ISOLATION AND ANTIMICROBIAL RESISTANCE OF *Escherichia coli* O157: H7 FROM GOAT MEAT, SELECTED FRUIT AND VEGETABLES IN MAYA CITY, EASTERN ETHIOPIA

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LIST OF ABBREVIATIONS

A/EEC	Attaching and effacing *E. coli*
CAC	CODEX Alimentarius Commission
DAEC	Diffuse adherent Escherichia Coli
E. COLI	*Escherichia Coli*
EAEC	*Enteroaggregative Escherichia Coli*
EC	European Commission
EHEC	*Enterohaemorrhagic Escherichia Coli*
EIEC	*Enteroinvasive Escherichia Coli*
EPEC	*Enteropathogenic Escherichia Coli*
ETEC	*Enterotoxigenic Escherichia Coli*
EMB	Eosin Methylene-Blue agar
GAIN	Global Alliance for Improved Nutrition
Gb3	Globotriaosylceramide
GIT	Gastrointestinal Tract
GIS	Geigraphical Information System
HC	Haemorrhagic Colitis
HUS	Hemolytic Uraemic Syndrome
ICMSF	International Commission on Microbiological Specification for Foods
IMViC	Indole, Methyl red, Vogesproskauer and Citrate
LMICs	low- and middle-income countries
LT	Liable Toxin
MUG	Methyl UmbelliferylGlucoronide
NA	Nutrient agar
SMAC	Sorbitol MacConkey Agar
STEC	Shigatoxin producing Escherichia Coli
Stx	Shigatoxin
TSB	Tryptone Soya Broth
TTP	Thrombocytopenic Purpura
VTEC	Verotoxigenic Escherichia Coli

BIOGRAPHICAL SKETCH

The author's name is Dursitu Teha. She was born on September 12, 1998, from her father Teha Hassen and her mother Alfiya Aliyi in Burka Dintu district, West Hararghe zone, Oromia region. She started her elementary education at Kurfa Roka primary school and she continued her secondary education at Boke Tiko secondary school. Then, after successfully passing National organization for Examinations, she joined Alage Agricultural Technical Vocational Education and Training College in September, 2011 and graduated with a diploma in Animal Health in July, 2013. After graduation, the author was employed at Teyife clinical center. After three years, she joined Jimma University in February, 2017 and graduated with a BV.Sc in Animal Health (summer) in May, 2021. After her graduation, she joined Haramaya University in October 2022 to attend her M.Sc. Degree in Veterinary Public Health.

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ABSTRACT

Escherichia coli O157:H7 is a cause of food-borne disease and global public health issues especially in developing countries. In Ethiopians most people prefer to eat raw or undercooked meat and fresh vegetables and fruits. A cross-sectional study was carried out from September 2024 to February 2025 to study the prevalence of E. coli O157:H7, evaluate its antimicrobial resistance, and assess hygienic practices in goat meat, selected fruits and vegetables at Maya city, Eastern Ethiopia. A total of 224 samples were collected, isolated, and confirmed using selective enrichment media, biochemical tests, and latex agglutination tests. The overall occurrence of E. coli O157:H7 was estimated to be 34(15.18%) and from total positive result, 12/34 (35.3%) of was found in goat meat swabs from butcher shop, 3/34 (8.8%) in tomato, 3/34 (8.8%) in cabbage, 5/34 (14.7%) in carrot, 6/34 (17.7%) in lettuce, 3/34 (8.8%) in banana and 2/34 (5.9%) in orange samples from open market. Hygiene and sanitation data were collected using questionnaire and observational checklist. The antimicrobial susceptibility study of 7 E. coli O157:H7 isolates using 8 commercially available antimicrobial discs revealed that all isolates were resistant to amoxicillin, clindamycin and penicillin antimicrobial disks and 6(85.7%) isolates were resistant to ampicillin and vancomycin. Also, 5(71.4%) isolates was resistant to erythromycin. However, 6(85.7%), 4(57.1%) and 1(14.3%) isolates were susceptible to ciprofloxacillin, kanamycin and vancomycin antimicrobial disks respectively. The results of this study demonstrate the presence of drug-resistant E. coli O157:H7 in goat meat, selected fruits and vegetables at Maya city. Results also showed multiple antimicrobial resistant profiles of E. coli O157:H7 isolates, poor personal hygiene practices among meat, fruit and vegetables handlers, and general hygiene measures in place. The current study needs for implementation of E. coli O157:H7 prevention and control strategies from butcher shops and open market.

Keywords: Drug resistance, Ethiopia, Foodborne disease, Fruit, Meat, Vegetables

1. INTRODUCTION

1.1. Background

Food-borne diseases are diseases caused by consuming food and drinks contaminated by various microorganisms or pathogenic microbes (foodborne pathogens) (Junillon *et al*., 2012). Foodborne diseases and cases of food poisoning are of wide concern in public health, especially in developing countries such as Indonesia (Carbas *et al.*, 2013). One of the key goals of national food safety programs is to reduce the number of cases of foodborne disease (Azevedo *et al.*, 2014). The causes of food-borne diseases in human often follow the consumption of contaminated food-stuffs especially from animal products such as meat from infected animals or carcasses contaminated with pathogenic bacteria (Nouichi and Hamdi, 2009).

Foodborne disease has numerous linkages with poor nutrition, including through its common manifestation as diarrhea, which is strongly associated with stunting (Checkley *et al*., 2008). Every year, one in 10 people fall ill and 33 million lose their healthy in years due to the consumption of unsafe foods. Children under five years of age are particularly vulnerable, accounting for almost 40% of all foodborne diseases and 30% of total deaths related to unsafe food annually (Gain, 2021). Most of the public health burden due to foodborne diseases falls on low and middle-income countries (LMICs), with sub-Saharan Africa presenting the highest per capita burden among all ages (WHO, 2015). Poor food handling and sanitation practices, insufficient food safety management, weakened monitoring systems, weakened infrastructure, and illegal slaughtering practices were the cause for outbreak of foodborne diseases in developing nations such as Ethiopia (Nigatu *et al.,* 2017). In addition, foodborne illness is associated with a wide range of economic costs due to disease, treatment, food recalls, food safety governance, lost productivity, and risk-reducing practices. The World Bank estimates economic losses in LMICs of up to US$95 billion a year associated with productivity loss alone (Jaffee *et al*., 2018).

A wide range of pathogens plays a role in food-borne disease, most of which have a zoonotic origin and have carriers in healthy food animals from which they spread to an increasing variety of foods of animal origin and are considered as major vehicles of foodborne infections (Sánchez-Vargas *et al.*, 2011; Ejo *et al*., 2016). Among the bacterial pathogens, *Escherichia coli* O157: H7 has frequently been associated with food-borne illness (Bedasa *et al.*, 2018). *E. coli*

O157:H7 is a major food borne and zoonotic pathogen responsible for hemorrhagic colitis and hemolytic uremic syndromes in humans. Transmission to human occurs through consumption of undercooked meat, unpasteurized dairy products, vegetables and water contaminated by feces of carrier animals (Songer and Post, 2005).

There are many ways to detect *E. coli* O157:H7, including biosensors, culture-based, immunological-based, and nucleic acid-based approaches. Laboratory tests and an epidemic of hemorrhagic colitis were used to establish the utility of a latex agglutination test for the quick presumptive detection of *E. coli* serotype O157:H7. The latex test was discovered to be a straightforward, incredibly effective, and trustworthy test for identifying *E. coli* O157:H7with a perfect sensitivity and specificity (March *et al*., 2017).

Domestic animals, including goats, are natural reservoirs for *E. coli* O157:H7; therefore, they play a significant role in the epidemiology of human infections. The pathogen is carried in the intestinal tract and excreted in the feces. During slaughter, the pathogen may be present on the skin or in the feces of the animal, and may get transferred to the carcass during evisceration or skin removal. Therefore, poor slaughter techniques, particularly poor hygienic practices during slaughter greatly increase the risk of meat contamination with *E. coli* O157:H7. The risk of meat contamination also depends on the *E. coli* O157:H7 carriage status of the slaughter animals (McEvoy *et al.*, 2003). Therefore, assessment of slaughter hygiene and the carriage status of the pre-slaughter animal population are essential in determining the risk of exposure of meat consumers to *E. coli* O157:H7(Dulo *et al.*, 2015).

Vegetables are a vital part of every healthy diet and balanced meal due to their high nutritional value, which includes vitamins, minerals, and phytonutrients as well as their support of the body's metabolic processes (Sharma *et al*., 2023). Vegetables can become contaminated with pathogenic microorganisms from harvesting equipment, transport containers and domestic animals (Uzeh and Adepoju, 2013). The pathogenic microorganisms which reside in intestinal tracts of animals or humans are more likely to contaminate vegetables through faeces, sewage, untreated irrigation water or surface water (Harris *et al.*, 2003).There is a great variation in the number of microorganisms on vegetables, and factors like nutritional substances, microbial competition, structural damages on plant (wounds) and the potential in internalization of

pathogens, affect the proliferation of pathogens on vegetables. There is always a chance of *E. coli* O157:H7 being present in vegetables grown in manure-fertilized soils (Islam *et al*., 2004).

Fresh fruits are essential components of the human diet and there is considerable evidence of the health and nutritional benefits associated with the consumption of fresh fruits (Ahmed *et al.,* 2010). Most fruits contain bacterial counts up to 1.0 X 10^5 cm^2 on their surfaces. Improper washing of fruits adds these bacteria to extracts leading to contamination. In addition, use of unhygienic water preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust can also act as sources of contamination. Such fresh fruits have shown to be potential sources of bacterial pathogens notable *E. coli* O157:H7 (Babiye, 2017).

1.2. Statement of Problem

In developing countries like Ethiopia, most people prefer to eat raw or undercooked meat (Locally called *kitfo, dulet* and *kurt*). Contaminated meat is the most common route of *E. coli* O157:H7 infection in the community (Sebsibe and Asfaw, 2020).

In the last few decades, consumption of fresh food has dramatically increased by 30% (Stea *et al.,* 2020). Bacterial communities can be abundant and diverse in fresh fruits and vegetables (Leff and Fierer, 2013). Such leaf plants are frequently consumed raw or only minimally cooked in order to maintain flavour; however, this approach may increase the risk of contracting foodborne illnesses (Fröder *et al.*, 2007).

In Ethiopia, a number of studies have been conducted about the occurrence of *E. coli* O157:H7 isolates from food of animal origin, animal surfaces, and samples taken from slaughter houses, retail shops, and restaurants (Abdissa *et al*., 2017; Beyi et al., 2017; Feleke and Wubshet, 2017; Hailu *et al.,* 2017; Ayenew *et al.*, 2021; Shumi et al., 2021; Abebe *et al.*, 2023; Engidaw *et al*., 2023). Since, people eat tomato, lettuce, carrot and cabbage as uncooked salad ingredients in many parts of the Ethiopia in combination with other food item like with raw or undercooked meat in Ethiopia (Sebsibe and Asfaw, 2020). Due to the consumption of contaminated ready-to-eat salad vegetables, food borne epidemics are likely to happen everywhere. In Maya city, also fruits such as orange, banana, and tomato were distributed to consumers under poor food

handling and sanitation practices from open market and also most of people eat these fruits without washing properly and carried foodborne disease like *E. coli* O157: H7.

However, there was no previous study conducted on antimicrobial resistance profile of *Escherichia coli* O157: H7 isolated from goat meat, selected fruit and vegetables items at Maya City, Eastern Ethiopia. Thus, the current study was conducted to assess on antimicrobial resistance profile of *Escherichia coli* O157: H7 isolated from goat meat, selected fruit and vegetables items at Maya City, Eastern Ethiopia.

General Objective

- To assess the occurrence and antimicrobial resistance of *Escherichia coli* O157:H7 from various food items at Maya City, Eastern Ethiopia

Specific objectives

- To isolate *Escherichia coli* O157: H7 from goat meat at selected butcher shop in Maya City, Eastern Ethiopia
- To isolate *Escherichia coli* O157: H7 from fruits (Tomato, orange and banana) in Maya City open market.
- To isolate *Escherichia coli* O157: H7 from vegetables (cabbage, lettuce and carrot) sold at different open market in Maya City, Eastern Ethiopia.
- To assesses hygienic handling practices of selected food items in Maya City, Eastern Ethiopia
- To investigate the antibiotic resistance of *Escherichia coli* O157:H7 after being isolated from goat meat, selected fruit and vegetables samples in the study area

2. LITERATURE REVIEW

2.1. General Characteristic of *Escherichia coli*O157:H7

As a normal flora, gram-negative, rod-shaped, facultative anaerobic bacteria predominate in the gastrointestinal tracts of both humans and animals. Somatic (O) antigen 157 and flagella (H) antigen 7 are both expressed by *E. coli* O157:H7. *E. coli* O157:H7 is distinct from other strains in that it ferments D-sorbitol more slowly (over 24 hours) and is incapable of manufacturing the enzyme glucuronidase, which may break down the synthetic compound. Therefore, Sorbitol MacConkey (SMAC)agar should be utilized for the detection of *E. coli* O157:H7. A commercially available latex agglutination assay can be used to confirm *E. coli* O157:H7 (Lim *et al.,* 2010)**.**

Escherichia coli are a member of the enterobacteriaceae family, found normally in the intestinal tract of goat, other animals and humans. This species can be differentiated from other enterobacteriaceae by its ability to utilize sugars and to cause a range of other biochemical reactions such as Indole production and formation of acid and gas from lactose and other carbohydrates, which takes place at 37 ºC. Most strains ferment lactose and grow over a wide range of temperature (15 ºC – 45 ºC). This species encompasses a variety of strains that cause disease in man and animals and some are haemolytic, a characteristic associated with pathogenicity (Mwai, 2011).

There are six classic pathotypes: enteropathogenic (EPEC), enterohemorrhagic (EHEC), enteroaggregative (EAEC), enterotoxigenic (ETEC), enteroinvasive (EIEC) and diffusely adherent *E. coli* (DAEC). A new pathotype has been recently reported: adherent invasive E. coli (AIEC). Both biotic and abiotic factors can influence *E. coli* growth and survival in natural environments (Jang *et al*., 2017). Temperature, water and nutrition availability, pH, and solar radiation are all abiotic variables. The presence of other microorganisms, as well as *E. coli* ability to acquire nutrients, compete with other microorganisms, and form biofilms in natural environments, are all biotic factors. The optimal temperature is 37 °C, with a temperature range of 30-42 °C (Alemneh and Temesgen, 2016).

2.2. Food Hygiene and Food Safety

Food hygiene is the conditions and measures necessary to ensure the safety of food from production to consumption. It is a fundamental requirement of any food process that the food produced should be safe for consumption. Food safety is a basic need but there is a danger that it may be overlooked in the development of effective and efficient processes. Unsafe food has been a human health problem since history was first recorded, and many food safety problems encountered today are not new. Although governments all over the world are doing their best to improve the safety of the food supply, the occurrence of foodborne disease remains a significant health issue in both developed and developing countries. Food can become contaminated at any point during slaughtering or harvesting, processing, storage, distribution, transportation and preparation. Proper food preparation can prevent most food borne diseases. More than 200 known diseases are transmitted through food (Kamboj *et al.*, 2020).

Food safety does not only protect the food production process, but it can also improve health and provide nutrition. Two organizations involved in the formulation of food safety standards are International Commission on Microbiological Specification for Foods (ICMSF) and CODEX Alimentarius Commission (CAC). Based on the definition of food safety given, food safety includes: - response to the hazard separating aspects food safety from food quality aspects others that cause food not suitable for human consumption, although not harmful to health, the concept of food safety and management is based on the standards provided to guarantee that the food is safe, the preparation process and / or the use of food products should consider food security and vice versa. Food products are considered safe if they are provided and / or used in accordance with the policy measures of food safety. These are necessary steps emphasized by producers, manufacturers, vendors and buyers (Mohamad *et al.*, 2015).

In the 21st century, foodborne diseases have become one of the important issues all over the world (Scott, 2003). Due to poor infrastructure and low level of awareness, this problem is worse in developing countries. Major pathogenic microorganisms that frequently have been associated with foods of animal origin, fruit and vegetables include Enterohemorrhagic *Escherichia coli* O157:H7. Human infections with *E. coli* O157:H7 have been mostly associated with the consumption of contaminated and improperly cooked meat. Butcher houses and restaurants are frequently incriminated as sources of *E. coli* O157:H7 for human infections (Pennington, 2010).

2.2.1. Ethiopian Food Hygiene and Food Safety Practices

In Ethiopia, the handling practice and production system is traditional, the meat, vegetables and fruits are mostly contaminated with external sources which are bacteria and categorized as poor quality, and this is mainly due to less attention for hygiene (Reda *et al*., 2014). Food-borne pathogens are the leading causes of food-borne human illness and death in the world (Agüeria*et al.*, 2018). The severity is higher among developing countries, including Ethiopia (Abdissa *et al*., 2017; Beyi *et al*., 2017). It could be attributed to changes in eating habits, mass catering, complex and lengthy food supply procedures with increased international movement, poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources, and awareness about proper food handling which creates a conducive environment for the spread of food-borne and food poisoning etiologic agents (López-Alonso, 2012).

2.2.2. Raw food consumption trend with risk of infection in Ethiopia

Escherichia coli O157:H7 can infect humans via various routes; however, a large proportion of infections and human outbreaks have occurred following the consumption of contaminated food products of animal origin, such as poorly or under cooked meat (Sebsibe and Asfaw, 2020), vegetables and fruits and their products like salad and juice which are generally regarded as a high-risk commodity in respect of pathogen contents, natural toxins, and other possible contaminants and adulterants (Haileselassie *et al*., 2013; Frehiwot and Fufa, 2019).

2.3. Risk association of *E. coli* O157:H7 on human

Growing evidence suggests that vegetables consumption is getting attraction by many people particularly in the urban community (Raaijmakers *et al*., 2018; Roberts and Shackleton, 2018). The recent popularity of vegetables could be its health advantage and low energy density (Shrestha *et al.*, 2017; Hölzel *et al.*, 2018). However, most urban farmers use untreated or

partially treated waste water. Hence, microbiologically contaminated water could affect the health of farmers on the one hand and consumers of fresh vegetables that are irrigated with contaminated water on the other hand (Mengesha *et al.*, 2021).

Apart from pre-weaning infants, no group of the population is excluded from eating raw vegetables and fruits (Jaja *et al.*, 2020; Haile *et al.*, 2021). The young, the old, the pregnant and the immune-compromised consumers potentially have a higher risk of bacterial infection than other groups. This factor is important in risk assessment relating to the consumption of fruits and vegetables. A particular concern relates to infection of young children with *E. coli* O157:H7 and the potential for these infections to progress to HaemolyticUraemic Syndrome (HUS) (Parry and Palmer, 2000).

2.4. Clinical sign

E. coli O157: H7 infections are associated with a range of illness in humans, although a proportion may be asymptomatic. Where symptoms do occur, the incubation period is 2 to 10 days, with most cases occurring in 3 days. The range of clinical disease includes: Mild diarrhea, fever, abdominal pain, vomiting, hemorrhagic colitis (HC), which consists of inflammation of the large bowel, with severe blood. Haemolytic Uraemic syndrome (HUS), a combination of anaemia, acute kidney failure and low platelet count which may be accompanied by fever, Thrombotic thrombocytopenic purport (TTP) characterized by fever, via skin and central nervous system involvement, resulting from aggregation of platelets in various organs. HUS largely affects children and TTP largely affects adults. TTP is a rare syndrome of *E. coli* O157:H7 infection (Fernandez, 2008).

2.5. Food item possible contaminated with *Escherichia coli* O157:H7

2.5.1. Water

Poor sanitation and hygiene conditions as well as lack of or little environmental awareness among people is considered as a major cause of source water contamination (Atnafie *et al.*, 2017;

Jaja *et al.*, 2020). Another practice is uncontrolled waste disposal, bathing and swimming in water sources such as rivers and dams, which serve as sources to municipal water supplies, answering calls of nature and grazing of cattle next to catchment areas. In developing countries, most of the families have no toilet facilities (Zamxaka *et al*., 2004).

Furthermore, living in a rural household was associated with a 10% increase in the risk of water contamination compared to an urban household, after adjusting for water source, sanitation infrastructure, wealth, and other household characteristics. Unimproved water sources such as surface water and unprotected wells and springs have the highest contamination levels (Santos *et al.*, 2023).

2.5.2. Meat and meat products

Transmission of *E. coli* O157:H7 to humans caused mainly by consumption of raw or undercooked meat which get contaminated during slaughter, handling and preparation of meat (Karmi, 2019). *Escherichia coli* O157:H7 have been isolated from cattle and goat and their carcasses, hides and faeces. In Ethiopia also, the occurrence of *E. coli* in foods of animal origin is arguably high due to many reasons like unhygienic slaughtering practices in the abattoirs, illegal slaughtering of animals in open fields, poor meat transport, and display conditions at butcher shops (Dulo *et al*., 2015).

2.5.3. Milk and milk products

Milk is considered a complete and nutritious food for the new-born mammal and human beings, but it is considered as a good medium for many microorganisms (Leedom, 2006). Raw untreated milk is still used by large number of farm families and workers and by a growing segment of the general population who believe that the milk is not only safe but also imparts beneficial health effects that are destroyed by pasteurization (Lejeune and Rajala-Schultz, 2009). For this reason, utilization of both raw untreated milk and raw milk cheeses has frequently been associated with food-borne illness. Especially, developing countries are mostly affected by food-borne infections because of the prevailing poor food handling and sanitation practices, inadequate food safety

regulatory systems, lack of financial resources to invest in safer equipment, and lack of education for food-handlers (Carbas *et al*., 2013).

In Ethiopia, as in other countries, foodborne diseases are frequently unreported, and there is no surveillance program for foodborne pathogens. Likewise, it is difficult to demonstrate the extent of contamination of milk and milk products, which is a challenge to food safety (Eshetu, 2016). Foodborne infections continue to be a major public health concern in Ethiopia because of the poor sanitary conditions, malnutrition, and lack of adequate medical services. Several reports from Ethiopia and other countries showed higher multidrug resistant *E. coli* and *E. coli* O157 H7 in milk samples (Atnafie *et al*., 2017; Jaja *et al*., 2020; Mesele *et al.*, 2023).

2.5.4. Fruits and vegetables and their products

The common type of fruits and vegetables that have always been implicated in *E. coli* O157:H7 outbreaks are tomato, lettuce, cabbage, banana, orange and carrot especially those used in salads and ready-to-eat (Mukherjee *et al.,* 2004). Most researchers have established that the commonest cause of *E. coli* O157:H7 on vegetables is the use of cattle manure or irrigation waters that are potentially contaminated with *E. coli* O157:H7 in the growing of fruits and vegetables. And also, the Outbreaks of diseases caused by shiga toxigenic *Escherichia coli* have been associated with the consumption of leafy vegetables (Johannessen *et al*., 2005).

2.6. Epidemiology

E. coli O157:H7 infections pose a major risk and great public health concern globally. The total number of cases due to infections by *E. coli* O157:H7 are beneath other intestinal pathogenic bacteria, such as *Campylobacter* and *Salmonella* infections (Banatvala *et al*., 2001; Dahmen *et al.,* 2012). However, infections posed by *E. coli* O157:H7 have recorded more hospitalization, including mortality in severe cases. Infections in humans posed by the *E. coli* O157:H7 strain may have a comprehensive clinical range originating in patients that do not show any clinical symptoms of mortality. Oftentimes, it starts with non-bloody diarrhoea and is self-solving in the absence of any additional complexity. In some cases, patients develop bleeding diarrhoea or haemorrhagic colitis (HC) in a few days, about one to three days (Banatvala *et al*., 2001).

Furthermore, in about 5–10% of patients with HC, the illness can advance to the life-threatening effect of thrombocytopenic purpura (TTP) or HUS. Prescription utilization of antimicrobial agent has a possibility to be controversial (Banatvala *et al*., 2001).However, the remedy primarily helps limit or prevent periods of symptoms and systemic complications. Therefore, it is recommended to implement proper measures to effectively prevent and control *E. coli* O157:H7 infections. *E. coli* O157:H7 strain has a delayed sorbitol fermentation (>24 hours) production of β-glucuronidase capable of hydrolysing the synthetic molecule 4-methylumbelliferyl-D-glucuronide (MUG). Sorbitol-MacConkey (SMAC) agar medium complemented with MUG is utilized to detect *E. coli* O157:H7. Furthermore, to strengthen the selectivity of *E. coli*, tellurite potassium, cefixime and vancomycin were included in the SMAC agar plate to suppress other bacteria that have the same Gram-negative characteristics. Latex agglutination assay that is commercially available can be used to further confirm serotypes O157 and H7 (Dahmen *et al*., 2012).

2.6.1. Diagnosis

Detection of *E. coli* O157:H7 is based on phenotypic differences from most other serotypes: its inability to ferment sorbitol on MacConkey sorbitol agar and absence of b-glucuronidase activity in most strains (Lupindu *et al.,* 2014). Presumptive *E. coli* O157:H7 from these tests must then be confirmed serologically for which a latex agglutination kit is commercially available. Identification of diarrhoeagenic *E. coli* can be based on detection of their associated virulence factors. For example, procedures are available to detect the ST and LT of ETEC serologically, and the LTI and Stx genes in ETEC and EHEC using gene probes and the polymerase chain reaction (PCR) (Dulo, 2014).

2.6.2. Treatment

Escherichia coli infections are often treated with antibiotics; however, STEC is treated symptomatically (Lupindu *et al.*, 2014). Antibiotics are ineffective in treating complications, such as hemolytic uremic syndrome (HUS), which is treated symptomatically (Adefisoye *et al.*, 2016). Antibiotic treatment is not recommended for STEC-HUS because it increases the secretion of Shiga toxins (STX), and thus, the risk of developing HUS after the elimination of

STEC (Lupindu *et al*., 2014; Odo *et al*., 2021). Other studies have shown their dis-agreement to the important role played by the class of antibiotic or bactericidal antibiotics, for example, the use of ciprofloxacin increase the risk for children to develop the disease. Studies in animal models have reported that azithromycin reduces STX release from STEC isolates and mortality in vitro. During the diarrhea phase, nephrotoxin use should be discontinued, and the dose of drugs excreted by the kidneys should be adjusted. Narcotics should be used cautiously in patients with renal failure because their metabolites can cause seizures. Therefore, symptomatic treatment requires hospitalization in specialized centers for managing of acute renal injuries (Moola *et al*., 2015).

2.6.3. Prevention and Control

In general, strategies for the prevention and control of the spread of *E. coli* should include access to safe water, good handling practices to reduce the risk of food contamination, sanitation measures, public education and vaccination (Seib *et al*., 2012). Access to safe water is the primary target for the prevention of *E. coli* infections. Measures to prevent infections from food products include appropriate storage and cooking temperatures. Hospital measures that limit risk of the spread of multiresistant pathogens include prevention of cross-contamination by implementing strict hygienic standard protocols as well as control over the use of antimicrobial drugs (Mielke, 2010).

The main vehicles for pathogens' spread are the hands of hospital workers and medical devices. Proper hand hygiene is critical for the prevention of cross-contamination. Antibiotics are essential for the control and treatment of *E. coli* infections in humans and animals. However, it is generally accepted that antimicrobial resistance is associated with the quantity of antibiotic consumption (Van Duijn *et al*., 2011). The inappropriate use and misuse of antimicrobials increased the resistance in pathogens as well as in normal human bacterial flora in both. Animal reservoir is also an important source for resistance strains. Furthermore, the wide spread of antimicrobial therapy also results in the environmental release of antibiotics and antibiotic resistance genes with consequent selection of resistant bacteria.(Allocati *et al*., 2013)To reduce the risk of acquiring an *E. coli* O157:H7 infection are: - Cook ground beef thoroughly (minimum 160 °F) before eating, drink only pasteurized milk and apple juice, wash fresh fruits and

vegetables thoroughly before eating, Wash hands thoroughly after handling animals, wash hands thoroughly after changing diapers or after providing care to children or adults suffering from a diarrhoeal disease, do not use fresh manure from ruminants to fertilize vegetables or fruits and avoid swimming in lakes or ponds used by cattle and drinking surface water that has not been properly treated to eliminate pathogens.

2.7. Pathogenesis and Virulence

The production of Shiga toxin is fundamental to the pathogenesis of bloody diarrhea and hemolytic uremic syndrome. *E. coli* O157:H7 strains have the locus of enterocyte effacement genes (Law, 2000) but other serogroup strains without these genes have also caused haemolytic uraemic syndrome. The pathogenicity of STEC is determined by several virulence factors that are encoded by chromosomal pathogenicity islands, phage chromosomes integrated in the bacterial genome as well as plasmids. Shiga toxins are members of a toxin family that share many common features. The Shiga toxins identified in EHEC are classified in two distinct subgroups: Stx1 and Stx2. The toxins are produced by the pathogen in the colon and cause local damage (Ståhl *et al*., 2009). The ability to pass through the bloodstream to the kidney plays a role in causing HC and HUS. *E. coli* O157:H7 can produce two different Shiga toxins encoded by bacteriophage. Stx1 is very similar to the type 1 toxin of Shigelladysenteriae; Stx2 is genetically and immunologically distinct with 55–60% similarity in genetic and amino acid sequences (Law, 2000). The possession and expression of the Stx2 gene and the variant Stx2c (which often occurs with Stx2) correlate strongly with the causation of bloody diarrhoea and haemolyticuraemic syndrome (Persson *et al.,* 2007).

Shiga toxins bind to glycosphingolipid globotriaosylceramide (Gb3), a cell surface receptor. They are then internalized by clathrin-dependent endocytosis, and go on to specifically depurinate 28S eukaryotic rRNA, inhibiting protein synthesis (Heredia and García, 2018). This step induces a ribotoxic stress response that can lead to glycosphingolipid globotriaosylceramide e release and apoptotic cell death. In the human kidney, Gb3 is present on glomerular endothelial cells, podocytes, and various tubular epithelial cell types. Shiga toxin binds to these cells in renal sections from patients with haemolyticuraemic syndrome, and damage markers from these cells can be detected in their urine; biopsy samples from these patients show apoptosis of glomerular and tubular cell types and fibrin-rich glomerular microangiopathy (Tarr *et al.,* 2005). Blood from

patients with haemolyticuraemic syndrome showed an increase in microparticles with surface-bound tissue factor and in functional tissue factor. Tissue factor can contribute to a pro thrombotic state (Ståhl *et al.*, 2009).

Other structures that help EHEC in adhering to host cells are fimbriae and fimbrial adhesins, thread-like structures that extend out from the bacterial surface (Heredia and García, 2018). Type 1 fimbriae are the first adhesins described in *E. coli* and are the most common adhesins produced. These adhesins mediate the adherence of the pathogen to mannose-containing glycoproteins found on the surfaces of eukaryotic cells. EHEC clinical isolates from HUS patients have been found to have a distinct virulence profile. Strains capable of producing both Shiga toxins have been found to be highly associated with bloody diarrhea or HUS, while strains with only Stx1 are rarely found in HUS patients. In addition, clinical strains associated with HUS have also been found to be more enterohemolytic and are more likely to possess intimin. The high virulence of STEC strains like *E. coli* O157:H7 is not only dependent on virulence factors but partially also on the ability to survive environmental unfavorable conditions, such as resistance to low pH levels found in the gastrointestinal tract which contributes to very low infectious dose of 50–100 cells or lower (Law, 2000).

2.8. *E. coli* O157:H7 isolation

Buffer peptone water was used as transporting media. The sample was enriched using buffer peptone water at 37 ℃ for 24 hours, inoculated on MacConkey agar, and then incubated at 37 ℃ for 24 hours. Typical colonies on MacConkey agar (pink, due to their ability to ferment lactose) were stained using gram stain and observed for their staining and morphological characteristics and transferred to eosin methylene-blue (EMB) agar. The colonies with metallic green sheen on EMB agar which is typical feature of *E. coli* were transferred to sorbitol MacConkey agar to check the presence of *E. coli* O157:H7 phenotype (inability to ferment sorbitol). Then the confirmed pure cultures considered as *E. coli* positive were transferred to nutrient agar to be used for additional confirmatory biochemical tests (IMViC tests) as described below (Disassa *et al*., 2017).

2.8.1. Basic Biochemical Examinations

For primary and secondary biochemical tests, pure cultures of a single colony from MacConkey agars were transferred onto a nutrient agar plate. Tests such as Oxidase, Catalase, Indole, Methyl red, Voges-Proskauer (VP), and Citrate (IMViC) tests were done to confirm the presence of E. coli in the test samples. Colonies that are positive for tryptophan utilization (indole test) (red ring), positive for Methyl red, negative for citrate utilization (green slant), and negative for Voges-Proskauer (VP) test were considered to be *E. coli* positive. Moreover, the Gram staining of the bacterial colony was done on a sterile glass slide as described by Chees brough. Isolates of presumptive *E. coli* for all biochemical tests were cultured on sorbitol MacConkey agar for further test on latex agglutination test (Ababu *et al*., 2020).

2.8.2. Confirmation Test

Confirmation of non-sorbitol fermenting *E. coli* O157:H7 were done by latex agglutination test using a latex kit. The latex kit consists of four components: Latex test reagent, latex control reagent, positive controls, and negative controls. The test reagent contains blue latex particles sensitized with a specific antibody against the *E. coli* O157:H7 antigen and the control reagent consist of latex particles sensitized with rabbit globulin. The positive controls are suspensions of inactivated *E. coli* O157:H7 cells, whereas the negative controls are suspensions of inactivated non-specific *E. coli* cells. The latex kit was first checked for its performance by using the control suspensions in the kit, the test was continued after the positive control reacts with the test latex showing a positive result. Briefly, one drop of 0.85% saline water and latex test were dispensed into the reaction card separately. Using a sterile wire loop, a few presumptive colonies of *E. coli* O157:H7 were taken and emulsified into the saline water on the latex card, then slowly mixed with the test latex and checked for agglutination within 1 minute. A result was positive if agglutination of the latex particles occurred within 1 minute. The negative result was obtained if no agglutination occurred and a smooth blue suspension remained after 60 seconds in the test area. Test positive isolates were stoked in glycerol using cryovials for further antimicrobial resistance determination (Abunna *et al*., 2023).

2.9. Antimicrobial Resistance

Antimicrobial Resistance (AMR) occurs when microorganisms including bacteria, viruses, fungi, and parasites become able to adapt and grow in the presence of medications that once impacted them (Founou *et al.,* 2017). Infection with AMR leads to serious illnesses and prolonged hospital admissions, increases in healthcare costs, higher costs in second-line drugs, and treatment failures (Chokshi *et al*., 2019). For instance, just in Europe, it has been estimated that antimicrobial resistance has been correlated with more than nine billion euros per year. Furthermore, according to the Centers for Disease Control and Prevention (CDC), antimicrobial resistance adds a 20-billion-dollar surplus in direct healthcare costs in the United States, which is exclusive of about 35 billion dollars in loss of productivity annually (Llor and Bjerrum, 2014). Over the past 20 years, the importance of antimicrobial resistance as a global public health concern has increased dramatically. This development of antimicrobial resistance resulting from agricultural use of antibiotics that could impact on the treatment of diseases affecting the human population that require antibiotic intervention has become a significant global public health concern (Rahimi *et al*., 2012). Different antibiotic resistance profiles have been detected in *E. coli* O157:H7 isolates from different sources, including humans, animals and foods. Streptomycin, sulfisoxazole, and tetracycline resistance is the most commonly reported resistance phenotype of *E. coli* O157:H7 isolates (Atlaw, 2021).

2.10. Public health importance

E. coli O157:H7 has been isolated from ill people around the world. It tends to be reported more often from more developed countries but this may be an artifact caused by the paucity of sophisticated diagnostic laboratories in developing countries. Food Net data indicate that *E. coli* O157:H7 causes significantly more cases of sporadic infections than cases linked to an outbreak. For example, in 2004, only 9% of 402 confirmed cases of infection with *E. coli* O157:H7 were associated with outbreaks. Sporadic infections appear to be associated with some of the same factors that cause outbreaks: undercooked hamburgers and exposure to farms and cattle. Some sporadic infections are also associated with use of immunosuppressive medications and dining at Table service restaurants (Kassenborg *et al*., 2004). Vehicles of infection, suspected or confirmed, have been identified for most outbreaks. A large outbreak in the world was associated with contaminated fruit juices, melon, and salad greens. Finally, direct person-to person infection

occurs particularly among children and their caregivers, such as in day care facilities and also within families (Pal and Ayele, 2017).

2.11. Status of *Escherichia coli* O157:H7 in Goat Meat and Vegetables Samples in Ethiopia

In Ethiopia, foodborne disease is a common problem. Microbial contamination of meat may originate from the feces and skin of animals presented for slaughter and can be transferred to the carcass during skin removal and evisceration. Also, the poor handling and unhygienic sanitation practice of vegetables was the source of microbial contamination of vegetables (Abreham *et al*., 2019).

Table1: Current status of *E. coli* O157:H7 isolated from goat meat and vegetables samples in Ethiopia

Location	**Sample source**	**Prevalence (%)**	**Strain isolated**	**Refences**
Somalia region	Goat meat	0.51	O157:H7	(Dulo *et al.*, 2015)
Addis Ababa	Goat meat	2.6	O157:H7	(Bekele *et al.*, 2014)
Dire Dawa	Goat meat	7.8	O157:H7	(Dulo, 2014)
Modjo	Goat meat	2.55	O157:H7	(Hiko *et al.*, 2008)
Modjo town	Goat meat	30.2	O157:H7	(Mersha *et al.*, 2010)
Addis Ababa	Lettuce	0.51	O157:H7	(Haile *et al.*, 2021)

2.12. Status of Antimicrobial Resistance Profile of *Escherichia Coli* O157:H7 Isolates Goat Meat and Vegetables Samples in Ethiopia.

The pathogenic microorganisms which reside in intestinal tracts of animals or humans are more likely to contaminate vegetables through faeces, sewage, untreated irrigation water or surface water. Outbreak of food infections associated with consumption of ready-to-eat vegetables has been increasing in recent times. There is always a chance of *E. coli* O157:H7 being present in vegetables grown in manure-fertilized soils (Reuben and Makut, 2014). In Ethiopia, the occurrence of *E. coli* O157: H7 including multi drug resistance in food of animal origin is

extremely increased due to many reasons like unhygienic slaughtering practices in the slaughter houses, indiscriminate use of antimicrobial agents for growth promotion and treatment of diseased animals may leads to development of resistant bacterial strains. The *E. coli* O157: H7 isolates showed different antimicrobial resistance profile against selected antimicrobial agents in Ethiopia. A summary of previously reported antimicrobial resistance profile *E. coli* O157:H7 isolates from goat meat and a vegetables sample among various regions of Ethiopia is presented in Table 2.

Table 2: Status of Antimicrobial resistance profile of E. coli O157:H7 isolates from goat meat and vegetables sample in Ethiopia

Location	**Sample source**	**No. positive sample and isolate tested**	**Antimicrobial resistance profile**				**References**
			AMP	AML	CIP	KA	
Somalia region	Goat meat	6	3	2	0	-	(Dulo *et al.*, 2015)
Addis Ababa	Goat meat	10	-	0	0	-	(Bekele *et al.*, 2014)
Dire Dawa	Goat meat	6	4	1	0	0	(Dulo, 2014)
Modjo	Goat meat	5	0	-	-	0	(Hiko *et al.*, 2008)
Addis Ababa	Lettuce	2	2	0	0	0	(Haile *et al.*, 2021)

Key: Not determined (-), Ampicillin (AMP), Amoxicillin(AML), Ciprofloxacin(CIP) and Kanamycin(KA).

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in Maya city (Fig. 1) from September 2024 to February 2025. The area lies between 09° 10'-09°30' N latitude and 41° 50'-42°20' E longitude with an elevation range from 2047 meters above sea level. It is found at 497 km east of Addis Ababa and the highest and lowest temperatures are 23.40 °C and 8.25 °C, respectively and have three sub-cities (Haramaya, Aweday and Adele sub-cities). Its annual average rainfall is 790 mm and the soil in Maya city is mostly clay type and, in some area, black soil is abundant. The agro-ecological zone of the Maya city contains, Woina Dega (Dagne, 2020). Many types of vegetables and fruits are grown in study area. Also, goats are found in and around the study area for meat production mostly.

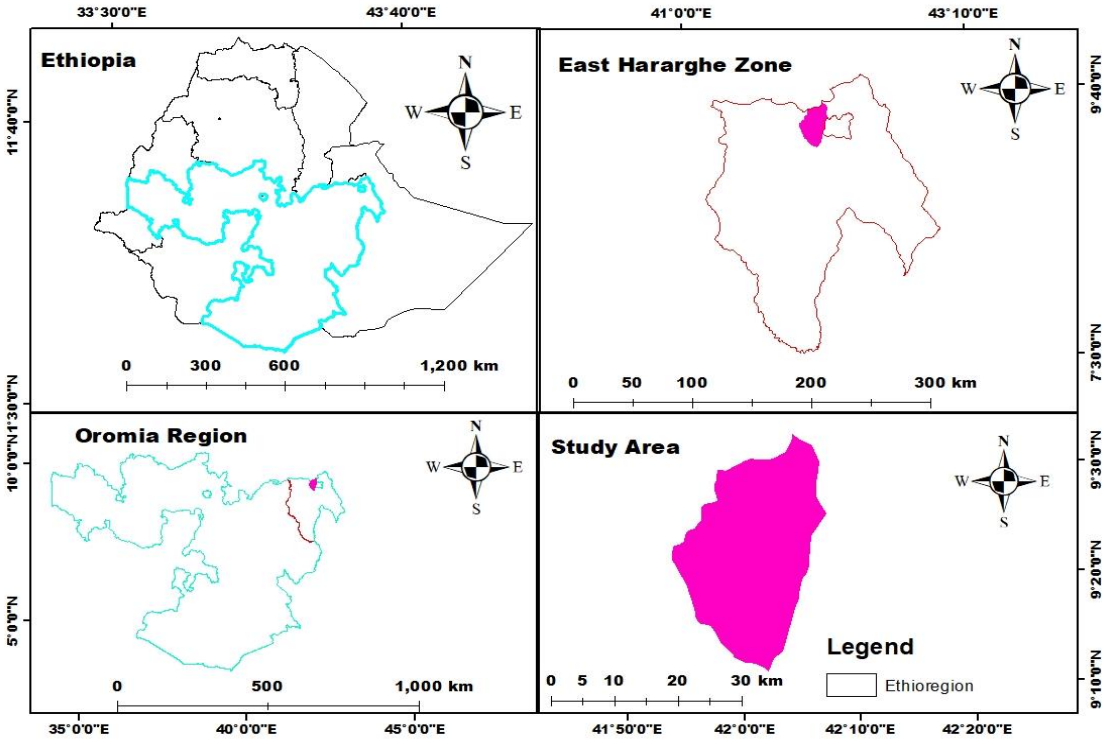

Figure 1. Map of study area

Source: from GIS

3.2. Study Samples and Population

The goat meat samples were brought from randomly selected butcher shop and fruit and vegetables samples were brought from randomly selected open market in Maya City to isolate *E coli* O157:H7. Goat meat, fruits and vegetables were the study population.

3.3. Study design and sample size determination

A cross-sectional study design was conducted from September 2024 to February 2025 in Maya city to isolate the prevalence of E. coli O157: H7 in goat meat, fruit and vegetables samples. A simple random sampling method was use to sample goat meat, fruits and vegetables. Sample size was determined by using the formula given by (Thrusfield, 2018). Thus, the sample size was calculated using averaged of the expected 50% prevalence in fruit (since there was no study on *E. coli* O157: H7), 0.51% in Lettuce representing vegetables (Haile *et al*., 2021) and 2.55% in goats meat at Dire Dawa (Dulo, 2014). The total prevalence was calculated by dividing the total summation of three prevalence to three ((50%+0.51%+2.55%)/3=17.7%) and becoming expected prevalence of 17.7% at 95% CI (Thrusfield, 2018).

$$\boldsymbol{n = \frac{z^2 \times pexp\,(1-pexp)}{d^2}} \qquad n = \frac{1.96^2 \text{x } P_{exp}\,(1- P_{exp})}{d^2}$$

Where, n= required sample size; Z= statistic for level of confidence = 1.96; d=desired absolute precision of 0.05, Pexp= expected prevalence.

$$N= \frac{(1.96)^2 \times 0.177\,(1-0.177)}{(0.05)^2} = 223.75 \approx 224$$

The minimum sample size of each of the seven food items were proportional distribution as shown on Table **3**. Questionnaire survey was also determined when a population's mean was of interest, the total number of respondents needed (n) using the formula provided (Arsham and Lovric, 2011) $n = 0.25 / SE^2$. The overall sample size (n) was therefore 100.

Table 3: Proportional Distribution of collected sample types from butcher shop and open market of the study area

Food types	Fresh food item	Study area			
		Haramaya	Aweday	Adele	Total
Meat	Goat meat	15	13	12	40
Fruits	Tomato	16	10	8	34
	Orange	14	10	6	30
	Banana	14	10	6	30
Vegetables	Cabbage	14	10	6	30
	Lettuce	14	10	6	30
	Carrot	14	10	6	30
Total		**101**	**73**	**50**	**224**

3.4. Technique for Sampling

A total of 224 samples were collected from September 2024 to February 2025 from Haramaya, Aweday and Adele sub-cities. 40 numbers of goat meat samples, 34 numbers of tomato samples and 30 numbers of samples for each (cabbage, orange, banana, lettuce and carrot) were sampled. A total of 35 (5 from each samples) were taken per a day and one times per a week.

A simple random sampling technique was employed to select the study unit at each butcher shops and open markets. The slaughtered animal was followed from the slaughter house to the butcher shop to confirm that meat was goat meat. After that the meat swab sample were taken from abdomen or flank, thorax (lateral) and breast (lateral) where considered to high risk of contamination were swabbed thoroughly.

3.5. Sample Collection

3.5.1. Goat meat swab sampling

The aseptically acquired fresh swab samples were collected from the butcher shops in the early morning (7:00–9:00 am). The cotton tipped swabs used to capture the swab samples were sterile

and aseptic. A method based on (ISO 17604, 2015) was used to obtain swabs over a measured surface area utilizing a sterile template and a viscose tip swab. 10 × 10 cm of flesh was rubbed on the goat meats many times, first horizontally and then vertically (Appendix figure **2c**). By pressing the cotton swap against the inner wall of the test tube that contained 10 ml buffered peptone water, the cotton swap was broken when the rubbing process was complete (ISO 17604, 2015). The swab was shaking in the tubes to help wash the bacteria off its surface. Each sample had a clearly labeled with the relevant identifying details. Then the sample was processed for microbiological investigation at the Central Laboratory Food Microbiology of Haramaya University while being properly labeled and transported in an icebox.

3.5.2. Vegetables and fruit sampling

Fresh vegetables and fruits including lettuce, cabbage, carrot, banana, orange and tomato samples were collected randomly and packed in separate sterile plastic bags together with ice in cardboard boxes (Appendix figure **1a-1f**). Each sample had a clearly labeled with the relevant identifying details. Then the sample was processed for microbiological investigation at the Central Laboratory Food Microbiology of Haramaya University while being properly labeled and transported in an icebox.

3.6. Questionnaire Survey

For butcher shop employees and fruit and vegetables seller, a standardized questionnaire pre-coded response options that were used to evaluate the likely cause contamination of meat, fruit and vegetables on selling process was prepared. Hygiene and sanitation were determined by the use of structured interview and through direct observations of the hygienic status and practices of butcher shop workers and fruit and vegetables sellers.

The questioners were classified into 3 categories depend on the types of food items they sells. These categories are denoted as the questions only for greengrocers, the questions for both greengrocers and butchers and the questions only for butchers. The target population constituted all the butcher shop workers and fruit and vegetables sellers. An approximate sample size was determined using a 5% standard error (SE) and 95% confidence intervals. Socio-demographic

profiles, training experiences, educational level, source of food item, wearing appropriate protective clothing and glove, disinfect of balance and various questions related to hygienic practices employed during the selling process and meat, fruit and vegetables handling were part of a semi-structured questioning survey that was primarily directed at butcher shop workers and fruit and vegetables sellers. Observational survey was also conducted on the hygienic status and practices by butcher shop workers and fruit and vegetables sellers' conditions of all sub-cities. Direct observation of general cleanliness and hygienic practices with reference to the state of goat meat, fruits and vegetables were also conducted during the study period. The questioners covered every procedure in butcher shop and open market that might have an impact on the way goat meat, fruits and vegetables are handled hygienically (Appendix figure **2a and 2b**).

3.7. *E. coli* O157:H7 Isolation

Upon arrival at Central Laboratory Food Microbiology of Haramaya University for selected vegetables and fruit samples, the sample preparation and enrichment were conducted by rinsing selected vegetables and fruits sample with buffered peptone water 0.1%. For the rinsing procedure, the necessary amount (cover all part of vegetables and fruit sample) of buffered peptone water 0.1% was added to sterile plastic bag that have vegetables and fruit samples, and gently shaken for 5 min. The solution was incubated overnight at 37 ℃ (Leff and Fierer, 2013). For goat meat samples were incubated aerobically at 37 °C for 24 h.

Then each sample was streaked on MacConkey agar (Himedia), which was a selective and differential medium of *E. coli* and incubated aerobically at 37 °C for 24 h for primary isolation. After incubation, the plates were checked to see if any pink colonies (lactose fermenter) had grown (Appendix figure **2f**). Then, to create a metallic sheen, a single, isolated colony was selected and sub-cultured on Eosin Methylene Blue (EMB Oxoid, Basingstoke, UK) agar for 24 hours at 37 °C. Then, suspected *E. coli* colonies were sub cultured onto nutritional agar (HiMedia Laboratories Pvt. Ltd., India) at 37 °C for 24 hours. The suspected E. coli colonies had a pinkish color look on MacConkey agar and a metallic sheen on EMB (Appendix figure **2g**).
For further identification the suspected *E. coli* colonies were carried out on nutrient agar for relevant biochemical experiments, such as those for indole, methyl red, the Voges-Proskauer

reaction (IMViC), were carried. Kovac's reagent, methyl red, and alpha-naphthol chemicals were employed as the test reagents for the indole test, the methyl red test, Voges-Proskauer and the citrate utilization tests respectively. Isolates were identified (+ + − −) IMViC patterns for indole, methyl red, Vogues Proskauer and citrate utilization tests, respectively, were considered as *E. coli* O157:H7 (Valderrama *et al*., 2016). The indole positive was producing pink to red color ring (Appendix figure **2h**).

Pure colony from nutrient agar streaked on CT-SMAC agar (Oxoid Ltd., Hampshire, England) containing 0.05 mg/l cefixime and 2.5 mg /l potassium tellurite (Dynal Biotech ASA, Oslo, Norway). Culturing was carried out carefully to obtain pure colonies and plates were incubated at 37 °C for 20–24 h. Plates of CT-SMAC agar were examined for the presence of non-sorbitol fermenting colonies. That a typical *Escherichia coli* O157:H7 colony is slightly transparent, almost colorless with pale appearance (Appendix figure **3b**). Pick *Escherichia coli* O157:H7 suspect colonies from the CT-SMAC agar plates. Streak the colonies onto non-selective media (nutrient agar) plates for confirmation test (ISO 16654, 2001).

3.8. *E. coli* O157:H7 Confirmation

Latex agglutination test was performed for confirmation of *E. coli* O157:H7 using latex kit (Thermo Fisher Scientific, Oslo, Norway). The latex kit consists of seven components indicated in: *E. coli* H7 tests latex, *E. coli* O157 tests latex, E. coli control latex, *E. coli* O157:H7 positive control and *E. coli* (not O157:H7) negative control, plastic stirring sticks and latex card. The test reagent is latex particles sensitized with specific rabbit antibody against O157 antigen and the control reagent consists of latex particles sensitized with rabbit globulin. The positive and negative controls are suspension of inactivated *E. coli* O157:H7 cells and inactivated non-specific *E. coli* cells respectively (ISO 16654, 2001). The test was performed according to the manufacturer instructions (Thermo Fisher Scientific, Oslo, Norway). First the latex kit was checked for its performance by using the control suspensions in the kit, the test was continued after the positive control reacts with the test latex showing positive result. The test was done for the presence of the O157 and the H7 antigens by agglutination with *E. coli* O157 and H7 latex reagent. Drop O157 latex regent on to latex card then few presumptive colonies (an average of 2 colonies) were taken and, then slowly mixed with the test latex and checked for appearance agglutination within 1 min. The same procedure was followed for presence of H7 antigen. The

sample which precipitates or agglutinated within a minute form were positive of *E. coli* O157: H7 (Appendix figure **3c, 3d**). In the event that there was no agglutination, the test was considered negative.

3.9. Test for Antimicrobial Resistance

The antimicrobial susceptibility test for 8 antimicrobial agents that are regularly used that may be important for public health, and are recommended by the National Committee for Clinical Laboratory Standards (CLSI, 2020) was conducted using the standard disc diffusion technique. Single colonies were transferred from nutrient agar plates into tubes containing 5 ml of saline water of (Oxoid, England). The broth culture was incubated for 4 hours at 37 °C in order to reach the required 0.5 McFarland turbidity levels. To evenly cover the surface of the Muller Hinton agar plate (Oxoid, England), a sterile cotton swab was dipped into the suspension, turned several times, and pressed firmly against the inside wall of the tube above the level to remove extra inoculums. To allow drying, the plates were kept at room temperature for 30 minutes.

A total of eight antimicrobials were evaluated on each *E. coli* O157; H7 isolate. Ampicillin (AMP), Amoxicillin (AML), Kanamycin(K), Ciprofloxacillin (CIP), Clindamycin (DA), Erythromycin (E), Pencillin (PG) and Vancomycin (VA) were spaced at least 15 mm apart from the edge of the plate to avoid the inhibition zones overlapping, antibiotic discs were properly positioned on the swabbed (MHA) plates (Appendix figure **3e**). The plates were incubated for 24 hours at 37 °C. Following incubation, the measured diameters of the control organism were compared to the diameters of the inhibitory zones (Appendix figure **3g**) accordance to the clinical laboratory standards institute's interpretative guidelines, classified as resistant, intermediate, or susceptible (CLSI, 2020).

3.10. Data Analysis and Management

Before analysis, the gathered data were entered into Microsoft Excel and analyzed using Stata SE 15. Descriptive statistics were expressed in in terms of frequency and percentages to evaluate the data and summarize the findings for the occurrence of *E. coli* O157. Comparisons between

each variable were also analyzed using Pearson chi square test, then, confirmed. Statistically significant associations between variables were considered at a 95% confidence interval and a 5% level of significance by calculating chi square values. The ($P < 0.05$) was considered statistically significant.

4. RESULTS

4.1. The prevalence of *E. coli*

From the total of 224 samples examined, 83 (37.05 %) were found to be contaminated with *E. coli* as indicated on Table **4**. There is no statistically significant variation in the prevalence of *E. coli* among the sample site ($p>0.05$) but significant variation among sample type ($p <0.001$)

Table 4: Proportion of *E. coli* among sample site and sample type

Sample site	**No. Examined**	**Positive No. (%)**	**$\chi 2$**	**P-Value**
Haramaya	101	39(38.61)	2.3740	0.305
Aweday	73	30(41.09)		
Adele	50	14(28.00)		
Sample type				
Meat	40	26(65.00)	22.2714	<0.001
Tomato	34	9(26.47)		
Cabbage	30	13(43.33)		
Carrot	30	11(36.67)		
Lettuce	30	11(36.67)		
Banana	30	8(26.67)		
Orange	30	5(16.67)		
Total	**224**	**83(37.05)**		

Statistically significant variation in the prevalence of *E. coli* between the sample sources and among selling method were observed ($p <0.001$) as shown on the table **5**.

Table 5: Proportion of *E. coli* among sample source and selling method

Sample source	No. Examined	Positive No. (%)	χ2	P-Value
Butcher shop	40	26(65.00)	16.3058	<0.001
Open market	184	57(30.98)		
Selling Method				
Weight Balance	40	26(65.00)	21.0324	<0.001
Bare Hand	90	35(38.89)		
Mixed	94	22(23.40)		
Total	**224**	**83(37.05)**		

4.2. The prevalence of *E. coli* O157:H7

Out of the total of 224 samples examined, 34 (15.18 %) were found to be contaminated with *E. coli* O157:H7 and from 83 *E. coli* positive samples, 34(40.96%) were positive with *E. coli* O157:H7 as indicated on Table **6**. There was no statistically significant variation in the prevalence of *E. coli* O157:H7among the sample site and sample type (p>0.05).

Table 6: Proportion of *E. coli* O157:H7 among sample site and sample type

Sample site	No. Examined	Positive No. (%)	χ2	P-Value
Haramaya	101	19(18.81)	2.2013	0.333
Aweday	73	10(13.70)		
Adele	50	5(10.00)		
Sample type				
Meat	40	12(30.00)	11.4229	0.076
Tomato	34	3(8.82)		
Cabbage	30	3(10.00)		
Carrot	30	5(16.67)		
Lettuce	30	6(20.00)		
Banana	30	3(10.00)		
Orange	30	2(6.67%)		
Total	**224**	**34(15.18)**		

From sample source and selling method, both were showing statistically significant ($p<0.05$) (table 7).

Table 7: Proportion of *E. coli* O157:H7 among sample source and selling method

Sample source	**No. Examined**	**Positive No. (%)**	**$\chi 2$**	**P-Value**
Butcher shop	40	12(20.00)	8.3087	0.004
Open market	184	22(11.96)		
Selling Method				
Weight Balance	40	12(20.00)	10.0811	0.006
Bare Hand	90	14(15.56)		
Mixed	94	8(8.51)		
Total	**224**	**34(15.18)**		

4.3. The prevalence of *E. coli* O157:H7 out of the *E. coli* isolates

Out of the total 83 *E. coli* positive samples, 34(40.96%) were positive with *E. coli* O157:H7. Of 34 positive *E. coli* O157:H7 isolates, the proportion was ranged from 23.08% in Cabbage to 54.55% in Lettuce samples from open market (Table **8)**.

Table 8: Proportion of *E. coli* O157:H7 out of the *E. coli* isolates among studied food items by studied variables

Sample type	**No. E. coli *isolates***	**Positive No. (%) of E. coli *O157:H7***
Meat	26	12(46.15)
Tomato	9	3(33.33)
Cabbage	13	3(23.08)
Carrot	11	5(45.45)
Lettuce	11	6(54.55)
Banana	8	3(37.50)
Orange	5	2(40.00)
Total	**83**	**34(40.96)**

4.4. Antimicrobial Profile of *E. coli* O157:H7

The antimicrobial susceptibility and resistance of *E. coli* O157:H7 was tested toward eight antimicrobial disks (Table **9**). Out of the eight antimicrobials tested for susceptibility pattern, all isolates were resistant to amoxicillin, clindamycin and penicillin antimicrobial disks. Moreover, 6(85.7%) isolates were resistant to ampicillin and vancomycin. Also, 5(71.4%) isolates was resistant to erythromycin. On the other hand, 6(85.7%), 4(57.1%) and 1(14.3%) isolates were susceptible to ciprofloxacillin, kanamycin and vancomycin antimicrobial disks respectively.

Also, all *E. coli* O157:H7 isolate samples were shown MDR (Table **10**). All isolates were resistant to three antimicrobial discs (AML, DA and PG) and six isolates were resistant to two antimicrobial discs (AMP and VA) and five isolates were resistant to one antimicrobial discs (E) and one isolate was resistant to one antimicrobial disc (K).

Table 9: Antimicrobial Resistance of *E. coli* O157:H7 isolates

Antimicrobial disc	**Unit**	**Antimicrobial Resistance of *E. coli* O157:H7 isolate (n=7) (%)**		
		Resistant	Intermediate	Susceptible
Ampicillin (AMP)	10 µg	6(85.7)	1(14.3)	0(0.0)
Amoxicillin (AML)	2 µg	7(100)	0(0.0)	0(0.0)
Ciprofloxacillin (CIP)	10 µg	0(0.0)	1(14.3)	6(85.7)
Clindamycin (DA)	10 µg	7(100)	0(0.0)	0(0.0)
Erythromycin (E)	15 µg	5(71.4)	2(28.6)	0(0.0)
Kanamycin (K)	30 µg	1(14.3)	2(28.6)	4(57.1)
Penicillin (PG)	10µg	7(100)	0(0.0)	0(0.0)
Vancomycin (VA)	30µg	6(85.7)	0(0.0)	1(14.3)

Table 10: Multidrug-Resistant Patterns of *Escherichia coli* O157:H7 Isolates

Isolates	**No of tested isolate**	**Drug Develop Resistance**	No of resistant isolate
Banana	1	AMP, AML, DA, PG,	1
Cabbage	1	AMP, AML, DA, E, PG, VA	1
Carrot	1	AMP, AML, DA, E, PG, VA	1
Lettuce	1	AMP, AML, DA, E, PG, VA	1
Meat	1	AMP, AML, DA, E, PG, VA	1
Orange	1	AMP, AML, DA, K, PG, VA	1
Tomato	1	AML, DA, E, PG, VA	1

Note: Ampicillin (AMP), Amoxicillin (AML), Clindamycin (DA), Erythromycin (E), Kanamycin (K), Penicillin (PG) and Vancomycin (VA).

4.5. Questionnaire Survey

Demographic Characteristics of butcheries and greengrocer in study area was summarized in (Table **11**). Noting, the questions were categorized into the questions for fruits and vegetables, the questions for all food items; and the questions for goat meat.

Table 11: Socio-Demographic Characteristics of the Participants

Characteristic		**Respondents**			**Percent (%)**		
		Only Greengrocer	**Greengrocer and Butcher**	**Only Butcher**	**Only Greengrocer**	**Greengrocer and Butcher**	**Only Butcher**
Site	Haramaya	44	43	7	52.4	43	43.75
	Aweday	26	35	5	31	35	31.25
	Adele	14	22	4	16.6	22	25
	Total	**84**	**100**	**16**	**100**	**100**	**100**
Sex	Male	2	18	16	2.4	18	100
	Female	82	82	0	97.6	82	0
	Total	**84**	**100**	**16**	**100**	**100**	**100**
Age (years)	10 up to 25	42	38	6	50	38	37.5
	26 up to 39	36	48	6	42.9	48	37.5
	Above 40	6	14	4	7.1	14	25
	Total	**84**	**100**	**16**	**100**	**100**	**100**
Occupational status	Butcheries	0	16	16	0	16	100
	Greengrocer	84	84	0	100	84	0
	Total	**84**	**100**	**16**	**100**	**100**	**100**
Level of education	Illiterate	33	37	4	39.3	37	25
	1 up to 6	48	53	7	57.1	53	43.75
	up to 12	3	8	3	3.6	8	18.75
	Certificate	0	2	2	0	2	12.5
	Total	**84**	**100**	**16**	**100**	**100**	**100**

The butcheries and greengrocers were also interviewed on question related to way to clean, disinfect and use of balance, washing food items before sale, advising customer to clean or cook the food item before consummation, work-related training, clean the butcher shop and open

market environment, awareness about carcass contamination, wear hair cover while working and checking their health status to collect information which can directly or indirectly contribute to the contamination of meat.

Majority of butcheries and greengrocers (69%) had not been trained on any job-related issues. More than half (51.2%) of greengrocers are not washing fruits and vegetables before sale with water. Most of them (76%) did not disinfectants the working surfaces and cutting material and also more than half butcheries and greengrocers (55%) are not disinfecting their balance after use. Out of the total butcheries (62.5%) have no any awareness about meat contamination (Table **12**).

Table 12: Questionnaires result to assess their knowledge and hygienic Practices.

Questionnaires	Responses	Questionnaires Result								
		Meat	Tomato	Cabbage	Carrot	Lettuce	Banana	Orange	Total	
									No	%
Would you use the same balance at selling of all items?	Yes	-	11	7	5	4	10	8	45	53.6
	No	-	3	7	9	10	4	6	39	46.4
	Total	-	14	14	14	14	14	14	84	100
Are you washing them before sale with water?	Yes	-	8	4	7	6	9	8	41	48.8
	No	-	6	10	7	8	5	6	43	51.2
	Total	-	14	14	14	14	14	14	84	100
How often you clean your balance?	between selling	8	4	-	-	-	5	5	22	22
	Daily	7	3	-	-	-	3	3	16	16
	weekly	1	1	-	-	-	1	-	3	3
	Not use	-	6	14	14	14	5	6	59	59
	Total	16	14	14	14	14	14	14	100	100
Would you disinfect your balance?	Yes	16	9	0	0	0	10	10	45	45
	No	0	5	14	14	14	5	5	55	55
	Total	16	14	14	14	14	14	14	100	100
Are you advice your customer to clean or cook the food item before consummation?	Yes	16	14	14	12	11	14	14	95	95
	No	0	0	0	2	3	0	0	5	5
	Total	16	14	14	14	14	14	14	100	100
Did you receive any training on hygienic food handling before?	Yes	8	4	4	4	4	3	4	31	31
	No	8	10	10	10	10	11	10	69	69
	Total	16	14	14	14	14	14	14	100	100
Are you clean the butcher shop and open market environment?	Yes	16	14	14	14	14	14	14	100	100
	No	0	0	0	0	0	0	0	0	0
	Total	16	14	14	14	14	14	14	100	100
Do you think food contamination could bring serious health problem to consumers?	Yes	11	8	9	10	8	11	11	68	68
	No	5	6	5	4	6	3	3	32	32
	Total	16	14	14	14	14	14	14	100	100
Do you wear hair cover while working?	Yes	11	9	13	12	12	10	11	78	78
	No	5	5	1	2	2	4	3	22	22
	Total	16	14	14	14	14	14	14	100	100
Do you use disinfectants for cleaning of working surfaces and cutting material?	Yes	4	4	4	2	3	3	4	24	24
	No	12	10	10	12	11	11	10	76	76
	Total	16	14	14	14	14	14	14	100	100
Do have any awareness about meat contamination?	Yes	6	-	-	-	-	-	-	6	37.5
	No	10	-	-	-	-	-	-	10	62.5
	Total	16	-	-	-	-	-	-	16	100

The observational survey revealed that besides the lack of training on food safety, the shortage of facilities to maintain the minimum acceptable hygienic practices. Most of the butcheries and greengrocers (77%) not cut and clean their fingernails. Majority of butcheries and greengrocers (96%) not wash and disinfection of their operational tools and floor after each working interval which is commonly observed at all butcher shops. All most the butcheries and greengrocers (97%) did not use glove upon selling food items. There was also no specifically separate meat and GIT content. There was no access water supply and soap for hand washing in the around butcher shop and open markets. Also, upon purchasing, 94% of customers touch the food item by their hand (Table **13**).

Table13: Researcher observation questionnaires result

Questionnaires	**Responses**	**Questionnaires Result**								
		Meat	**Tomato**	**Cabbage**	**Carrot**	**Lettuce**	**Banana**	**Orange**	**Total**	
									No	**%**
Degrees of damage to the vegetables and fruit:	Intact	-	9	7	8	4	8	10	39	46
	Few	-	5	7	6	10	6	5	45	54
	High	-	-	-	-	-	-	-	-	-
Are they place different food items in the same container?	Yes	-	4	6	5	7	7	6	35	58
	No	-	10	8	9	7	7	8	49	42
Is there access water supply and soap for hand washing	Yes	5	0	0	0	0	0	0	5	5
	No	11	14	14	14	14	14	14	95	95
Up on selling, did you use	Glove	3	0	0	0	0	0	0	3	3
	Bare hand	13	14	14	14	14	14	14	97	97
Are the consumer customers touching the food item by their hand?	Yes	10	14	14	14	14	14	14	94	94
	No	6	0	0	0	0	0	0	6	6
Are they wash and disinfection of their operational tools and floor after each working interval?	Yes	4	0	0	0	0	0	0	4	4
	No	12	14	14	14	14	14	14	96	96
Fingernails short and clean?	Yes	6	3	2	3	3	2	4	33	33
	No	10	11	12	11	11	12	10	77	77
Meat and GIT content kept separately?	Yes	7	-	-	-	-	-	-	7	44
	No	9	-	-	-	-	-	-	9	56
Method of meat dressing	Mixed	5	-	-	-	-	-	-	5	31
	Hanging on the floor	11	-	-	-	-	-	-	11	69

5. DISCUSSION

The prevalence of *E. coli* O157:H7 was not statistically significant ($p > 0.05$) among the sub-cities and food types. This might be due to outlets across the city source their goat, fruits and vegetables from the same markets and farm lands. The present study revealed that the overall occurrence of *E. coli* O157:H7 in Maya city was 15.18%. Out of result,30% from goat meat, 8.82% from tomato, 10% from cabbage, 16.67% from carrot, 20% from lettuce, 10% from banana and 6.67% from orange samples. The goat meat prevalence of this study was found comparable with other reports with, 30.2% in Modjo town (Mersha *et al.*, 2010). In this study, the prevalence of *E. coli* O157:H7 goat meat (30%) were higher than the previous study done 2.6% in Somali region of Ethiopia (Dulo *et al*., 2015), 2.55% in goat at Dire Dawa (Dulo, 2014), 7.8% from goat meat in Addis Ababa (Bekele *et al.,* 2014), 2% in Debre-Zeit and Modjo town (Hiko *et al.,* 2008) from goat meat in different parts of Ethiopia. Additionally, lower findings were also reported from other countries such a 11.8%in Bangladesh (Islam *et al.*, 2008), 5.7% in Nigeria (Ojo *et al.*, 2010), 1.7% from Iran (Rahimi *et al.,* 2012). On the other hand, other scholars reported higher results than the present findings 50% prevalence for goat meat in India (Gomashe *et al.*, 2011).

The overall variations in the prevalence of *E. coli* O157:H7 might be due to different sampling techniques, laboratory methodology used agroecology of the study area, and hygienic conditions used and due to the identification technique of this study and the biology identification of the others, which was a highly sensitive automated machine. Another reason for high prevalence of this study may be due to the storage time of the meat on the floor of butcher shop and the was no refrigerator used for cooling purpose. Moreover, there could be risk of carcass contamination and cross and subsequent contamination, during transportation, environment, handling of meat at butcher shops. In this study slightly, higher prevalence (30%) was observed for *E. coli* O157:H7 on goat meat swab samples in comparison with other samples. This seems to be quite logical as the main source of contamination is the skin of the animal which found its way to the surface of the carcass due to poor hygienic conditions during slaughtering process of the animals or it might be related to cross contamination during the slaughter process which in overall reflect the general unhygienic conditions in employees, utensils and environmental sanitation of the butcher shop under study.

Furthermore, the consumption of goat meat is much more frequent in the Maya city compared to other areas of Ethiopia, with fresh goat meat reportedly purchased by the predominantly Muslim population from butcher shops 1–3 times a week. However, there are no regulations in Ethiopia to protect meat consumers from food-borne pathogens such as *E. coli* O157:H7. This is especially problematic because of the widespread practice of raw meat consumption throughout the country. Therefore, avoiding carcass contamination during the slaughter process should be of utmost importance in order to protect the public from food-borne illness due to consumption of contaminated meat.

Our study is the first report on the presence of *E. coli* O157:H7 in fruits and second report in vegetables next to Haile *et al.* (2021) in Addis Ababa on the presence of *E. coli* O157:H7 in Ethiopia. In this study, the prevalence of *E. coli* O157:H7 in vegetables 10%-20% were similar with the previous study 17.5% in Nigeria (Reuben and Makut, 2014), (15.7 %) in Greece (Pinaka *et al.*, 2013) and also lower prevalence has been reported in 0.51% in Addis Ababa (Haile *et al*., 2021), 3.96% in Peru (Mora *et al*., 2007). In this study, the prevalence of *E. coli* O157:H7 in fruit 6.67% -10% were similar with the previous study 14-0% in India (Verma *et al.,* 2018).

E. coli O157:H7 could contaminate the vegetables and fruits from different source either from animal manures used as soil fertilizer, animal inadvertently enter fields, or through contaminated transportation vehicles. Farmers use bovine manure and untreated water on their farms to grow vegetables and fruits and the vegetables and fruits are sold in open-air markets. These vegetables and fruits are displayed either on top of wooden racks or on the ground in these open markets. Studies have shown that *E. coli* O157:H7 can get internalized in vegetables and fruits through contaminated irrigation water and soil. However, the occurrence of this pathogen in vegetables and fruits may have been a direct reflection of sanitary quality of the cultivation water, harvesting, transportation, storage, processing and poor handling practices (Reuben and Makut, 2014). Especially, the farmer in and around Maya city have been using untreated sewage water, untreated Haramaya and Adele lake water, and animals' dung on their farm lands. The consumers purchase these vegetables and fruits and eat without properly washing or cooking as salads or unpasteurized juice in home, restaurants and cafeteria and can be easily *E. coli* O157:H7 carrier.

In this study, *E. coli* O157:H7 was most frequently isolated in carrot (16.67%), followed by banana (10%) and orange (6.67%). Incidentally, these are among the most consumed ready-to-eat fresh fruits and vegetable in Maya city. It has been observed that fruits and vegetables that get in contact with the soil have a higher risk of being contaminated, especially if the soil is contaminated with animal/human waste or manure that is not well-composted. It is instructive to note that fruits and vegetables comprise a diversed range of plant parts (leaves, roots, tubers, fruits, and flowers) and any of these parts are consumed directly, partially cooked or otherwise. Production practices, growth conditions and the location of the eatable part during growth (soil, soil surface, and aerial part) will in combination with harvesting and processing factors affect their microbial status at the time of consumption (Moses *et al*., 2016). Also, the contamination of ready to eat food by calf manure was linked to another outbreak of *Escherichia coli* O157:H7 infections (Kiranmayi *et al.,* 2010).Hence, any part that hosts microbial pathogens becomes a risk to humans especially the ready-to-eat ones such as carrot, and others that may require no boiling process before being consumed.

Nevertheless, the present study is the first to report the existence of *E. coli* O157:H7 in fruit samples and second to report the existence of *E. coli* O157:H7 in vegetables in Ethiopia and its antimicrobial resistant patterns. This information calls for larger and fittingly powered studies to understand the sources, the points of contamination, and define appropriate risk mitigation strategies for the country.

This study has also considered interview questionnaires and an observational survey to assess the hygiene and sanitary practices of butcher shop and open market and the overall marketing process of goat meat, fruits and vegetables. From face to face interview, all of butcheries and greengrocers are cleaning the butcher shop and open market environment and 95% of them were advising their customers to clean and cook the food item before consummation. On the other hand, butcheries and greengrocers have less concern on hygienic practice from observation and interview. From the survey conducted, more of the respondent focus on working quickly is more important rather than keeping hygiene. In open market most of greengrocers were sold fruit and vegetables on dirty cloth or carton. Also, most of butchers placed their equipment or knife on dirty floor and no frequent washing of equipment was observed. This study agreed with study

done on factors associated with food safety practices among food handlers: facility-based cross-sectional study at Gondar city (Azanaw *et al*., 2019) and at Nigeria (Miner *et al*., 2020) which discovered that persons who had received training have excellent food handling practices than those who had not receive training. This finding suggests that individuals who receive regular training might have access updated knowledge.

Lack of water for washing floor and operational tools, selling without glove, sharing of knife, placing different food items in one container, customers were touching all food items upon purchasing without washing/sterilizing their hands, lack of water and soap for hand washing, uncleaned protective cloths and containers (except in a few butcher and greengrocers) and infrequent hand washing were bad practices observed at the butcher shops and open markets. Personal and general hygienic practice is extremely vital to ensure production of safe food to consumers (Sani and Siow, 2014). However, (97%) of the butcheries and greengrocers did not use the glove upon selling food items and also 77% of them were not shorten and clean their finger snails. Also, in many butcher shops (56%), the meat and GIT were not kept separately. Thus, the incomplete separation still can make cross contamination (table **13**).

The relatively higher prevalence of *E. coli* O157:H7 on goat meat 12/34(35.29%) in this study might be due to most of the butchers had not use glove and touching the meat, their cloth and money without proper hand washing using soap and disinfection of their operational tools and floor after each working interval and the cross contamination of carcass with GIT at many butcher shops. This finding calls for the policy framework to include pre-service trainings and set educational requirements for butchers such consumable food in public services.

Multidrug resistance has been a common problem among gram-negative bacterial species (Bekele *et al.*, 2014). Antimicrobial resistance may arise due to antimicrobial misuse by humans or overuse in feeding or treatment of animals by farmers. Resistance development also might be related to exchange of resistance factors between related bacteria (Dulo, 2014). In this study, the antimicrobial resistance of *E. coli* O157:H7 isolates to the eight antimicrobials were tested and the antimicrobial of the isolates were graded according to clinical laboratory standards institute's (CLSI, 2020).

In this study 6(85.7%) isolates were susceptible to ciprofloxacin and 4(57.1%) isolates were susceptible to kanamycin. This finding was comparable with reported by (Ashenafi *et al*., 2017). All *E. coli* O157:H7 isolates in the present study exhibited resistance to at least four or more of the eight antimicrobial agents tested. Resistance of amoxicillin, clindamycin, penicillin and vancomycin were the most common resistance profiles identified among our study isolates. This study was in line with the report 51 (100) for penicillin and 51 (100) for vancomycin (Rubab and Oh, 2020). The *E. coli* O157:H7 isolates in this study showed resistance to ampicillin (85.7%). The resistance to the same antibiotics has been reported in studies in Iran for ampicillin (100%) (Shakerian *et al.,* 2016) and in Pakistan for ampicillin (87%) (Shah *et al.*, 2015). The resistance pattern might be related the broad use of ampicillin in management of various infections in Ethiopia (Eguale *et al.,* 2015). The moderate rate of resistance to erythromycin 5(71.4%) obtained in this study also in close agreement with report of 5(83.3%) in the Somali Region of Ethiopian (Dulo *et al.*, 2015).

Low resistance also observed to kanamycin (14.3%) with similarly reported by (Dulo, 2014). No resistance was observed to the newer generation of antimicrobials such as ciprofloxacin which are important in the treatment of human cases of gastroenteritis. The goat and human population in this region have access to antimicrobials, even though often through informal channels with neither diagnosis nor proper recommendations for use. Such misuse is conducive to the rise of antimicrobial resistance; thus, the origin of resistance may have been drugs used to treat human infections.

5.1. Limitation of the study

Limited food items were studied, due to seasonal availability, frequency of local consumption and available on the market during the study period. Due to shortage and lack of availability of antimicrobial discs, only one isolate per types of studied food items consisting of seven (7) were tested.

6. CONCLUSION AND RECOMMENDATIONS

This study showed that higher isolation rate of *E. coli* O157:H7 in goat meat, selected fruit and vegetables destined for human consumption in the studied area with some antimicrobial resistance pattern. In addition, the results showed the risk of this pathogen to consumers due to unhygienic meat, fruit and vegetables processing most commonly practiced in Maya city. This study has also attempted to features about the knowledge and hygienic practices of butcher and greengrocer relating food safety and general hygiene. The results indicated that there were poor personal and general hygiene measures in place and that the butchers and greengrocers not focus on hygienic practice.

Generally, this study confirmed a need for preventative approach to control *E. coli* O157:H7 in goat meat, fruit and vegetables from farm to fork. Based on the findings of the present study, the following recommendations are made:

- Training should be given on hygiene practices and production process of goat meat, fruit and vegetables to all butchers and greengrocers.
- Implementing appropriate hygienic measures, like Good Hygiene Practice (GHP), implementing of HACCP principles and standard operating procedure for slaughter to the greatest extent practicable.
- Maya city administration should be give the suitable market place for greengrocers.
- Veterinarians and agriculture professionals should work together for the possibility of awareness creation to the public about severe health consequences associated with under cooked meat, fruit and vegetables consumption.
- Further studies should have to be conducted on isolation of *E. coli* O157:H7 from other fruit and vegetables items in this study area.

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8. APPENDICES

Appendix 1: Questioners for butcheries and greengrocer workers to assess their knowledge and hygienic Practices at the butcher shop and open market

Date_____

Questionnaire code _____

Respondent Name: _____ Sex _____ Age _____ Educational Status 1) Illiterate 2) 1 up to 6 3) up to 12 4) Certificate 5) Above Certificate

S.no	Question	Response	Skip to
1	Would you use the same balance at selling of all items?	1. Yes [ ] 2. No [ ] If No skip	
2	Are you washing them before sale with water?	1. Yes [ ] 2. No [ ] If No skip	
3	How often you clean your balance?	1. Between selling of the food item 2. Once a day 3. Once a week 4. Not used	
4	Would you disinfect your balance?	1. Yes [ ] 2. No [ ] If No skip	
5	Did you advice your customer to clean or cook the food item before consummation?	1. Yes [ ] 2. No [ ] If No skip	
6	Did you receive any training on hygienic food handling before?	1. Yes [ ] 2. No [ ] If No skip	
7	Are you clean the butcher shop and open market environment?	1. Yes [ ] 2. No [ ] If No skip	
8	Do you think food contamination could bring serious health problem to consumers?	1. Yes [ ] 2. No [ ] If No skip	
9	Do you wear hair cover while working?	1. Yes [ ] 2. No [ ] If No skip	
10	Do you use disinfectants for cleaning of working surfaces and cutting material in order to reduce contamination?	1. Yes [ ] 2. No [ ] If No skip	
11	Do have any awareness about meat contamination?	1. Yes [ ] 2. No [ ] If No skip	

Appendix 2: Researcher observation

S.no	Question	Response	Skip to
1	Degrees of damage to the vegetables and fruit:	1. Intact 2. Few 3. High	
2	Are they place different food items in the same container?	1. Yes [ ] 2. No [ ] If No skip	
3	Is there access water supply and soap for hand washing?	1. Yes [ ] 2. No [ ] If No skip	
4	Up on selling, did you use	1. Glove 2. Bare hand	
5	Are the customers touch the food item by their hand?	1. Yes [ ] 2. No [ ] If No skip	
6	Are they wash and disinfection of their operational tools and floor after each working interval?	1. Yes [ ] 2. No [ ] If No skip	
7	Fingernails short and clean?	1. Yes [ ] 2. No [ ] If No skip	
8	Meat and GIT content kept separately?	1. Yes [ ] 2. No [ ] If No skip	
9	Method of meat dressing	1. Hanging on the floor 2. Mixed	

Appendix 3: Composition and Method of Preparation the Media Used for Laboratory Work

A. Buffered peptone water (Oxoid England; CM 0509)

Composition (g/Litre):

Enzymatic digest of casein	10.0 g,
Sodium chloride	5.0 g,
Disodium hydrogen phosphate, dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)	9.0 g,
Potassium dihydrogen phosphate (KH_2PO_4)	1.5 g
Water	1000 ml.

Preparation: Add 15 gram of the components in the 1000 ml of distilled water, mix well and distribute into universal bottle of suitable capacity to obtain the portions necessary for the test. Sterilize by autoclave set at 121 °C for 15 minutes.

B. MacConkey Agar

Composition (g/Litre)

Pancreatic Digest of Gelatin	17.0
Peptone from Meat	1.5
Peptone from Casein	1.5
Lactose	10.0
Sodium Chloride	5.0
Bile Salts	1.5
Agar	15.0
Neutral Red	0.03
Crystal Violet	0.001
Ph	7.1 ± 0.2 at 25°C

Preparation: Suspend 50 gram in 1 liter of distilled water. Mix thoroughly, boil for 1 minute to dissolve the media completely and sterilize in autoclave at 121 °C for 15 minutes.

C. Nutrient agar (HiMedia Laboratories Pvt.Ltd., India)

Composition (g/Litre):

Lab-Lemco powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0
Agar	15.0
pH:	7.4 ± 0.2

Preparation: Dissolve 28g of the components or the dehydrated complete medium in 1 liter of distilled water, by heating if necessary. Sterilize for 15 min in the autoclave at 121 °C.

D. Eosin Methylene Blue (EMB) (Oxoid, Basingstoke, UK)

Composition g/liter

Peptone	10.0
Lactose	10.0
Di-potassium hydrogen phosphate	2.0
Eosin Y	0.4

Methylene –blue	0.06
Agar	15.0

Preparation: Suspend 37.5 gram in 1 liter of distilled water. Mix thoroughly, boil to dissolve the media completely and sterilize in autoclave at 121 °C for 15 minutes.

E. IMViC tests

1.Indole test

Test principle; the indole test is based on the formation of a red color complex when indole reacts with the aldehyde group of p – dimethylaminobenzaldehyde which is the active chemical in kovac‟s reagent.

Test procedure; 5 ml of the TSB medium was inoculated with the test organism and was incubated at 37 ℃ for 24 hours. Then after 24 hours of incubation, 3 drops (0.2ml) of kovac‟s reagent was added and then the tube was gently shake, kept for a minute and was observed for any red color formation at the surface of the media.

2.Methyl red (MR) test

Test principle;The methyl red test is a quantitative test for acid production, requiring positive organisms to produce strong acids (lactic, acetic and formic) from glucose through the mixed acid fermentation pathway.

Test procedure; A bacterial colony was picked up and inoculated into MR-VP broth medium. It was incubated at 37 ℃ for 48 hours and then 5 drops of Methyl red was added. Finally, the result was observed for the development of a sTable red color in the surface of the medium.

3. Voges -Proskauer (VP) test

Test principle; In this test bacteria can be distinguished on the basis of their production of acetoin (Acetyl-methyl carbinol), a neutral end product, after incubation in buffered pepton glucose media. If acetoin is present, it is oxidized in the presence of air and KOH to diacetyl. Diacetyl then reacts with guanidine components of peptone, in the presence of alpha naphthol to produce red color.

Test procedure; The MR-VP broth was inoculated with a pure culture of the test organism and was incubated at 37 ℃ for 48 hours. Then two reagents, 3 ml of alpha-naphthol followed by 1 ml

of 40% KOH were added. Then the tube was shaked gently and allowed to remain undistributed for 10-15 minutes.

F. Cefiximetellurite Sorbitol MacConkey agar (CT- SMAC)

Complete medium (CT- SMAC Oxoid Ltd., Hampshire, England)

Composition Ingredients	Per mL
Base media	1000 mL
Potassium tellurite solution	2.5 mg/L
Cefixime	0.05 mg/L

Preparation; Suspend 51.5 g of the powder in 1 liter of distilled water. Mix well. Heat to boil for 1-minute shaking frequently until completely dissolved. Sterilize in autoclave at 121 °C for 15 minutes. Then mix and pour about 15 mL amounts in to sterile Petri dish and allow it to solidify.

Appendix 4: Latex Test Kit confirmation for *E. coli* O157:H7

Test Principle

1. Dispense one drop approximately (30µL) of sample diluents on to two separate wells of a clean, dry agglutination slide card with in circle place, one drop contains O157 antibody suspension the other drop contains H7 antibody suspension.

2. Pick several suspected colonies from nutrient agar from the top by using stick which was prepared with latex kit for this purpose.

3. Emulsified the colonies in the drops of sample diluents on the test slide to produce a heavy, smooth suspension. Spread the suspension, over the entire surface of the wells.

4. Rock the slide gently for 30 seconds and observe for auto agglutination or clumping to occur.

5. Discard the used slides and mixing sticks into a suiTable disinfectant.

McFarland: Standard Composition: 1.17% of $BaCl.2H_2O$ solution and 0.36N of 1% sulfuric acid (H_2SO_4).

Preparation: Add approximately 85 mL of 1% H_2SO_4 to a 100 mL of volumetric flask, using a 0.5 mL pipette add 0.5 mL of 1.17% $BaCl.2H_2O$ drop-wise to H_2SO_4 while constantly swirling

the flask. Bring to 100 mL with 1% H_2SO_4. Place a magnetic stirring in the flask and place on the magnetic stirrer approximately three to five minutes. Examine solution visually to make certain it appears homogeneous and free of visible clumps. Dispense three to seven ml, cub tube tightly and seal with paraffin and keep at dark and room temperature.

Mueller-Hinton Agar (OXOID, UK)

Composition Ingredients g/liter

Beef, dehydrated infusion 300

Casein hydrolysate 17.5

Starch 1.5

Agar 17.0

Preparation: Suspend 38 gram in 1000 mL of distilled water. Mix thoroughly, boil to dissolve the medium completely and sterilize by autoclaving at 121 ℃ for 15 minutes.

Appendix 5: Biochemical test result interpretation

Tests	**Positive**	**Negative**
Methyl Red	Red color at the surface	Yellow color
VP	Pink-red color	Yellow
Indole	Red ring on surface	Yellow ring
Citrate	Blue	Green

Key Results: Indole and MR were positive VP and Citrate were negative

Appendix 6: Sample collection and laboratory activities work sheet for laboratory analysis

Data collection					Laboratory analysis				
No	Date	Sample source	Sample type	Sample ID	Pre-enrichment	Enrichment CT-SMAC	Colony Char.	latex test	Antimicrobial Susceptibility Test

Appendix 7: Table of Antimicrobial Susceptibility Test range

S/N	Antimicrobials	Strength	Resistance (mm)	Intermediate (mm)	Susceptible (mm)
1	Ampicillin (AMP)	10 µg	≤ 13	14-16	≥17
2	Amoxicillin (AML)	2 µg	≤ 19	-	≥20
3	Ciprofloxacillin (CIP)	10 µg	≤ 15	16-20	≥21
4	Clindamycin (DA)	10 µg	≤ 15	16-18	≥19
5	Erythromycin (E)	15 µg	≤ 13	14-22	≥23
6	Kanamycin (K)	30 µg	≤ 13	14-17	≥18
7	Penicillin (PG)	10µg	≤ 19	20-27	≥28
8	Vancomycin (VA)	30µg	≤ 9	10-11	≥12

Appendix 8: Pictures taken during the study

1a 

1b 

1c 

1d 


1e


1f

Appendix Figure 1: 1a-1f fruits and vegetables sample collection from open market.

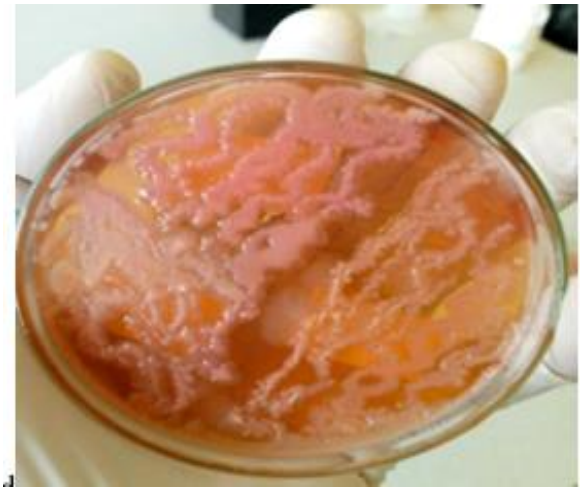

2a


2b


2c


2d

2e 

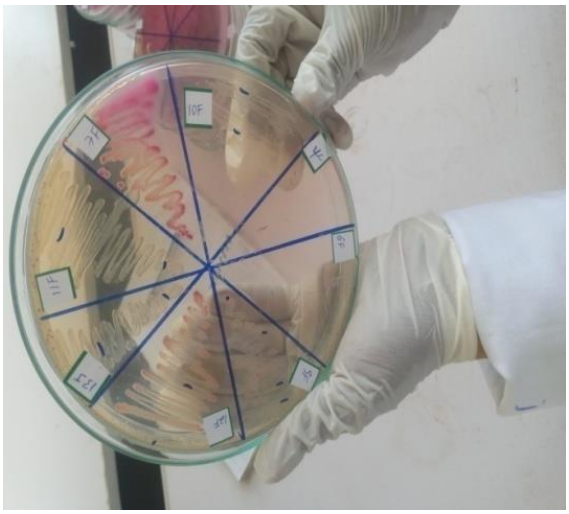
2f 

2g 

2h 

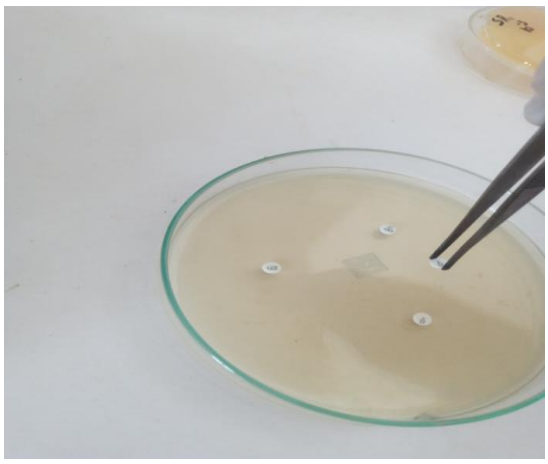
Appendix Figure 2: 2a and 2b, questioner (face to face interview) taking from fruit and vegetables sellers 2c, goat meat swab sample, 2d, pouring media to sterile Petri dish, 2e, heating EMB media. 2f,*E. coli* on MacConkey agar, 2g, *E. coli* on EMB, 2h, *E. coli* colony indole positive result

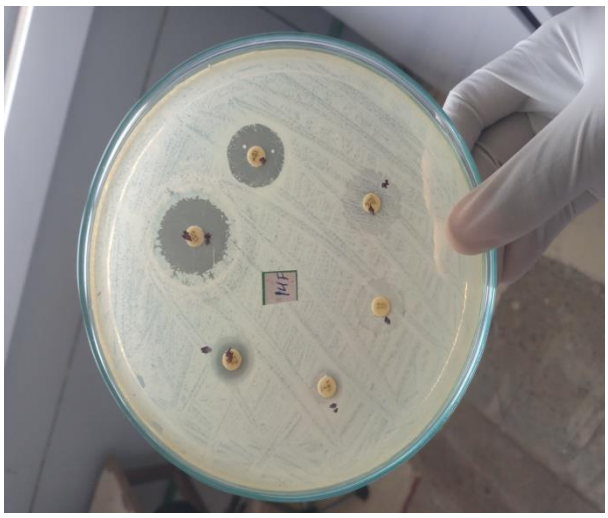
3a 

3b 

3c 

3d 

3e 

3f 

3g

Appendix Figure 3:3a, observation of result, 3b, pure *E. coli* O157:H7 on Sorbitol MacConkey agar, 3c and 3d, latex agglutination test result, 3e, properly placing of antibiotic discs were on the swabbed (MHA) plates 3f, Anti- microbial susceptibility test result and 3g, Measuring zone of inhibition.