Thesis Ref. No:_____________

ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF VETERINARY MICROBIOLOGY, IMMUNOLOGY AND PUBLIC HEALTH

MASTER OF VETERINARY SCIENCE IN VETERINARY PUBLIC HEALTH

MVSc THESIS

BY

SEGNI BEDASA GUDINA

MOLECULAR CHARACTERIZATION AND ANTIBIOGRAM OF *ESCHERICHIA COLI* O157:H7 FROM CATTLE CARCASS IN ABATTOIR AND BUTCHER SHOPS OF BURAYU TWON, OROMIA, ETHIOPIA

JULY 2023

BISHOFTU, ETHIOPIA

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A thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa university in partial fulfillment of the requirements for the degree of Master of Veterinary Science in Veterinary Public Health

BY

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JUNE 2023

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As MVSc research advisors, we certify that we have read and evaluated the thesis prepared under our guidance by **Segni Bedasa Gudina** entitled: "M**olecular characterization and antibiogram of *Escherichia coli* O157:H7 from cattle carcass in abattoir and butcher shops of Burayu town, Oromia, Ethiopia**." We recommend that it be accepted as fulfilling the thesis requirement for the degree of Master of Science in Veterinary Public Health.

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As members of the examining board of the final MVSc open defense, we certify that we have read and evaluated the thesis prepared by **Segni Bedasa Gudina** entitled: "**Molecular characterization and antibiogram of *Escherichia coli* O157:H7 from cattle carcass in abattoir and butcher shops of Burayu town, Oromia, Ethiopia**." And recommend that it be accepted as fulfilling the thesis requirement for the degree of Master of Science in Veterinary Public Health.

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Final approval and acceptance of the thesis dissertation is contingent upon the submission of its final copy to the CGS/FGC through the departmental graduate committee (DGC) of the candidate's major department.

I hereby certify that I have read the revised version of this thesis prepared under my direction and recommend that it be accepted as fulfilling the thesis/dissertation requirement.

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STATEMENT OF AUTHOR

First, I confirmed that this thesis is my novel work and that all sources of materials used for this thesis have been properly acknowledged. The thesis has been submitted in partial fulfillment of the requirements for an MVSc degree on veterinary public health at Addis Ababa University, College of Veterinary Medicine and agriculture and it's been deposited at the university or college library to be made available to borrowers under the rules of the library. I strongly declare that this thesis is not submitted to any other institution for the award of any academic degree, diploma, or certificate.

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Date of Submission: ________________________

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ACKNOWLEDGMENTS

First, my sincere gratitude goes out to my advisor, **Dr. Bedaso Mammo** (Associate Professor), for allowing me the opportunity to collaborate with him and for his continual follow-up, direction, material-providing, encouragement and advice during my MSc thesis work. The completion of this work would have hardly been possible without his support. I will always be appreciative of the idealistic paragon of goodness he showed me as a brother, not only as an academic advisor.

Next, I appreciate the helpful suggestions from my co-advisors, **Dr. Zerihun Assefa** and **Dr. Beksisa Urge**, regarding the work. I value your help and advice with my thesis. I thank you for having significantly contributed to getting the job done.

The workers of the Burayu abattoir and butcher shops, and livestock office in Burayu Town have my deepest gratitude and respect for their willingness to participate in this study. Additionally, I want to express my gratitude to all my friends who supported me in one way or another when I was conducting the research.

Ms. Tesfaynesh Moges and **Ms. Tsedale Teshome** from Addis Ababa University, CVMA and **Mr**. **Melaku Sombo** and **Mr. Kemal Imiyu** from the Animal Health Institute deserve my utmost respect and gratitude because they were essential to the completion of my thesis.

I would like to thank the staff of Holeta Research Center, **Dr. Fikadu Gutama, Dr. Tamirat Siyum, Dr. Temesgen, Mr. Ashebir Abagas, Ms. Elsabet Yahanis** and **Ms. Bayise Abdisa**, for their support of ideas and for providing laboratory equipment during microbiology laboratory work.

Finally, I am greatly thankful to **Addis Ababa University** and **Ambo University (my home university)** for their assistance in completing the MSc program, particularly the research work. Ethiopian Agricultural Research Institute, **Holeta Research Center (HRC)**, and Agriculture Minister, **Animal Health Institute (AHI),** for allowing me to work in their microbiology laboratory.

LIST OF ABBREVIATIONS

A/E	Attaching and Effacing
AMS	Antimicrobial susceptibility
CI	Confidence Interval
CLSI	Clinical Laboratories and Standard Institute
DNA	Deoxy Ribonucleic Acid
eae	Intimin gene
EHEC	Enterohemorrhagic *Escherichia coli*
EMB	Eosin Methyl Blue
EPEC	Enteropathogenic *Escherichia coli*
HUS	Hemolytic Uremic Syndrome
IMViC	Indole Methyl Voges Proskauer Citrate
LEE	Locus of enterocyte effacement
MDR	Multidrug Resistance
MR-VP	Methyl Red Voges Proskauer
NSF	Non-sorbitol fermenting
PCR	Polymerase Chain Reaction
PPE	Personal Protective Equipment
SMA	Sorbitol MacConkey agar
Stx	Shiga Toxin
Tir	Translocated Intimin Receptor
TSB	Tryptone soya broth
UV	Ultra Violet

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ABSTRACT

Escherichia coli O157:H7 is the most important foodborne pathogen in countries like Ethiopia, where raw beef meat consumption is common. However, the occurrence of this pathogen in beef carcasses has not yet been studied in Burayu Town. Therefore, a cross-sectional study design was conducted from October 2022 to June 2023 to generate relevant information on the occurrence and antibiogram of *E. coli* O157:H7 from cattle carcasses as well as assess the hygienic and sanitary practices of meat handling in abattoir and butcher shops in Burayu Town. For this study, a total of 254 samples (121 carcass swabs, 37 knives, 37 personnel hands, 22 cutting boards, 6 water, 6 sewage and 25 cecal contents) were randomly collected from abattoir and butcher shops. *E. coli* O157:H7 isolation and identification were carried out using primary culture and biochemical tests. Conventional PCR was conducted to detect the *eae* gene from positive isolates. The antibiogram of *E. coli* O157:H7 isolates was assessed using the Kirby-Bauer disk diffusion method. Data analysis was carried out using STATA Version 14.0. The overall prevalence of *E. coli and E. coli* O157:H7 was 22.05% (56/254) and 5.5% (14/254) with higher distribution in butcher shops (17.11%-27.65%% and 2.8%-12%) than in abattoir (13.16%-27.67% and 1.8%-10%), respectively. From PCR amplification of *eae* gene in 12 isolates, 490 bp target gene was amplified in 10 isolates. The antibiogram of *E. coli* O157:H7 indicated that 78.6% (11/14) of the isolates had developed resistance against tetracycline, whereas 64.3% (9/14) had developed resistance against both amoxicillin and penicillin. On the other hand, 78.6% (11/14) and 71.4% (10/14) of isolates were susceptible to gentamycin and sulfamethoxazole/trimethoprim, respectively. Resistance to three or more antimicrobials was detected in 78.6% of the isolates, which signifies a public health risk. The results of this study revealed that the circulation of MDR *E. coli* O157:H7 isolates at the beef value chain is a potential public health hazard in Burayu abattoir and butcher shops. Increasing awareness on hygienic practice, strictly following sanitary rules at the abattoir and butcher shops, and antimicrobial stewardship are highly recommended to halt the transmission dynamics of *E. coli* O157:H7 at the human-animal-environment interface.

Keywords: *Abattoir, Antimicrobials, Burayu, Butcher shop, Carcass, eae, E. coli O157:H7*

1. INTRODUCTION

Foodborne infections are the world's leading cause of human illness and mortality (Assefa, 2019), and they are associated with the ingestion of food contaminated by bacteria, viruses, parasites and chemicals. Even though viruses cause half of all foodborne diseases, bacteria are the leading cause of hospitalizations and deaths from foodborne infections (Teplitski, *et al*., 2009). Such risks are exacerbated if animal feces come in contact with food products and the food is mishandled during preparation and processing, where pathogens could multiply exponentially under favorable conditions (Loretz *et al*., 2011). In humans, undercooked consumption of foods of animal origin is a major source of exposure to foodborne pathogens (Li *et al*., 2019).

Pathogenic *Escherichia coli (E. coli* O157:H7*)* is the most common cause of bacterial foodborne diseases (Bélanger *et al*., 2011). *E. coli* O157:H7 is an emerging public health concern in most countries around the world (Kiranmayi and Krishnaiah, 2010; Asfaw and Regassa, 2021). Foodborne *E. coli* O157:H7 is predicted to cause 2.8 million acute infections worldwide each year. According to Scallan *et al*. (2011), the pathogen is responsible for over 60,000 illnesses, 2,000 hospitalizations, and 20 fatalities per year in the United States. This causes a financial burden of $607 million (Scharffner, 2012), which includes $370 million for premature deaths, $30 million for medical care, and $5 million in lost productivity (Frenzen *et al*., 2006; Havelaar *et al*., 2015). Additionally, according to Majowicz *et al*. (2014) meta-analysis in Africa the estimated annual burden of *E. coli* O157:H7 is 2,801,000 acute infections, 3890 cases of hemolytic uremic syndrome and 230 deaths.

Escherichia coli O157:H7 is frequently associated with the consumption of contaminated foodstuffs, particularly meat from infected animals or carcasses contaminated with pathogenic microorganisms (Loretz *et al*., 2011). Meat surfaces are likely to be exposed to invasion by a wide range of microorganisms found in the meat's production and processing environment (Mohammed *et al.,* 2014). Cattle are the primary reservoirs of *E. coli* O157:H7 (Ayenew *et al*., 2021). Carcass contamination occurs because of pathogen transfer from skin to the carcass or from feces to carcass during the slaughter process at processing plants by handlers or processing materials. The removal of the gastrointestinal tract during food animal slaughter is regarded as one of the most significant sources of carcass and organ contamination with bacteria at abattoirs (Abdissa *et al*., 2017).

The most common way for the *E. coli* O157:H7 infection to spread is through the consumption of contaminated food and water, particularly uncooked and contaminated cattle products (Sodha *et al*., 2015). Meat is passed through different contacts starting from slaughterhouse until it is consumed as food, which increase its chance of microbial contamination (Abayneh *et al.*, 2019). Furthermore, due to unsanitary slaughtering practices in abattoirs, illegal slaughtering of animals in open fields, poor meat transport, and display conditions in butcher shops, antimicrobial resistance among enteric bacteria is becoming a growing global public health concern (Sebsibe and Asfaw, 2020).

Furthermore, the indiscriminate use of antibiotics to treat diseased animals and boost livestock development may lead to the emergence of a number of resistant bacterial strains that can be transmitted from animals to people through the food chain in countries like Ethiopia, where the habit of consuming raw and/or undercooked meat is common (Okeke *et al.*, 2005). Given the longstanding tradition of eating raw meat in Ethiopia, it could enhance the likelihood of getting a foodborne illness. When microorganisms like *E. coli* O157:H7 develop antimicrobial resistance, it becomes a major global concern for public health around the world (Hubaílek and Rudolf, 2010).

In addition to common factors such as overcrowding, poverty, inadequate sanitary conditions and poor general hygiene, unhygienic raw meat handling is public health challenging in developing countries like Ethiopia. Raw meat is sold in open air local retail shops without proper temperature control, and it is purchased by households. Despite Ethiopia's tremendous meat production, quality control is either absent or underdeveloped. Historically, most abattoirs have operated without quality control systems (Thomas *et al*., 2015). Furthermore, outbreaks of infections linked to poor hygiene due to animals being slaughtered and dressed under unhygienic conditions and the consumption of contaminated food have been reported in Ethiopia (Dulo *et al*., 2015). Lack of surveillance of foodborne pathogens, lack of awareness among abattoir and butcher shop workers and poor hygiene practices among food handlers are major factors contributing to the high-risk exposure of Ethiopians to foodborne pathogens like *E. coli* O157:H7 (Assefa, 2019).

Studies on *E. coli* O157:H7 conducted from different samples in Ethiopia (Abdissa *et al.*, 2017; Atnafie *et al*., 2017; Bedasa *et al*., 2018; Bekele *et al.*, 2014; Beyi *et al*., 2017; Geresu and Regassa, 2021; Sebsibe and Asfaw, 2020; Tadese *et al*., 2021; Tilahun and Engdawork, 2020) showed the occurrence of the pathogen. Most of these studies were based on primary culture and serological methods, which are not confirmatory methods. So, PCR-based molecular characterization and antibiogram of *E. coli* O157:H7 from cattle carcasses at abattoirs and butcher shops is useful to better understand the circulating pathogen and its transmission dynamics as well as design feasible control strategies. Additionally, there is a lack of information on the status of *E. coli* O157:H7 contamination in cattle carcasses and its antibiogram at abattoirs and butcher shops, as ewell as sanitary and hygienic meat processing practices in Burayu Town, Oromia, Ethiopia.

1.1 General objective

- The overall aims of the study were to generate relevant information on the occurrence and public health risk of *E. coli* O157:H7 from cattle carcasses in abattoir and butcher shops, Burayu, Oromia, Ethiopia.

1.2 Specific objectives

- To isolate and characterize *E. coli* O157:H7 using PCR amplification of specific virulence genes from cattle carcasses, worker hands and environmental samples of abattoir and butcher shops in Burayu Town.
- To determine the antimicrobial susceptibility profile of *E. coli* O157:H7 isolates.
- To assess the hygienic and sanitary practices of meat handling in abattoir and butcher shops in the study area.

2. LITERATURE REVIEW

2.1 Historical background of *Escherichia coli* O157:H7

In 1982, *Escherichia coli O157:H7* was first identified as a human pathogen after two outbreaks in Oregon and Michigan (Riley, 2014; Sewlikar and D'Souza, 2017). Since its first description in 1982, *Escherichia coli* 0157:H7 has emerged as an important global zoonotic food and water-borne pathogen that produces serious illnesses in humans such as hemorrhagic colitis, Hemolytic Uremic Syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP) (Chekabab *et al*., 2013; Pal and Mahendra, 2016). In more recent years, *E. coli* O157:H7 has caused major human illness outbreaks worldwide with considerable morbidity and mortality (Constable *et al*., 2017).

2.2 Classification and taxonomy of *Escherichia coli* O157:H7

The infection is caused by *Escherichia coli,* a gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium in the phylum proteobacteria, class gamma proteobacteria, order Enterobacteriales, family Enterobacteriaceae, and genus *Escherichia* (Farrokh *et al*., 2012; Xia *et al*., 2010). The family Enterobacteriaceae can be divided into those with uncertain significance for animals, pathogens of animals such as Salmonella species, *Escherichia coli* species and three of the Yersinia species and opportunistic pathogens that are known occasionally to cause infections in animals based on their pathogenicity for animals (Garrity *et al*., 2004).

Escherichia coli is a common bacterium of the intestinal microflora and a significant pathogen in both animals and humans (Tayh *et al*., 2016). The pathogenic strains that cause enteric disease are grouped into six categories: enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAggEC), and diffuse-adherent *E. coli* (DAEC). The most important category of zoonoses is enterohemorrhagic *E. coli*, which is also the most severe, whereas the last two categories are not yet well defined (Hashish *et al.*, 2016).

These categories differ in their pathogenesis and virulence properties, and each comprises a distinct group of O:H serotypes on their cell membrane (Figure 1**)** (Riemann and Cliver, 2006). Serotyping based on the O and H antigens are considered the 'gold-standard' as only limited laboratories can type the K-group (DebRoy *et al*., 2011). *E. coli* O157:H7 is the most predominant and virulent serotype in a pathogenic subset of EHEC. *E. coli* O157:H7 is so named because it expresses the 157th O antigen identified and the 7th H antigen (Chapman *et al.,* 2001).

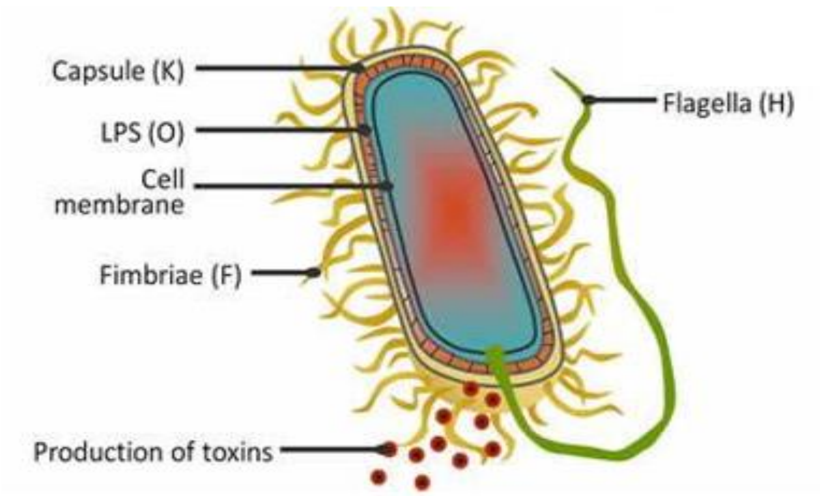

Figure 1: Schematic presentation of *Escherichia coli* surface antigens
Source: (*Escherichia coli* Reference Laboratory, 2004)

2.3 Virulence factors and mechanisms of *E. coli* O157:H7 pathogenicity

A number of requirements must be met for an *E. coli* O157:H7 infection to occur, one of which is a complex interaction between the components of the host and the bacteria. This pathogen has proven its ability to persist in difficult environments. *E. coli* O157:H7 poses a threat to humans' environment and can infect them through the consumption of food that has been contaminated (Chekabab *et al*., 2013). Stx, which has two variants, stx1 and stx2, is one of the key virulence factors for *E. coli* O157:H7 and is encoded with stx1 and stx2 genes (Lv *et al*., 2010). Disease cannot occur only by the production of toxins by Stx. Two additional elements contribute to the pathogenicity of *E. coli* O157:H7. The first factors harbor a 60 megadalton virulence factor, pO157, which encodes hemolysin. The other factor is the locus of enterocyte effacement (LEE), which encodes intimin (*eae*) (Ogierman *et al*., 2000; Perera *et al*., 2015).

Stx is made in the colon and then circulates through the blood to the kidney, where it causes renal inflammation and causes renal endothelial cells to die. According to Kaper *et al.* (2004), Stx of EHEC cleaves ribosomal RNA, interrupting protein synthesis and killing the intoxicated epithelial or endothelial cells. It also obstructs the microvasculature by inducing the production of cytokines and chemokines locally (Obrig and Diana, 2012). According to Kaper *et al*. (2004) and Melton-Celsa (2014), this damage can result in HUS, which is characterized by hemolytic anemia, thrombocytopenia, and possibly deadly acute renal failure.

It is believed that the development of an A/E lesion causes the intestinal mucosa's absorptive capacity to decrease, which consequently results in a disruption of the electrolyte balance and, ultimately, diarrhea. According to Clarke *et al*. (2003), a variety of physical and environmental factors influence the development of A/E lesions. The LEE, a well-known pathogenicity island presents in EPEC, EHEC, and other A/E. *E. coli* that are pathogenic in human and animal species, is the genetic component responsible for the lesions. Intimin, which facilitates close attachment to the host cell, and Tir (Translocated Intimin Receptor) are encoded in this LEE's essential region. Tir-intimin interaction mediates tight binding of EPEC and EHEC to the intestinal epithelia, resulting in the formation of effacing lesions on intestinal epithelia (Yoon and Hovde, 2008). Initial profiling of the plasmids presents in *E. coli* O157:H7 demonstrated the presence of multiple plasmids and the high prevalence of the pO157.

Ingested bacteria must survive in the acidic environment of the stomach and then compete with other gut microflora to establish intestinal colonization. Once colonization has occurred, the bacteria produce Stxs in the intestinal lumen, which must be absorbed by the intestinal epithelium and must move to the blood stream. A three-stage model for EPEC and EHEC has been proposed, including (i) initial adherence, (ii) signal transduction, and (iii) intimate adherence (Yoon and Hovde, 2008). The genes involved in intimate adherence are *eae* and *Tir* (Paton and Paton, 1998).

2.4 Epidemiology of *E. coli* O157:H7

2.4.1 Geographical distribution

Pathogenic *E. coli* is responsible for numerous outbreaks of gastrointestinal diseases around the world (Parsons *et al*., 2016). It has grown in importance in North America, Europe, South Africa, Japan, South America, and Australia since its original conception in 1982. *E. coli* O157:H7 is the serotype most associated with clinical disease in people, particularly in North America, Japan, and the United Kingdom. High rates are found in South American countries, particularly Argentina, where HUS is endemic (Constable *et al*., 2017). The meta-analysis study on the global prevalence of *E. coli* O157:H7 estimated that the agent's prevalence in cattle was 5.68%, with the prevalence of world regions revealed in Table 1 (Islam *et al*., 2014).

Table 1: Estimated pooled prevalence of *E. coli* O157:H7 from cattle in the world

World region	**No. of study**	**No. of cattle sampled**	**No. of positive sample**	**Pooled estimate (%)**
Global estimate	140	220,427	12,683	5.68
Africa	4	626	118	31.20
Asia	22	14,916	937	4.69
Europe	53	88,643	5,425	5.15
Latin America and Caribbean	11	4,313	73	1.65
Northern America	46	110,641	6,059	7.35
Oceania	4	1,288	71	6.85

Source: (Islam *et al*., 2014)

2.4.2 Reservoirs of Escherichia coli

Most *Escherichia coli* from the *Enterobacteriaceae* family are normal commensals found in both human and animal intestinal tracts, while others are pathogenic to humans (Kaper *et al.* 2004). Ruminants have been identified as the primary reservoir of *E. coli,* with cattle being the primary source of human infections (Abreham *et al*., 2019). The presence of *E. coli* and other *Enterobacteriaceae* in food, such as *Enterococcus faecalis,* indicates fecal contamination (Gelsomino *et al*., 2002) and suggests poor hygiene during food preparation, handling, and storage, as well as a lack of reheating and an incorrect heating temperature of food.

Recently, in the 2016 outbreak of *E. coli* O157:H7, slaughtered cattle animals were the primary sources of infection, resulting in the illness of eleven people (CDC, 2016). Sheep, goats, and deer are among the other ruminants known to harbor these bacteria. Shiga toxin *Escherichia coli* bacteria are occasionally isolated from other animals, but it is believed that the bacteria are present as transients and that the animals including humans acquired these bacteria from meat, foods, or water contaminated by fecal material from ruminants (Caprioli *et al*., 2005).

2.4.3 Occurrence of E. coli O157:H7 in carcass

During the slaughter process at processing factories, butchery hands and utensils can transfer *E. coli* from the skin to the carcass or from the feces to the carcass (Abdissa *et al.*, 2017; Mersha *et al.*, 2010). One of the primary methods used in abattoirs to contaminate organs and carcasses with bacteria is the removal of the gastrointestinal system during the slaughter of food animals (Atsbha *et al.*, 2018). Additionally, lymph nodes that remained at the carcass act as sources of carcass contamination in addition to being gastrointestinal contamination sources by acting as habitats for *E. coli* O157:H7 persistence. The sources of *E. coli* O157:H7 infections in humans are frequently believed to be butcher shops and restaurants (Pennington, 2010). Because meat is exposed to numerous contacts from the slaughterhouse until it is served as food on the table, butcher shops have the highest prevalence of bacterial contamination (Abayneh *et al.*, 2019).

2.4.4 Escherichia coli O157:H7 transmission and clinical signs

Most of foodborne outbreaks including *E. coli* O157:H7 have been linked to cattle-derived foods, particularly ground beef. In the 1980s, most outbreaks were linked to undercooked hamburgers and unpasteurized milk (Doyle, *et al*., 2006). Additionally, *E. coli* O157:H7 can survive in water and the fact that it can spread to people through contaminated food makes it a risk for humans (Chekabab *et al*., 2013). In both outbreaks and sporadic cases, it has been the most commonly bacterial isolated serotype in association with asymptomatic fecal shedding of the organism, abdominal cramps, bloody diarrhea, thrombotic thrombocytopenic purpura, hemorrhagic colitis, and hemolytic uremic syndrome (Karmali *et al.*, 2010). With a few exceptions, such as diarrhea in calves, Shiga toxigenic *E. coli* (STEC) bacteria do not typically cause illness in animals (Kang *et al*., 2004). The incubation period can range from 1 to 10 days and fewer than 40 cells of *E. coli* O157:H7 can cause illness in some people (Strachan *et al*., 2005).

2.4.5 Detection of Escherichia coli O157: H7

There is no single technique that can be used to detect *E. coli* O157:H7. Clinical cases can be diagnosed by finding the organisms in fecal samples. Food and environmental samples may also be tested to determine the source of the infection. Many diagnostic laboratories can identify *E. coli* O157:H7. Infection with this agent is associated with a broad spectrum of illness ranging from mild diarrhea and hemorrhagic colitis to the potentially fatal HUS. These clinical symptoms are used as one diagnostic technique (Rahal *et al*., 2012; Elhadidy *et al*., 2015).

Isolation by primary culture on selective media like MacConkey agar and EMB and biochemical tests were used to identify *E. coli*. In order to identify *E. coli* O157:H7, Sorbitol MacConkey (SMAC) agar supplemented with MUG has been employed. Cefixime, potassium tellurite, and vancomycin have been added to SMAC agar plates to inhibit other gram-negative bacteria and improve the selectivity for *E. coli* O157:H7. A commercially available latex agglutination assay can be used to further confirm the serotypes O157 and H7 (Lim *et al.*, 2013; Dulo *et al.*, 2015; Shecho *et al*., 2017).

Immunoassays and polymerase chain reaction technology have led to more rapid and reliable but expensive detection of *E. coli* O157:H7 in stools, food, and water. Techniques included in this category are PCR and DNA-based techniques, immunomagnetic separation, and enzyme-linked immunosorbent assays (Bavaro, 2009). The most trustworthy approaches for distinguishing pathogenic strains from non-pathogenic members continue to be molecular genetic methods. It can be used to identify pathogenic *E. coli* after phenotypic identification of *E. coli* isolates using species-specific PCR and DNA hybridization (Stenutz *et al*., 2006). Targeting several virulence genes that encode for various virulence factors allows PCR to be used to characterize different *E. coli* strains (Casey and Bosworth, 2009).

2.4.6 Status of Escherichia coli O157:H7 in Ethiopia

Despite the fact that raw meat and its byproducts are frequently consumed in traditional Ethiopian diets, *E. coli* O157:H7 is not as frequently investigated as it is in other nations (Bekele *et al*., 2014). Additionally, the majority of studies that looked at the occurrence of *E. coli* O157:H7 used non-confirmatory techniques like sorbitol MacConkey agar and latex agglutination assays. However, some studies were done to determine the occurrence and proportion of *E. coli* O157:H7 in the beef meat chain using different methods of detection from different sample types in different study areas (Table 2).

Table 2: Research findings on the prevalence of *E. coli* O175: H7 from beef in Ethiopia**.**

Study area	Sample unit	Sample type	Preva-lence	Detecting technique	References
Jimma	Cattle	Carcass swab Cecal content	9.3% 7.3%	SMA and LAT	Feleke *et al*., 2017
Addis Ababa Debre Berhan	Cattle Butcher shop	Fecal	2%	SMA and LAT	Abdissa *et al*., 2017
		Skin swab	0.5%		
		Intestinal mucosal swabs	0.8%		
		carcass swabs	0.5%		
Dire Dawa	Cattle	Raw meat	2.06%	SMA and LAT	Edget *et al*., 2017
Hawassa	Cattle	Carcass & environmental swabs	2.4%	SMA and Omni log	Atnafie *et al*., 2017
Debre Zeit Addis Ababa		Carcass Swab	5.5%	SMA and LAT	Tassew, 2015
Arsi		Food of animal origin	2.1%	SMA and LAT	Asfaw & Regassa, (2021).
Addis Ababa	Cattle	Beef	10.2%	SMA and Omnilog	Bekele *et al*., 2014
Addis Ababa	Cattle	Raw Beef sample	3.64%	SMA and LAT	Haile *et al*, 2022
Haramaya	Cattle	Carcass swab	2.65%	SMA and LAT	Taye *et al*., 2013
Modjo	Cattle	Raw meat	4.2%	SMA and LAT	Hiko *et al*., 2008
Ambo	Abattoir		7.2%	SMA and LAT	Tadese, *et al.*, 2021.
	Retailers		19.4%		
Central Ethiopia	Abattoir	Carcass swab	4.5%	SMA and LAT	Beyi *et al.*, 2017
	Butcher	Board swabs	3.6%		
		Minced beef	0%		
Bishoftu	Restaurant	Beef (carcass)	13.84%	SMA and LAT	Bedasa, *et al*., 2018

SMA=Sorbitol MacConkey agar, LAT= Latex Agglutination Test

2.4.7 Treatment, prevention and control

Antimicrobial treatment of *E. coli* O157:H7 infection is associated with an increased risk of severe health consequences, such as HUS (Rahal *et al*., 2012) and may aggravate the patient's condition by increasing the release of preformed Shiga toxins (Stx) upon cell lysis. Certain management practices, such as avoiding antibiotics during the pre-hemolytic uremic syndrome phase and hospitalization (Davis *et al*., 2013), and patients with complications who may require intensive care such as dialysis, transfusion, and/or platelet infusion in addition to kidney transplant, improve the likelihood of good outcomes (CFSPH, 2009). However, early administration using some antimicrobials is effective in the course of infection (Nassar *et al*., 2013).

Management interventions, infection control and immunization techniques are among the most effective means of preventing and controlling *E. coli* infections (Kabir, 2010). Adequate sanitation and food processing are critical; cook steaks thoroughly at a temperature of at least 160 °F/70°C and avoid raw meat, milk, and unpasteurized dairy products (Mathusa *et al*., 2010). Keeping cattle away from water sources, properly disposing of infected faces, and practicing good kitchen hygiene may all help to reduce the occurrence of *E. coli* O157:H7 human infection. People who have been exposed to Shiga toxigenic *E. coli* should be isolated to contain the outbreak (Dagne *et al.,* 2019). Testing and withholding contaminated material before releasing it to the market is one method of preventing human infection and illness (CFSPH, 2009).

2.5 Public health and economic significance of *E. coli* O157: H7

According to Tadesse *et al.* (2012); Minda and Shimelis (2021), *E. coli* O157:H7 are an emerging and significant zoonotic foodborne pathogen that could cause sporadic cases or serious outbreaks around the globe. Antimicrobial resistance (AMR), particularly of *E. coli* O157:H7, is another of the most serious global public health threats in this century (Murray *et al.,* 2022). So, the presence of antibiotic-resistant bacteria in food can also pose a serious threat to public health. Antibiotic resistance determinants can spread to other pathogenic *E. coli*, thereby jeopardizing the treatment of serious bacterial infections (Ababu *et al.,* 2020).

According to Muniesa *et al*. (2006), enterohemorrhagic *E. coli* O157:H7 and other serotypes are zoonotic pathogens that are connected to serious diseases in humans. Following its identification as the cause of human disease, *E. coli* O157:H7 has been linked to multiple outbreaks and isolated cases of illness in Argentina, Australia, Belgium, Canada, China, and many other nations (Bach *et al*., 2002). Adugna *et al.* (2015) reported a hospital-based cross-sectional study at Bahirdar that found 28.90% of *E. coli* serotype O157:H7 isolates. Another study looked at the prevalence of *E. coli* O157:H7 among food handlers in food-handling facilities in southern Ethiopia, and the prevalence was reported to be 6.03% (Wada *et al*., 2017). According to Getaneh *et al*. (2021), Children from families with livestock were four times more likely than children from households without livestock to get *E. coli* O157:H7.

Costs are significant due to the severity and long-term effects of infections with *E. coli* O157:H7 and other verocytotoxin-producing *E. coli.* Abe *et al*. (2002) assessed the direct and indirect economic costs of the 1996 *E. coli* O157:H7 outbreak in Japan that was linked to the lunches at primary schools. The outbreak had an estimated economic cost of 82,686,000 yen. The laboratory charges represented the largest portion of the outbreak's overall cost (approximately 26%). According to Frenzen *et al.* (2006), the annual cost of disease caused by O157 STEC in the United States in 2003 was $405 million. This figure included $370 million for premature deaths, $30 million for medical care, and $5 million for lost productivity.

In addition to the direct costs to human health, the threat posed by the *E. coli* O157:H7 infection is shared by cattle and dairy farmers, meat packers and dairy processors, meat and milk distributors, etc. The cost of *E. coli* O157:H7 to the food industry in the United States alone is estimated to be in the billions of dollars as a result of recalls, destroyed food, control measures, and lost demand due to a loss of customer trust (Frenzen *et al*., 2006; Roberts *et al*., 2000). According to Asfaw and Regassa (2021), *E. coli* O157:H7 is a growing public health risk in the majority of countries due to the development of an antibiotic resistance gene that makes the infection challenging to cure.

3. METHODOLOGY

3.1 Study area description

The study was carried out in Burayu's abattoir and butcher shops. Burayu is a town in the special zone of the Oromia Region, Ethiopia (Figure 2). Burayu town has six kebeles (Burayu Gefersa, Burayu Keta, Gefersa Nono, Leku Keta, Gefersa Guje, and Melka Gefersa). Burayu Town's abattoir is found in Burayu Gefersa and is owned by a Private limited company. During the study period, there were 126 legally registered butcher shops and beef carcasses were supplied to these butcher shops from the Burayu abattoir.

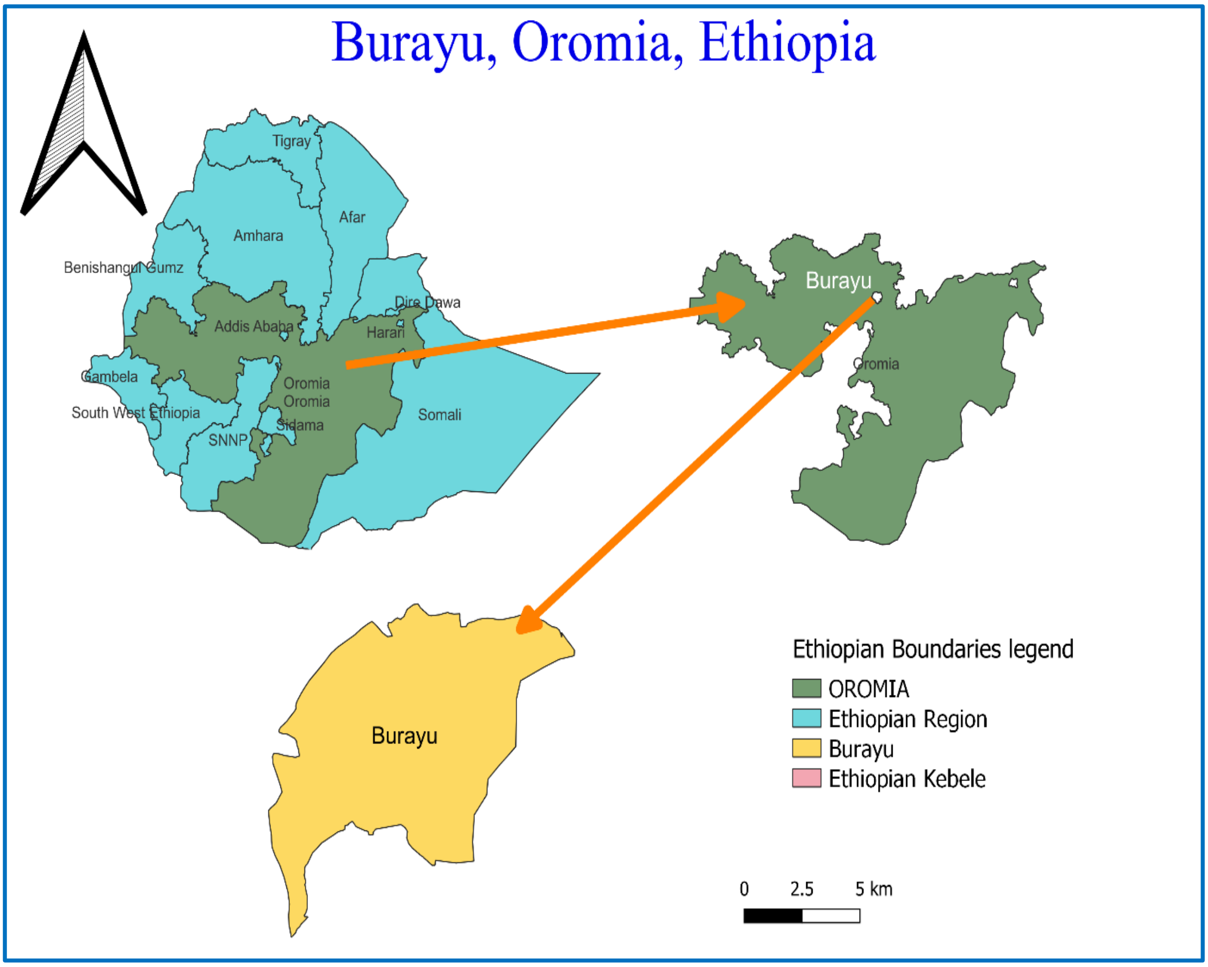

Figure 2: Map of Ethiopia and Oromia region depicting the location of the study area

3.2 Study design

A cross-sectional study was conducted from October 2022 to June 2023. Isolation, characterization and antibiogram of *E. coli* O157:H7 were performed following a standard operating procedure recommended by Quinn *et al.* (2002) from swab samples collected from carcass, worker hands and environmental samples of abattoir and butcher shops. An antimicrobial susceptibility profile was also carried out for each isolate using eight different antimicrobial drugs which were selected based on their availability and use for treatment. A structured and pretested questionnaire, and personal observations were used to collect information on operational facilities and hygienic handling practices, as well as meat handlers' hygienic practices at abattoir and butcher shops.

3.3 Samples source

The carcasses of cattle slaughtered at the Burayu abattoir destined for local consumption, and slaughterhouse and butcher shop workers were parts of the study**.** Water from borehole source used to wash the carcasses in the abattoir was sampled directly from the tap. Swabs from carcasses, slaughterhouse workers hand, butcher shop workers hand and environmental swabs (knife, cutting board, water and sewage) and cecal contents were collected as study samples.

3.4 Questionnaire

A pretested semi-structured questionnaire and observational checklist, prepared in English, were developed based on information gathered from relevant literature, and national and international guidelines to obtain hygienic status and practices that the abattoir and butcher shops workers were practicing to assess the operational and hygienic practices of participants in the study area. A total of 110 respondents in the study area were interviewed (55 abattoir workers and 55 butcher workers). This sample size was calculated based on the central limit theorem (n = 0.25/SE2). Where SE is the standard error, which is 0.05 with a 95% CI according to Arsham (2002). Personal observations were also used based on Observational check lists. All questions were answered as indicated in Appendix 9 (B and C)**.**

4.5 Sample size determination

The number of samples was determined based on the expected prevalence of *E. coli O157:*H7 and the desired absolute precision stated in Thrusfield, (2005). An expected prevalence of 9.1% of *E. coli* O157:H7 in Ambo town abattoir and butcher shops reported by Tadese *et al. (*2021) was used to calculate the sample size using the following formula.

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

Where: n=required sample size

P exp =Expected prevalence

d = desired absolute precision

So, using a 95% confidence interval and 5% absolute precision, the sample size was 127. A total of 254 samples were collected from the Burayu abattoir (127) and butcher shops (127).

3.6 Materials, equipment, chemicals and media used

Ice box, test tubes, wooden cotton swabs, labeling paper, biosafety cabinet, Petri dish, autoclave, refrigerator, incubator, transparent ruler, wire loop, Bunsen burner, aluminum foil, plastic bag, gloves, marker, gram staining reagents (Crystal violet, lugos' iodine, alcohol and safranin), slides, oil immersion, peptone water, 3% H2O2, Kovac's reagent, 40% KOH, Methyl red reagent, 5% alpha nepthanol, MRVP broth, TSB , citrate agar, MacConkey agar, Eosin methyl blue agar (EMB), SMA, Brain Heart Infusion broth, Muller Hinton agar, antibiotic disks, pipette, Eppendorf (mixmate), Eppendorf centrifuge, digital dry bath, Eppendorf tube**,** PCR machine (FlexCycler2), PCR components (*EAE* primer(R/F), IQ super mix, Rnase free water, DNA templates), UV cabinet for PCR operations), 2% agarose gel**,** a gel stain, UV for the band reader were all used. All medias were prepared according to manufactures guidelines (Appendix 5).

3.7 Sampling techniques

Butcher shops found at three kebeles (Burayu Keta, Gefersa Burayu, and Guje Gafarsa) were selected based on the number of butcher houses distributed to collect carcass swabs and environmental samples because most butcher shops legally registered were found in these three listed kebeles. About 50–150 cattle were slaughtered at the abattoir found in Gefersa Burayu Kebele based on market demand and customer requests five days a week except for Tuesday and Thursday. Simple random sampling technique was used to collect swab samples from butcher shops carcass and a systematic random sampling method was used for abattoir carcass swab sample. whereas workers hands and environmental samples were purposively selected to determine the occurrence of *E coli* O157:H7. sample collection, a support letter was sent to the study area and concerned bodies. Consent was obtained from carcass owners, butcher shops, and working personnel to collect samples and be interviewed for the questionnaire (Appendix 9 A). For two months, the abattoir was visited once per week on Wednesday, and butcher shops were visited once per week on Thursday.

3.8 Sample collection procedures and processing

3.8.1 Sample collection and transportation

Carcasses at the abattoir and butcher shops were swabbed from the shank, rump, midline and brisket area (Appendix 1) according to the methods described by McEvoy *et al*., (2003). All samples except cecal contents, water and sewages were swabbed using a sterile cotton swab with 10 ml of buffered peptone water, first horizontally and then vertically. Twenty-five gm of cecal content was collected and transferred into a sterile stomacher bag (Seward, England), containing 225 ml of TSB (Himedia, India) and homogenized using a homogenizer (Stomacher 400, Seward Medical, England) at 260 rpm for 2 minutes. The resulting homogenate was incubated at 37°C for 24 hours (Ethelberg *et al*., 2009). Ten ml of sewages and water samples were collected and enriched in 90 mL of modified TSB (HiMedia, India) and incubated at 37°C for 24 hours. All the samples were identified by sample number, date of sampling, source of sample and sample type. And then transported to the microbiology laboratory of the Holeta Agricultural Research Center using an icebox and kept chilled until microbiological analysis was done within 24 hours.

3.8.2 Isolation and identification of Escherichia coli O157:H7

Isolation and identification of *E. coli* were performed using techniques recommended by Quinn *et al.,* (2002); Tozzoli *et al*., (2019). A loopful of all samples from abattoir (carcass swab, workers hands and environmental swab samples (knife, sewage and water)) and butcher shops (carcass swab, workers hand and environmental swab samples (knife, cutting board)) were inoculated on MacConkey agar (Appendix 2A) for primary isolation of *E. coli* (Difco Laboratories, USA) and incubated aerobically at 37 °C for 24 h. The plates were observed for the growth of *E. coli* (pink colony; lactose fermenter). A single, isolated colony (pinkish, lactose fermenter) was picked and sub-cultured on EMB for the formation of a metallic sheen (characteristic of *E. coli*) (Appendix 2B). Simultaneously, another single colony with similar characteristics was picked and stained with Gram stain (Appendix 6). The isolate was examined for staining and morphological characteristics using bright-field microscopy. A suspected colony of *E. coli* (pinkish color appearance on MacConkey agar and metallic sheen on EMB) was then inoculated on nutrient agar (OXOID) (non-selective media) (Appendix 2E) for further tests.

3.8.3 Biochemical identification and serotyping of Escherichia coli O157:H7

A colony from nutrient agar was taken and biochemical tests were performed to confirm the *E. coli using the* catalase test, Indole Production test, Methyl Red test, Voges Proskauer test and Simon's Citrate test on tryptone soya broth, MR-VP medium and Simon citrate agar, respectively (Appendix 7) (ISO, 2003).Then the bacterium that was confirmed as *E. coli* (Catalase test positive, Indole production test positive, Methyl red positive, Voges Proskauer test negative and Simmons citrate test negative) (Appendix 3) was sub cultured onto SMA (Himedia, India) from nutrient agar (OXOID). SMA (Himedia, India) plates were incubated at 35 °C for 20 to 22 h. *E. coli* O157:H7 does not ferment sorbitol and, therefore, produces colorless colonies (Appendix 2C). In contrast, most other *E. coli* strains ferment sorbitol and form pink colonies (Soomro *et al*., 2002). All non-sorbitol fermenting colonies from the SMA were sub cultured on Brain Heart Infusion media and subjected to PCR for confirmation of a specific gene.

3.8.4 Molecular detection of Escherichia coli O157:H7

To determine whether *E. coli* O157:H7 colonies contained the virulence gene *eae*, a conventional PCR assay was carried out in accordance with the procedures previously published by Hasan *et al.* (2016). As previously described by Wasilenko *et al*. (2012); Firoozeh *et al*. (2014), the standard boiling method of DNA extraction was used to first extract DNA from the *E. coli* O157:H7 isolates. Thus, suspected fresh colonies were selected and suspended separately in 100 µl of sterile distilled water in Eppendorf tubes; the suspensions were then boiled in a water bath at 92.5 °C for 17 minutes. After centrifuging at 13000 rpm for 10 minutes, the supernatant containing the template DNA was transferred into nuclease-free Eppendorf tubes. The extracted DNA was used as a template for conventional PCR to amplify the virulence genes using a specific *eae* primer.

Amplification of DNA was conducted using initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and 30 seconds, extension at 72 °C for 1 min, and final extension at 72 °C for 7 min, and finally putting at 4 °C until the machine turned off. Visualization of the amplified product was done by mixing the 4µL gel red with loading dye, 10µL PCR products, and 10µL markers (ladder) onto a 1.5% agarose el. Electrophoresis was conducted at 125 V for 1:20 hours. A 100-1000 base pair molecular weight marker was used to identify the amplified products as a ladder, which was visualized by UV illumination (Appendix 4). The sequences of the primers and amounts of reagents required for one reaction master mix preparation are shown in Table 3 below.

Table 3: Primer’s sequence used in conventional PCR for amplification of *eae* genes

Types of reagents	**Volume**
RNase free water	3µL
Primer **EAE1**-forward-5pm/µl (5’-AAACAGGTGAAACTGTTGCC-3’)	2µL
Primer **EAE2**-reverse-5pm/µl (5’-CTCTGCAGATTAACCTCTGC-3’)	2µL
IQ super mix	10µL
DNA template	3µL
Total volume	**20µL**

EAE (intimin)

3.9 Antimicrobial susceptibility profile of *Escherichia coli* O157:H7

The antimicrobial susceptibility profile of the *E. coli* O157:H7(non-fermenting sorbitol) isolates was determined by the disk diffusion method (Kirby-Bauer) according to CLSI M100-ED32 (CLSI, 2022) using commercially available antimicrobials and frequently used for treatment. For the susceptibility testing, the following eight antimicrobial drugs were used: Gentamycin (GN, 10 μg), Kanamycin (K30, 30 μg) and Amikacin (AK30, 30 μg) from the aminoglycosides group; Amoxicillin (AML2, 2 μg) and Penicillin (P10, 10 unit) from the beta-lactams group; Tetracycline (TE, 30 μg) from tetracyclines group; Erythromycin (E15, 15 μg) from the macrolides group and Sulfamethoxazole-Trimethoprim (SXT25, 25 μg) from the sulfonamides group.

Each isolated bacterial colony from pure fresh culture was transferred into a test tube of 5 ml TSB (OXOID, England) and incubated at 37 °C for 6 h. The test broth was adjusted to McFarland 0.5 turbidity to obtain the desired bacterial population. Mueller-Hinton agar (Bacton Dickinson and Company, Cockeysville, MD, USA) plates were prepared according to the manufacturer's guidelines. A sterile cotton swab was immersed in the inoculum suspension, rotated against the side of the tube to remove the excess fluid and then swabbed in three directions uniformly on the surface of Mueller-Hinton agar plates. After the plates dried, antibiotic disks were placed on the inoculated plates using sterile forceps. The antibiotic disks were gently pressed onto the agar to ensure firm contact with the agar surface and incubated at 37 °C for 24 h. Following this, the diameter of the inhibition zone formed around each disk was measured using a black surface, reflected light, and a transparent ruler by lying it over the plates (Appendix 2D). The results were classified as sensitive, intermediate, or resistant according to the standardized table (Appendix 8) supplied by CLSI, (2022). According to Selim *et al.* (2013), the multidrug resistance of an individual isolate was calculated by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics to which the isolate was exposed.

3.10 Inclusion and exclusion criteria

3.10.1 Inclusion criteria

Cattle slaughtered at the Burayu abattoir and carcasses at butcher shops outlet as well as workers who were interested in taking in the study, were included.

3.10.2 Exclusion criteria

Carcasses other than beef carcasses, abattoir and butcher shop workers whose were unwilling to participate in the investigation based on the questionnaire as well as persons who refused to provide hand swab samples were excluded.

3.11 Quality control

Confidence in the quality of test results was increased by strengthened quality control processes. First, sample collection materials were sterilized in a hot air oven to sterilize them. The sterility of the culture medium was checked by incubating the prepared media for 24 hours at 37°C and then checking for the growth of any contaminants. Media dispenses, culture and sub culture, and biochemical tests were carried out in biosafety cabinet to reduce contaminations. Additionally, the entire process and interpretation of the findings were carried out in accordance with standard operating procedures. All PCR analysis procedures were carried out in accordance with protocol, and the accuracy of the positive and negative controls were used to determine the test's validity. The questionnaire and observational lists were checked for completeness by advisors.

3.12 Data management and statistical analysis

The questionnaire and laboratory data were entered into a Microsoft Excel spreadsheet. The data was analyzed using STATA 14.0 software. Using descriptive statistics, the prevalence of *E. coli and E. coli* O157:H7 and AMS profiles in all samples were determined. The frequency of sanitation and hygiene habits among the respondents was also identified from the questionnaire data. The Fisher exact test and chi-square test were used to determine whether the prevalence of *E. coli and E. coli* O157:H7 varied among various sample types and between abattoir and butcher shops. In all cases, the confidence level was held at 95% and a p-value < 0.05 was considered indicative of statistical significance. To map the location of the study area, QGIS version 3.30 GIS software was used. The percentages of antimicrobials of each pattern (Susceptible, Intermediate and Resistance) were calculated and presented in percentages.

3.13 Ethical clearance

A request for approval was submitted was submitted to the Addis Ababa University College of Veterinary Medicine and Agriculture's Animal Research Ethics and Review Committee, explaining the purpose of the study. The research was carried out after receiving approval from Animal Research Ethics and Review Committee with ref. no: VM/ERC/10/02/15/2023 (Appendix 10).

4. RESULTS

4.1 Prevalence of *E. coli* and *E. coli* O157:H7 in abattoir and butcher shops of Burayu

4.1.1 Prevalence of E. coli in abattoir and Butcher shop of Burayu

Of the total 254 samples examined, 56 were positive for *E. coli* using selective culture. Out of 56 total *E. coli* positives, 19.68% (25/127) (95% CI: 13.16–27.67%) and 24.4% (31/127) (95% CI: 17.11%–27.65%) were isolated from samples collected from abattoir and butcher shops, respectively. There was no statistically significant difference in the occurrence of *E. coli* between abattoirs and butcher shops (p>0.05) (Figure 3).

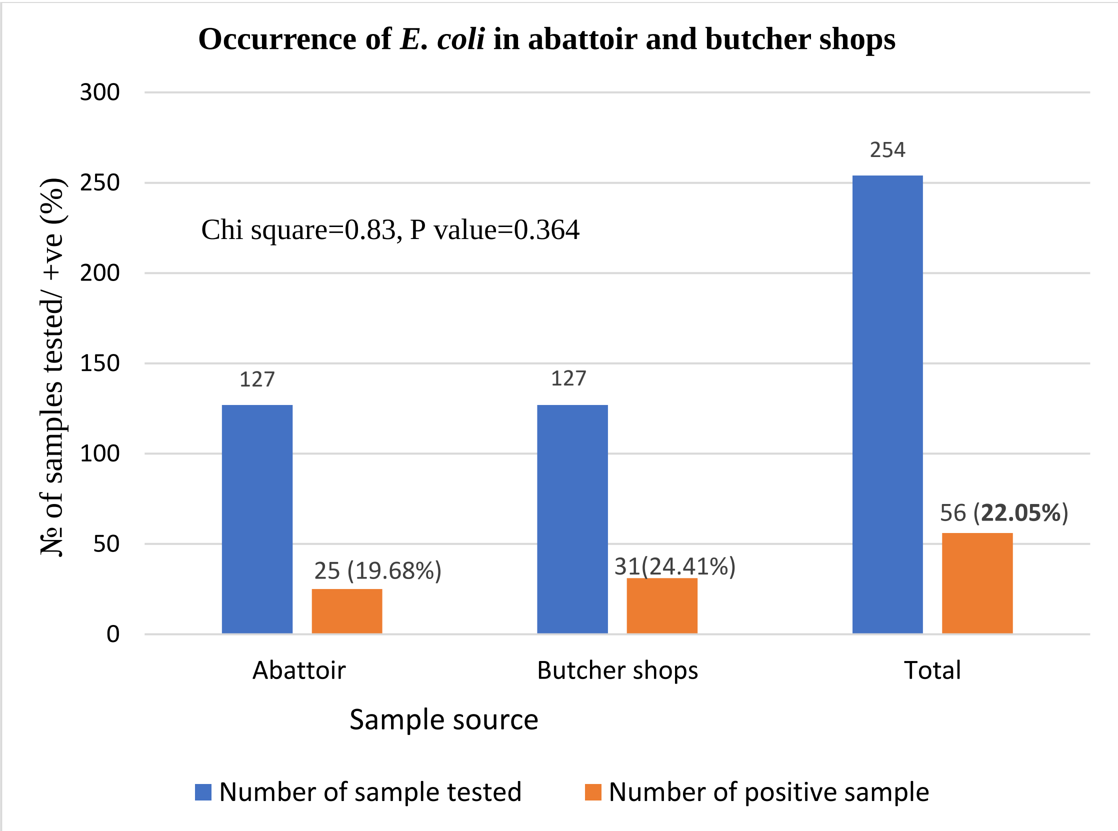

Figure 3: Occurrence of *E. coli* in Burayu abattoir and butcher shops

4.1.2 Prevalence of E. coli among different sample types from Burayu abattoir and butcher shops

Out of 56 *E. coli* isolates, the highest proportion (52 %) was recovered from cecal contents followed by samples from abattoir sewage (50%), butcher shop carcass 32.7% (20/61), butcher shop cutting board (27.3%), abattoir worker hand swabs (20%), butcher shop knife 13.6% (3/22), abattoir carcass (10%) and butcher shop hand (9%), respectively, but *E coli* was not detected from abattoir knife and water samples. (Table 4).

Table 4: Prevalence of *E. coli* among abattoir and butcher shops sample types

Sample source	**Sample type**	**№ of samples**		**Prevalence (95% CI)**	**Fisher's exact**
		Tested	***E. coli* + Ve**		
Abattoir	Carcass swab	60	6	10(3.7 – 20.5)	0.449
	Hand swab	15	3	20(4.3 – 48.0)	
	Knife	15	0	0(00)	
	Sewage	6	3	50(11.8 - 88)	
	Water	6	0	0(00)	
	Cecal content	25	13	52(31.3 – 72.2)	
Butcher shop	Carcass swab	61	20	32.7 (21.3 – 46)	
	Knife swab	22	3	13.6(2.9 – 34.9)	
	Cutting board	22	6	27.3(10.7 – 50)	
	Hand swab	22	2	9(1.1 – 29.0)	
Overall prevalence		254	56	22.5 (17.1 – 27.7)	

CI= Confidence interval, p value= probability value, №=Number

4.1.3 Prevalence of E. coli O157:H7(NSF) in abattoir and Butcher shop of Burayu

Out of 56 *E. coli* isolates subjected to SMA, 14 isolates showed the likelihood characteristics of *E. coli* O157:H7 (colorless, non-sorbitol fermenting) in both the abattoir and butcher shops. The highest proportion of *E. coli* O157:H7 (6.3%, CI=2.8% - 12.0%) isolates were encountered in samples collected from butcher shops when compared with the proportion of *E. coli* O157:H7 isolates found in abattoir (4.7%, CI=1.8% –10%). There was no statistical significance in the distribution of *E. coli* O157:H7 between abattoir and butcher shops ($p>0.05$) (Figure 4).

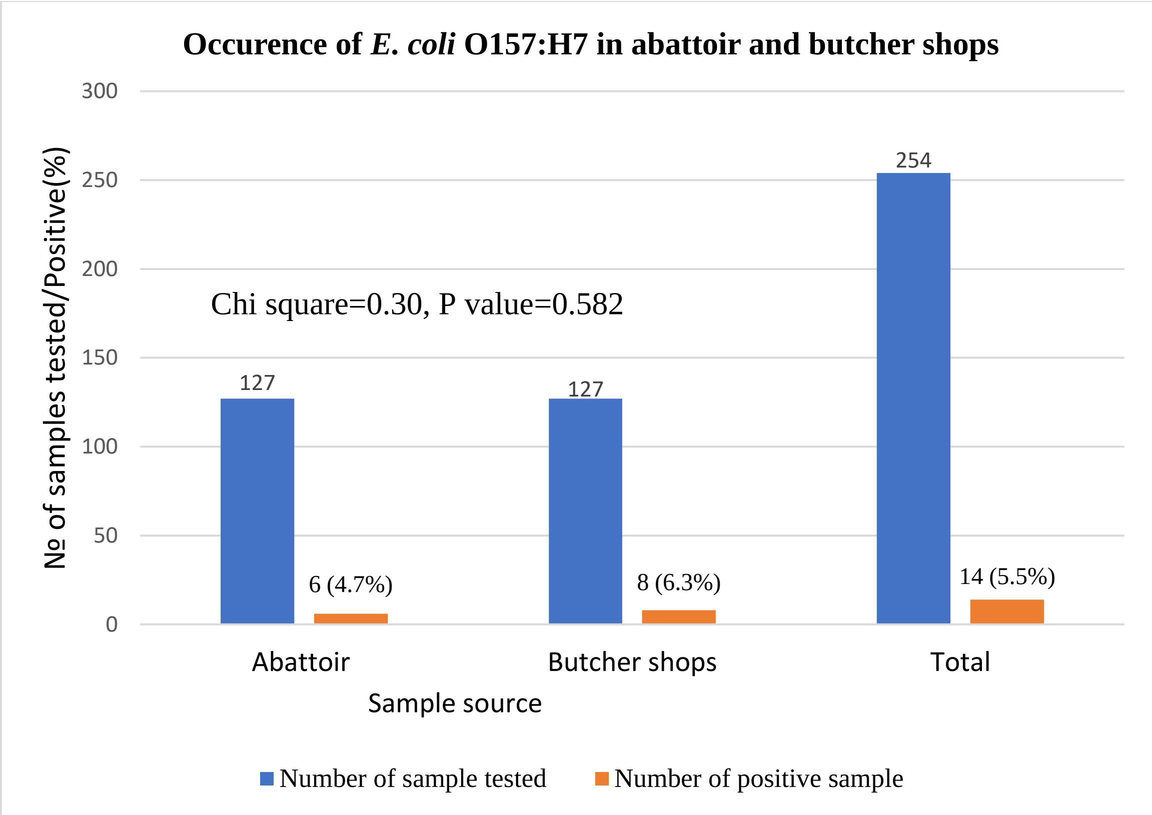

Figure 4 Occurrence of *E. coli* O157:H7 in Burayu abattoir and butcher shops

4.1.4 Prevalence of E. coli O157:H7 (NSF) among different sample types

Of the total 254 samples collected, the overall prevalence of non-sorbitol-fermenting isolates (believed to be *E. coli* O157:H7) was 5.5% (3.1%–9.1%). The highest proportion was isolated in sewage (16.7%), followed by cutting boards (13.6%), cecal contents (12%), abattoir hand swabs and butcher shop carcass swabs (6.7%), butcher shop knife swabs (4.5%) and abattoir carcass swabs (1.7%), but abattoir knives, borehole source water and butcher shops hand swabs were negative for *E. coli* O157:H7 (Table 5).

Table 5: Prevalence of *E. coli* O157:H7(NSF) in abattoir and Butcher shops of Burayu

Sample source	**Sample type**	**№ of samples**		**Prevalence (95% CI)**	**Fisher's exact**
		Tested	***E. coli* O157:H7**		
Abattoir	Carcass swab	60	1	1.7(.04– 8.9)	0.785
	Hand swab	15	1	6.7(.2 – 31.9)	
	Knife	15	0	0.00(00)	
	Sewage	6	1	16.7(.4 – 64.1)	
	Water	6	0	0.00(00)	
	Cecal content	25	3	12(2.6 – 31.2)	
Butcher shop	Carcass swab	61	4	6.7(1.8 – 15.9)	
	Knife swab	22	1	4.5(.12 – 22.8)	
	Cutting board	22	3	13.6(2.9 – 34.9)	
	Hand swab	22	0	0.00	
Overall prevalence		**254**	**14**	**5.5 (3.1 – 9.1)**	

CI= Confidence interval, p value= probability value, №=Number

4.2 Molecular detection of *E. coli* O157:H7 virulence Genes (*eae*)

From the total of 14 NSF *E. coli* O157:H7 isolates 12 were subjected to conventional PCR analysis. The result indicated that 10 (83.3 %) had the *eae* virulence gene. *E. coli* O157:H7 isolates were detected using intimin (*eae)* virulence gene. The distribution of PCR positive isolates among the different sample types and the results of molecular analysis indicating amplification of a target gene of 490bp fragment (Abreham *et al*., 2019) were shown on Table 6 and Figure 5, respectively.

Table 6: Distribution of intimin virulence gene among *E. coli* O157:H7 isolates

Sample type	**№ of *eae* gene positive isolates**
Abattoir carcass	1
Abattoir worker hands	1
Cecal content	3
Abattoir sewage	1
Butcher shop cutting boards	1
Butcher shop knife	1
Butcher shop carcass	2
Total	**10**

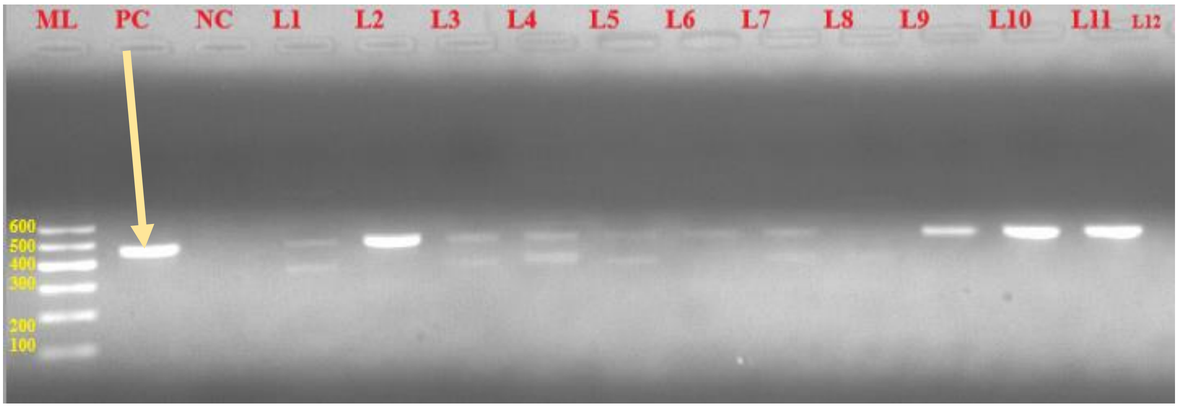

Figure 5: Gel electrophoresis of amplified products of *eae* genes in *E. coli* O157:H7 strains isolated from abattoir and butcher shops in Burayu Town, Oromia, Ethiopia

Key: *Lane ML (DNA ladder), PC (Positive control), NC (negative control), arrow (indicator) and L (sample), all samples were positive for eae gene except L8, 12, 13 and 14.*

4.3 Antibiogram of *E. coli* O157:H7

The antimicrobial susceptibility profile of *E. coli* O157:H7 isolates was determined by disk diffusion methods using eight antimicrobials. Out of 14 *E. coli* O157:H7 (NSF) isolates, 78.57% (11/14) and 71.43% (10/14) of isolates were highly susceptible to gentamycin and sulfamethoxazole/trimethoprim, whereas 78.57% (11/14) and 64.28% (9/14) were resistant to tetracycline and both amoxicillin and penicillin, respectively. Antimicrobial susceptibility profile of *E. coli* O157:H7 was summarized on Figure 6.

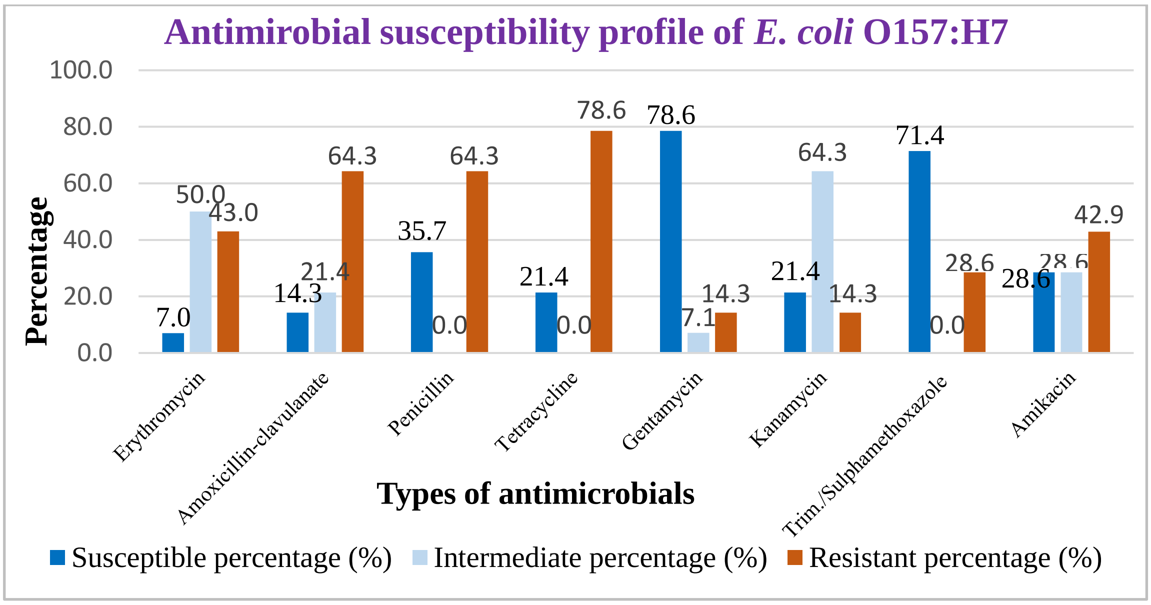

Figure 6: Antimicrobial susceptibility profiles of *E. coli* O157:H7 isolates

4.3.1 Multidrug resistance pattern of E. coli O157:H7

Multidrug resistance is when a single isolate of microorganism is resistant to two or more than two antimicrobials' classes. From the total isolates, 78.57% (11/14) of *E. coli* O157: H7 (NSF) isolates showed multidrug resistance to two or more classes of drugs used (Table 7), whereas only two isolates exhibited resistance to a single drug (Tetracycline).

Table 7: Multidrug resistance profile of *E. coli* O157:H7 (NSF)

Classes	Multidrug resistance (µg)	№ of isolates	Percent
Two drugs classes	Beta lactams and Aminoglycosides	1	
	Beta lactams and Tetracycline group	1	21.42%
	Beta lactams and sulfonamides	1	
Three drugs' classes	Beta lactams, Macrolides and TE group	1	
	Macrolides, aminoglycosides and TE group	1	
	Sulfonamides, beta lactams and TE group	1	21.42%
Four and more than four drugs	Beta lactams, sulfonamides Macrolides and TE group	1	
	Macrolides, Beta lactams, aminoglycosides and TE group	3	35.71%
	Macrolides, sulfonamides Beta lactams, aminoglycosides and TE group	1	
Total isolates developed MDR		**11**	**78.6** %

TE= Tetracyclines group

4.4 Personal observation and questionnaire on sanitation and hygienic practices

4.4.1 Personal observation during the study period

During the study period, all activities, including different facilities and infrastructures of the abattoir and butcher shops, were observed to assess the hygienic practices, infrastructure and waste management (leftovers and sewage) with international standards according to check lists on Appendix 9B (Table 8).

Table 8: Personal observations made during the study period

Source	Personal observations	Status
Abattoir	Source of water	Borehole
	Hygiene of premises	Full of leftovers (bones, rumen, contents etc.)
	Toilet	Defecation and urination out of the toilet hole
	Slaughterhouse partition	No at all
	Floor and ceiling	The slopy concrete floor and ceiled roof
	Waste management	Sewage leakage was being dumped into the environment
		Leftovers were being throwed to the premises
	Carcass dressing	Everything was being done on the floor
	Sink	Available
	Wash hands with soap	No practice of hand washing with soap during visit
	Carcass transportation	Closed vehicle
Butcher shop	Source of water	Municipal
	Hygiene of premises	leftovers like bone, fat part of carcass were thrown into the environment
	Collect money	Almost all
	Floor and ceiling	The concrete floor and ceiled roof
	Wasta management	Sewage leakage was being dumped into the environment
		Leftovers were being thrown to the premises
	Bulbs	Present
	Sink	Absent
	Cutting board	Wooden

4.4.2 Questionnaire data result on sanitation and hygienic practices of workers

The sociodemographic characteristics of abattoir and butcher shops workers were assessed (Table 9). Fifty four percent (54%) of abattoir workers aged between 22–30 years, 42% were above 31 years old and 4% were less than or equal to 21 years, whereas 54.55%, of butcher shop workers aged above 31 years, 43.64% and 1.82% of the workers between 22-30 years and ≤21 years old respectively. Ninety five percent of abattoir workers were male, whereas all the butcher shops workers were male. More than 64% of abattoir and 94% of butcher shop workers' educational status was primary education or above, whereas 9% and 5.45% of them were unable to read and write, respectively. In the butcher shop, more than 80% of workers had a maximum of ten years of experience and about 60% of employees had been there for at least six years in the abattoir.

Table 9: Sociodemographic characteristics of abattoir and butcher shop workers in Burayu Town, Oromia, Ethiopia

Variables	**Categories**	**Abattoir worker**		**Butcher shop**	
		Frequency	Percent	Frequency	Percent
Age	≤21 years	2	4%	1	1.82%
	22-30 years	30	54%	24	43.64%
	≥31 years	23	42%	30	54.55%
Sex	Female	3	5%	0	0%
	Male	52	95%	55	100%
Educational status	Unable to read &write	5	9%	3	5.45%
	Primary school	35	64%	31	56.36%
	Secondary school	10	18%	21	38.18%
	Above secondary school	5	9%	0	0%
Role	Veterinarian	2	4%	-	-
	Butcher	33	60%	55	100%
	Cleaner	14	25%	-	-
	Loader	6	11%	-	-
Work experience	≤5 years	22	40%	19	34.55%
	6-10 years	26	47%	26	47.27%
	≥11 years	7	13%	10	18.18%

More than 80% of abattoir workers wore head covers and boots, whereas most butcher shops workers (72.73%) used white coats as personal protective cloth. More than 56% of abattoir and butcher shop workers were wash their PPE twice per week. Although most of them (80%) do not use soap, practically all of the workers washed their hands before going to work and after work with tap water. In the butcher shops, 52.73% did not wash their hands with or without soap (Table 10).

Table 10: Use of personal protective equipment and Personal hygienic practice among 110 Burayu abattoir and butcher shop workers

Variables	Categories	**Abattoir (55 workers)**		Butcher shop **(55 workers)**	
PPE		**Frequency**	**Percent**	**Frequency**	**Percent**
Use of apron	No	41	75%	3	5.45%
	Yes	14	25%	52	94.54
White coat	No	46	84%	15	27.27%
	Yes	9	16%	40	72.73%
Head cover	No	6	11%	42	76.36%
	Yes	49	89%	13	23.64%
Glove	No	51	93%	55	100%
	Yes	4	7%	0	0%
Boots	No	10	18%	NA	NA
	Yes	45	82%	NA	NA
PPE wash frequency	Once per day	11	20%	9	16.36%
	Twice per week	34	61.82%	31	56.36%
	Once per week	10	18.18%	15	27.27%
Wash hands before and after work	No	1	2%	29	52.73%
	Yes	54	98%	26	47.27%
Wash hands with soap	No	44	80%	33	60%
	Yes	11	20%	22	40%

NA= Not Applicable

Fifty nine percent of butchers did not use the same knife for carcass and evisceration, then and 56% of the butchers washed the carcass, whereas the rest used the same knife and did not wash the carcass. More than 72% of abattoir workers had received training and aware that feces, boots, hides, the floor, a knife, the worker's hand, and the hanging hook could all contaminate carcasses. About 73% of butcher shop workers collect money while handling meat. Information on medical checkup intervals, diseases caused from raw meat consumption and symptoms was summarized in Table 11.

Table 11: Cross contamination assessment, training, medical checkups and knowledge of disease caused by consumption of raw meat at the abattoir and butcher shop

Variables	Categories	Abattoir (55 workers)		Butcher shop (55 workers)	
		Frequency	Percent	Frequency	Percent
Use the same equipment for meat and offal	No	23	59%	22	40%
	Yes	16	41%	33	60%
Wash hands after evisceration	No	1	3%	NA	NA
	Yes	38	97%	NA	NA
Carcass wash	No	18	44%	NA	NA
	Yes	23	56%	NA	NA
Source of contamination	Feces	5	9%	-	-
	Hides	2	3.6%	-	-
	Hand	2	3.6%	-	-
	Knife	1	2%	-	
	Floor	3	5.4%	-	-
	Hanging hook	1	2%	-	-
	Boots	1	2%	-	-
	Combination	40	72.7%	-	-
Do you collect money while handling meat	No	NA	NA	13	23.64%
	Yes	NA	NA	42	73.36%
Training	No	13	23%	39	70.91%
	Yes	42	77%	16	29.09%
Medical checkup Frequency	No at all	5	9%	14	25.45%
	Once per year	20	36.36%	23	41.82%
	Every 3 months	8	14.54%	12	21.82%
	Every six months	22	40%	6	10.9%
Information on diseases caused by consuming raw meat	No	8	14.55%	16	29.1%
	Yes	47	85.45%	39	70.9%
Symptoms of the disease	Diarrhea	29	67.44%	22	40%
	Colic	2	4.65%	3	5.45%
	Diarrhea & colic	7	16.28%	-	-
	Worms with feces	5	11.63%	14	25.45%
	Have no idea	-	-	16	29.09%

NA= Not Applicable

In contrast to the 7.27% of workers who cleaned once a week, more than 50% of butcher shop workers cleaned equipment using cold water with powder detergent at least once at every two days interval. Fly control was absent in about 78% of butcher shops, while spray and smoking were used in the remaining 22%. Plastic bags were used in nearly 89% of the outlets (butcher shops) for meat that customers took home, whereas 11% of the outlets used newspaper. More than half (58.18%) of butcher shops used the same place for storage and display of meat and offal, and about 81.82% of butcher shops were not using cover display whereas 18.18% did. Overall hygienic practices were summarized in Table 12.

Table 12: Sanitation and hygienic practices of butcher shops

Variables	Categories	Butcher shop worker (n=55)	
		Frequency	**Percent**
Washing	Once per day in the morning	3	5.45%
	Once per day in the evening	29	52.73%
	Once every two days	13	23.63%
	once per week	4	7.27%
	Twice per week	6	10.91%
Type of detergent used for equipment	Powder (Omo)	16	29.09%
	Liquid (Largo)	37	67.27%
	Bleach	2	3.64%
Cleaning butcher shop equipment	Using cold water with soap	38	69.09%
	Clean by cold water only	8	14.55%
	Clean by wiping only	9	16.36%
Routine fly control and how?	No control	43	78.18%
	Yes, control by smoking	10	18.18%
	Yes, control by spray	2	3.64%
Meat cover display	No	45	81.82%
	Yes	10	18.18%
Meat stored in butchery before it is over	Within 24 hours	14	25.45%
	Stay for one day	19	34.54%
	Stay for two days	22	40%
The material used to wrap meat for sale	Plastic	49	89.1%
	Newspaper	6	10.9%
Different storage/display for offal and meat	No	32	58.18%
	Yes	23	41.82%

5. DISCUSSION

In the present study, the overall prevalence of *E. coli* was 22.05%, which is in line with the reports of 20.2% by Sebsibe and Asfaw's (2020) from Jima Town, 20.8% by Abayneh *et al.* (2019) from Jima, 23.7% by Abebe *et al*. (2023) from Addis Ababa, 23.4% by Tadese *et al.* (2021) from Ambo and 21.6% by Messele *et al.* (2017) from Addis Ababa and Bishoftu. However, Atnafie *et al.* (2017) from Hawassa and Mohammed *et al.* (2014) from Dire Dawa reported a prevalence of 12.38% and 15.89%, respectively, which is lower than the current findings. On the other hand, Bersisa *et al*. (2019), Endale *et al.* (2014) and Gugsa *et al.* (2022) reported a prevalence of 35.2%, 61.7% and 43.7%, respectively, which is higher than the current finding. The significant discrepancy in prevalence between the current finding and those reported by different investigators may be due to differences in sanitation and hygienic practices, sample size, sampling methodology and agroecological conditions.

The highest prevalence of *E. coli* was isolated from butcher shops (24.41%), followed by abattoirs (19.68%), which is in accordance with the result of Sebsibe and Asfaw's (2020), which reported 21.4% from butcher shops and 19.3% from abattoir in Jimma town. The current finding of *E. coli* from abattoir (19.68%) is slightly higher than the report of Kebede *et al.* (2014) and Fikadu *et al.* (2023), which was 15% from Tigray and 11.3% from Bedelle Ethiopia, respectively. A higher prevalence of *E. coli* from butcher shops (35%) was reported by Zerabruk *et al*. (2019) from Addis Ababa as compared to the present study which was 24.41%. The highest prevalence in butcher shops is due to the fact that meat is exposed to numerous contacts from the slaughterhouse until it arrives on the table as food, increasing the likelihood of microbial contamination (Abayneh *et al.*, 2019).

In this study, there was no *E. coli* detected from the knife, which disagrees with the 16.7% and 4.3% prevalence reported by Sebsibe and Asfaw (2020) and Fikadu *et al.* (2023), respectively, whereas, 10% prevalence by abattoir carcass swabs and 20% from abattoir butcher hand swabs were lower and higher than 20% and 13.3%, respectively. Additionally, 32.7%, 9%, 13.6%, and 27.3% prevalence of *E. coli* were encountered in meat, hand swabs, knives, and cutting boards in retail shops, which is somewhat close to 28.5%, 12.5%, 16.7%, and 23.3% of the current findings from butcher shops in the study area. Nevertheless, Fikadu *et al.* (2023) found a prevalence of *E. coli* from hand swabs and cecal content samples of 7.5% and 15%, respectively, which is lower than the current study's findings of hand swabs

(20%) and cecal contents (52%). whereas a 3.3% prevalence in water is contrary to the current finding (no water contamination by *E. coli*). The variation is might be due to sample size, sampling techniques, laboratory protocol and status of hygienic practices among different study areas.

The current study revealed that the overall prevalence of *E. coli* O157:H7 was 5.5% which is consistent with the report of Sebsibe and Asfaw's (2020) (5.4%) from Jima town. However, the prevalence of *E coli* O157:H7 from beef carcass in this study was higher than the previous study finding in various parts of Ethiopia (Abdissa *et al*., 2017 (3.49%), Assefa, 2019 (4%), Atnafie *et al.*, 2017 (2.4%), Beyi *et al*., 2017 (4.5%), Fikadu *et al.,* 2023 (2.7%) and Mengistu *et al.,* 2017 (2.5%)). In another hand, the prevalence of *E. coli* O157:H7 in the current study was lower than Bekele *et al.* (2014) (13.3%), Ayenew *et al*. (2021) (9.8%), Haile *et al.* (2017) (8.3%), and Tadese *et al.* (2021) (9.1%). The variations may result from differences in sanitation, hygienic practices and hygienic settings that could put meat at risk of cross-contamination in different study areas.

The distribution of *E. coli* O157:H7 in abattoir and butcher shops was 4.7% and 6.3%, respectively. The prevalence of *E. coli* O157:H7 between abattoir and butcher shops was statistically not significant (p value=0.582). These results were slightly in line with the report of Sebsibe and Asfaw (2020), which was 3.7% from abattoir and 6% from butcher shops. In contrast to the current findings, a lower prevalence of *E. coli* O157:H7 from abattoir and butcher shops was reported by Atnafie *et al.* (2017), which is 2.8% and 1.7%, respectively. Additionally, Tadese *et al.* (2021) reported a prevalence of *E. coli* O157:H7 from abattoir (7.2%) and butcher shops (19.4%) which was much higher than the current findings. The higher prevalence in butcher shops than abattoir may be because there is a greater risk of meat cross-contamination during transportation in cars, meat handlers, the duration of meat on display and displaying without cover (Callaway *et al*., 2009).

In the current study, the prevalence of carcass contamination by *E. coli* O157:H7 was 4.2%. This result is in line with the reports of Beyi *et al*. (2017) and Fikadu *et al. (*2023*)*, who reported a 4.5% and 4.7% prevalence of *E. coli* O157:H7, respectively. However, the slightly lower prevalence of 3.2% and 2.65% *E. coli* O157:H7 from carcass samples reported by Dulo *et al*. (2015) and Taye *et al*. (2013), respectively. Differences in the handling of meat, the use of hygienic utensils, the way in which individuals are cleaned up, and other hygienic practices may be the reason for the disparity in prevalence.

Regarding water samples from the abattoir, no *E. coli* O157:H7 was encountered; this result is similar to the result of Fikadu *et al*. (2023). however, opposes the findings of Dulo *et al.* (2015) and Mersha *et al*. (2010), in which they reported 7.1% and 4.2%, respectively. Unlike Beyi *et al*. (2017), who reported a 3.6% prevalence of *E. coli* O157:H7 on butcher shop cutting boards, the current finding is 13.6% prevalence of *E. coli* O157:H7. In the present study, no *E. coli* O157:H7 was isolated from abattoir workers hands, which disagrees with the report of Fikadu *et al*. (2023), who reported 1.1%. The proportion of *E. coli* O157:H7 isolated from butcher knives was 1/22 (4.5%) and no isolate was isolated from abattoir knives, which is slightly in agreement with the proportion of 1/30 (3.3%) of *E. coli* O157:H7 isolated from butcher shop knives (Sebsibe and Asfaw, 2020).

The recto anal junction of cattle is the principal site of colonization for *E. coli* O157:H7 (Cobbold *et al.,* 2007). Therefore, in the present study, the prevalence of *E. coli* O157:H7 from cecal content was 12%, which is higher than Haile *et al.* (2017) and Dulo *et al.* (2015), who found 7.3% and 2.2% prevalence, respectively. A high prevalence in cecal contents implies that there was a high shedding pattern of *E. coli* O157:H7. Additionally, the prevalence of sewage in abattoir was 16.7%, which is slightly in line with the reports of Ayaz *et al.* (2014) and Oluwawemimo *et al*. (2016), who reported 20.8% and 16%, respectively. However, Barel *et al*. (2022) reported a lower prevalence of *E. coli* O157:H7 from sewage/wastewater (11%) than the current finding.

The presence of bacteria in the hands of personal workers and environs (knife, cutting boards, cecal contents) strongly suggests the circulation of pathogens in the meat chain and could be regarded as a cause of meat contamination, which has public health implications. The variations in agroecology, methodology, laboratory protocols, sampling techniques, sample sizes, status of sanitary and hygienic standards, and types of samples utilized in various study locations may be the cause of these contradictions in the prevalence of *E. coli* O157:H7 among different study samples.

It has been discovered that a number of virulence factors contribute to the pathogenicity of NSF *E. coli* O157:H7. Production of at least one of the two Shiga toxins (stx1 and/or stx2), intimin (*eae*), and enterohemolysin (EHEC-hlyA) are some of these factors (Fashina *et al*., 2018). In this study, *eae* virulence gene was investigated to further characterize the isolates. The *eae* gene was detected in 83.3 % of the isolates. This result is somewhat lower than the reports of Ayenew *et al.* (2021) and Gutema *et al*. (2021), who reported that all of the isolates subjected to PCR were positive for *eae*. Intimin gene-positive isolates are typically associated with life-threatening infections such as hemolytic-uremic syndrome and hemorrhagic colitis (Ramachandran *et al*., 2003). The characterization of the isolates in the current study using this virulence gene capitalizes the public health risk of *E. coli* O157:H7 as one of the major food contaminants.

Despite the fact, some antimicrobial drugs are effective, the occurrence of antimicrobial resistance among foodborne pathogens is increasing (Hassan *et al*., 2018). In the current study, all non-sorbitol-fermenting isolates displayed a variety of drug susceptibility profiles. Among the aminoglycosides group, 78.57% of *E. coli* O157:H7 (non-sorbitol fermenting) isolates were susceptible to gentamycin, which is in accordance with Ababu *et al*. (2020) from Holeta district, Abebe *et al*. (2023) from Dessie and Kombolcha towns, Atnefie *et al*. (2017) from Hawassa, Gugsa *et al*. (2022) from Mekele, Hiko *et al.* (2008) from Ethiopia, Abayneh *et al*. (2019) from Jima and Sebsibe and Asfaw (2020) from Jima town, who reported more than 78% of the isolates were susceptible to gentamycin.

In the current study, from Aminoglycoside group, the susceptibility of the isolates to kanamycin was 85.71%, which is somewhat consistent with the reports of Abdissa *et al.* (2017), Atnefie *et al*. (2017), Beyi *et al*. (2017), Tassew *et al.* (2010) and Taye *et al*. (2013), in which they reported that all the isolates were susceptible from different study areas. However, Asfaw and Regasa (2021) from Arsi and Hiko *et al*. (2008) reported 100% resistance to kanamycin, which contradicts the current finding. In addition, more than 57% of isolates from the current study were susceptible to amikacin, which contradicts the report of Abayneh *et al.* (2019), who reported that 100% of the isolates were susceptible.

Additionally, among sulfonamides groups, Hailu (2020) from central Ethiopia, Gugsa *et al*. (2022) from Mekele, Ethiopia Beyi *et al. (*2017) from central Ethiopia reported that 78.79%, 79.2% and 71% of the isolates were susceptible to sulfamethoxazole/ trimethoprim, respectively, which is in line with the current finding where 75% of the isolates were susceptible to sulfamethoxazole/ trimethoprim. However, more than 54% of the isolates were reported to be resistant to sulfamethoxazole/trimethoprim by Ababu *et al.* (2020) from the Holeta district, which contradicts the current finding. Abebe *et al.* (2023) from Dessie and Kombolcha towns and Abayneh *et al.* (2019) from Jima reported that 92% and 100% of the isolates were resistant to erythromycin, respectively, which disagrees with the current finding in which only 42.85% of the isolates were resistant to erythromycin from macrolide groups. This could be due to indiscriminate use and genetic variation among the isolates in different geographical areas.

In another study, among tetracycline group, Gugsa *et al*. (2022) and Messele *et al. (*2017) reported that 41.7% and 47.6% of the isolates were resistant to tetracycline, respectively, which is a lower percentage of resistance than the current finding. However, Abdissa *et al. (*2017*)* from Addis Ababa and Debre Berhan cities, Bekele *et al.* (2014) from Addis Ababa and Carvalho *et al.* (2020) from Brazil reported that 100%, 94.9% and 96% of the isolates were highly susceptible to tetracycline respectively, which opposes the current finding. Moreover, among beta lactams group, Abayneh *et al.* (2019) reported that penicillin was resistant to all the samples (100%). This result contradicts the current finding, in which only 64% of the samples were resistant. In the present study, 78.57% and 64.28% of the isolates were resistant to tetracycline and amoxicillin clavulanate, which contradicts the findings of Atnefie *et al.* (2017), who reported 73.7% and 80% of the isolates were susceptible to tetracycline and amoxicillin clavulanate, respectively, from Hawassa. The differences might be due to laboratory procedures applied, the brand of drugs used, or the indiscriminate use of drugs in different study areas.

Multidrug resistance occurs when a single isolate of a microorganism is resistant to two or more antimicrobial classes. MDR patterns in the current study showed that 11/14 (78.57%) of tested isolates were resistant to three or more antimicrobials, which has a serious public health implication. This agrees with the reports of Gugsa *et al*. (2022) from Mekele and Atnefie *et al.* (2017) from Hawassa, which showed more than 75% of the isolates were multidrug resistant. The occurrence of MDR in this study was higher than the findings of other studies conducted in Addis Ababa (22.6%) by Bekele *et al*. (2014) and in Jima Town (44.2%) by Sebsibe and Asfaw (2020). However, the proportion (78.57%) of the MDR observed in the current finding is lower than the report of Mekuria and Beyene (2014), who reported 93.2% proportion of the isolates showed MDR. This might be due to indiscriminate use of drugs, the number of isolates, or the genetic variation of the isolates among different geographical locations.

The study conducted by Bedasa *et al*. (2017) from Bishoftu and Shecho *et al.* (2017) from eastern Ethiopia showed that 92.5% and 92.3% of the isolates were multidrug resistant, respectively, which is slightly higher than the present finding (78.57%). In the current study findings, 21.42%, 21.42% and 35.71% of the isolates subjected to antimicrobials were resistant to two, three or more drugs of classes, respectively. This finding is in line with the report of Bedasa *et al.* (2018) where 21.6% of the isolates were resistant to three drugs and 14.6% to four drugs. However, Geresu and Regassa, (2021) reported that 50%, 25% and 25% of the isolates developed resistance to three, four or more drugs, respectively, which is slightly higher than the current findings.

The higher resistance rate might be due to inappropriate and excessive use of these antimicrobials for therapeutic and prophylactic purposes, or it could also be due to the insufficiency or lack of antimicrobial resistance observation programs (Gebrekirstos *et al*., 2017), both in human and animal infections. A change in the resistant genes of *E. coli* O157:H7, which may be a result of natural resistance in which the pathogen possesses characteristics that inhibit the action of the antibiotics or acquired resistance in which there is a change in the genetic characteristics of the pathogen, plays a crucial role in variation and the formation of MDR (Arber, 2014). The frequent use and misuse of antibiotics in humans and food animals are intricately linked to the recent emergence of MDR bacteria. AMR harms human health by making it more difficult and expensive to treat serious illnesses (Aker *et al.*, 2012; Gebremedhin *et al*., 2021).

During the study period, abattoir premises were full of leftovers, there was improper toilet use, no partition at the slaughterhouse (all activities were performed on the floor), and no workers washed their hands with soap. These all have the potential to contaminate carcasses with pathogenic organisms like *E. coli* O157:H7, which in turn affects public health. Additionally, carcasses could be contaminated with pathogens through contact with the digestive tract of slaughtered animals, as well as through contaminated worker hands, tools, or utensils (Brusa *et al.,* 2019). Similarly, in butcher shops, leftovers were thrown to the premises; cutting boards were wooden type (wooden type of cutting board may retain moisture and are vulnerable to the growth of bacteria, which can contaminate carcasses) and there was no sink in the butcher shop at all. Improper sewage management (sewage leakage from an abattoir's safety tanker) has also the potential to harm the environment and lead to the emergence of drug-resistant strains of microorganisms. All these in the abattoir and butcher shops could have significant potential for the occurrence of pathogenic *E. coli*.

During the study time, it was noted that the floor was sloped (in the abattoir case) and made up of concrete. Because of the concrete and impenetrable nature of the abattoir's floor, less filth was tracked into the abattoir and drainage and easy cleaning were made possible. However, the vicinity of the abattoir and most of the butcher shops were full of leftovers including gastrointestinal content, horns, shanks, and bones, which is against WHO and FAO guidelines (WHO, 2009). This could make it easier for *E. coli* O157:H7 to spread from the abattoir to the outside or vice versa, which may have an impact on the emergence of antibiotic resistance which in turn disturb the health of animal, human and environment.

In the current study, about 85% (abattoir) and 70.9% (butcher shop) of workers were aware of illnesses caused by consuming raw meat, and 67.44% (abattoir) and 70.9% (butcher shop) knew that anyone exposed to consuming unhygienic meat can experience diarrhea, while the other workers suggested colic, worms in feces, or a mix of these, but around 15% of abattoir and 29% of butcher shop workers did not know about diseases caused by consumption of raw meat. Number of human infections by *E. coli* O157:H7 peaks by Houseflies and blow flies can carry relatively high concentrations of potentially virulent *E. coli* O157:H7 (Bach *et al.*, 2002). However, more than 78% of butcher shops did not control flies, which have the potential to cross contamination of meat. Abattoir is one of the food industries that contribute to the problem of possible foodborne diseases and health hazards associated with food unless the principles of foodborne hygiene practices are implemented (Roberts *et al.,*2009). Workers used the toilet in between operations and then came back to work without washing their hands with soap or using any disinfectants. This habit will exacerbate the contamination of meat by microorganisms like *E. coli* and violate the standards of meat hygiene and safety.

International standards state that a retaining room, sterilizer, and hot and cold water should all be easily accessible for cleaning equipment and workers' hands in abattoirs (CAC, 2005). However, the Burayu abattoir lacked hot water, sterilizers, retention rooms (cooling facilities), change rooms and proper restrooms for use, like what Tadese *et al*. (2021) observed in the Ambo municipal abattoir. Additionally, observations made for the current study indicate that the same area is used for both slaughter and postmortem processes on the floor and 41% of abattoir and 60% of butcher shop workers use a single knife continuously despite contact with dirty or contaminated surfaces, which is a potential risk of contaminating carcasses with pathogenic microorganisms like *E. coli* O157:H7. This goes against what the Codex Alimentarius Commission advises (CAC, 2005).

In the present study, except for head covers and boots (a white coat in the case of the butcher shop), more than 75% of the workers were not wearing protective clothing, which may possibly contaminate the meat. Even though PPE should be washed once a day or every two days, however, more than half (61.82% of abattoir and 56.36% of butcher shops) of employees only did so twice per week. Meat at abattoir and retail outlets are primarily contaminated by unhygienic protective equipment (Fasanmi *et al*., 2010; Adetunde *et al*., 2011) and in butcher shops collecting cash while handling meat, the duration meat stayed at the retail shop (In the current study, meat was stored in the butchery for 1-2 days before it was sold out) (Zerabruk *et al*., 2019). Therefore, a beef meat chain with unclean handling practices and cash collection in between touching meat has a high risk of meat contamination by *E. coli* O157:H7. Part of the cause of meat contamination during processing is a lack of awareness about how to make the meat industry safer.

Additionally, the Ethiopian Ministry of Agriculture declares that personal clothes can transport microorganisms (germs) acquired from a number of sources into the meat or meat handling facility (MOA, 2010). So, the practice of wearing protective clothes helps reduce the burden of contaminants in meat. Therefore, to protect meat and meat handling facilities from contamination because of personal clothing, protective overalls or hair covers should be clean and always worn when handling meat (Bersisa *et al*., 2019). However, it was not properly implemented for the workers of the butcher shops and abattoirs in Burayu. This might be an important possible cause of microbial contamination of carcasses.

In addition to their clothes, the workers themselves can be a probable source of contamination due to illness. Respondents of abattoir (9%) and butcher shops (25.45%) had no general health checkup, while others were checked at varied intervals, ranging from once a year to four times a year. It was recommended that workers be examined clinically and bacteriologically before they are employed and at regular intervals afterwards (Bersisa *et al*., 2019). Moreover, the water used in the abattoir for cleaning and meat processing must meet drinking water standards; otherwise, it may contaminate the meat during washing (Adebowale *et al*., 2010). In the Burayu abattoir, borehole water without treatment was the source of water supply, which could be a potential contaminant. Furthermore, 80% of the abattoir and 60% of butcher shop workers had no interest in washing their hands with soap, which is consistent with the findings of Tadese *et al*. (2021), who found that all respondents were reluctant to do so during slaughtering procedures and meat handling.

At the Burayu abattoir, most workers (64%) had only completed elementary school; 18% completed secondary school and only 9% had training in higher education. These results are slightly like those of Bersisa *et al*. (2019), who reported 71%, 16%, and 13%, respectively. In butcher shops, 5.45%, 56.36%, and 38.18% were unable to write and read, primary, and secondary school were completed, respectively. Regarding educational status, as the educational status of workers increases, the capability of training increases, which can play a significant role in maintaining good sanitation and hygienic practices by giving them training on meat safety.

Unlike Bersisa *et al.* (2019) and Tadese *et al*. (2021), who reported that almost half of abattoir workers were not trained in the Bishoftu town municipal abattoir and none of the workers from the Ambo municipal abattoir, respectively, the current findings report that more than 77% of the Burayu abattoir and 29.09% of butcher shop workers were trained at varied intervals and the rest 23% and 70.9% were not. Studies also highlighted that individual with proper training regarding meat safety have significantly better practices compared to the untrained (Jianu and Goleţ, 2014). In the current study, more than 72% of workers knew where the contamination came from, which may have been feces, hides, a worker's hand, a knife, the floor, a hanging hook, or boots. Generally, the current study revealed that abattoir and butcher shop establishments in the town of Burayu did not adhere to the necessary sanitation and hygiene practices.

There are some limitations to the current investigation: Isolation, identification and confirmation of microorganisms should be done with updated technology like OmniLog, MALDI-TOF mass spectrometry and sequencing of the strains. But the current study was done in a wet laboratory, including culture and biochemical tests, which are laborious and prone to contamination. The identification of *E. coli* O157:H7 was performed by culturing on SMA (a colorless colony was believed to be *E. coli* O157:H7) due to a lack of latex agglutination test kits, but it would have been preferable to use a latex agglutination test kit. Additionally, due to financial limitations, we could only include 254 carcass and environmental swab samples, but in order to increase precision, it would be better to include samples from each environmental sample and carcass swabs in the study area. The antibiogram was done only by measuring the zone of inhibition, but it would be better if virulence-resistant genes were identified.

6. CONCLUSION AND RECOMMENDATIONS

According to the current investigation, there were significant distributions of *E. coli* O157:H7 circulating in the Burayu abattoir and butcher shops. The presence of *E. coli* O157:H7 isolates in carcasses provided evidence of cross-contamination from worker hands and their environs (sewage, cecal content, cutting board and knife). Additionally, abattoir and butcher shop establishments in the town of Burayu did not adhere to the necessary sanitation and hygienic standards; in the case of the abattoir, all activities were done on the same floor. The distribution of multidrug resistant *E. coli* O157:H7 isolates among the different sample types is an indication that the pathogen is circulating at human -animal-environment interface posing a public health hazard. Moreover, the distribution of the pathogen along the beef value chain demands a collaborative One Health approach to safeguard public health.

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

- Training on the possible ways of prevention and controlling contamination should be given to all abattoir and butcher shop workers.
- Continuous surveillance of AMR patterns should be needed, especially for organisms contained in food originating from animals. And there should be stringent enforcement of the laws governing veterinary antimicrobials.
- Abattoir and butcher shop workers should adhere to and be monitored for sanitation and hygienic standards.
- Separate places (partitions) for slaughtering, evisceration and post-mortem examination to minimize cross-contamination should be implemented.
- Further characterization using different virulent genes and sequencing of pathogenic *E. coli* in food chains should be undertaken to determine the molecular epidemiology of *E. coli* O157:H7 for appropriate and effective control measures.

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8. APPENDIXES

Appendix 1: Pictures taken during sample collection and labeling

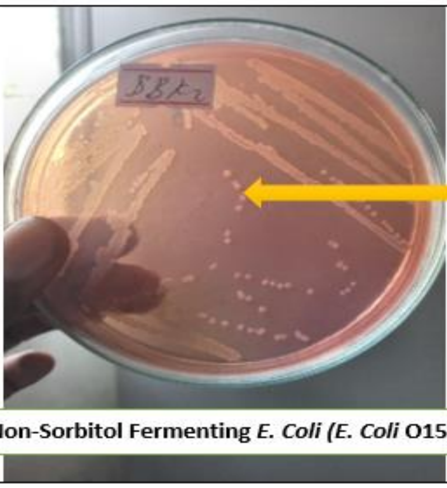
Sample collection from cattle carcass

Sample coding (sample type, date, source)

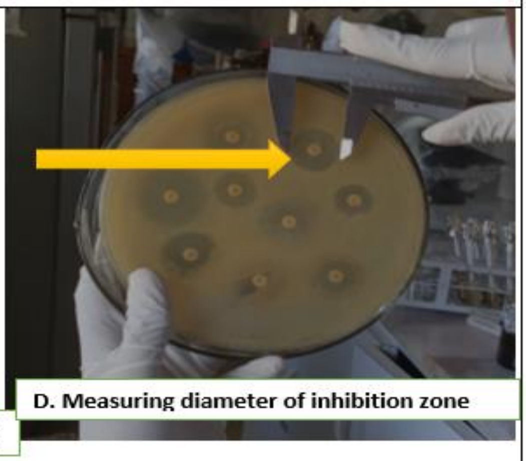
Appendix 2: Pictures taken during Microbiology laboratory work

A. Pinkish colony in MacConkey agar

B. Metallic sheen characteristic of E. Coli on EMB



C. Non-Sorbitol Fermenting *E. Coli (E. Coli* O157:H7)

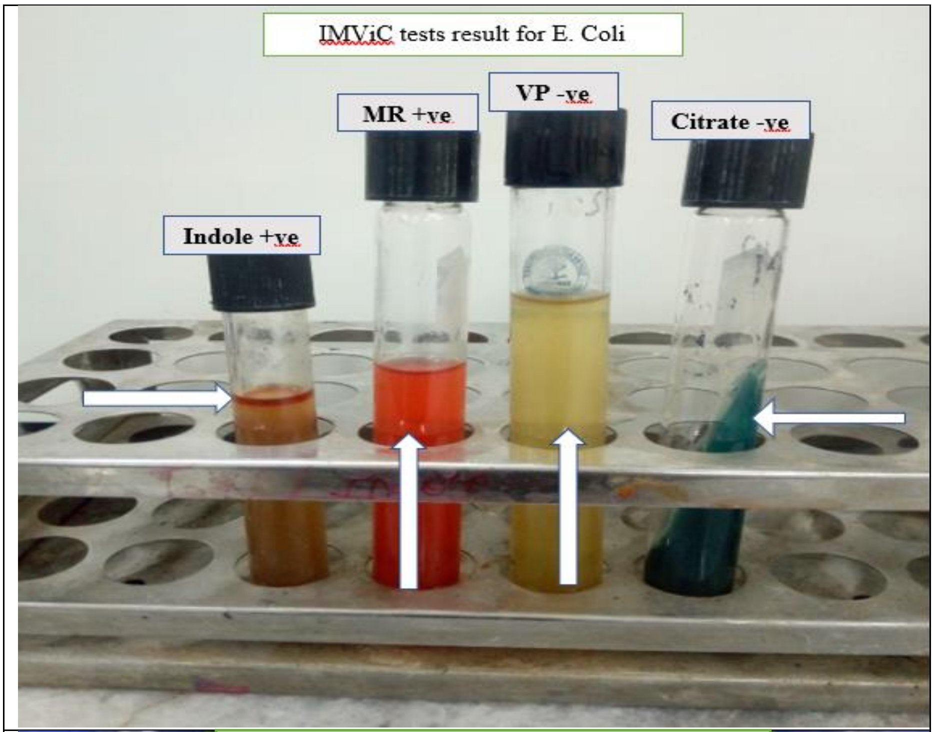


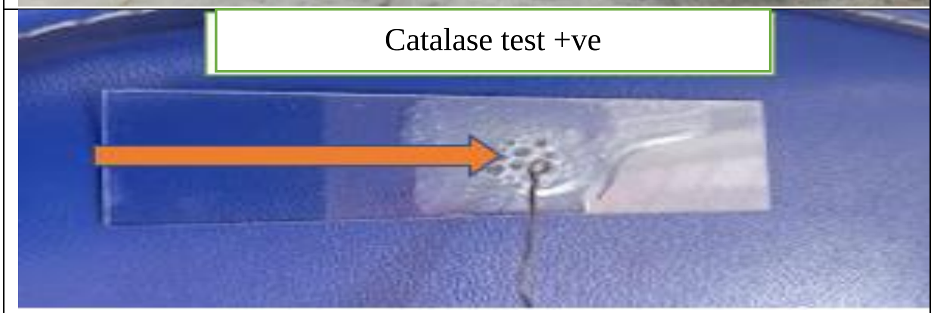
D. Measuring diameter of inhibition zone

Laboratory work in biosafety cabinet

E. *E. coli* colony on nutrient agar

Appendix 3: Biochemical tests (IMViC) and catalase test used for *E. coli* identification





Appendix 4: Molecular laboratory work at Animal Health Institute

DNA extraction from pure colony

Appendix 4: Molecular laboratory work (Continued)

Appendix 5: Media preparation for Isolation of *Escherichia coli*

A. MacConkey agar preparation

Procedures

1. The agar powder was suspended (See in the agar bottle and generally 50 gram) in 1 L of purified water and mix thoroughly.
2. Heated with frequent agitation and boiled for 1 minute to completely dissolve the powder.
3. Autoclaved at 121°C for 15 minutes.
4. Poured (15ml-20ml) cooled MacConkey Agar into sterile petri dishes on a level, horizontal surface to give uniform depth.

B. EMB agar preparation

Procedures

1. The agar powder was suspended (See in the agar bottle and generally 50 gram) in 1 L of purified water and mix thoroughly.
2. Heated with frequent agitation and boiled for 1 minute to completely dissolve the powder.
3. Autoclaved at 121°C for 15 minutes.
4. Poured (15ml-20ml) cooled Eosin methyl blue agar into sterile petri dishes on a level, horizontal surface to give uniform depth.

C. Nutrient Agar Preparation (CM 0003, OXOID, Basingstoke, England)

Procedures

1. Suspended 28 grams in 1000ml distilled water.
2. Heated to boil to dissolve the medium completely.
3. Sterilized by autoclaving at 15 Ibs pressure (121℃) for 15 minutes.
4. Mixed well and poured in to sterile Petri dishes.

D. Muller Hinton agar preparations for drug sensitivity test

Procedures

1. 38 gm of the medium was suspended in one litter of distilled water.
2. Heated with frequent agitation and boiled for one minute to completely dissolve the medium.

3. Autoclaved at 121°C for 15 minutes then cooled to room temperature.
4. Poured cooled Mueller Hinton Agar into sterile petri dishes on a level, horizontal surface to give uniform depth.
5. Check for the final pH 7.3 ± 0.1 at 25ºC. Store the plates at 2-8 ºC.

Appendix 6: Principle and procedures of gram staining

Principle

Gram positive bacteria will resist decolorization by acetone and retain the primary dye (crystal violet) while Gram negative bacteria get decolorized by a decolorizer and then get counterstained by gram's safranin.

Procedures

1. The inoculation loop was heated with Bunsen burner until it becomes red hot and allowed to cool.
2. An isolated colony was touched with inoculating loop and smear was made on a clean glass slide
3. The smear was air dried and heat fixed by passing through flame
4. The smear flooded with crystal violate and waited for 1 minute
5. The slide was rinsed with cold tap water and drained
6. The slide flooded with gram's iodine and allowed to remain for 1 minute
7. Rinsed with tap water and drained
8. It was decolorized with acetone for 20 seconds, then rinsed with tap water
9. Counterstained with gram's safranin for 1 minute; rinsed with tap water and dried
10. It was examined under 100x oil immersion
    - ❖ Gram negative bacteria stain safranin dye
    - ❖ Gram positive bacteria stain crystal violet (deep purple) dye.

Appendix 7 Principle and procedures of biochemical tests (IMViC) for *E. coli*

A. Principle and procedures of catalase test.

The catalase enzyme works on breaking of the hydrogen peroxide into water and oxygen. Therefore, the hydrogen peroxide is added on bacterial colony on the glass slide if it has catalase enzyme, it breaks the H_2O_2 into H_2O and O_2 and then bubble is produced.

Procedure:

A. A pure colony of bacteria cultured on nutrient agar was taken by sterilized loop and placed on glass slide
B. The two drops of 3% H_2O_2 was added onto colony of bacteria on the glass slide
C. The slide was then observed for bubble formation
D. Bubble was observed after a few second.

B. Principle and procedures of Indole test

The test was performed to examine the ability of bacteria to breakdown amino acid tryptophan into indole.

1. Inoculated the tube of tryptone broth with a small amount of a pure culture.
2. Incubated at 37°C for 24 to 48 hours.
3. To test for indole production, 5 drops of Kovac's reagent was added directly to the tube.
4. A positive indole test is indicated by the formation of a pink to red colour ("cherry-red ring") in the reagent layer on top of the medium within seconds of adding the reagent.
5. If a culture was indole negative, the reagent layer was remained yellow or be slightly cloudy.

   Indole positive bacteria: *E. coli, Vibrio cholera*

   Indole negative bacteria: *Klebsiella, Salmonella, Shigella* spp.

C. Principle and procedures for methyl red test

The methyl red test was used to test the ability of an organism to produce and maintain stable acid end products from glucose fermentation. Some bacteria were fermented a glucose and form stable acid product at 4.4 PH.

1. An isolate was inoculated into a tube with a sterile transfer loop.
2. The tube was incubated at 35 ºC for 2–5 days.
3. After incubation, 2.5 ml of the medium are transferred to another tube. Five drops of the pH indicator methyl red were added to this tube.
4. The tube was gently rolled between the palms to disperse the methyl red.
5. Enteric that subsequently metabolize pyruvic acid to other acids lower the pH of the medium to 4.2. At this pH, methyl red turned red, a positive test.
6. Enteric that subsequently metabolize pyruvic acid to neutral end products lower the pH of the medium to only 6.0. At this pH, methyl red was yellow, a negative test.

D. Procedures for Voges Proskauer (VP) test

1. One colony from the pure culture was suspended in VP/MR medium.
2. Incubated at 30-37ºC during 24-48 h.
3. 0.2 ml of 40% KOH and then 0.6 ml of alpha-naphthol solution were added.
   - Positive test result: color change to pink.
   - Negative test result: no color change

E. Procedures for citrate test

1. A fresh (16- to 18-hour) pure culture was used as an inoculation source.
2. A single isolated colony was picked and lightly streak the surface of the slant. A needle was the preferred sampling tool in order to limit the amount of cell material transferred to the agar slant.
3. Liquid cultures as the inoculum source were avoided. Citrate utilization requires oxygen and thus screw caps, if used, should be placed loosely on the tube.
4. Incubated at 35 ºC (+/- 2 ºC) for 18 to 48 hours. Some organisms may require up to 7 days of incubation due to their limited rate of growth on citrate medium. Citrate

positive: growth was visible on the slant surface and the medium was an intense Prussian blue.

5. The alkaline carbonates and bicarbonates produced as by-products of citrate catabolism raise the pH of the medium to above 7.6, causing the bromothymol blue to change from the original green colour to blue.
6. Citrate negative: trace or no growth was visible. No color change will occur; the medium will remain the deep forest green color of the uninoculated agar.
7. Only bacteria that can utilize citrate as the sole carbon and energy source was able to grow on the Simmons citrate medium, thus a citrate-negative test cultures were virtually indistinguishable from an uninoculated slant.
    - Citrate positive bacteria: *Klebsiell*a spp.
    - Citrate negative bacteria: *E. coli.*

Appendix 8: Antibiotic discs used during the AMS profile of *E. coli* O157:H7 with their respective concentrations and its interpretation.

Antimicrobial agent	**Symbol**	**Disc Content**	**Zone of inhibition in millimeter (mm) with its interpretation**		
			Susceptible	**Intermediate**	**Resistant**
Erythromycin	E15	15 µg	≥23	14-22	≤13
Amoxicillin	AML2	2 µg	≥18	14-17	≤13
Penicillin	P10	10 unit	≥15	-	≤14
Tetracycline	TE30	30 µg	≥15	12-14	≤11
Gentamycin	CN10	10 µg	≥15	13-14	≤12
Kanamycin	K30	30 µg	≥18	14-17	≤13
Sulfamethoxazole/ Trimethoprim	SXT25	25 µg	≥16	11-15	≤10
Amikacin	AK30	30 µg	≥17	15-16	≤14

Source: (CLSI, 2022)

Appendix 9: Informed consent, observational check lists and questionnaire to assess hygienic and sanitary practices of meat handling in Burayu abattoir and butcher shops.

A. Informed Consent

Isolation and antibiogram of *E. coli* O157:H7 from cattle carcass in abattoir and butcher shops Burayu town, Oromia, Ethiopia."

CONSENT FORM

==

Good morning/good afternoon!

I am an MSc student at the College of Veterinary Medicine and Agriculture at Addis Ababa University and I am conducting research for my thesis on ''**Isolation and antibiogram of *E. coli* O157:H7 from cattle carcass in abattoir and butcher shops Burayu town, Oromia, Ethiopia**." The purpose of this study is to know the occurrence of *E. coli* O157:H7, effective drug to treat *E. Coli* O157:H7 and to assess hygienic and sanitary practices of meat handling in abattoir and retailer shops in Burayu. I would like to know your thoughts on hygienic and sanitary practices of meat handling in abattoir and retailer shops. I believe that this study will be very helpful in knowing the Occurrence and hygienic practices of meat handler and factors that contribute to it. It will produce original data that will be a tremendous help in informing future action by the public sector and others stakeholders. In light of this, it is crucial that you provide honest answers to all of the questions. Your participation in this study is entirely voluntary, and there are no personal gains or risks for you as a result. I guarantee that your response will be kept private and that any data gathered about your personal identify won't be disclosed to a third party as is standard research procedure. During the report or presentation of this study, no one will learn your identity in relation to specific questions and answers.

Respondent statement: I have understood the above statements:

1. Yes (Agree to participate)
2. No (Not agree to participate)

Name of respondent: ____

Signature: __________________

Date: ______________________

B. Observational check lists

Source	Personal observations	Status
Abattoir	Source of water	Borehole /municipal/river
	Hygiene of premises	Good /medium/poor hygiene
	Toilet	Proper defecation and urination to the hole/not
	Slaughterhouse partition	Partition /partially partition/ No at all
	Floor and ceiling	Concrete and roof/other type and no roof
	Wasta management	Good /medium/poor sewage management
		Leftovers on the premises/ properly managed
	Carcass dressing	On the floor /hanging
	Sink	Available /not available
	Wash hands with soap	Yes/ no
	Carcass transportation	Closed vehicle /cart/open car
Butcher shop	Source of water	Borehole /municipal/river
	Hygiene of premises	Good /medium/poor hygiene
	Collect money	Yes/no
	Floor and ceiling	Concrete and roof/other type and no roof
	Wasta management	Good /medium/poor sewage management
		Leftovers on the premises/ properly managed
	Bulbs	Present /absent
	Sink	Absent /present
	Cutting board	Wooden /plastic/others

C. Basic information

1. Questionnaire for meat handlers on hygienic practices at ABATTOIR

Date: ______________________________ Questionnaire Code: _______________

NO.	Questions	Answers
	1. General characteristics of individuals	
2.1	Age	_________________
2.2	Sex	Male [ ] Female[ ]
2.3	Level of Education:	Illiterate [ ] Informal Education [ ] Primary Education [ ] Secondary Education [ ] degree [ ] Other (Specify)………………
2.4	Your role at the abattoir/?	Veterinarian/meat inspector [ ] Butchers [ ] cleaner [ ] , load carcass [ ] Other (specify)
2.5	Work experience in years/month	---------------------
	2. Possible risk factors for contamination of carcass during slaughter process	
3.1	Stunning before slaughter	Yes [ ] No[ ]
3.2	How long waited to start flaying after stunning?	Hrs---------------------------------
3.3	Method of carcass dressing?	Vertical (hanging) [ ] Horizontal(on floor)[ ]

3.4	Do you use the following protective materials while working in the abattoir? (observe)	

Response	Protective materials				
	Apron	White coat	Head cover	Gloves	Boots
Yes					
No					

3.5	Do you have sink for washing hands in the abattoir?	Yes [ ] No [ ]
3.6	Do you wash your hands before touching the carcass?	Yes [ ] No [ ]
3.7	Do you wash your hands with soap?	Yes [ ] No [ ]
3.8	Do you use the same knife for flaying and evisceration?	Yes [ ] No [ ]
3.9	Do you wash your hands after evisceration?	Yes [ ] No [ ]
3.10	Do you sink knife in hot water in between flaying and evisceration?	Yes [ ] No [ ]
3.11	Is carcass washed after evisceration?	Yes [ ] No [ ]
3.12	What do you think the possible sources of contamination of carcass?	Feces during evisceration [ ] hides during flaying [ ] handlers hand [ ] knife[ ] floor [ ] hanging hook[ ] Others(specify)
3.13	What is your source of water for use in the abattoir?	City/Municipal council [] borehole [ ] rain collected water [ ] River [ ] others (specify) [ ]
3.14	Have you ever received any training on hygienic handling of carcass?	Yes [ ] No[ ]
3.15	Have you gone for medical checkups to work at the abattoir?	Yes [ ] No [ ]
3.16	How frequent you go for medical checkup?	Once per year [ ] Every three months [ ] Every six months [ ] others(specify)--

3.17	Do you know any disease caused by consumption of raw meat?	Yes [ ] No [ ]
3.18	What type of symptoms you observe?	Diarrhea, headache, kidney disease, any other (specify)
3.19	Do you think improvement needed to avoid contamination of carcass at the abattoir?	Yes [ ] No [ ]

2. Questionnaire for meat handlers on hygienic practices at BUTCHER SHOPS

Date______________________ Questionnaire Code__________

No	**Questions**	**Response**
	1. General characteristics of individuals	
2.1	Age	----------
2.2	Sex	Male [ ] Female[ ]
2.4	Level of Education:	Illiterate [ ] Informal Education [ ] Primary Education [ ] Secondary Education [ ] Other (Specify)………
2.5	Duration of selling meat in retail outlet? /Experience	
	2. Possible risk factors for contamination of meat at retail market	
3.1	What is the means of transporting meat from abattoir to the retail shop?	Open vehicle [ ] Closed vehicle[ ] Cart horse) [ ]
3.2	Is there any cover on display case?	Yes [ ] No [ ]
3.3	Is retail shop floor is made of concrete?	(observe) [ ]Tile [ ]wood earthen material[ ] others(specify)

<table>
<tr><td>3.4</td><td>Wall and ceiling are clean or free of dust</td><td>(observe) Yes [ ] No [ ]</td></tr>
<tr><td>3.6</td><td>If yes, is there sign of dirty on the wall?</td><td>Yes [ ] No [ ]</td></tr>
<tr><td>3.7</td><td>What is the ventilation status of display case and butchery</td><td>(observe) Good [ ] Fair [ ] Poor [ ]</td></tr>
<tr><td>3.8</td><td>Is there use of bulbs at the display case</td><td>((observe) yes [ ] No [ ]</td></tr>
<tr><td>3.9</td><td>Are there meat cooling facilities at the display cabinet?</td><td>(Observe) Yes [ ] No [ ]</td></tr>
<tr><td>3.10</td><td>Do you have a refrigerator for storage of the meat remains from daily sale?</td><td>Yes [ ] No [ ]</td></tr>
<tr><td>3.11</td><td colspan="2">
<table>
<tr><td rowspan="4">Do you use the following protective materials while selling or handling meat? (observe)</td><td rowspan="2">Response</td><td colspan="3">Protective materials</td></tr>
<tr><td>Apron/white coat</td><td>Head cover</td><td>Gloves</td></tr>
<tr><td>Yes</td><td></td><td></td><td></td></tr>
<tr><td>No</td><td></td><td></td><td></td></tr>
</table>
</td></tr>
<tr><td>3.12</td><td>How frequent do you wash the protective (white coat and Apron)?</td><td>Once per day in the evening [ ] Twice per day, morning and evening [ ] once after every two days [ ] once per week [ ] others [ ]</td></tr>
<tr><td>3.13</td><td>Do you have sink for washing hands</td><td>Yes [ ] No [ ]</td></tr>
<tr><td>3.14</td><td>Do you wash your hand before touching the meat?</td><td>Yes [ ] No [ ]</td></tr>
<tr><td>3.15</td><td>Do you wash your hand with soap</td><td>Yes [ ] No [ ]</td></tr>
<tr><td>3.13</td><td>What is your source of water for use in the butchery?</td><td>Municipal water [ ] borehole [ ] rain collected water [ ] River [ ]</td></tr>
<tr><td>3.14</td><td>What kind of cutting board you are using?</td><td>(Observe) Wood [ ] plastic [ ] Metal [ ] concrete [ ] Marble [ ]</td></tr>
<tr><td>3.15</td><td colspan="2">How often do you wash the following butchery surfaces and equipment’s?</td></tr>
</table>

Frequency of wash	**Equipment's /surfaces**					
	Knife	Cutting boards	Saw/Axes	Display cabinet	Hooks	Floors
Once per day in the morning						
Once per day in the evening						
Twice per day						
More than twice						
Once in every two days						
Others (specify)						

3.16	Do you use detergent/disinfectant for cleaning the butchery utensils?	Yes [ ] No [ ]
3.17	If "Yes" what types of detergent/ disinfectant do you use	
3.18	Do you sterilize your equipment's	Yes [ ] No [ ]
3.19	If "Yes" what are the methods used to sterilize the equipment	____________________
3.20	Do you have any hot water baths for dipping of knives?	Yes [ ] No [ ]
3.21	Ways of cleaning butchery equipment's	cold water only [ ], cold water with soap [ ] hot water only [ ] hot water with soap [ ] wiping with pieces of fabrics [ ] others (specify)……..
3.22	Do you have routine control of flies in your butcher?	Yes [ ] No [ ]
3.23	If "Yes" what are the methods used to control flies?	_____________________
3.24	How long does the meat stay in your butchery before it is over?	Less than 12 hours [ ] one day [ ] Two days [ ]

3.25	Material to wrap meat for sale.	Newspaper [ ] Plastic [ ] Used paper[ ] Others[ ]
3.26	Do you collect money while handling or selling meat?	Yes [ ] No [ ]
3.27	Have you ever received any training on hygienic handling of meat?	Yes [ ] No [ ]
3.28	Do you ever receive complaints from the consumers on the quality of your meat?	Yes [ ] No [ ]
3.29	Do you know any disease caused by consumption of raw meat?	Yes [ ] No [ ]
3.30	What type of sign and symptoms?	Diarrhoea, headache, kidney disease, any other (specify)
3.31	How frequent you go for medical check-up?	Once/ year [ ] every 3 months [ ] every six months [ ]
3.32	Do you have different storage and display cabinets for offal's and meat?	(observe) Yes [ ] No [ ]
3.33	Do you use the same equipment while handling meat and the offal's?	Yes [ ] No [ ]
3.34	Do you believe that your butchery some improvement for better handling?	Yes [ ] No [ ]
3.35	If yes, what kind of improvement?	________________

Appendix 10: Ethical clearance

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/10/02/15/2023

Name and affiliation of applicant: **Segni Bedasa Gudina (DVM, MSc student)**
Department of Microbiology, Immunology and Veterinary Public Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *isolation and molecular characterization and antibiotic susceptibility profile of Escherichia coli O157:H7 from abattoir carcasses and butcher shops in Burayu town, Oromia-Ethiopia*

Date of application:	**December, 2022**
Nature of the project:	**Field investigation**
Target animal species:	**No live animal involved**
Number of animals involved:	**None**
Study area:	**Burayu, Ethiopia**

Minutes No. and date of review: **VM/ERC/02/15/022, 23/12/2022**

The Animal Research Ethical Review Committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University has reviewed the above research project and unanimously approved the application of **Segni Badasa.**

Professor Getachew Terefe (DVM, PhD)
Chairman

The Federal Democratic Republic of Ethiopia
Addis Ababa University
College of Veterinary Medicine and Agriculture

Signature

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Please quote Our Ref. No. When replying

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