (Rosser *et al.*, 2008).

Furthermore, the first outbreaks caused by *E. coli* O157 occurred in Oregon and Michigan, USA, in 1982, when it was isolated from individuals who developed bloody diarrhea and severe abdominal cramps after eating hamburgers in a restaurant chain (Pennington, 2010). In addition, investigations indicated that *Escherichia coli* O157:H7 is an emerging cause of food-borne illness and young dairy cattle are a reservoir for it. Therefore, infection with the strains often associated with food-borne outbreaks traced to milk, dairy products, under cooked ground meat products and other foods that lead to hemorrhagic colitis (bloody diarrhea) and hemolytic uremic syndrome in humans (Desmarchelier and Fegan, 2011; Nguyen and Sperandio, 2012).

## **2.2. Epidemiology of EHEC O157:H7**

### **2.2.1. Geographic distribution**

Enterohemorrhagic *Escherichia coli* infections occur worldwide. However, the lineages of this organism are reported to differ between regions, potentially influencing the incidence and severity of human disease (CFSPH, 2016). The numbers of case and outbreak due to STEC O157 have been reported worldwide since the first reported outbreak in the USA. It was the causative agent of many outbreaks worldwide. Therefore, serotype O157:H7 has been isolated in outbreaks

in Canada, Great Britain, and the United States. It has also been isolated in Argentina, Australia, Belgium, former Czechoslovakia, China, Germany, Holland, Ireland, Italy and Japan (Xia *et al.*, 2010). Also, reports from Africa have shown that the rates of infections by the pathogen but in countries lacking diagnostic capabilities might be underestimated (Tarr *et al.*, 2005). Thus, microbiologically culture had proven *E. coli* O157:H7 diarrheal cases have been reported from some African countries including South Africa, Swaziland, Central African Republic, Kenya, Ethiopia, Uganda, Gabon, Nigeria and Ivory Coast (Raji *et al.*, 2006). Annual incidence rates of 0.4 to 2.74 per 100,000 inhabitants per year have been reported in the region of Australia, USA, Mexico and Japan by Sakuma *et al.* (2006), Vally *et al.* (2012), Canizalez-Roman *et al.* (2013) and Sodha *et al.* (2015). Furthermore, a prevalence of 5.68% of *E. coli* O157:H7 in cattle at the global level have been reported by Islam *et al.* (2014).

#### 2.2.2. Susceptibility and reservoirs of *E. coli* O157:H7

Understanding the epidemiology of this organism requires knowledge of where these bacteria live and grow in nature (their reservoir) and of how humans come into contacts with them. Ruminants, especially cattle, sheep and possibly goats, are the major reservoirs for EHEC O157:H7, but are not normally affected by this organism (Naylor *et al.*, 2005; Ragione *et al.*, 2009; CFSPH, 2016). All ages of cattle are susceptible to colonization with the pathogen, although peak shedding is observed in sub adult cattle from weaning to 24 months of age (Joris *et al.*, 2012). Also, the strain is occasionally isolated from other animals but it is believed that the bacteria are present as transients and that the animals acquired these bacteria from meat, foods or water contaminated by faecal material from ruminants (Dharmasiri *et al.*, 2010). Furthermore, people of all ages are susceptible to infection with STEC. However, the young and the elderly are more susceptible and are more likely to develop more serious symptoms (FDA, 2012).

#### 2.2.3. Factors affecting survival and growth of *E. coli* O157:H7 in the environment

The organism can survive and persist in numerous environments, such as soil, water, and food as well as in animal reservoirs (Chekabab *et al.*, 2013). It has been shown to survive for a year in manure-treated soil and for 21 months in raw manure that had not been composted. Meanwhile,

composting manure is effective in destroying the pathogen if the temperature is maintained above 50 °C for six days (Lim *et al.*, 2010). Also, it can survive for a long time in the water, especially at cold temperatures. Thus, water trough sediments contaminated with bovine faeces can serve as a long-term (> 8 months) reservoir of *E. coli* O157:H7 and the surviving bacteria in contaminated troughs is a source of infection (Avery *et al.*, 2008; Liu *et al.*, 2008).

Additionally, to survive in varied environments, the pathogen requires the ability to adapt to variations or extreme changes in temperature, pH, and osmolarity conditions commonly encountered in nature. For example, its exopolysaccharide (EPS) production is associated with heat and acid tolerance, and the alteration of lipid composition in membranes is induced by heat stress (Yuk and Marshall, 2004; Oh *et al.*, 2009). Thus, the ability to be acid resistant varies among strains and is influenced by growth phase and other environmental factors. Once induced, acid resistance is maintained for long periods of time during cold storage. The stationary-phase are more resistant than growing cells to acid (Oh *et al.*, 2009).

Therefore, these environmental adaptations of *E. coli* O157:H7 play an important role in the persistence and dissemination of this microorganism on farms and increasing transfer from cattle to cattle. Also, the ability to survive outside the host reservoir increases the risk that the pathogen may contaminate crops via bovine manure contamination, irrigation with contaminated water, or direct contact with infected animals (Lim *et al.*, 2010).

#### 2.2.4. Source of infection and mode of transmission

*Escherichia coli* O157:H7 has been isolated from the faeces or gastrointestinal tract of cattle, sheep, horses, pigs, turkeys, dogs, and a variety of wild animal species (Kiranmayi *et al.*, 2010). However, epidemiologic studies have found that cattle manure is the source of most human infections. It has also been isolated from bodies of water sources like ponds and streams, wells, and water troughs and has been found to survive for months in manure and water trough sediments (Fairbrother and Nadeau, 2006). Also, it presents in purchased animal feeds. Therefore, such feeds may be an important route by which the pathogen is disseminated to farms (Hancock *et al.*, 2001).

Enterohemorrhagic *Escherichia coli* are transmitted by the faecal-oral route. It can spread between animals by direct contact or via water troughs, shared feed, contaminated pastures or other environmental sources. In addition, birds and flies are potential vectors. Moreover, in one experiment, EHEC O157:H7 was transmitted in aerosols when the distance between pigs was at least 10 feet. The organism was thought to have become aerosolized during high-pressure washing of pens, but normal feeding and rooting behavior may have also contributed (CFSPH, 2016). Furthermore, people mainly become infected with EHEC O157:H7 by ingesting contaminated food and water, or during contact with animals (especially ruminants) faeces and contaminated soil (Fairbrother and Nadeau, 2006; Gyles, 2007). Thereby, the infectious dose for humans is estimated to be less than 100 organisms, and might be as few as 10 (CFSPH, 2016). Additionally, food-borne outbreaks caused by the pathogen are often associated with undercooked or unpasteurized animal products, particularly ground beef, but also other meats and sausages and unpasteurized milk and cheese (Ateba and Bezuidenhout, 2008; CFSPH, 2016).

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

In Ethiopia, there were few studies conducted by some researchers to determine the prevalence of *E. coli* O157:H7 in raw milk of cow in different areas of the country. Disassa *et al.* (2017) have been reported a prevalence of 2.9% of *E. coli* O157:H7 from traditionally marketed raw cow milk in and around Asosa town, western Ethiopia. Similarly, a prevalence of 6.9% of the pathogen has been reported from raw milk in selected commercial dairy farms of Holeta district by Ayano *et al.* (2013). On the other hand, a contamination frequency of 3.27% also reported from raw milk and milking equipment samples collected from different dairy farms in Bishoftu town by Zemenu (2017). In addition, an isolation frequency of 12% of the pathogen was reported from raw milk sample collected from open markets in Bishoftu town by Bedasa *et al.* (2018).

### 2.3. Pathogenesis and clinical manifestations

The pathogenicity of *Escherichia coli* O157:H7 is encoded by a variety of plasmid, bacteriophage and chromosomal genes (Kiranmayi *et al.*, 2010). On the other hand, it has several virulence

factors such as Shiga toxins (Stx 1 and Stx 2), intimin (*eaeA*) and enterohemolysin (*hlyA*) (Rey *et al.*, 2006; Bentancor *et al.*, 2012). Thus, Shiga toxin is composed of two major subunits, designated as A and B. The B subunit forms a pentamer that binds to globotriaosylceramide-3 (Gb3) and this specificity determines where Shiga toxin mediates its pathophysiology (Nguyen and Sperandio, 2012). While, the A subunit exhibits an RNA N-glycosidase activity against the 28S rRNA that inhibits host protein synthesis (Karmali *et al.*, 2010) and can cause eukaryotic cell injury and death (Rey *et al.*, 2006; Bentancor *et al.*, 2012). Thus, the production of Shiga toxin is central to the pathogenesis of bloody diarrhea and haemolytic uraemic syndrome (Pennington, 2010). Also, intimin, encoded by the *eaeA* gene, is responsible for adherence on the intestinal mucosa and is important for causing intestinal lesions (Mora *et al.*, 2007; Bentancor *et al.*, 2012). Alternatively, enterohemolysin which is encoded by *hlyA* and may contribute to virulence by lysing of erythrocytes and therefore providing a source of iron to the bacterium to survive in the intestine (Fu *et al.*, 2005; Rey *et al.*, 2006).

In humans, EHEC O157:H7 colonizes the large intestine. Thus, Shiga toxin released by the pathogen binds to endothelial cells expressing Gb-3, allowing absorption into the bloodstream and dissemination of the toxin to other organs (Nguyen and Sperandio, 2012). The tissues and cell types expressing Gb-3 varies among hosts, and the distribution of Gb-3 targets the pathology of toxin-mediated disease to cells expressing Gb-3 (Callaway *et al.*, 2009). For example, renal glomerular endothelium expresses high levels of Gb-3 in humans (Nguyen and Sperandio, 2012).

The clinical manifestation of acute phase associated with this organism is named hemorrhagic colitis in humans. Thus, the symptoms characteristic to this disease are watery and/or bloody diarrhea, fever, nausea, severe abdominal cramping and vomiting (Callaway *et al.*, 2009; Walker *et al.*, 2012). Furthermore, from the point of ingestion, the incubation period of the infection ranges from 8 hours to 16 days, but the typical incubation period is three to four days and the illness usually lasts 5-10 days (Robinson and McKillip, 2010).

Additionally, life-threatening complications on some victims, particularly the very young, the elderly and those who are immunosuppressed or have debilitating illnesses, may develop haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Karmali,

2009; Martorelli *et al.*, 2017). Thus, haemolytic uraemic syndrome is characterized by three features, acute renal failure, haemolytic anemia (reduction in the number of red blood cells) and thrombocytopenia (a drop in the number of blood platelets), sometimes preceded by a bloody diarrhea (Adam and Moss, 2008) and occurs in up to 15% of hemorrhagic colitis victims and can lead to permanent loss of kidney function (FDA, 2012). In addition, thrombotic thrombocytopenic purpura is a less common complication which is largely confined to adults. It is related to HUS but causes less kidney damage and includes fever and neurological symptoms resulting from blood clots in the brain (Adam and Moss, 2008; Chekabab *et al.*, 2013).

Moreover, in clinical cases the mortality rate varies with the syndrome in humans. Thus, hemorrhagic colitis alone is usually self-limiting, although deaths can occur. The case fatality rate of the infection associated with HUS is estimated to be 1-10% and 50% in children and elderly, respectively. In European surveillance, the case fatality rate in all reported EHEC O157:H7 infections was < 0.5% (CFSPH, 2016).

#### **2.4. Diagnosis**

Infection with this agent is associated with a broad spectrum of illness ranging from mild diarrhea and hemorrhagic colitis to the potentially fatal haemolytic uraemic syndrome (HUS). Thus, these clinical symptoms used as one diagnosis technique (Rahal *et al.*, 2012). Besides, clinical cases can be diagnosed by finding the organisms in faecal samples, food and environmental samples may also be tested to determine the source of the infection (CFSPH, 2016). Therefore, the conventional samples is diarrhetic faeces in animals, predictable food item in both animal and human food (Elhadidy *et al.*, 2015).

Moreover, detection of *E. coli* O157:H7 is based on phenotypic differences from most other serotypes, its inability to ferment sorbitol on Sorbitol MacConkey (SMAC) agar and absence of B-glucuronidase activity in most strains. A presumptive diagnosis of the strain from these tests must then be confirmed serologically by *E. coli* O157:H7 latex agglutination test, by which the kit is commercially available (Adam and Moss, 2008). Also, immunoassays and polymerase chain reaction technology have led to the more rapid detection of this *E. coli* strain in stools,

food, water and other environmental samples. Thus, techniques included in this category are PCR and DNA-based techniques, immunomagnetic separation and enzyme-linked immunosorbent assays (ELISAs) (Bavaro, 2009). Also, molecular based techniques are distinctly advantageous because of their sensitivity, selectivity and their rapid results. However, molecular-based methods are appreciably more expensive than traditional plating techniques and are also more novel and unfamiliar. Therefore, the integration of molecular-based approaches into quality control procedures depends on the overall needs and resources of the food processing plant (Robinson and McKillip, 2010).

## **2.5. Treatment**

Treating *E. coli* O157:H7 infection with antimicrobial agents are associated with an increased risk of severe sequel such as HUS (Rahal *et al.*, 2012; Goldwater and Bettelheim, 2012) and may exacerbate the patient's condition by increasing either the release of preformed Shiga toxins (Stx) upon cell lysis. However, early administration using some antimicrobials is effective (Nassar *et al.*, 2013). Moreover, in an epidemiology study conducted by the Centers for Disease Control and Prevention, patients treated with antibiotics for EHEC enteritis had a higher risk of developing HUS. Additionally, antibiotics promote Shiga toxin production by enhancing the replication and expression of Shiga toxin genes that are encoded within a chromosomally integrated lambdoid prophage genome. Shiga toxin induction also promotes phage-mediated lysis of the pathogen cell envelope, allowing for the release and dissemination of Shiga toxin into the environment (Nguyen and Sperandio, 2012). Therefore, treatment of hemorrhagic colitis is supportive and may include fluids and a bland diet (Thorpe, 2004). Thus, antibiotics are controversial and are usually avoided, they do not seem to reduce symptoms, prevent complications or decrease shedding, and as they may increase the risk of HUS (CFSPH, 2016).

## **2.6. Prevention and control**

One Health approaches are the opportunity to implement control programmes that reduce the multiple impacts of zoonoses in both human and animal populations. Thus, interventions that

may control zoonotic infection in animal populations or prevent disease transmission from animals to people may offer more effective and economically viable approaches to disease management than those focusing on the human population alone (Halliday *et al.*, 2015). Therefore, environmental management targeted at reducing bovine exposure to *E. coli* O157 through biosecurity and environmental niche management such as feed and drinking water hygiene, reduced exposure to insects or wildlife, and improved cleanliness of the bedding or pen floor are of the essences (LeJeune and Wetzel, 2007; Nastasijevic *et al.*, 2008).

In human, since the infection primarily occurs via faecal-oral route, the preventive measures include food hygiene measures like proper cooking of meat, consumption of pasteurized milk, washing fruits and vegetables especially those to be eaten raw and drinking chlorine treated water and personal hygiene measures like washing hands after toilet visits (Kiranmayi *et al.*, 2010). Moreover, the prevention of infection requires control measures at all stages of the food chain from agricultural production on the farm to processing, manufacturing and preparation of foods in both commercial establishments and the domestic environment (WHO, 2010). Thus, the HACCP system continues to be the most effective means for systematically developing food safety protocols that can reduce the risk of *E. coli* O157:H7 infections. Therefore, an important component of HACCP application in animal production at farms is reducing the carriage of the pathogen in animals. Furthermore, the two approaches that have the potential for prevention are competitive exclusion and vaccination (Sargeant and Smith, 2003).

Vaccines against EHEC O157:H7 for cattle may reduce shedding, and have received full or conditional approval in some countries including the U.S. and Canada, but are not in wide use but there is no human vaccine against infections induced by the pathogen (Smith, 2015). Likewise, different vaccination strategies have been tested as a means to control *E. coli* O157:H7 infection in cattle. Most experimental vaccines are based upon type three secretion systems (T3SS) proteins or H7 antigens and show limited and variable effects. Moreover, econiche was the first commercially available vaccine for use in cattle and unfortunately, this vaccine is insufficiently capable of limiting the duration of infection and shedding in cattle, even after three vaccine doses. However, another vaccine strategy is based upon the bacterial needs for iron as a nutrient, a siderophore receptor and porin-protein (SRP) based vaccine formulation disrupts the iron

transport system, causing bacterial cell death and has shown to be effective in some cattle feedlot settings (Fox *et al.*, 2009; Thomson *et al.*, 2009).

## **2.7. Public health and economic importance**

*Escherichia coli* O157:H7 is an emerging public health concern in most countries of the world and it is the most common serotype of shiga toxin producing *E. coli* (Kiranmayi *et al.*, 2010). Worldwide the food-borne *E. coli* O157:H7 estimated to cause 2.8 million acute illnesses each year (Majowicz *et al.*, 2014). Meanwhile, an estimated 73,480 illnesses due to *E. coli* O157:H7 infection occur each year in the United States, leading to an estimated 2,168 hospitalizations and 61 deaths annually (Rangel *et al.*, 2005; Scallan *et al.*, 2011) resulting in an economic burden of 607 million US dollars (Scharff, 2012), including 370 million US dollars for premature deaths, 30 million US dollars for medical care and 5 million US dollars in lost productivity (Frenzen *et al.*, 2005). In addition, patients who develop HUS often require prolonged hospitalization, dialysis and long term follow up, which are expensive in all directions (CFSPH, 2016). Furthermore, the infection costs billions of US dollars as a result of recalls, destroyed food, control measures and lost demand associated to loss of consumer confidence (Frenzen *et al.*, 2005).

## **2.8. Antimicrobial resistance**

In animal production antimicrobial drugs are used for therapy, prophylaxis and growth promotion. The use of such drugs causes a selective pressure to be imposed on bacterial populations and antimicrobial resistances are selected. Thus, the pool of resistance genes is thus spread in the environment (Bruno and Carolissen-Mackay, 2012). However, antimicrobial resistance in *E. coli* has been reported worldwide, and increasing rates of resistance among *E. coli* is a growing concern in both developed and developing countries. Thus, a rise in bacterial resistance to antibiotics complicates treatment of infections (Erb *et al.*, 2007). Moreover, development of antibiotic resistance among bacteria such as *E. coli* poses an important public

health concern. The effectiveness of treatments and ability to control infectious diseases in both animals and humans may be severely hampered (Thaker *et al.*, 2012).

In Ethiopia, some studies have been done on the prevalence and antimicrobial resistance patterns of *E. coli* from various clinical sources (Gebre-Sealssie, 2007). The recent research findings of Hiko *et al.* (2008), Taye *et al.* (2013), Bekele *et al.* (2014) and Mohammed *et al.* (2014) have confirmed that *E. coli* O157:H7 has developed already in different degrees of resistance against various commonly used antibiotics including erythromycin, amoxicillin-clavulanic acid, sulfonamides, ampicillin and tetracycline, some strains have also developed multidrug-resistance.

In addition, from the recent research done by Disassa *et al.* (2017) and Zemenu (2017) from raw cow milk samples in and around Asosa and Bishoftu towns of Ethiopia, respectively showed the presence of  $\geq 50\%$  resistance of *E. coli* O157:H7 against tetracycline, streptomycin, kanamycin, cefoxitin and chloramphenicol. On the other hand, 14.4% resistance isolates to drugs of ampicillin, vancomycin, streptomycin and tetracycline have been reported from milk and milk product samples collected from the open market and supermarket sources in Bishoftu town (Bedasa *et al.*, 2018).

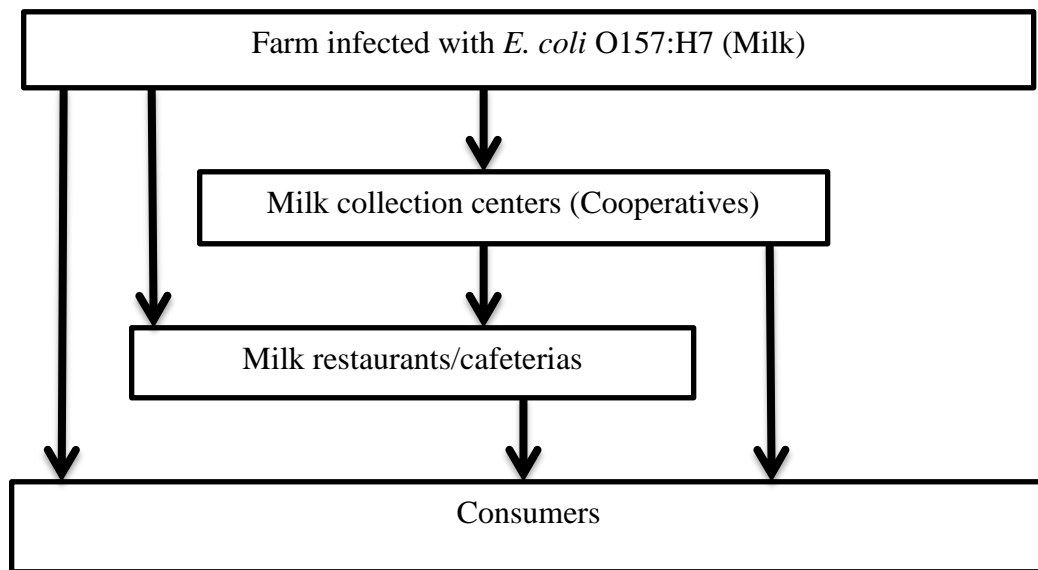
## **2.9. HACCP practices in dairy supply chains**

Unsafe food is still an important threat in most developing countries, especially in the African region (WHO, 2007b). Microbial contamination represents the main potential risk, and food-borne diseases caused by microorganisms are a large and growing public health issue. In fact, most countries with case-reporting systems have documented significant increases over the past few decades in the incidence of food-borne diseases by pathogenic microorganisms (WHO, 2014). Therefore, microbial contamination of milk is a growing concern in our country.

Meanwhile, milk is essential components to the human diet, but it is also well known to be potentially hazardous food items due to several contamination sources (equipment, personnel and water) during their production in nonconforming conditions (Peristeropoulou *et al.*, 2015). Also, the health and hygiene of the cow, the environment in which the cow is housed and milked,

hygiene during milking and storage equipment influence microbial contamination of milk (Ibtisam and Mahboba, 2007). Therefore, the implementation of the HACCP system from farm to consumption of milk promotes food safety and prevention of food-borne diseases (Fotopoulos *et al.*, 2011; Wallace, 2014; Peristeropoulou *et al.*, 2015).

Hazard analysis critical control point (HACCP) is developed in the early 1970s and introduced in the United States in 1971 at the conference of food protection where it was recommended for widespread use (Robert, 1995). The call for change was galvanized in the early 1990s with a tragic outbreak of *Escherichia coli* O157:H7 food-borne illness in Northwest of United States (Omer and Abdelgadir, 2014). Therefore, if HACCP is applied to milk supply chain, it should underpin the approach to milk safety at all stages of the chain. Thus, controlling the risk of human infection with the zoonotic disease can then be controlled by CCPs at different stages, resulting in a sustained reduction of pathogens at relevant points (Herath *et al.*, 2007; Jin *et al.*, 2008; Fotopoulos *et al.*, 2011).



**Figure 1:** Risk pathway of *E. coli* O157:H7 along the dairy supply chains in the study areas

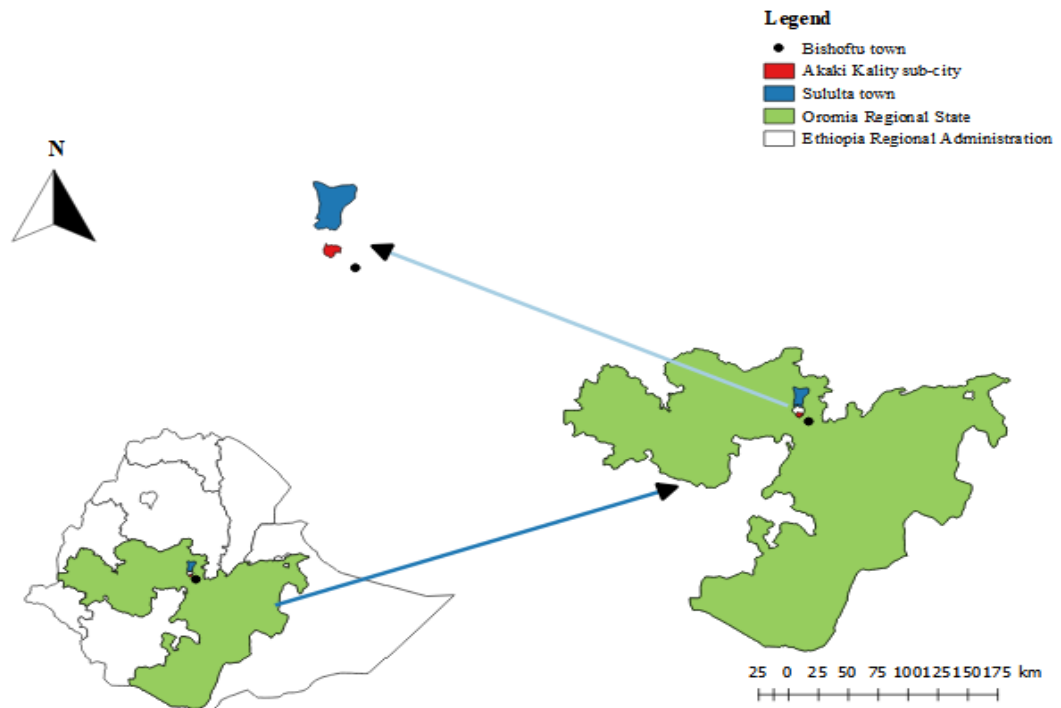
**Source:** (Swai and Schooman, 2013)

### 3. MATERIALS AND METHODS

#### 3.1. Study areas

The study was carried out at Akaki Kality sub-city of Addis Ababa, Bishoftu and Sululta towns of central Ethiopia. Samples were collected from dairy farms, milk collection centres and cafeterias located at the aforementioned towns. The study areas are known for their high potential for dairy production and located in the central highlands of Ethiopia. Bishoftu and Sululta towns are located in Oromia National Regional State located at distance of 47 km southeast and 23 km northwest of the capital Addis Ababa, respectively. Bishoftu town is situated between 8°44'4.74" North latitude and 39°0'30.72" East longitude. The altitude of the town ranges from 1900-1995 meters above sea level. Thus, it belongs to *woina-dega* (agro-climatic zone). Its average temperature and rainfall are 18 °C and 816 mm, respectively. Sululta is situated between 9°11'00" North latitude and 38°44'59.99" East longitude. It lies at an altitude of 2500 meters above sea level. The annual average maximum and minimum temperature is 25 °C and 10 °C, respectively and it has annual average rainfall of 1500 mm (CSA, 2015).

Akaki Kality sub-city is one of the ten sub-cities of Addis Ababa. It is the southernmost suburb of the city and borders with the sub-city of Nifas Silk-Lafto and Bole. It is situated at a latitude of 8°53'40.92" North and 38°46'23.52" longitude of East. It lies at an altitude of 2140 meters above sea level. It has an average rainfall of 1800 mm per annum. The annual average maximum and minimum temperatures are 26 °C and 11 °C, respectively with an overall average of 18.7 °C (NMSA, 2017).



**Figure 2:** Map of the study areas

### 3.2. Study design

A cross-sectional study design was carried out from November 2017 to May 2018 to determine the epidemiology, antimicrobial susceptibility pattern and assessment of critical control points of *E. coli* O157:H7 from farm to fork and to determine the handling and hygienic practices of milk along the dairy supply chains in central Ethiopia.

### 3.3. Sample size determination

The sample size was calculated using the equation (1) described by Thrusfield (2005). The considerations were 95% confidence interval and 5% desired absolute precision. The required sample size was calculated considering a previously published prevalence estimate of 6.9% by

Ayano *et al.* (2013) reported from a study on the bacterial isolates from raw cow milk in Holeta district, which is located at a distance of 44 km south of the capital.

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

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

Therefore, the required sample size was calculated as 98 based on the prevalence estimate described above (6.9%). Thus, 98 raw cow milk samples were collected from dairy supply chains of each study areas.

### 3.4. Study units

The study was conducted on a total of 450 samples which were meticulously collected from raw cow milk (294), milker hand swab (65) and water (91) on ward the dairy supply chains.

### 3.5. Sampling strategy

In this study, stratified random sampling method was used to classify dairy farms based on their herd size into three stratum (small, medium and large-scale commercial dairy farms) for the collection of raw cow milk sample from dairy farms. As described by Ezezew (2014), farms which keep 1-5 improved dairy cows (crossbred and pure exotic) was classified as a small-scale dairy farms. While, farms which keep 6-30 improved dairy cows were classified as medium-scale dairy farms. Also, farms which keep 31 or more improved dairy cows were classified as large-scale dairy farms.

Accordingly, there were 780 lactating cows (191 from large scales, 246 from medium scales and 343 from small scales), 700 (234 from large scales, 296 from medium scales and 170 from small scales) and 541 (144 from large scales, 297 from medium scales and 100 from small scales) in

Akaki Kality sub-city, Bishoftu and Sululta towns, respectively. This information was based on the data obtained from the respective livestock and fishery office of each study areas. Moreover, proportionate stratified random sampling method was used to allocate the number of samples to be collected from each stratum in proportion to the total number in the stratum as described by Dohoo *et al.* (2003). Thus, the total elements in each stratum were divided by the total number of elements in the study areas, after that, it was multiplied by the number of samples to be collected from each study areas. Therefore, 78 (19, 25 and 34 from large, medium and small-scale farms, respectively), 70 (23, 30 and 17 from large, medium and small-scale farms, respectively) and 78 (21, 43 and 14 from large, medium and small-scale farms, respectively) milk samples were collected using simple random sampling method in Akaki Kality sub-city, Bishoftu and Sululta towns, respectively. Likewise, the samples were collected from 22 (2 large, 7 medium and 13 small-scale) dairy farms in Akaki Kality sub-city, 19 (3 large, 8 medium and 8 small-scale) dairy farms in Bishoftu and 17 (3 large, 8 medium and 6 small-scale) dairy farms in Sululta towns.

There are 2, 3, and 2 milk collection centers in Akaki Kality sub-city, Bishoftu and Sululta towns, respectively and were involved in the investigation. Thus, simple random sampling technique was employed to collect a milk sample from the collection container in the milk collection centers at the time of sample collection. Accordingly, 12, 18 and 12 milk samples from the milk collection centers were collected in Akaki Kality sub-city, Bishoftu and Sululta towns, respectively. In addition, there are 10, 12 and 10 cafeterias in Akaki Kality sub-city, Bishoftu and Sululta towns, respectively which purchase milk from the milk collection centers. Thus, simple random sampling technique was employed to select cafeterias. Accordingly, 8, 10 and 8 cafeterias were selected in Akaki Kality sub-city, Bishoftu and Sululta towns, respectively and milk samples were collected from the selected cafeterias. Water samples were collected from each dairy farm, milk collection centers and cafeterias. Furthermore, milker hand swab samples were also collected from the aforementioned dairy farms.

### **Inclusion criteria**

Those owners of improved breeds of lactating cows, owners of cafeteria and milk collection centers who were willing to participate in this study were included.

### Exclusion criteria

Unwilling dairy farm, cafeteria and milk collection center owners and dairy farmers who hold only dry and local breeds of cows were excluded from this study.

**Table 1:** Number, type and sources of sample collected in the study areas

Sample type	Source	Sampling site and number			Total
		Akaki Kality	Bishoftu	Sululta	
<b>Milk</b>	Farm	78	70	78	226
	Collection center	12	18	12	42
	Cafeteria	8	10	8	26
<b>Water</b>	Farm	22	19	17	58
	Collection center	2	3	2	7
	Cafeteria	8	10	8	26
<b>Milker hand swab</b>	Farm	24	21	20	65
<b>Total</b>		<b>154</b>	<b>151</b>	<b>145</b>	<b>450</b>

### 3.6. Data collection

#### 3.6.1. Questionnaire survey

Verbal consent was obtained, and the objective of the survey was also explained to the respondents. A total of 91 (58 from dairy farms, seven from milk collection centers and 26 from cafeterias) owners of dairy farms, milk collection centers and cafeterias were interviewed parallel to milk and environmental samples collection using structured questionnaire. The questionnaire was prepared and translated in to the local language (Amharic and Afaan Oromo) (Annex 1, 2 and 3). Data obtained were about milking system, milking hygienic practices (washing of milkers hand and udder), sources of water and housing management. Also, milk collection center and cafeteria owners were also interviewed regarding the sources of water and type of storage container of milk, type of milk test employed and the source of milk.

### 3.6.2. Sample collection and transportation

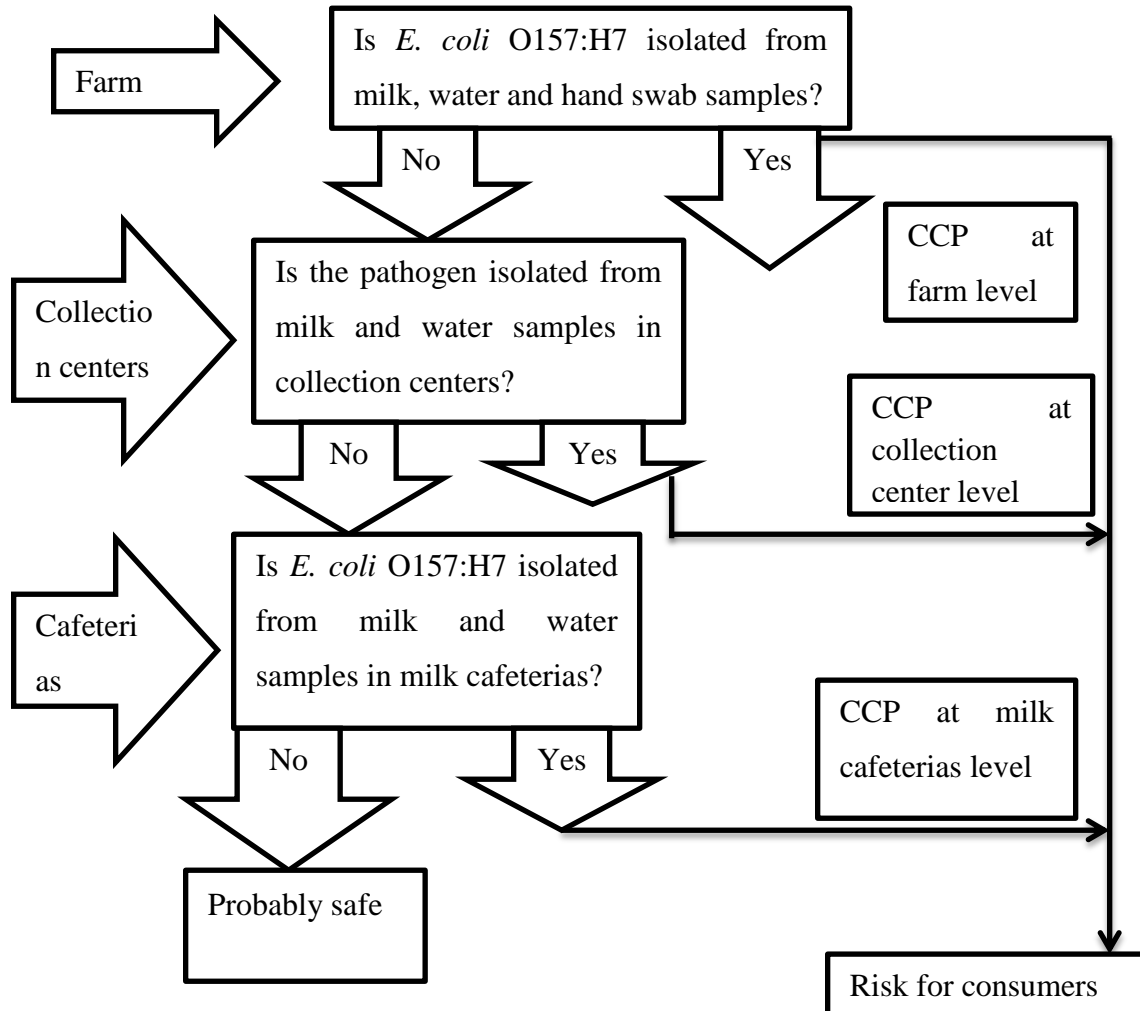
Milk samples were collected aseptically from bulk milk buckets and tanks, immediately after milking following complete agitation while in bulk, using a sterile syringe at the levels of dairy farms and from tanks in milk collection centers and cafeterias. Thus, approximately 5 ml of raw cow milk was aseptically collected and handled within a sterile screw-capped falcon tube. Similarly, 5 ml of water samples were collected from a water tank that can be used for routine sanitation purpose of equipment and workers in the dairy farms, milk collection centers and cafeterias, and handled within a sterile screw-capped falcon tubes. Also, for swab samples, sterile cotton swabs with breakable sticks were used by immersing it in a tube containing 10 ml of dilution fluid, buffered peptone water, for sampling and transporting the milker hand swab samples (ISO 18593, 2004).

All samples were coded with random numbers for identification purpose and stored in a cool box with ice packs during fieldwork. After that, the samples were transported to microbiology laboratory, College of Veterinary Medicine and Agriculture of Addis Ababa University and kept in a refrigerator at 4 °C until the time of analysis and culturing was conducted within 24 hours as described by Quinn *et al.* (2004).

### 3.6.3. Assessment of critical control points of *E. coli* O157:H7 along the dairy supply chains

Assessment of critical control point of *E. coli* O157:H7 was conducted to identify the CCPs of *E. coli* O157:H7 along the dairy supply chains. Therefore, the critical control points of *E. coli* O157:H7 was assessed in the dairy supply chains (Figure 2). Thus, three CCPs were identified to collect samples and qualitatively indicate the presence of the pathogen along the dairy supply chains. Meanwhile, for this assessment, those dairy farms which deliver milk directly to collection centres and collection centres which deliver milk to cafeterias were used as the inclusion criteria. While, those dairy farms which deliver milk directly to house hold consumption and cafeterias were used as the exclusion criteria. Therefore, for this assessment, 18 (2 large, 6 medium and 10 small-scale), 17 (3 large, 7 medium and 7 small-scale) and 12 (3 large, 7 medium and 2 small-scale) dairy farms; 2, 3 and 2 milk collection centres, and 8, 10 and 8

cafeterias in Akaki Kality sub-city, Bishoftu and Sululta towns, respectively were involved. Accordingly, a total of 86, 91 and 84 milk samples; 28, 30 and 22 water samples, and 20, 19 and 15 milkers' hand swab samples from Akaki Kality sub-city, Bishoftu and Sululta towns, respectively were included in the assessment.



**Figure 3:** Assessment of critical control points of *E. coli* O157:H7 along the dairy supply chains

**Source:** (Swai and Schooman, 2013)

### 3.7. Laboratory investigation

Laboratory investigations were carried out at Addis Ababa University, College of Veterinary Medicine and Agriculture, microbiology laboratory.

#### 3.7.1. Isolation and identification of *E. coli* O157:H7

##### *Stage 1: Liquid enrichment media*

Each raw milk and water samples (1 ml) were enriched using 9 ml of buffered peptone water (Sisco Research Laboratories, India) (Annex 4) and incubated at 37 °C for 18-24 hours. Similarly, swab sample in 10 ml BPW was incubated at 37 °C for 18-24 hours (Quinn *et al.*, 2004). The non-selective pre-enrichment technique was used to effectively recover low levels of stressed *E. coli* O157:H7. After that, the enrichment broths were pre-warmed to prevent cold shocking of the organisms and to slow their initial growth (Clifton-Hadley, 2000).

##### *Stage 2: Culturing of the enriched sample*

Ten microliters (10 µl) of the enriched samples were spread onto Sorbitol MacConkey agar (Oxoid, England) (Annex 4), after thoroughly mixing the enrichment. Then, the plates were incubated at 37 °C for 24 hours. The SMAC agar plates were examined for the presence of non-sorbitol fermenting colonies (pale colonies). Then, the confirmed pure cultures considered as *E. coli* O157:H7 positive was sub-cultured on SMAC to get pure colonies. After that, pure cultures were transferred to nutrient agar (Oxoid, England) (Annex 4) to be used for additional biochemical and serological confirmation (Quinn *et al.*, 2004).

##### *Stage 3: Confirmation of *E. coli* O157:H7*

Biochemical (indole and KIA tests) and serological tests (*E. coli* O157:H7 latex agglutination test) were performed to the well-isolated colony from nutrient agar plates to confirm the presence of *E. coli* O157:H7 in the test samples (Annex 5).

### 3.7.2. Antimicrobial susceptibility testing

Antimicrobial Susceptibility pattern of *E. coli* O157:H7 isolates were carried out following the Kirby Bauer disc diffusion method on Mueller Hinton agar (Oxoid, England) (Annex 4) as described by the Clinical and Laboratory Standards Institute (CLSI, 2015) (Annex 6). The criteria used to select the antimicrobials agents tested were based on the availability and to some extent from Clinical and Laboratory Standards Institute (CLSI, 2015) suggested guideline for antimicrobial susceptibility testing of *Enterobacteriaceae*.

Thus, antimicrobial discs impregnated with ampicillin (AMP/10 µg) (Oxoid, England), cefoxitin (FOX/30 µg) (Oxoid, England), streptomycin (S/10 µg) (Oxoid, England), tetracycline (TE/30 µg) (Oxoid, England), ciprofloxacin (CIP/5 µg) (Oxoid, England), gentamicin (GEN/10 µg) (Oxoid, England), sulfamethoxazole (RL/100 µg) (Oxoid, England), trimethoprim (TR/25 µg) (HiMedia, India) and doxycycline (DO/30 µg) (Oxoid, England) was used for sensitivity testing of isolated *E. coli* O157:H7 strains. Then, isolates bacterial suspension equivalent to McFarland standard, were uniformly streaked on Mueller Hinton agar and plates with antimicrobial disks were incubated for 18 to 24 hours. Finally, the diameters of the clean zone of inhibition formed by impregnated disks were measured and recorded to the nearest millimetre using vernier calliper. Then, isolates were classified as resistance, intermediate and susceptible according to Clinical and Laboratory Standards Institute (CLSI, 2015) interpretive criteria for *Enterobacteriaceae* (Table 15). Moreover, isolates showing resistance to two or more antimicrobials were considered as multidrug-resistant (Hill *et al.*, 2005).

### 3.8. Data analysis

The data obtained was classified, filtered and coded using Microsoft Excel® 2010. The data was then be exported to STATA version 12 (Stata Corp., Texas, USA) for appropriate statistical analysis. Then, descriptive statistical analysis of various risk factor and dependent variables were presented in tables using percentage. The total prevalence was calculated by dividing the number of positive samples by the total number of samples tested. The Fisher's exact test was used to

measure the association of *E. coli* O157:H7 occurrence with incriminated categorical risk factors. Also, logistic regression analysis was utilized to measure the degree of association between the risk factors and *E. coli* O157:H7 occurrence. The data was interpreted as significant when the *p*-value is less than 0.05. Meanwhile, for CCPs assessment the result was described qualitatively. For antimicrobial susceptibility test, the results were interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2015) interpretive criteria for *Enterobacteriaceae* (Table 15).

### **3.9. Ethical considerations**

Before any attempt to collect sample the protocol was approved by Addis Ababa University College of Veterinary Medicine and Agriculture animal research ethical committee with reference number VM/ERC/21/05/10/2018 (Annex 8). Official permission was also obtained from livestock and fishery office of the study areas as well as dairy farm owners. Necessary changes and revision were carried out as per the feedback from the committee board before moving ahead to the data collection scheme.

## **4. RESULTS**

### **4.1. Milk handling practices along the dairy supply chains**

Out of the total 58 dairy farms involved in this study, the result indicated that 12.1% of the producers were practicing udder washing before and after milking. However, 84.2%, 72.7% and 64.7% of the producers in Bishoftu, Akaki Kality sub-city and Sululta towns, respectively were washing udder only before milking. The result also showed that, 36.8%, 31.8% and 29.4% of the producers in Bishoftu, Akaki Kality sub-city and Sululta towns used a collective towel to dry the udder and teats, respectively. Similarly, 75.9% of the producers in the study areas used pipe water sources to clean the udder, hand and milking utensils. The result also indicated that, 13.8% of the producers did not manage hygienic practices of hand and udder washing. In addition, 52.6%, 36.3% and 23.5% of the milker personnel's in Bishoftu, Akaki Kality sub-city and Sululta towns washed their hand before and after milking, respectively (Table 2).

In addition, all the milk collection centers used pipe water for sanitation practices. Likewise, 57.1% of the milk collection centers have conducted a milk quality test, alcohol and lactoscan tests (Table 3). Also, 84.6% of the cafeterias used plastic milk storage containers. As well, 60%, 37.5% and 25% of the cafeterias in Bishoftu, Sululta towns and Akaki Kality sub-city, respectively used pipe water to clean milk storage utensils. However, 30%, 25% and 12.5% of the cafeterias in Bishoftu, Akaki Kality sub-city and Sululta towns have stored milk in the refrigerator before it was delivered to the consumers, respectively (Table 4).

**Table 2:** Frequency distribution of milking related hygienic practices taken by dairy farms at different study areas

Hygienic practices	Study areas						Total (n = 58)	
	Akaki Kality (n = 22)		Bishoftu (n = 19)		Sululta (n = 17)		Freq.	%
	Freq.*	%	Freq.	%	Freq.	%		
<b>Udder washing</b>								
Washing udder before and after milking	2	9.1	2	10.5	3	17.6	7	12.1
Washing udder only before milking	16	72.7	16	84.2	11	64.7	43	74.1
No hygienic practices	4	18.2	1	5.3	3	17.6	8	13.8
<b>Source of water for farm activities</b>								
Pipe	14	63.6	19	100	11	64.7	44	75.9
Well	8	36.4	0	0.0	6	35.3	14	24.1
<b>Use of towel</b>								
Collective towel	7	31.8	7	36.8	5	29.4	19	32.8
Individual towel	1	4.5	6	31.6	3	17.6	10	17.2
With bare hand	10	45.5	5	26.3	6	35.3	21	36.2
No hygienic practices	4	18.2	1	5.3	3	17.6	8	13.8
<b>Milker hand washing practices</b>								
Washing hand before and after milking	8	36.3	10	52.6	4	23.5	22	37.9
Washing hand between milking	0	0.0	3	15.8	0	0.0	3	5.2
Washing hand only before milking	10	45.5	5	26.3	10	58.8	25	43.1
No hygienic practices	4	18.2	1	5.3	3	17.6	8	13.8

\* → Frequency

**Table 3:** Frequency of water source and tests employed at milk collection centers

Hygienic practices	Study areas						Total (n =7)	
	Akaki Kality (n = 2)		Bishoftu (n = 3)		Sululta (n = 2)		Freq.	%
	Freq.*	%	Freq.	%	Freq.	%		
<b>Source of water</b>								
Pipe	2	100	3	100	2	100	7	100
Well	-	-	-	-	-	-	-	-
<b>Type of milk test</b>								
Only alcohol test	1	50	0	0.0	1	50	2	28.6
Alcohol test and Lactoscan	0	0.0	3	100	1	50	4	57.1
There is no test	1	50	0	0.0	0	0.0	1	14.3

**Table 4:** Frequency of milk handling related hygienic practices taken by cafeterias

Hygienic practices	Study areas						Total (n = 26)	
	Akaki Kality (n = 8)		Bishoftu (n = 10)		Sululta (n = 8)		Freq.	%
	Freq.*	%	Freq.	%	Freq.	%		
<b>Type of milk storage container used</b>								
Plastic	7	87.5	8	80	7	87.5	22	84.6
Steel	1	12.5	2	20	1	12.5	4	15.4
<b>Source of water for sanitation</b>								
Pipe	2	25	6	60	3	37.5	11	42.3
Well	6	75	4	40	5	62.5	15	57.7
<b>Storage of milk</b>								
In refrigerator	2	25	3	30	1	12.5	6	23.1
With container at room temperature	6	75	7	70	7	87.5	20	76.9

\* → Frequency

#### **4.2. The overall prevalence of *Escherichia coli* O157:H7**

Out of the total of 450 samples examined, the overall prevalence of *Escherichia coli* O157:H7 was 6.0% (95% CI 0.04, 0.08). Accordingly, a relatively higher prevalence of the pathogen was observed in Akaki Kality sub-city 2.67% when compared with Sululta 2.0% and Bishoftu 1.33% towns. The result described in Table 5 showed that, from the total milk samples examined at the different sampling point, a relatively higher prevalence of *E. coli* O157:H7 was observed in the milk samples collected from cafeterias (34.62%) when compared with milk samples collected from dairy farms (1.33%) and milk collection centers (0.0%). Also, 23.08% and 5.17% of the water samples collected from cafeteria and dairy farms were found to be contaminated by the pathogenic bacteria, respectively (Table 5).

Accordingly, from the total milk samples examined in the dairy supply chains of Akaki Kality sub-city, 1.28% and 50% of the milk samples were contaminated by *E. coli* O157:H7 from dairy farm and cafeterias, respectively. Also, from the water samples examined, 37.5% of the samples from cafeteria and 9.09% from dairy farms were found to be contaminated by the pathogen in Akaki Kality sub-city. The pathogen also contaminates 20% of the milk samples collected from cafeterias in Bishoftu town. The study also revealed that, 5.26% and 20% of the water samples collected from dairy farm and cafeterias in Bishoftu town were contaminated by *E. coli* O157:H7, respectively. In addition, the result indicated that, 2.56% and 37.5% of the milk samples from dairy farm and cafeterias in Sululta town were contaminated by the pathogen, respectively. Similarly, 12.5% of the water samples from cafeterias and 15% of milker hand swab samples from dairy farms in Sululta town were contaminated by the pathogen (Table 5).

**Table 5:** Prevalence of *E. coli* O157:H7 from different sample types in dairy supply chains, central Ethiopia.

Sources	Sample types	Study areas									Total		
		Akaki Kality sub-city			Bishoftu			Sululta			No of examined	No of positive	Percent age
		No of examined	No of positive	Percen tage	No of examined	No of positive	Percen tage	No of examined	No of positive	Percent age			
Farm	Milk	78	1	1.28	70	0	0.0	78	2	2.56	226	3	1.33
	Water	22	2	9.09	19	1	5.26	17	0	0.0	58	3	5.17
	Milker hand swab	24	2	8.33	21	1	4.76	20	3	15	65	6	9.23
Collection center	Milk	12	0	0.0	18	0	0.0	12	0	0.0	42	0	0.0
	Water	2	0	0.0	3	0	0.0	2	0	0.0	7	0	0.0
Cafeteria	Milk	8	4	50	10	2	20	8	3	37.50	26	9	34.62
	Water	8	3	37.50	10	2	20	8	1	12.5	26	6	23.08
<b>Total</b>		<b>154</b>	<b>12</b>	<b>7.79</b>	<b>151</b>	<b>6</b>	<b>3.97</b>	<b>145</b>	<b>9</b>	<b>6.21</b>	<b>450</b>	<b>27</b>	<b>6.00</b>

The result described in Table 6 indicated that, there was statistically significant difference observed ( $p < 0.05$ ) between the occurrences of *E. coli* O157:H7 with the source of sample, sources of water used, sampled material and type of containers. However, there was no statistically significant difference observed ( $p > 0.05$ ) between the occurrence of the pathogen with the study area, type of sample and scales of dairy farms (Table 6).

**Table 6:** Association of risk factors with *E. coli* O157:H7 occurrence along dairy supply chains

<b>Risk factors</b>	<b>No. examined</b>	<b>No positive (%)</b>	<b>Fisher's exact p-value</b>
<b>Study areas</b>			0.357
Akaki Kality	154	12 (7.79)	
Bishoftu	151	6 (3.97)	
Sululta	145	9 (6.21)	
<b>Farm scales</b>			0.193
Large	85	1 (1.18)	
Medium	145	4 (2.76)	
Small	119	7 (5.88)	
<b>Source of samples</b>			0.000*
Cafeterias	52	15 (28.85)	
Collection centers	49	0 (0.0)	
Farm	349	12 (3.44)	
<b>Type of samples</b>			0.057
Milk	294	12 (4.08)	
Swab	65	6 (9.23)	
Water	91	9 (9.89)	
<b>Source of water</b>			0.000*
Pipe	62	1 (1.61)	
Well	29	8 (27.59)	
<b>Sampled materials</b>			0.000*
Bucket	226	3 (1.33)	
Collection containers	68	9 (13.24)	
<b>Type of container</b>			0.001*
Plastic	217	19 (8.76)	
Stainless steel	168	2 (1.19)	

\* → Statistically significant

Out of the total 294 milk samples examined, 4.08% ( $n = 12$ ) of the samples were contaminated by *E. coli* O157:H7. The result described in Table 7 indicated that, there was no statistically significant difference observed ( $p > 0.05$ ) between the occurrences of *E. coli* O157:H7 with the study area and dairy farm scales. However, the occurrence of the pathogen was 3.1 (95% CI 0.27, 34.67) times more likely to occur in the small-scale than medium-scale dairy farms. Also, there were statistically significant difference observed ( $p < 0.05$ ) between the occurrence of the pathogen with the source of samples, sampled materials and source of water used. Similarly, the occurrence of the pathogen was 11.34 (95% CI 2.98, 43.21) times more likely to occur in the milk samples collected from collection container (cafeteria and milk collection centers) than milk samples collected from bucket (in the dairy farms). In addition, the occurrence of the pathogen was 8.85 (95% CI 2.18, 35.95) times more likely to occur from sampling points which use well than pipe water sources (Table 7).

**Table 7:** Univariable logistic regression analysis based on prevalence of *E. coli* O157:H7 in the milk samples and associated risk factors

<b>Risk factors</b>	<b>No. examined</b>	<b>No of positive (%)</b>	<b>OR (95% CI)</b>	<b>p-value</b>
<b>Study areas</b>				
Akaki Kality	98	5 (5.10)	Ref.	
Bishoftu town	98	2 (2.04)	0.39 (0.07, 2.05)	0.264
Sululta town	98	5 (5.10)	1 (0.28, 3.57)	1.000
<b>Farm scales</b>				
Medium	98	1 (1.02)	Ref.	
Large	63	0 (0.0) **		
Small	65	2 (3.10)	3.1 (0.27, 34.67)	0.363
<b>Sampled materials</b>				
Bucket	226	3 (1.33)	Ref.	
Collection containers	68	9 (13.24)	11.34 (2.98, 43.21)	0.000*
<b>Source of samples</b>				
Cafeterias	26	9 (34.62)	Ref.	
Collection centers	42	0 (0.0) **		
Farm	226	3 (1.33)	0.03 (0.006, 0.10)	0.000*
<b>Source of water</b>				
Pipe	62	3 (4.84)	Ref.	
Well	29	9 (31.03)	8.85 (2.18, 35.95)	0.002*
<b>Type of container</b>				
Plastic	168	12 (7.14)	Ref.	
Stainless steel	126	0 (0.0) **		

\* → Statistically significant, OR → Odds ratio, CI → Confidence interval, Ref → Reference

\*\* → Variables for which comparison doesn't compute due to zero value (isolation)

On the other hand, out of the total 91 water samples examined, 9.89% (n = 9) of the samples were found to be contaminated by *E. coli* O157:H7. The result described in Table 8 indicated that, there was no statistically significant difference observed ( $p > 0.05$ ) between the occurrence of the pathogen with the study areas and type of containers used. However, there was statistically

significant difference observed ( $p < 0.05$ ) between the occurrence of the pathogen with the sources of samples and sources of water used. Also, the occurrence of *E. coli* O157:H7 was significantly ( $p = 0.004$ ) higher (27.59%) in the well water with 23.2 (95% CI 2.74, 196.9) times more likely to occur than pipe water sources (Table 8).

**Table 8:** Univariable logistic regression analysis based on prevalence of *E. coli* O157:H7 in the water samples and associated risk factors

<b>Risk factors</b>	<b>No examined</b>	<b>No of positive (%)</b>	<b>OR (95% CI)</b>	<b>p-value</b>
<b>Study areas</b>				
Akaki Kaliti sub-city	32	5 (15.63)	Ref.	
Bishoftu town	32	3 (9.38)	0.56 (0.12, 2.57)	0.454
Sululta town	27	1 (3.70)	0.21 (0.02, 1.9)	0.164
<b>Source of samples</b>				
Cafeterias	26	6 (23.10)	Ref.	
Collection centers	7	0 (0.0) **		
Farm	58	3 (5.20)	0.2 (0.04, 0.79)	0.024*
<b>Source of water</b>				
Pipe	62	1 (1.61)	Ref.	
Well	29	8 (27.59)	23.2 (2.74, 196.9)	0.004*
<b>Type of container</b>				
Plastic	49	7 (14.28)	Ref.	
Stainless steel	42	2 (4.76)	0.3 (0.06, 1.53)	0.148

\* → Statistically significant, OR → Odds ratio, CI → Confidence interval, Ref → Reference

\*\* → Variables for which comparison doesn't compute due to zero value (isolation)

Out of the total 65 milker hand swab samples examined, 9.23% ( $n = 6$ ) of the samples were positive for *E. coli* O157:H7. As shown in Table 9, there was no statistically significant difference observed ( $p > 0.05$ ) between the occurrences of *E. coli* O157:H7 with the study areas, dairy farm scales and sources of water used. However, the occurrence of the pathogen was 1.94 (95% CI 0.29, 12.95) times more likely to occur in the milkers hand from dairy farm in Sululta than dairy farms in Akaki Kaliti sub-city dairy. Also, the occurrence of the pathogen was 1.63

(95% CI 0.15, 17.24) times more likely to occur in the milkers from the small-scale than large-scale dairy farms (Table 9).

**Table 9:** Univariable logistic regression analysis based on prevalence of *E. coli* O157:H7 in the milkers hand swab samples and associated risk factors

<b>Risk factors</b>	<b>No examined</b>	<b>No of positive (%)</b>	<b>OR (95% CI)</b>	<b>p-value</b>
<b>Study areas</b>				
Akaki Kality sub-city	24	2 (8.33)	Ref.	
Bishoftu town	21	1 (4.76)	0.55 (0.05, 6.54)	0.636
Sululta town	20	3 (15)	1.94 (0.29, 12.95)	0.493
<b>Farm scales</b>				
Large	14	1 (7.14)	Ref.	
Medium	24	2 (8.33)	1.2 (0.09, 14.35)	0.896
Small	27	3 (11.11)	1.63 (0.15, 17.24)	0.687
<b>Source of water</b>				
Pipe	50	3 (6.0)	Ref.	
Well	15	3 (20.0)	3.92 (0.7, 21.9)	0.120

OR → Odds ratio, CI → Confidence interval, Ref → Reference

The result of multivariable logistic regression analysis showing an association of incriminated risk factors and the occurrence of *E. coli* O157:H7 in the milk samples (Table 10). After removing a variable which were insignificant and has collinearity effect from univariable logistic analysis the final model for multivariable logistic regression analysis contains the variable source of samples and sources of water. The result indicated that, there was statistically significant difference observed ( $p < 0.05$ ) between the occurrences of *E. coli* O157:H7 with the source of sample and sources of water used, which were statistically significant association with the occurrence of the pathogen in the univariable logistic regression analysis. Also, the occurrence of *E. coli* O157:H7 was 4.92 (95% CI 1.1, 21.8) times more likely to occur in the well than pipe water sources (Table 10).

**Table 10:** Multivariable logistic regression analysis identifying the association of potential risk factors for the occurrence of *E. coli* O157:H7 in the milk samples

<b>Risk factors</b>	<b>No. examined</b>	<b>No positive (%)</b>	<b>OR</b>	<b>95% CI</b>	<b>p-value</b>
<b>Source of samples</b>					
Cafeterias	26	9 (34.62)	Ref.		
Collection centers	42	0 (0.0) **			
Farm	226	3 (1.33)	0.15	(0.035, 0.68)	0.013*
<b>Source of water</b>					
Pipe	62	3 (4.84)	Ref.		
Well	29	9 (31.03)	4.92	(1.1, 21.8)	0.036*

\* → Statistically significant, OR → Odds ratio, CI → Confidence interval, Ref → Reference

\*\* → Variables for which comparison doesn't compute due to zero value (isolation)

The result described in Table 11 showed, the multivariable logistic regression analysis that associates the incriminated risk factors with the occurrence of *E. coli* O157:H7 in the water samples. There was no statistically significant difference observed ( $p = 0.198$ ) between the occurrence of the pathogen and sources of sample, which were statistically significant association with the occurrence of the pathogen in the univariable logistic regression analysis.

**Table 11:** Multivariable logistic regression analysis identifying the association of potential risk factors for the occurrence of *E. coli* O157:H7 in the water samples

<b>Risk factors</b>	<b>No. examined</b>	<b>No positive (%)</b>	<b>OR</b>	<b>95% CI</b>	<b>p-value</b>
<b>Source of samples</b>					
Cafeterias	26	6 (23.10)	Ref.		
Collection centers	7	0 (0.0) **			
Farm	58	3 (5.20)	0.35	(0.07, 1.73)	0.198
<b>Source of water</b>					
Pipe	62	1 (1.61)	Ref.		
Well	29	8 (27.59)	15.3	(1.7, 136.0)	0.014*

\* → Statistically significant, OR → Odds ratio, CI → Confidence interval, Ref → Reference

\*\* → Variables for which comparison doesn't compute due to zero value (isolation)

However, there was statistically significant difference observed ( $p = 0.014$ ) between the occurrence of *E. coli* O157:H7 and source of water used. Also, the occurrence of the pathogen was 15.3 (95% CI 1.7, 136.0) times more likely to occur in the well water than pipe water sources (Table 11).

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

Antimicrobial susceptibility testing result showed that, of the total 27, all of the isolates were found to be resistant to ampicillin and cefoxitin. On the other hand, 70.37% (n = 19), 59.26% (n = 16), 37.04% (n = 10), 25.93% (n = 7), 22.22% (n = 6) and 14.81% (n = 4) were found to be resistant to streptomycin, tetracycline, doxycycline, gentamicin, sulfamethoxazole and trimethoprim, respectively. None of the isolates was resistant to ciprofloxacin (Table 12).

**Table 12:** Overall antimicrobial susceptibility profile of *E. coli* O157:H7

Antimicrobials	Resistance	Intermediate	Susceptible
	N (%)	N (%)	N (%)
<b>Ampicillin</b>	27 (100)	0 (0.0)	0 (0.0)
<b>Cefoxitin</b>	27 (100)	0 (0.0)	0 (0.0)
<b>Ciprofloxacin</b>	0 (0.0)	2 (7.41)	25 (92.59)
<b>Doxycycline</b>	10 (37.04)	6 (22.22)	11 (40.74)
<b>Gentamicin</b>	7 (25.93)	4 (14.81)	16 (59.26)
<b>Streptomycin</b>	19 (70.37)	5 (18.52)	3 (11.11)
<b>Sulfamethoxazole</b>	6 (22.22)	6 (22.22)	15 (55.56)
<b>Tetracycline</b>	16 (59.26)	7 (25.93)	4 (14.81)
<b>Trimethoprim</b>	4 (14.81)	0 (0.0)	23 (85.19)

All the isolates were resistant to at least two antimicrobials (Table 13). Three isolates (11.11%) were found to be resistant to seven antimicrobial drugs tested. Moreover, the most common pattern found in multidrug-resistance isolates was ampicillin, cefoxitin, tetracycline, streptomycin

and doxycycline. The details of the multidrug-resistant patterns of all the isolates were described below (Table 13).

**Table 13:** The fortitude demonstration of multidrug-resistance pattern of *E. coli* O157:H7 isolates

<b>Drug types</b>	<b>Number of isolates</b>	<b>%</b>
<b>Resistance to one drug</b>	0	0.0
<b>Resistance to two drugs</b>	4	14.81
AMP*FOX	4	14.81
<b>Resistance to three drugs</b>	5	18.52
AMP*FOX*TE	2	7.41
AMP*FOX*S	2	7.41
AMP*RL*FOX	1	3.7
<b>Resistance to four drugs</b>	8	29.63
AMP*RL*FOX*S	3	11.11
AMP*FOX*DO*S	1	3.7
AMP*FOX*TE*S	3	11.11
AMP*FOX*GEN*TE	1	3.7
<b>Resistance to five drugs</b>	2	7.41
AMP*FOX*DO*TE*S	2	7.41
<b>Resistance to six drugs</b>	5	18.52
AMP*FOX*GEN*DO*TE*S	4	14.81
AMP*RL*FOX*TR*TE*S	1	3.7
<b>Resistance to seven drugs</b>	3	11.11
AMP*RL*FOX*DO*TR*TE*S	1	3.7
AMP*FOX*GEN*DO*TR*TE*S	2	7.41
<b>Total</b>	<b>27</b>	<b>100.0</b>

**Key:** AMP (Ampicillin), FOX (Cefoxitin), TE (Tetracycline), RL (Sulphamethoxazole), S (Streptomycin), GEN (Gentamycin), DO (Doxycycline), TR (Trimethoprim)

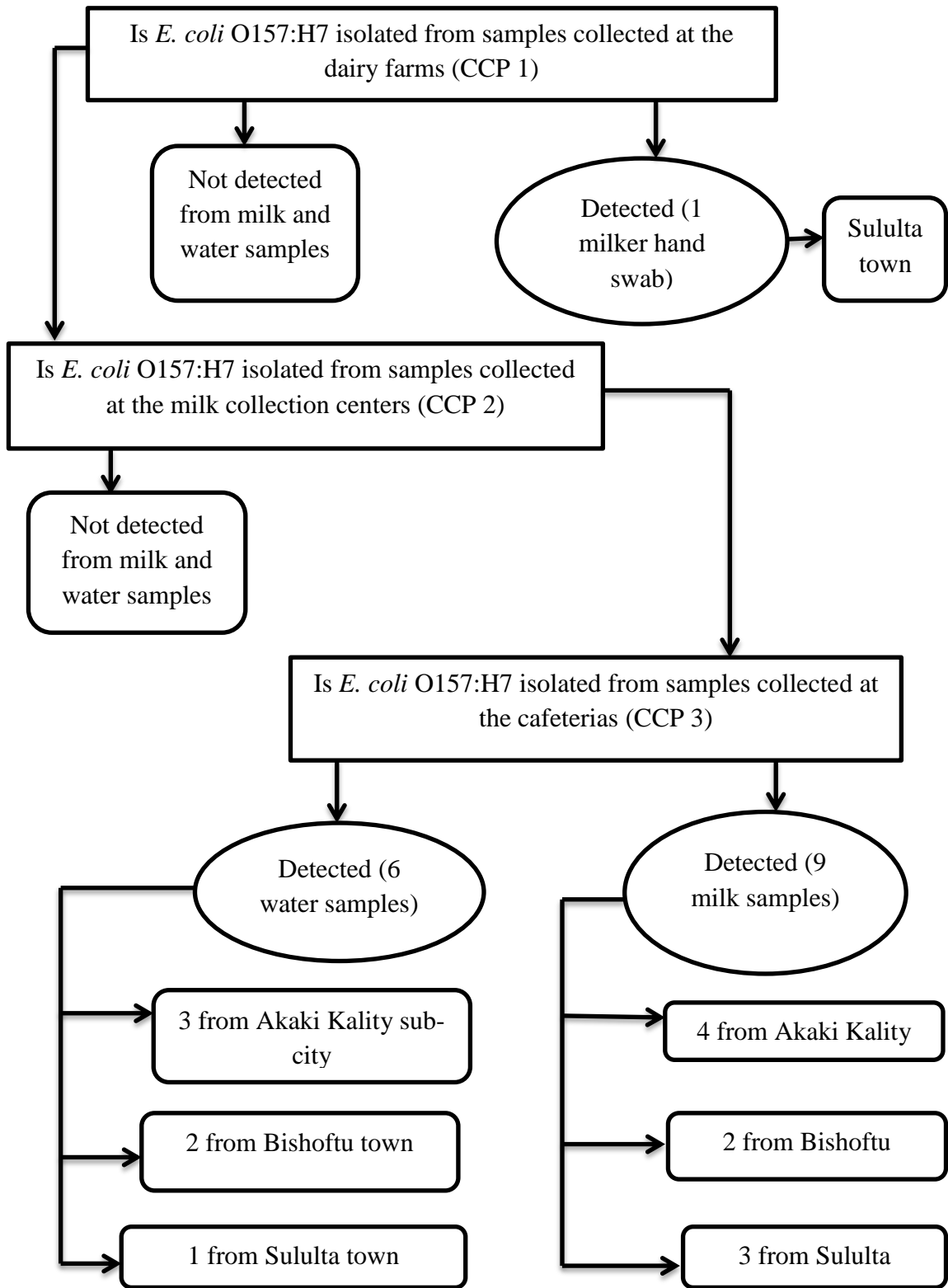
#### 4.4. Assessment of critical control points of *E. coli* O157:H7 along the dairy supply chains

Out of the total 395 samples evaluated from three critical points of the dairy supply chains in this assessment, sixteen *E. coli* O157:H7 were isolated. The result also showed that, the pathogen was only isolated from one milker hand swab sample from a Sululta dairy farm. Similarly, the pathogen was isolated from 4, 3 and 2 milk samples collected from cafeterias in Akaki Kality sub-city, Sululta and Bishoftu towns, respectively. The result also indicated that, 3, 2 and 1 water samples from cafeterias in Akaki Kality sub-city, Bishoftu and Sululta towns, respectively were found to be contaminated by *E. coli* O157:H7 (Table 14).

**Table 14:** Isolation of *E. coli* O157:H7 at the level of CCPs along the dairy supply chains

Study areas	Sources (CCP)	Type of sample	Results	No of isolates
<b>Akaki Kality</b>	Farm	Milk	ND	
		Water	ND	
		Swab	ND	
	Milk collection center	Milk	ND	
		Water	ND	
	Milk cafeterias	Milk	Detected	4
	Water	Detected	3	
<b>Bishoftu</b>	Farm	Milk	ND	
		Water	ND	
		Swab	ND	
	Milk collection center	Milk	ND	
		Water	ND	
	Milk cafeterias	Milk	Detected	2
	Water	Detected	2	
<b>Sululta</b>	Farm	Milk	ND	1
		Water	ND	
		Swab	Detected	
	Milk collection center	Milk	ND	
		Water	ND	
	Milk cafeterias	Milk	Detected	3
	Water	Detected	1	

ND → Not detected



**Figure 4:** Isolation of *E. coli* O157:H7 at the level of CCPs along the dairy supply chains

## 5. DISCUSSIONS

### 5.1. Handling practices of milk along with the dairy milk supply chains

Hygienic practices are major pathways to produce safe and quality products for the consumers there by reduces microbial contamination (Mesfine *et al.*, 2015). Therefore, the source and type of water used for washing the hand and milking utensil have a profound effect on microbial contamination of the milk. In this study, 75.9% of the producers in the study areas used pipe water for sanitation purpose. The current finding was in line with the reports of Kuma *et al.* (2015) in Sebeta town who have reported 76.7% of the producers used pipe water. However, the current finding was higher when compared with the reports of Asrat *et al.* (2016) in and around Wolaita Sodo and Disassa *et al.* (2017) in and around Asosa towns of Ethiopia who have reported 56.6% and 45% of the producers, respectively, used pipe water. The variation observed in the different studies may be due to the difference in geographical location and the availability of water between the different study areas.

On the other hand, in this study, 13.8% of the producers did not adopt hygienic practices like udder and hand washing before and after milking. The current finding was higher when compared with the reports of Duguma and Geert (2015) in Jimma and Kuma *et al.* (2015) in Sululta towns who have reported 3.7% and 3.2% of the producers did not follow hygienic practices of udder and hand washing, respectively. Similarly, 74.1% of the producers were washing the udder of the cow only before milking. In agreement with this finding, Addisu *et al.* (2016) in Gonder town and Wangalwa *et al.* (2016) in Uganda have reported that majority of the producers wash the udder of the cows only before milking. In addition, results of the present findings revealed that, 32.8% of the producers in study areas used a collective towel for wiping udder after washing. Comparable to this, Duguma and Geert (2015) have reported 61.1% of the producers in Jimma town used a common towel even though this was higher than the present findings. In addition, 17.2% of the producers in the study areas used an individual towel for cleansing of udder and teats. In contrast to this, Kuma *et al.* (2015) have reported 3.8% and 3.4% of the producers in Holeta and Sebeta towns used an individual towel, respectively and this was lower than the present finding. The variation observed in the different studies may be due to lack of training for

the producers and other milk handlers on the washing of their hands, udders, milk utensils and use of towel that mitigate the growth of microorganisms and maintaining the safety of the products. Allover, the production of good hygienic quality of milk for consumers requires good hygienic practices, such as washing milkers' hands, cleaning udder and use of individual towels during milking and handling, before delivery to consumers or processors is imperative (Oliver *et al.*, 2009).

Likewise, in this study, all of the milk collection centers use pipe water for sanitation practices. In contrary to this, Disassa *et al.* (2017) have reported that 30% of the milk collection centers in and around Asosa town use pipe water. Also, 57.1% of the milk collection centers in the study areas have conducted a milk quality test, alcohol and lactoscan tests. The result of this finding also revealed that, 84.6% and 57.7% of the cafeterias used plastic milk storage containers and well water for sanitation purpose, respectively. This finding was contrary to the reports of Disassa *et al.* (2017) in and around Asosa town who have reported that 66.6% of the cafeterias used plastic storage containers. The variation observed in the different study might be due to the difference in the size of the sample, accessibility of water, geographical location and awareness level about milk-borne zoonosis among the different studies and sample sources. Therefore, a range of factors can lead to food being unsafe, such as poor handling and storage conditions, contaminated water and lack of adequate temperature control. Such safety problems, in extreme cases, can have a negative impact on the food security status of a country (FAO, 2011).

## **5.2. The overall prevalence of *E. coli* O157:H7**

Food-borne infections are the major public health concerns in developing countries including Ethiopia. Human infections of *E. coli* O157:H7 have been mostly recognized to be originated from animal source foods (Jo *et al.*, 2004). Thus, domestic ruminants, mainly cattle, sheep, and goats, have been established as major natural reservoirs for STEC and play a significant role in the epidemiology of human infections (Kiranmayi *et al.*, 2010). Therefore, information on the incidence of these infections and their susceptibility to antimicrobials help the policy makers to develop appropriate strategies regarding prevention, treatment and control protocols.

Upon this study, from a total of 450 samples, 6.0% prevalence of *E. coli* O157:H7 were obtained from 27 milk, milker hand swab and water samples along the dairy supply chains in the study areas. However, the prevalence of the pathogen was 7.79%, 6.21% and 3.97% in Akaki Kality sub-city, Sululta and Bishoftu towns, respectively and there was no statistically significant difference observed ( $p = 0.357$ ) between the occurrence of the pathogen and study areas. The difference in the prevalence of the pathogen observed in the different study areas may be due to the difference in the management protocols, hygienic practices and water sources used for sanitation purpose among the different sampling points in the study areas.

Likewise, from the different sample types examined the prevalence of *E. coli* O157:H7 was higher in the water samples (9.89%) when compared with milkers hand swab (9.23%) and raw cow milk (4.08%) samples. Also, there were no statistically significant difference observed ( $p = 0.057$ ) between the occurrence of the pathogen and source of samples. This study showed a slightly higher rate of raw milk contamination by *E. coli* O157:H7 compared with the reports of Disassa *et al.* (2017) in and around Asosa and Zemenu (2017) in Bishoftu towns of Ethiopia who have reported as 2.90% and 3.70%, respectively. Similarly, the current finding from raw cow milk was higher when compared with the reports from abroad by Garbaj *et al.* (2016) in Libya (3.5%), Ivbade *et al.* (2014) in Nigeria (2%), Solomakos *et al.* (2009) in Greece (0.74%), Ahmed and Shimamoto (2014) in Egypt (0.5%), Sancak *et al.* (2015) in Turkey (0.0%), Swai and Schoonman (2011) in Tanzania (0.0%) and Addo *et al.* (2011) in Ghana (0.0%). The higher finding during this study might be due to the variation in the dairy farming type, related to the emphasis on livestock system in abroad, geographic location and the level of sanitary measures.

Meanwhile, the current finding from raw cow milk was lower when compared with the reports of Basil *et al.* (2012) in Iraq, Ayano *et al.* (2013) in Holeta and Bedasa *et al.* (2018) in Bishoftu towns who have reported prevalence estimates of 4.67%, 6.9% and 12%, respectively. The variation observed in the contamination frequency of *E. coli* O157:H7 in the different studies might be due to the difference in the sample size, dairy farming system, milking techniques, geography and hygienic practices employed among the different regions and study areas.

On the other hand, in the present study, a higher prevalence of the pathogen was observed in the milk samples collected from cafeterias (34.62%) compared with milk samples collected from dairy farms (1.33%) and milk collection centers (0.0%), by which the variation was statistically significant ( $p = 0.013$ ). The current findings from dairy farms (1.33%) were found higher than the reports of Disassa *et al.* (2017) and Zemenu (2017) who have reported 0.6% in and around Asosa and 2.26% in Bishoftu towns, respectively. In contrast to this, Ayano *et al.* (2013) have reported higher prevalence of the pathogen from dairy farms in Holeta district (6.9%). In addition, the finding from milk collection centers (0.0%) in this study, was converse to the reports of Zemenu (2017) and Disassa *et al.* (2017) who have reported 15.38% in Bishoftu and 5% in and around Asosa towns, respectively. Meanwhile, in this study, *E. coli* O157:H7 was isolated from 34.62% of the milk samples collected from cafeterias and compared to this study, a lower prevalence of the pathogen was reported by Zemenu (2017) in Bishoftu who have reported a prevalence of 4.17% from cafeterias. The variation observed in the different studies might be due to the difference in animal management, farm hygiene, milk handling practices, sample size and geographical location among the different study areas, milk handling equipment, transportation and difference in milk treatment during storage. Therefore, the presence of *E. coli* O157:H7 may not necessarily indicate direct faecal contamination of milk but is an indicator of poor hygiene and unsanitary practices during milking and further handling of milk and presents a potential hazard for people consuming such products (Arafa, 2013).

Similarly, upon evaluation of the containers in which samples were collected, a higher rate of contamination, the contamination frequency of *E. coli* O157:H7, was detected in the samples collected from plastic (8.76%) compared with samples collected from stainless steel (1.19%) containers, by which the variation was statistically significant ( $p = 0.001$ ). Meanwhile, the finding, level of contamination, from plastic containers was higher than the finding of Disassa *et al.* (2017) in and around Asosa town, western Ethiopia who have reported contamination frequency of 3.5% from plastic containers while the current findings obtained from stainless steel containers were slightly lower when compared with the reports of Disassa *et al.* (2017) who have reported contamination frequency of 1.6% from stainless steel containers. The variation observed in the different studies may be due to the variation in the level and awareness of hygienic methods adopted for milk and milking containers. Hence, improper hygienic management of the

narrow-necked milking and storage utensils and plastic containers used in keeping the milk which is difficult to clean and disinfect may be factors contributing to rapid bacterial multiplication and poor quality of the milk. Similar notions have been stated by Swai and Schoonman (2011), Gemechu *et al.* (2015) and Ndungu *et al.* (2016).

On the other hand, in this study, *E. coli* O157:H7 was isolated from 9.89% of the water samples. Also, from the different sampling points, a relatively higher contamination frequency of water by the pathogen was observed in water samples collected from cafeterias (23.10%) when compared with water collected from dairy farms (5.20%) and the variation was not statistically significant ( $p = 0.198$ ). However, this study showed a higher rate of water contamination (9.89%) by the pathogen compared with the reports of Mersha *et al.* (2010) in Modjo and Mekonnen *et al.* (2014) in Batu towns who have reported 4.3% and 3.3% contamination frequency, respectively. Similarly, the current findings also revealed that, *E. coli* O157:H7 was isolated from 27.59% and 1.61% of the well and pipe water, respectively, and the variation in the isolation frequency between well and pipe water samples were found to be statistically significance ( $p = 0.014$ ). In contrary to this finding, from pipe water, Mekonnen *et al.* (2014) in Batu town have reported 5.0% of the tap water were contaminated by the pathogen. The dissimilarities observed could be due to differences in methods utilized, absence of treatment or disinfection of well water, carelessness upon water usage, size of samples and geographical locations in which the studies were conducted. Consequently, the bacteriological quality of water destined sanitation and domestic uses is affected by both quality status at the source and the handling practices of water during collection, transportation, and storage; comparable thoughts were reflected by Rufener *et al.* (2010).

Similarly, in the current study *E. coli* O157:H7 was isolated from 9.23% of the milkers hand swab in the dairy farms. In addition, a higher contamination frequency of the pathogen was observed from dairy farms in Sululta town (15%) when compared with dairy farms in Akaki kality sub-city (8.33%) and Bishoftu town (4.76%), by which the variation was not statistically significant ( $p > 0.05$ ). However, compared to this study, Msolo *et al.* (2016) in South Africa and Zemenu (2017) in Bishoftu Ethiopia have reported 7% and 3.22% contamination frequency, respectively and this was lower than the present finding. The variation observed might be due to

the difference in the hygienic practices implemented, sample size, geographical location and the source of water used between the different regions and study areas.

### **5.3. Antimicrobial susceptibility profile of *E. coli* O157:H7**

The antimicrobial sensitivity tests results showed a high level of resistance to most of the antibiotics used. Thus, the development of antimicrobial resistance by the bacteria to these drugs poses a major challenge in both human and animal medicine since they are commonly used in the treatment of human patients and veterinary practice. Moreover, uncontrolled and indiscriminate usage of antibiotics in the treatment of animals and their incorporation in animal feeds has been alleged to account significantly to the increase in antibiotic resistance bacterial isolates (Reuben and Owuna, 2013).

In the course of the study, from the total 27 isolates tested  $\geq 50\%$  susceptibility to the antimicrobials was found to be ciprofloxacin (92.5%), trimethoprim (85.19%), gentamycin (59.26%) and sulfamethoxazole (55.56%). The current finding was in line with the reports of Reuben and Owuna (2013) in Nigeria, Alam *et al.* (2017) in Bangladesh and Bedasa *et al.* (2018) in Bishoftu town who have reported 89.5% and 78.9%; 50% and 66.67%, and 100% and 85.7% susceptibility of *E. coli* O157:H7 isolates to gentamycin and ciprofloxacin, from raw cow milk samples, respectively. However, the current finding was in contrary to the reports of Disassa *et al.* (2017) in and around Asosa town who have reported 36.4% and 18.2% susceptibility to gentamycin and trimethoprim-sulfamethoxazole, respectively. The variation observed might be due to the expression of resistant gene code by the pathogen which is associated with emerging and re-emerging aspects of the isolates with regards to the different agro-ecology (Reuben and Owuna, 2013).

On the other hand, the finding revealed that  $\geq 50\%$  of *E. coli* O157:H7 isolates were found to be resistant to ampicillin, cefoxitin, streptomycin and tetracycline. Which was in agreement with the findings of Zemenu (2017) in Bishoftu, Disassa *et al.* (2017) in and around Asosa and Bedasa *et al.* (2018) in Bishoftu towns who have reported  $\geq 50\%$  of resistance rate to ampicillin and cefoxitin. Similarly, Reuben and Owuna (2013), Disassa *et al.* (2017) and Alam *et al.* (2017)

have reported  $\geq 50\%$  resistance rate to tetracycline and Disassa *et al.* (2017) and Bedasa *et al.* (2018) have also reported  $\geq 50\%$  resistance rate to streptomycin. Thus, the occurrence of resistance might be due to inappropriate or indiscriminate use of antibiotics for therapeutic and prophylactic purpose both for *E. coli* and other infections.

Antimicrobial resistance is a global public health concern that is impacted by both human and non-human antimicrobial usage and the resultant development and spread of antimicrobial resistance (WHO, 2007a). Therefore, Multidrug-Resistance (MDR) is defined as the resistance of an isolate to more than two antimicrobials tested (Hill *et al.*, 2005). Thus, all *E. coli* O157:H7 isolates in the present study exhibited resistance to at least two or more of the nine antimicrobial agents tested. Compared to this finding, Bedasa *et al.* (2018) in Bishoftu town have reported 100% resistance of all the isolates to more than two drugs from milk. Also, Dulo *et al.* (2015) and Atnafie *et al.* (2017) have also reported 100% and 73.3% resistance rate of the isolates to two or more drugs, respectively in an abattoir based study conducted in Ethiopia and this was in agreement with this findings.

Likewise, in this study, the higher rate of multidrug-resistance was observed for four drugs (29.63%) followed by six (18.52%), three (18.52%) and two (14.81%) drugs. In contrast to this, the resistance of (57.1%) to two, (28.6%) to three and (14.3%) to four drugs were reported by Bedasa *et al.* (2018). Also, the resistance of 71.4% and 28.6%, and 40% and 13.3% for three and four drugs were also reported by Bekele *et al.* (2014) and Atnafie *et al.* (2017), respectively from an abattoir based investigation, Ethiopia. Meanwhile, in this study resistance to seven antimicrobials was recorded in (11.11%) of the isolates. The current finding was lower when compared with the reports of Reuben and Owuna (2013) in Nigeria who have reported (52.6%) and (15.8%) resistance rate to seven and eight drugs, respectively. Therefore, development of antibiotic resistance among bacteria such as *E. coli* poses an important public health concern. The variation in the development multidrug-resistance for the bacteria may be due to the variation in dose, route of administration, regimen and continuous and indiscriminate use of antimicrobials for treatment and feed additive in various study areas and level of awareness and geographic location for studies abroad. The effectiveness of treatments and ability to control infectious

diseases in both animals and humans may be severely hampered due rapid development of multidrug resistance (Thaker *et al.*, 2012).

#### **5.4. Assessment of CCPs of *E. coli* O157:H7 along the dairy supply chains**

The potential milk-borne hazards (*E. coli* O157:H7) can enter the milk chain at many points along the supply chain, depending on handling and the ethical attributes of the actors along the chains (Swai and Schooman, 2013). The result of the current findings revealed that *E. coli* O157:H7 was only isolated from one milker hand swab samples from a Sululta town dairy farm. Thus, this indicates that improper hygienic management or use of well water which possibly contaminated by the faeces of animal or human for the hygienic purpose might be the reason for the isolation of the pathogen from the milker personnel. The result also revealed that the agent was not isolated from milk and water samples from collection centers in the study areas.

Likewise, results of the current finding revealed that *E. coli* O157:H7 was isolated from 4, 3 and 2 milk samples collected from cafeterias in Akaki Kality sub-city, Sululta and Bishoftu towns, respectively. In addition, the pathogen was also isolated from 3, 2 and one water samples collected from cafeterias in Akaki Kality sub-city, Bishoftu and Sululta towns, respectively. The isolation of *E. coli* O157:H7 in the milk and water samples collected from cafeterias might be due to using plastic storage containers and well water, and improper hygienic practices. Also, the isolation of coliform especially *E. coli* from water sources is attributable to contamination by human and animal origin. Also, plastic containers used in keeping the milk which is difficult to clean and disinfect may be factors contributing to rapid bacterial multiplication and poor quality of the milk (Gemechu *et al.*, 2015; Ndungu *et al.*, 2016). Similarly, the water used during handling and processing of milk products can be potential sources of microbial contamination with possible negative consequences on food safety. Particularly, the water used in keeping the hygiene of milking and milk storage utensils is crucial to keep the quality and safety of the products (Amenu *et al.*, 2016).

## 6. CONCLUSION AND RECOMMENDATIONS

The present study showed that, 13.8% of the producers did not follow hygienic practices of hand and udder washing before and after milking. Also, 74.1% of the producers practice udder washing only before milking. In this study, *E. coli* O157:H7 contaminated raw cow milk, milker hand swab and water samples collected along the dairy supply chains suggests a potential risk to public health and food safety. The prevalence of the pathogen was higher in the samples taken from Akaki Kality sub-city. On the other hand, the CCPs assessment also revealed higher *E. coli* O157:H7 contamination in the samples collected from cafeterias. Importantly, the occurrence of multiple antibiotic resistant *E. coli* O157:H7 shows a risk for public health and food safety, as well as animal health and production. Generally, this study provided an initial baseline data regarding to the occurrence of *E. coli* O157:H7 in the dairy supply chains. Therefore, the following are recommended:

- Dairy farms should implement hygienic practices such as hand washing between milking, and udder washing before and after milking and use of teat dip with iodine tincture.
- Milk cafeterias should use clean pipe water, and stainless steel containers for the sanitation practices and storage of milk, respectively and the milk should be stored in a refrigerator before delivering to consumers.
- Awareness creation should be conducted to the public on the importance of the disease and avoidance of raw milk consumption
- Developing a preventive approach is needed to prevent contamination risks by the application of Hazard Analysis Critical Control Points (HACCP) practices in the dairy supply chains if possible.
- Further study should be conducted which links the public health and animal carriers of the disease in order to implement possible prevention measures of the disease.

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## 8. ANNEXES

### Annex 1: English version of questionnaire

Format 1.1: English version of questionnaire for owners of the dairy farm

Dear participant,

To ensure the hygienic handling practice of milk is essential in this town, examining the problem in detail is important. Thus, this survey is an investigation of sound hygienic practices of dairy farm owners and employees in contact with animals and their products and also milk collection centers and cafeterias owners and employees in contact with milk in order to collect the base line data which is necessary to identify the point of intervention and to take the important measurement to prevent food-borne infections. The results of this study will help public institutions in designing control strategies. All responses to this survey are completely confidential.

***Thank you for your participation in this study!***

Questionnaire No. \_\_\_\_\_

1. Farm ID \_\_\_\_\_ Address \_\_\_\_\_
2. Scale and type of the dairy farm \_\_\_\_\_ Government  Private
3. Where do the cows milked?  In barn  In milking room
4. How do you milk your cow?  By hand  By machine
5. Type of milk bucket used?  Plastic  Stainless steel  Others \_\_\_\_\_
6. How often the barn and/or the milking room are/is cleaned?  
 Twice a day  Once a day  Once per two day  Once a week
7. Do you wash your hands? Yes  No   
7.1.If yes, when you wash?  
 Before and after milking  Only after milking  
 Between milking  Not at all  
 Only before milking
8. Source of water for sanitation?  Pipe  Well  Both  others \_\_\_\_\_
9. Do you wash the udder and use towel? Yes  No

9.1.If yes, when do you wash the udder?

- Before milking  Before and after milking  
 After milking  Don't use any wash

9.2. If yes, use of towel practice?

- Collective towel  Individual towel  
 With bare hand  Don't use any towel

10. How do you keep the hygiene of milking buckets?

Washing with: Warm water  Cold water  Both alternatively

Use of detergents: Yes  No

11. Milkers' clothing: Boots  Clean outer garment  Own cloth  Apron

12. Where does the milk go?

To household consumption  To restaurants   
To collection centers  Others (Specify) \_\_\_\_\_

13. When does the collection center take the milk?

Every day  Every other day  Others (Specify) \_\_\_\_\_

Format 1.2: English version of questionnaire for milk collection center owners

Questionnaire No. \_\_\_\_\_

1. Collection center ID \_\_\_\_\_ Address \_\_\_\_\_

2. Collection center type? Private  Cooperative

3. Sources of milk for the collection center?

From farms  From own farm  Both

4. Type of milk containers used?

Stainless steel  Plastic   Others \_\_\_\_\_

5. Source of water for the sanitation of milk storage containers?

Pipe  Well  Both   Others \_\_\_\_\_

6. Do you have the processing plant? Yes  No

6.1. If yes, did you process the collected milk? Yes  No

6.2. What type of the processed product do you sell for the customers?

Processed milk  yoghurt  cheese  cream

7. Did you sell the collected raw milk to the cafeterias/restaurants?

Yes  No

8. Do you test the milk for contamination when you take from farms?

Yes  No

8.1. If yes, what type of test do you take? \_\_\_\_\_

8.2. If no, do you mix all milks together?

Yes  No

Format 1.3: English version of questionnaire for cafeteria owners

Questionnaire No. \_\_\_\_\_

1. Cafeteria/restaurant ID \_\_\_\_\_ Address \_\_\_\_\_

2. Source of milk for the cafeteria/restaurants? Collection center  Farms

3. Type of milk storage container used?

Plastic  Stainless steel   Others (specify) \_\_\_\_\_

4. Source of water for sanitation?

Pipe  Well  Both   Others (specify) \_\_\_\_\_

5. Which form of milk do you sell for the consumers?

Boiled  Raw  Yoghurt

6. Where do you store milk until used by the consumers?

In the refrigerator  With container at room temperature

**This completes the questionnaire. Do you have any question? Thank you, I really appreciate your participation in this important study.**

**Annex 2:** Amharic version of questionnaire

Format 2.1: Amharic version of questionnaire for owners of the dairy farm

ለ ወተት ላም ባለቤቶች የሚቀርብ ቃለመጠይቅ

የቃለ መጠየቁ ቁጥር \_\_\_\_\_

1. የድርጅቱ መለያ ቁጥር \_\_\_\_\_ አድራሻ \_\_\_\_\_

2. የ ወተት ድርጅቱ ደረጃና አይነት? \_\_\_\_\_ የመንግስት  የግል

3. የወተት ላሞች የት ይታለባሉ? በረት ውስጥ  በማለቢያ ክፍል ውስጥ
4. ላሞችን እንዴት ያልባሉ? በእጅ  በማለቢያ ማሽን
5. ምን አይነት የማለቢያ ዕቃ ይጠቀማሉ?  
 ከፕላስቲክ የተሰራ  ከብረት የተሰራ  ሌላ ካለ ይጥቀሱ\_\_\_\_\_
6. በረቱንና የማለቢያ ክፍሎችን በየስንት ጊዜው ያፀዳሉ?  
 በቀን ሁለት ጊዜ  በሁለት ቀን አንድ ጊዜ   
 በቀን አንድ ጊዜ  በሳምንት አንድ ጊዜ
7. እጅወን ይታጠባሉ? አወ  አልታጠብም
- 7.1. አወ ካሉ, መቸ ይታጠባሉ?  
 ከማለቢያ በፊትና ካለቡ በኋላ  ካለቡ በኋላ ብቻ   
 ከማለቢያ በፊት ብቻ  ሲያልቡ በመካከል
8. ለማፅጃ የሚጠቀሙበት ውሃ አይነት?  
 የቧንቧ  የጉድጓድ  ሁለቱምንም   ሌላ ካለ ይጥቀሱ\_\_\_\_\_
9. ጡት ማጠብ እና ለማድረቂያ ፎጣ ይጠቀማሉ?  
 አዎ እጠቀማለሁ  አልጠቀምም
- 9.1. አዎ ካሉ, ጡት ማጠቡን መቼ ይተገብራሉ?  
 ከማለቢያ በፊትና ከታለበ በኋላ  ከመታለቡ በፊት ብቻ   
 ከታለበ በኋላ ብቻ  አላጥብም
- 9.2. አዎ ካሉ, የፎጣ አጠቃቀም ምን ይመስላል?  
 አንድ ፎጣ ለሁሉም እንስሳ  ለእያንዳንዱ እንስሳ አንድ ፎጣ   
 በእጄ አደርቃለሁ  አልጠቀምም
10. የማለቢያ ባልዲዎን ንጽህና እንዴት ይጠብቃሉ?  
 የሚያፀዱት እንዴት ነው: በሙቅ ውሃ  በቀዝቃዛ ውሃ  ሁለቱን በመቀያየር   
 የማፅጃ ኬሚካል ይጠቀማሉ: አዎ  አልጠቀምም
11. አላቢው ምን ምን አይነት ልብሶችን ሲያልብ ይጠቀማል?  
 በቲ ጫማ  ንዱህ ጋውን  የራሱን ልብስ  ሽርጥ

12. የሚያገኙትን ወተት ለምን ጥቅም ያውሉታል?

ለቤት ፍጆታ እጠቀመዋለሁ

ለወተት ሰብሳቢ ማዕከል አስረክባለሁ

ለሆቴል አስረክባለሁ

13. ወተቱን ምን ለሰብሳቢ ማዕከል ያስረክባሉ?

በየቀኑ

አሳድሬ በማግስቱ

Format 2.2: Amharic version of questionnaire for milk collection center

ለወተት ሰብሳቢ ማዕከል ባለቤቶች የሚቀርብ ቃለመጠይቅ

የቃለ መጠየቁ ቁጥር \_\_\_\_\_

1. የሰብሳቢ ማዕከል ሙሉ ደረጃ ቁጥር \_\_\_\_\_

አድራሻ \_\_\_\_\_

2. የሰብሳቢ ማዕከል አይነት? የግል

የህብረት ስራ

3. ለሰብሳቢ ማዕከል የወተት ምንጭ?

ከወተት ላም ድርጅቶች

ከራስዎ የወተት ላም ድርጅት

ከሁለቱም

4. ለወተት ማጠራቀሚያ የሚጠቀሙበት ዕቃ አይነት?

ከብረት የተሰራ

ከፕላስቲክ የተሰራ

ሌላ ካለ ይጥቀሱ \_\_\_\_\_

5. ዕቃዎችን ለማጽዳት የሚጠቀሙበት ውሃ አይነት?

የቧንቧ

የጉድጓድ

ሁለቱንም

ሌላ ካለ ይጥቀሱ \_\_\_\_\_

6. የወተት ማቀናበሪያ አለዎት? አዎ

የለኝም

6.1. አዎ ካሉ የሰበሰቡትን ወተት ያቀናብራሉ? አዎ

አላቀናብረም

6.2. ምን አይነት የተቀነባበረ የወተት ውጤት ለደንበኛ ይሸጣሉ?

የተቀነባበረ ወተት

እርጎ

አይብ

ክሬም

7. የሰበሰቡትን ጥሬ ወተት ለወተት ቡና ቤቶች ወይም ሆቴሎች ይሸጣሉ?

አዎ

አልሸጥም

8. የሚረከቡትን ወተት መበላሸቱን ለማረጋገጥ ምርመራ ያደርጋሉ?

አዎ አደርጋለሁ

አላደርግም

8.1. የሚያደርጉ ከሆነ ምን አይነት ምርመራ ያደርጋሉ? \_\_\_\_\_

8.2. የማያደርጉ ከሆነ ሁሉንም ወተቶች አንድ ላይ ይደባልቃሉ?

አዎ

አልደባልቅም

Format 2.3: Amharic version of questionnaire for cafeteria owners

ለወተት ቡና ቤት ወይም ሆቴል ባለቤቶች የሚቀርብ ቃለመጠይቅ

የቃለ መጠየቁ ቁጥር\_\_\_\_\_

1. የወተት ቡና ቤቱ ወይም ሆቴሉ መለያ ቁጥር\_\_\_\_\_ አድራሻ\_\_\_\_\_
2. የቡና ቤቱ ወይም ሆቴሉ የወተት ምንጭ?  
 ከመሰብሰቢያ ማዕከል  ከወተት ላም ድርጅቶች
3. ለወተት ማስቀመጫ የሚጠቀሙበት ዕቃ አይነት?  
 ከብረት የተሰራ  ከፕላስቲክ የተሰራ  ሌላ ካለ ይጥቀሱ\_\_\_\_\_
4. ለድርጅት የሚጠቀሙት የውሃ አይነት?  
 የቧንቧ  የጉድጓድ  ሁለቱንም  ሌላ ካለ ይጥቀሱ\_\_\_\_\_
5. ምን አይነት ወተት ለደንበኞች ለሽያጭ ያቀርባሉ?  
 የተፈላ ወተት  ጥሬ ወተት  እርጎ
6. ወተቱን ለ ደንበኛ ከመሸጥ በፊት እንዴት ያስቀምጡታል?  
 ማቀዝቀዣ ውስጥ  በማስቀመጫ ቤት ውስጥ

**ይህ የቃለ መጠይቁ ማብቂያ ነው። ጥያቄ አላችሁ ለተሳትፎ አችሁ እናመሰግናለን።**

Annex 3: Oromiffa version of questionnaire

Format 3.1: Oromiffa version of questionnaire for owners of the dairy farm

Uunka Gaaffannoo Horsiiise Bultootaf

Lakk. Gaaffannoo\_\_\_\_\_

1. Lakk. Eenyummaa horsiisa\_\_\_\_\_ Teessoo\_\_\_\_\_
2. Sadarkaafi gosa horsiisa aannanii\_\_\_\_\_ Motummaa  Miti-motummaa
3. Saawwan eessatti elmamuu? Foonaa/ Moonaa  Mana aannan Iitti elmamutti
4. Akkamitti saawwan keessan elmituu? Harkaan  Mashinaan
5. Gosa okolee aannanii itti fayyadamtan? Laastika  Sibiila  Kanbiraa
6. Yeroo meeqa moona/ dallaa ykn mana aannani itti elmamu qulqulleessitu?  
 Guyyaatti al-lama  Guyyaatti al-tokko

- Guyyaa lamatti al-tokko  Torbanitti al-tokko
7. Harka keessan ni ququlqulleeffattuu? Eeyyee  Lakkii
- 7.1. Yoo deebiin keessan eeyyee ta'e, yoom dhiqattuu?
- Elmaamuun duraafi booda  Erga elmamee booda
- Elmaa gidduutti  Darbee darbee  Elmuun dura qofa
8. Madda bishaan qulqullinaaf oolu?
- Bool'a  Ujummoo  Lamaanu  kanbiraa \_\_\_\_\_
9. Mucha dhiquuf foomaa/huccuu ni fayyadamtuu? Eeyyee  Lakkii
- 9.1. Yoo deebiin keessan eeyyee ta'ee mucha yoom dhiqxuu?
- Elmuun dura  Elmaamuun duraafi booda
- Erga elmamee booda  Mucha hin dhiqnuu
- 9.2. Yoo huccuu/ foomaa fayyadamtanii dhiqxu ta'ee huccuu akkamiin fayyadamtu?
- Huccuu waliini  Harka qullaa
- Huccuu dhuunfaa dhuunfaan  Huccuu kamuu hin fayyadamu
10. Qulqullina okolee aannanii haala kamiin eeggatta?
- Kan dhiquu: Bishaan ho'aa  Bishaan qorraa  lamaan kessaa kan naaf mijateen
- Saamunaa daakuu/ dhangala'aa ni fayyadamtaa qulqulleessuuf? Eeyyee  Lakkii
11. Uffata namni aannani elmu uffatu: Boottii  Tuutaa  Marxoo
12. Erga elmamee booda aannani gara kam geeffama?
- Gara maatiitti  Dhaabbata aannani walitti qabuu
- Gara mana nyaataatti  kan biraa (ibsi) \_\_\_\_\_
13. Dhaabbatni aannani walitti qabu/ aannani oomishu aannani yoom fudhataa?
- Guyyaa hunda  Darbee darbee

Format 3.2: Oromiffa version of questionnaire for milk collection center

Gaafannoo dhaabbata aannan walitti qabuuf dhiyaatu

Lakk. Gaafannoo \_\_\_\_\_

1. Lakk. Eenyummaa dhaabbata aannani walitti qabu \_\_\_\_\_ Teessoo \_\_\_\_\_
2. Gosa dhaabbatichaa? Dhuunfaa  Waldaa
3. Madda aannanii dhaabbata kanaaf dhiyaatu?

- Horsiisa aannanii addaa addaa irraa                       Horsiisa aannanii kan ofii irraa
4. Gosa qabduu/ baattuu aannanii fayyadamtuu?  
Sibiila                       Laastikii                       kan biraa (ibsi) \_\_\_\_\_
5. Madda bishaan qulqullina baattuu/qabduu aannaniif oolu?  
Ujummoo                       Bool'a                       Lamaanu                       Kan biraa(ibsi)\_\_\_\_\_
6. Maashina oomisha aannanii qabduu?                      Eeyyee                       Lakkii
- 6.1. Yoo deebiin kessaan eeyyee ta'ee; aannani itti oomishtuu? Eeyyee  Lakkii
- 6.2. Gosa oomisha aannanii kamtu maamiltootaaf gurgurama?  
Aaannani raafame  Itittuu                       Saaqqanna/Qimixoo/Baadu  Qarruu
7. Aannani akkuma walitti qabameen mana nyaataaf/ gabaadhaaf ni dhiyeessitaa?  
Eeyyee     Lakkii
8. Aannan horsiisa aannanii addaa addaa irraa walitti qabamee qulqullina isaa ni ilaaltaa/qorattaa?                      Eeyyee                       Lakkii
- 8.1. Yoo deebiin kee eeyyee ta'ee qorannoo akkamii geggesita? \_\_\_\_\_
- 8.2. Yoo deebiin kee lakkii ta'ee aannani hundumaa walitti maktaa?  
Eeyyee     Lakkii

Format 3.3: Oromiffa version of questionnaire for cafeteria owners

Gaafannoo mana nyaataaf dhiyaatu

- Lakk. Gaafannoo \_\_\_\_\_
1. Lakk. Eenyummaa mana nyaata \_\_\_\_\_                      Teessoo \_\_\_\_\_
2. Madda aannani mana nyaataaf dhiyaatu?  
Dhaabbata aannani walitti qabu irraa                       Horsiisa aannanii irraa
3. Gosa meesha tajaajila aannan kuusuuf oolu?  
Laastiika                       Sibiila                       Kanbiraa (ibsi) \_\_\_\_\_
4. Madda bishaan qulqullinaaf gargaaramtanii?  
Ujummoo                       Bool'a                       Lamaanu                       Kan biraa (ibsi)\_\_\_\_\_
5. Akaakuu aannanii kamtu maamiltootaatti gurguramaa?  
Danfaa     Osoo hin danfisn     Itittuu
6. Aannani maamiltootaaf dhiyaachuu dura eessa ta'aa?

**Kun xumura gafannoowwaan kanaati. Gaaffii qabduu? Hirmaannaa qorannoo fi qo'annoo barbaachisa kana irraatti gootaniif kabaja guddaan isiniif qaba. Galatooma.**

**Annex 4:** Type and preparation of bacteriological medias used for isolation, identification and antimicrobial susceptibility test of *E. coli* O157:H7

#### **Nutrient agar**

Nutrient agar (CM0003, Oxoid Ltd., Basingstoke, Hampshire, England) is composed of 1 g/l of 'lab-lecno' powder, 2 g/l of yeast extract, 5 g/l of peptone, 5 g/l of sodium chloride and 15 g/l of agar, and final pH of  $7.4 \pm 0.2$  at 25 °C. It was prepared according to the manufacturer's instructions. Briefly, 28 g of the powder was dissolved in 1 liter of distilled water. The solution was boiled to dissolve completely and sterilized by autoclaving at 121 °C for 15 minutes. Before use, the media were cooled up to 45 °C and poured into sterile Petri dishes, and lastly allowed to solidify at room temperature, and stored upside down at 4 to 8 °C, refrigerator, for subsequent use.

#### **Buffered peptone water (BPW)**

The medium (BM020, Sisco Research Laboratories Pvt. Ltd., Mumbai, India) is composed of 10 g/l peptone, 5 g/l sodium chloride, 3.5 g/l di-sodium phosphate and 1.5 g/l di-hydrogen potassium phosphate. The medium was prepared according to the manufacturer's instructions. Thus, 20 g of the powdered medium was dissolved in 1 liter of distilled water. Then, the culture medium was mixed well and sterilized by autoclaving at 121 °C for 15 minutes and cooled to 25 °C before use. The prepared media was stored under the refrigeration temperature.

#### **Mueller-Hinton agar**

The medium (CM0337, Oxoid Ltd., Basingstoke, Hants, England) is composed of 300 g/l beef dehydrated infusion, 17.5 g/l casein hydrolysate, 1.5 g/l starch and 17 g/l agar, and final pH of  $7.3 \pm 0.1$  at 25 °C. The medium was prepared according to the manufacturer's instructions

whereby 38 g of the powdered medium was suspended into 1 liter of distilled water, mixed well and brought to boil to dissolve the medium completely. Then, the medium was sterilized by autoclaving at 121 °C for 15 minutes and cooled to below 45 °C before use. Thereafter, it was poured into sterile Petri dishes. The plates was left at room temperature for the media to solidify then put upside down in the incubator for 24 hours at 37 °C to check for the sterility and to dry the condensed vapour on the plate cover.

#### **Sorbitol MacConkey (SMAC) agar**

Sorbitol MacConkey (SMAC) Agar (CM0813, Oxoid Ltd., Basingstoke, Hampshire, England) is composed of peptone (20 g/l), sorbitol (10 g/l), bile salts No.3 (31.5 g/l), sodium chloride (5 g/l), neutral red (0.03 g/l), crystal violet (0.001 g/l) and agar (15 g/l), and finally adjusted at pH of 7.1 ± 0.2 at 25 °C. It was prepared according to the manufacturer's instruction and briefly, 51.5 g of the powder medium was suspended in one liter of distilled water and brought to the boil to dissolve completely. Then, it was sterilized by autoclaving at 121 °C for 15 minutes. Thereafter, it was allowed to cool to 50 °C and poured into sterile Petri dishes, and lastly allowed to solidify at room temperature, and stored upside down at 4 to 8 °C, refrigerator, for subsequent use.

#### **Kligler Iron agar**

Kligler iron agar (CM0033, Oxoid Ltd., Basingstoke, Hampshire, England) is composed of enzymatic digest of casein (10 g/l), enzymatic digest of animal tissue (10 g/l), lactose (10 g/l), dextrose (1 g/l), ferric ammonium citrate (0.5 g/l), sodium chloride (5 g/l), sodium thiosulfate (0.5 g/l), phenol red (0.025 g/l) and agar (15 g/l), and final pH of 7.4 ± 0.2 at 25 °C. It was prepared according to the manufacturer's instruction, whereby 52 g of the powder medium was suspended in one liter of distilled water and brought to the boil to dissolve the medium completely. Then, it was sterilized by autoclaving at 121 °C for 15 minutes and cooled to 45-50 °C before use. Thereafter, the medium was poured in to sterile test tubes, in so doing cooled in slanted position and finally stored in a refrigerator to ensure the shelf life.

#### **Brain Heart Infusion agar**

The medium (BM018, Sisco Research Laboratories Pvt. Ltd., Mumbai, India) is composed of calf brain infusion form (200 g/l), beef heart infusion form (250 g/l), protease peptone (10 g/l),

sodium chloride (5 g/l), dextrose (2 g/l), di-sodium phosphate (2.5 g/l) and agar (15 g/l), and finally adjusted at pH of  $7.4 \pm 0.2$  at 25 °C. The medium was prepared according to the manufacturer's directions, thus 33 g of the powdered medium was added into one liter of distilled water, mixed well, gently heated and brought to boil to dissolve the medium completely. Then, it was sterilized by autoclaving at 121 °C for 15 minutes and cooled to 45-50 °C before use. Thereafter, the medium was poured into sterile test tubes and allowed to cool in a slant position. After that, it was stored in a refrigerator to ensure the shelf life.

### **Tryptone/tryptophan medium**

The medium (HiMedia Laboratories Pvt. Ltd., Mumbai, India) is composed of sodium chloride (5 g/l), tryptone (10 g/l), DL-tryptophan (1 g/l) and final pH of  $7.3 \pm 0.1$  at 25 °C . The medium was prepared according to the manufacturer's instructions, whereby the above compositions was dissolved in 1 liter distilled water and boiling if necessary. Then, it was sterilized by autoclaving at 121 °C for 15 minutes and cooled to 45-50 °C before use. After that, 5 ml of the medium was dispensed into sterile test tube and stored in a refrigerator.

### **Annex 5: Biochemical and serological test procedures**

**Indole Test:** Fresh sterile loops was used to pick a well-isolated colony of the bacteria and inoculated into a test tubes which contains 5 ml of the tryptophan medium (HiMedia, India). Thereafter, the tubes were incubated at 37 °C for 24-48 hours. After incubation period, 0.5 ml of Kovac's indole reagent (TR008, Titan Biotech Ltd., Rajasthan, India) was added to the inoculated test tubes. The tubes was subjected to gentle shaking and examined for red colour in the surface layer within 10 minutes (Cheesbrough, 2006). A red ring on top of the tube indicated indole positive reaction.

**Dextrose and lactose fermentation test (KIA test):** A sterile straight inoculating needle was used to select an isolated colony from the culture plate and stab needle into the butt of the medium (Oxoid, England). Then, the inoculating needle was withdrawn to the slant and streak back and forth up the slant surface. After that, the tube was cap loosely and incubated aerobically

overnight for 18-24 hours at  $35 \pm 2$  °C. After the incubation period, the tube was examined for an acid, gas and hydrogen sulfide production. Thus, the presence of an acid slant-acid butt (yellow/yellow) and cracks, splits or bubbles (gas production) in the medium indicates positive result for *E. coli* (Tiwari *et al.*, 2009).

***E. coli* O157:H7 latex agglutination test:** The *E. coli* O157:H7 latex agglutination assay (DR0621M, Oxoid Ltd., Basingstoke, Hampshire, England) containing latex particles coated with antibodies specific for the *E. coli* O157, and *E. coli* H7 antigens. Identification of *E. coli* O157:H7 was carried out following the manufacturer's instructions; hence colonies that agglutinated were considered to be *E. coli* O157:H7. The control latex reagent identifies the non-specific agglutination. The test was done with the following procedures:

1. Suspected colonies (non-sorbitol fermenting colonies) isolated from Sorbitol MacConkey (SMAC) agar (Oxoid, England) which were sub cultured on the nutrient agar (Oxoid, England) was used from 18-24 old culture.
2. For each isolate to be tested dispense one drop of the *E. coli* O157 test latex was dispensed into the well of the test slide (reaction card).
3. In like manner, one drop of *E. coli* control latex was dispensed into a separate well of the test slide.
4. Then a drop of sterile saline solution was dispensed into each of the test slide.
5. Using a plastic stick (provided), a portion of the colonies was removed from the nutrient agar plate and emulsified in *E. coli* O157 test latex and sterile saline water on the slide as well it was spread over two-thirds of the reaction area. Lastly the plastic stick was discarded properly.
6. Once more using a fresh plastic stick, the process was repeated with the remaining colonies and emulsified in *E. coli* control latex on the slide.
7. Thereafter, the slide was rotated using circular motions for up to 1 minute and observe for the presence of precipitation on the *E. coli* O157 test latex and control latex. If agglutination occurred with the *E. coli* O157 test latex and the control latex was negative. Then, it indicates positive result for the *E. coli* O157 serogroup.

## **Annex 6:** Antimicrobial susceptibility test, the disc diffusion method

- Three to five well-isolated colonies of the same morphological type were selected from the nutrient agar medium (Oxoid, England) (non-selective medium), from 18 to 24 hours agar plate, was touched with the loop, and transferred into a tube containing 4 to 5 ml of sterile saline solution.
- The inoculum was prepared by making direct colony suspension and was adjusted to match the 0.5 McFarland turbidity standard.
- Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab.
- The dried surface of a Mueller-Hinton agar plate (Oxoid, England), already prepared media, was inoculated by streaking the swab over the entire sterile agar surface. The procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of the inoculum. Finally, the rim of the agar was swabbed.
- The lid was left ajar for 3 to 5 minutes to allow for any excess surface moisture to be absorbed before applying antimicrobial discs.
- Then after, antimicrobial discs were placed onto the surface of the inoculated agar plate by using sterile forceps, no closer than 24 mm from center to center. The discs were pressed gently down to ensure complete contact with the agar surface.
- The plates were inverted and incubated at 35 °C for 18 hours.
- After incubation, each plate was examined and the diameters of the zones of complete inhibition were measured, using sliding calipers (vernier calliper) on the back of the inverted petridish.

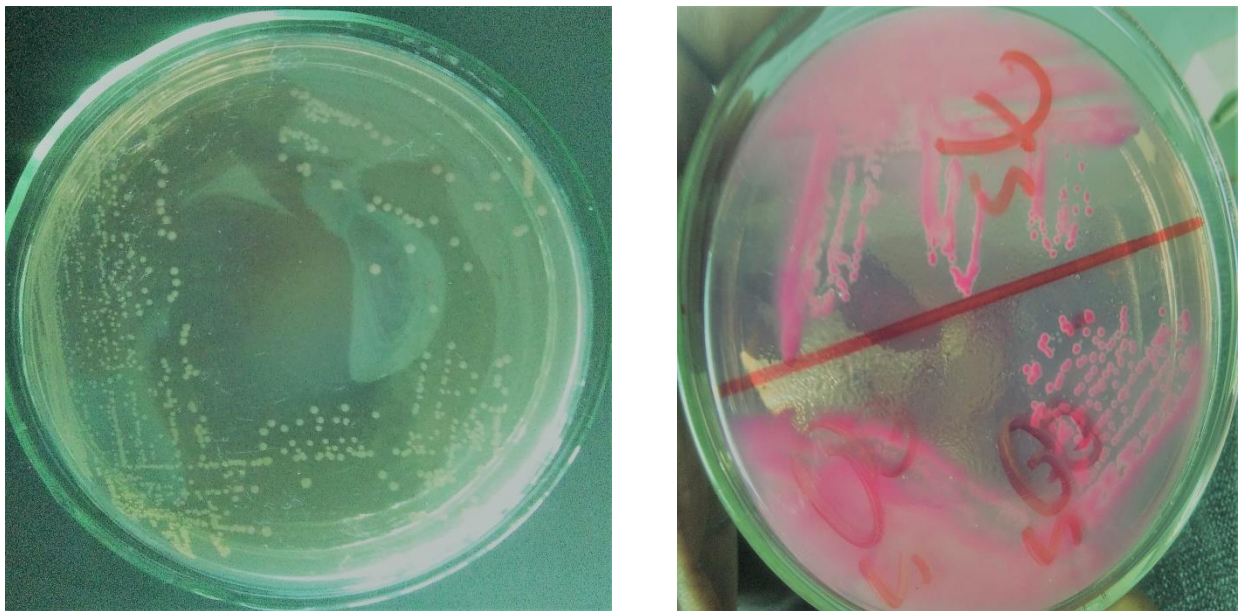
The sizes of the zones of inhibition, to the nearest whole millimeter, were interpreted according to CLSI (2015) criteria as described below (Table 15).

**Table 15:** Antimicrobial susceptibility test interpretive criteria for *Enterobacteriaceae*

Antimicrobial agent	Disk code (concentration)	Zone Diameter: Interpretive Criteria (nearest whole millimeter)			Expired date
		S	I	R	
Ampicillin	AMP (10 µg)	≥17	14-16	≤13	2019
Cefoxitin	FOX (30 µg)	≥18	15-17	≤14	2019
Ciprofloxacin	CIP (5 µg)	≥21	16-20	≤15	2020
Doxycycline	DO (30 µg)	≥14	11-13	≤10	2020
Gentamicin	GEN (10 µg)	≥15	13-14	≤12	2020
Streptomycin	S (10 µg)	≥15	12-14	≤11	2019
Sulfamethoxazole	RL (100 µg)	≥16	11-15	≤10	2020
Tetracycline	TE (30 µg)	≥15	12-14	≤11	2019
Trimethoprim	TR (5 µg)	≥16	11-15	≤10	2019

**Abbreviations:** I: Intermediate, R: Resistant, S: Susceptible

**Annex 7:** The portraits of bacterial colonies on bacteriological media, biochemical and agglutination test



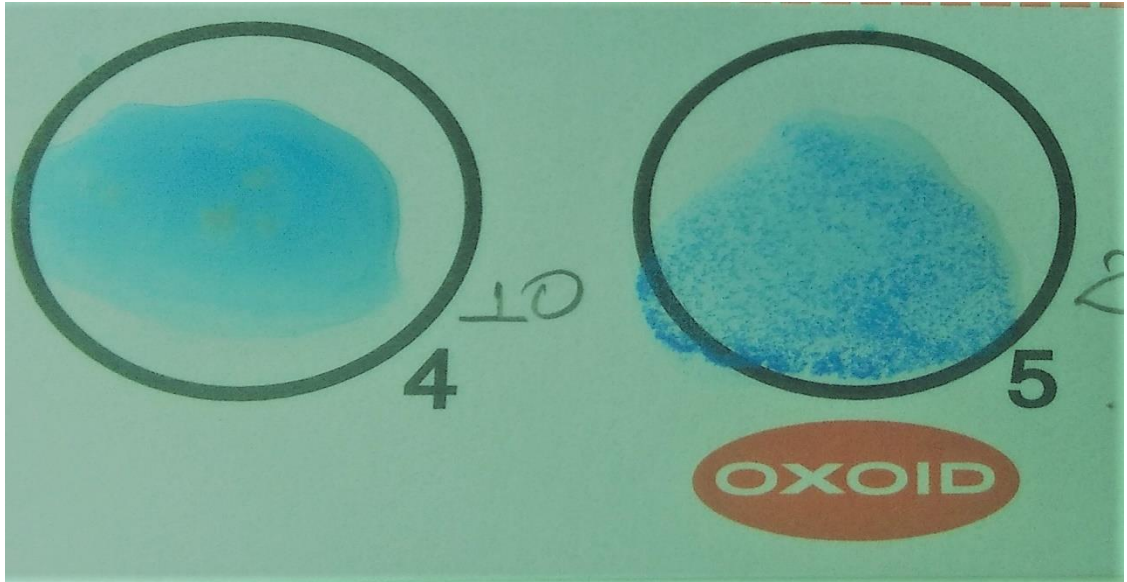
**Figure 5:** Non sorbitol fermenter colonies of *E. coli* O157:H7 (Pale colonies/left) and sorbitol fermenter non *E. coli* O157:H7 colonies (Pink colonies/Right) grown in SMAC agar



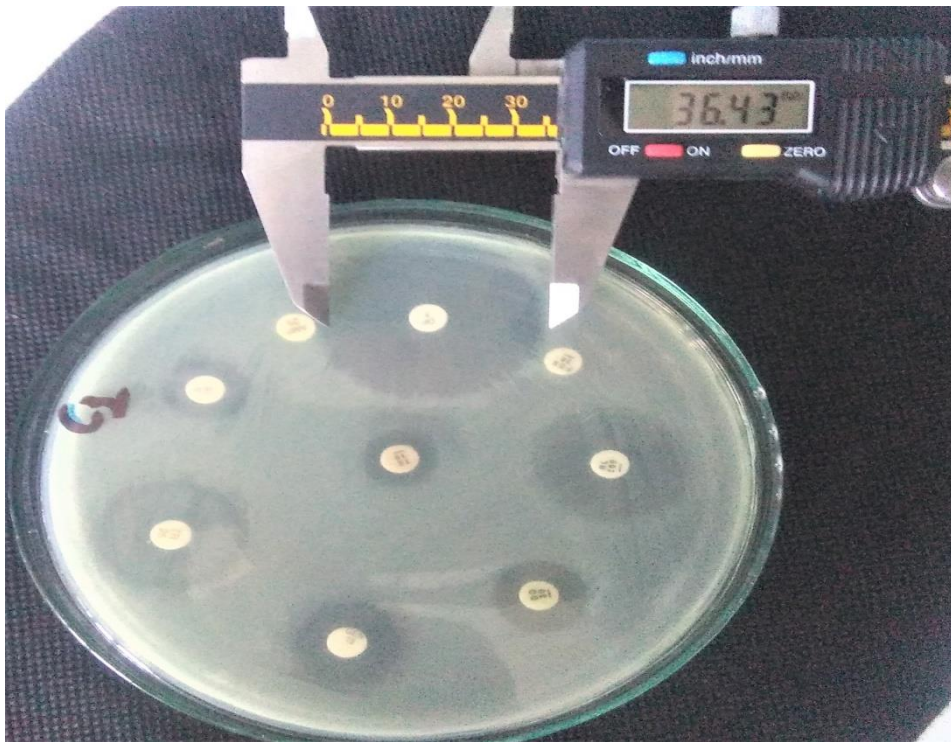
**Figure 6:** Dextrose and lactose fermentation test; positive reaction (yellow/yellow butt and slant with cracks or bubbles in the medium) and uninoculated medium (right side)



**Figure 7:** Indole test positive reaction (Red ring) and negative reaction (Yellow ring)



**Figure 8:** Latex agglutination test negative result (left/smooth blue suspension) and agglutination of NSF colonies with antibody specifically reactive with *E. coli* O157 serogroup (positive result/right)



**Figure 9:** Antimicrobial sensitivity test showing different degrees of zone of inhibition

**Annex 8: Ethical clearance certificate**

<p>አዲስ አበባ ዩኒቨርሲቲ የእንስሳት ሕክምናና ግብርና ኮሌጅ ቢሾፍቲ/ደብረ ዘይት</p>		<p>ADDIS ABABA UNIVERSITY College of Veterinary Medicine and Agriculture Bishoftu/Debre Zeit</p>
<p>Animal Research Ethical Review Committee</p> <p><i>Ethical clearance certificate</i></p>		
<p>Certificate Ref. No: VM/ERC/21/05/10/2018</p>		
<p><b>Name of Applicant:</b> Haileyesus Dejene (DVM, MVSc fellow)</p> <p><b>Address:</b> College of Veterinary Medicine and Agriculture, Addis Ababa University</p> <p><b>Title of the project:</b> research project: <b>Epidemiology and assessment of critical control points of <i>E. coli</i> 0157:H7 along dairy milk supply chain in Central Ethiopia</b></p> <p><b>Date of application:</b> 15/11/2017</p> <p><b>Nature of the project:</b> non-invasive  <b>Target animal species:</b> Cattle  <b>Number of animals involved:</b> 226  <b>Study area:</b> Central Ethiopia</p> <p><b>Minutes No. and date of review:</b> VM/ERC/05/10/018, 03/01/2018</p> <p>The above indicated research project is acceptable from ethical perspective, relevance, originality and technical competence points of view. Hence the project is allowed to be executed provided that:</p> <ol style="list-style-type: none"> <li>1. All procedures and conditions stipulated in the proposal are respected and any deviation or changes be reported to the committee</li> <li>2. The project activities be open for occasional supervision by the committee whenever this is deemed necessary</li> <li>3. Any major work on human subjects require a separate clearance from concerned authority</li> </ol>		
<p style="text-align: center;"> <u>Dr Getachew Terefe</u>              Chairman         </p> <div style="text-align: right; margin-top: 20px;">   </div>		
<p>መልስን በማጽናት ጊዜ እባክዎን የሩጅን ቁጥርን ይጠቀሙ              Please quote Our Ref. No. When replying</p>		
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