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EPIDEMIOLOGY OF *ESCHERICHIA COLI* O157: H7 AND ITS ANTIMICROBIALS SUSCEPTIBILITY PROFILE IN BEEF AT ADDIS ABABA, ETHIOPIA

MSc THESIS

BY

GEMECHIS TEGEGN TILAHUN

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BISHOFTU, ETHIOPIA

EPIDEMIOLOGY OF *ESCHERICHIA COLI* O157: H7 AND ITS ANTIMICROBIALS SUSCEPTIBILITY PROFILE IN BEEF AT ADDIS ABABA, ETHIOPIA

A Thesis Submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Veterinary Epidemiology

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by **Gemechis Tegegn Tilahun,** entitled '**Epidemiology of *Escherichia coli* O157:H7 and Its Antimicrobial Susceptibility Profile in Beef in Addis Ababa, Ethiopia',** And recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters of Veterinary Science in Veterinary Epidemiology.

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STATEMENT OF THE AUTHOR

I affirm that this research is solely my own work and that all sources of materials used in this thesis work have been properly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for the Master of Veterinary Science in Veterinary Epidemiology degree at Addis Ababa University's College of Veterinary Medicine and Agriculture, and it has been deposited in the College library to be made available to borrowers in accordance with the Library's rules. I solemnly declare that I am not submitting my thesis to any other institution anywhere for the award of any academic reward.

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LIST OF ABBREVIATIONS

CDC	Centers for Disease Control and Prevention
CFSPH	Center of Food Security and Public Health
CFU	Colony forming units,
DAEC	Diffuse-adherent
DALYs	Disability Adjusted life years
DNA	Deoxyribonucleic Acid,
EAggEC	Entero aggregative *E. coli*
EHEC	Enter hemorrhagic *E. coli*
EIEC	Entero invasive *E. coli*
ELISA	Enzyme-linked immunosorbent assays
EPEC	Enteropathogenic *E. coli*
ETEC	Eenterotoxigenic *E. coli*
EU	European Union
FAO	Food and Agriculture Organization
GMPs	Good Manufacturing Practices
HACCP	Hazard Analysis Critical Control Point
HUS	Hemolytic uremic syndrome
IMS	Immunomagnetic separation
LEE	Locus for enterocyte effacement
NSF	Non-Sorbitol fermenting
OIE,	World Organization for Animal Health
PCR	Polymerase chain reaction
RNA	Ribonucleic Acid
STEC	Shiga-toxin producing *E. coli*
Stx	Shiga toxins
Stx1	Shiga toxins 1
Stx2	Shiga toxins 2
TTP	Thrombotic Thrombocytopenic Purpura
VTEC	Verocytotoxin producing *E. coli*
WHO	World Health Organization

ABSTRACT

Escherichia coli O157:H7 spreads through the consumption of contaminated, raw beef. A cross-sectional study was carried out between November 2022 and June 2023 on 285 samples collected from butcher shops and abattoir to investigate the prevalence of *E. coli* O157:H7 and assess its antimicrobial susceptibility profile in slaughterhouse and butcher shops in Addis Ababa, Ethiopia. The 3M^{TM} Molecular Assay Detection E. coli O157 methods were used to detect *E. coli* O157 using the 3MDS protocol guide. Isolation and identification *E. coli* O157:H7 was by using Biolog GENIII Microplate system. Over all, the prevalence of *E. coli* O157:H7 in the abattoir and butcher shops was 6.32% at 95% CI [4.0–9.83] with a higher prevalence in the abattoir (12/150; 8.0%) than butcher shops (6/135; 4.44%). In the abattoir, *E. coli* O157:H7 was isolated from 12 (8%) at 95% CI [4.6, 13.6] samples, 10 (6.67%) from beef carcasses, 1 (0.67%) from workers' hands, and 1 (0.67%) from knife swabs. Animal-related risk factors (sex, age, breed, and body condition score) were not significantly associated with the occurrence of *E. coli* O157:H7 in the abattoir. The prevalence of *E. coli* O157:H7 in butcher shops was 4.44 % at 95% CI [1.99–9.63], and cutting board swabs (16%), carcasses (1.67%), and knife swabs (4%) were the most common sources. The test statistics association found a significant difference in the prevalence of *E. coli* O157:H7 among sample types from an abattoir and butcher shops (df = 7, p = 0.04). The disc diffusion method used to evaluate the antimicrobial susceptibility profiles of the *E. coli* O157:H7 isolates. Eight antimicrobials used to evaluate the antimicrobial susceptibility profile of 18 *E. coli* O157:H7 isolates. Accordingly, *E. coli* O157:H7 showed 100% resistance to amoxicillin, penicillin G, and vancomycin and 94% resistance to ampicillin. Moreover, both streptomycin and sulfamethoxazole trimethoprim showed 100% susceptibility. *E. coli* O157:H7 was also found to be susceptible to gentamicin and tetracycline (17/18) (94.44%) and 16/18 (88.9%), respectively. In the current study, samples from butcher shops and an abattoir were found to contain the human pathogenic *E. coli* O157:H7, which is resistant to many antibiotics. Therefore, coordination of efforts is required to minimize or eliminate the dangers that this organism poses at various points in the food chain. These can be prevented primarily by using antibiotics ethically and correctly handling and cooking animal products.

Key words: - ***E. coli O157:H7, beef, abattoir, butcher shop, prevalence, and antimicrobial susceptibility***

1. BACKGROUD

Foodborne infections are a major cause of disease and death, especially in developing nations. These infections result in substantial medical and economic expenses (Havelaar *et al*., 2015; Panwar *et al*., 2022). The risks of foodborne illnesses are increasing due to globalization, industry growth, and changing eating habits (Lineback *et al*., 2009; LaVeist *et al*., 2023). Meat can be contaminated with pathogenic bacteria, which can lead to health complications and pose food safety risks. Akbar *et al*. (2011); Ghattas (2014) Escherichia coli is a common component of the gut microflora of many normal animals and people. However, certain strains of *E. coli* have the potential to be pathogenic and result in infections that can be fatal (Belanger and colleagues, 2011).

Gram-negative *E. coli* O157:H7 is one of these pathogens and is classified as a food-borne pathogen. *Escherichia coli* O157:H7 typically results in human fatalities (Belanger and collaborators, 2011; Pang *et al*., 2018). *E. coli* O157:H7 is an emerging threat to public health in the majority of countries around the world, and cases have been documented on all continents except Antarctica (CFSPH, 2009; Kiranmayi *et al*., 2010). *E. coli* O157:H7 is thought to be the cause of 2.8 million acute foodborne illnesses annually, according to estimates (Majowicz *et al*., 2014).

E. coli O157:H7 is naturally found in the intestinal tracts of many farm animals, including healthy cattle, sheep, and goats, and it regularly leaks into the surroundings through waste effluent or feces (Ameer *et al*., 2018; Gambushe *et al*., 2022). The bacteria are mainly found in cattle (Martorelli *et al.,* 2015; Tourret *et al.,* 2016).

By handling the carcasses in an unhygienic manner during slaughter and distribution, microbes from the handlers, tools, and other carcasses might contaminate the meat (FAO, 1991). *Escherichia coli* can contaminate meat at any stage along the value chain, including during slaughter, transportation to retailers, handling by retailers' customers, and at retailers' stores (FAO, 2006).

Microbial contamination of carcasses may originate from feces (Fratamico *et al.*, 2006; Abdalla *et al.*, 2009). During handling and processing procedures such as skinning, storage, evisceration, and distribution at slaughterhouses and retail facilities, the skin of animals that are presented for slaughter may transfer to the corpse. According to several studies (Hiko and colleagues, 2008; Abdalla *et al*., 2009; Sodha *et al*., 2015), eating uncooked or undercooked meat of bovine origin has been the most common way to disseminate this bacterium. The high nutritional content of meat makes it the ideal environment for the development of bacteria like Escherichia coli (Feng and others, 2017).

Antimicrobial resistance is viewed as the third greatest public health threat of the twenty-first century from a larger viewpoint (Omulo *et al.,* 2015). The most commonly used veterinary medications to treat animal infections are antibiotics and anthelmintic. However, the widespread use of these medications might encourage the development of drug resistance (Tufa *et al.,* 2018). Bacterial resistance to antibiotics is an issue, and knowing how resistance develops and spreads can help create novel approaches to combat its clinical and socioeconomic implications, particularly in ruminant animals (Ebrahim & Farnaz, 2012). *E. coli* O157:H7's emergence as an antimicrobial-resistant strain is a growing concern and a new public health problem (Newell *et al.,* 2010). Through the beef food chain, pathogenic *E. coli* O157:H7 that is resistant to antibiotics that used in food cattle can infect people (Akbar *et al.,* 2014).
Escherichia coli O157:H7, which produces the Shiga toxin, has been found in animal products, particularly meat butchered in abattoirs, in Ethiopia (Abdissa et al., 2017; Haile *et al*., 2022).

According to studies, raw beef purchased from butcher shops in Ethiopia frequently contains *E. coli* O157:H7, with prevalence rates ranging from 0.8% to 21.9% (Haile et al., 2022). Among the reported prevalence are Hawassa (2.4% overall) by Atnafie *et al.* (2017), Addis Ababa (10.2%) by Bekele *et al.* (2014), Jimma (9.3%) from carcass swabs and 7.3% cecal content samples by Feleke *et al. (*2017), and Dire Dawa (2.06%) from cattle raw meat samples by Edget *et al.* (2017). In addition to these, there are also a few reports of antimicrobial resistance to the zoonotic *E. coli* O157:H7 strains isolated from raw meat samples at abattoirs. According to Darwish *et al. (2013),* tetracycline, beta-lactams, chloramphenicol, quinolones, nitro furans, and macrolides are among the antibiotics that overused in Ethiopia and other African nations. Tetracycline levels have been discovered to be very high and above WHO standards in meat and kidney samples from numerous abattoirs in Ethiopia. Feleke *et al.* (2017)

have reported resistance to various antibiotics, such as erythromycin (100%), ampicillin (83.3%), and nitrofurantoin (50%), on *E. coli* O157:H7 isolated from samples at Jimma.

According to Bello *et al.* (2015) and Dulo *et al.* (2015), animal slaughter frequently takes place in unhygienic settings in developing nations like Ethiopia. In Ethiopia, raw meat sold in local, open-air stores without proper temperature regulation, where customers can either buy it to take home or eat there. Throughout the nation, consumers serve minced meat, known locally as "Kitfo," raw or barely cooked (Avery, 2004). As a result, the microbiological quality and safety of the meat derived from the animals are further compromised (Bello *et al.,* 2015 and Dulo *et al.,* 2015).
Escherichia coli still poses a health risk despite the sanitation procedures municipal abattoirs have in place to lessen contamination (Atnafie *et al., 2017).* The availability of information on the use of antibiotics by people in central Ethiopia who raise food animals is very limited (Tufa *et al.,* 2018).

Knowing what factors increase the prevalence of *E. coli* O157:H7 in abattoirs and detecting drugs that are susceptible to the bacteria strain are two excellent ways to manage and reduce risks to both human and animal health. However, only a few studies have looked at *E. coli* O157:H7 epidemiology and antibiotic resistance patterns in Ethiopia. Therefore, the general objectives of the study were estimate the prevalence of *E. coli* O157: H7 and its antimicrobial susceptibility profile in beef at the Addis Ababa Ethiopia

The specific objectives of the study were:

- To estimate the prevalence of *E. coli* O157:H7 in the abattoir and butcher shops in Addis Ababa
- To identify the major risk factors for *E. coli* O157:H7
- To assess the antimicrobial susceptibility profile of *E. coli* O157:H7
- To identify the knowledge gaps of employees and evaluate the current hygienic practices in the main Addis Ababa abattoir and the beef retail butcher shops in Addis Ababa city

2. EPIDEMIOLOGY OF *ESCHERIA COLI* O157: H7

2.1. Historical background of *E. coli* O157:H7

German microbiologist Theodor Escherich first discovered Escherichia coli in 1885 after isolating it from a child's excrement under the name "Bacterium coli commune" (Escherich, 1885). According to Riley (2014) and Sewlikar and D'Souza (2017), two outbreaks of *E. coli* O157:H7 in Oregon and Michigan led to the discovery of the pathogen in 1982. The *E. coli* strain O157:H7 was responsible for three hemorrhagic colitis (HC) outbreaks in North America this year, two of which included ground beef sandwiches at fast food establishments in Oregon and Michigan and one at an Ontario, Canada, and nursing home. In nursing homes, two outbreaks with a common source presumably caused by food. 66 cases of hemorrhagic colitis, 12 cases of hemolytic uremic syndrome (HUS), and 17 fatalities were reported in Canada between the years 1983 and 1985 (31 cases in 1983 and 73 in 1985) (Carter *et al.,* 1987).

There was a report of an outbreak in central Scotland at the end of 1986, which led to the deaths of 21 persons and the illness of more than 500 others. In terms of human morbidity and mortality, this was one of the deadliest food born outbreaks ever recorded. According to Griffin and Tauxe (1991), items from cattle have been linked to about 52% of known human disease outbreaks. Since then, *E. coli* O157: H7 and a few other serotypes have been responsible for significant human sickness outbreaks with high rates of morbidity and mortality all over the world (Constable *et al.,* 2017).

2.2. Etiology

According to (Farrokh *et al. (2012),* Xia *et al. (2010),* and *the* CDC (2015) the infection is caused by Escherichia coli, gram-negative, which is a member of the phylum Proteobacteria and the family Enterobacteriaceae. The Enteric disease-causing pathogenic strains can be categorized into six groups, such as enteroaggregative (EAggEC), diffuse-adherent (DAEC), enter hemorrhagic (EHEC), enter toxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), and enteropathogenic (EPEC). Each of these groups has a unique collection of O-H serotypes, and they vary in terms of pathogenesis and severity. In a pathogenic subset of EHEC, *E. coli*

O157:H7 is the most prevalent and virulent serotype. According to Chapman *et al.* (2001), *E. coli* O157:H7 is known as such because it produces the 7th H antigen and the 157th O antigen. Escherichia coli O157:H7 is classified as non-sorbitol fermenting (NSF), indole positive, catalase +ve, oxidase (-ve), urease negative, Voges-Proskauer (-ve), and citrate (-Ve), according to Rosser *et al.* (2008).

2.3. *E. coli* O157:H7 food borne illness

Numerous unique diseases have emerged because of the food industry's changing production techniques. These comprise previously unidentified pathogens, pathogens that becoming known as a source of food-borne diseases, and pathogens that are growing and becoming more potent (Mor-Mur and Yuste, 2010). *E. coli* O157:H7 has caused serious illnesses in humans like hemorrhagic colitis, hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP) since it was first described in 1982 (Chekabab *et al.*, 2013; Pal and Mahendra, 2016). It is now a significant global zoonotic food- and water-borne pathogen.

Foodborne *E. coli* O157:H7 diseases are associated with food handling practices, including food processing and packing, or the importation of particular foods from a new region. According to Robinson *et al.* (2007), outbreaks involving ground beef were more common in settings like restaurants and schools.

Escherichia coli O157:H7 is a growing public health issue in most of the world's countries (Kiranmayi and colleagues, 2010). Food-borne *E. coli* O157:H7 estimated to cause 2.8 million serious infections annually, as reported by Majowicz *et al.* (2014). According to Scallan *et al.* (2011), the pathogen believed to be responsible for over 60,000 illnesses each year in the United States, 2,000 hospitalizations, and 20 fatalities. This causes a financial cost of $607 million (Scharff, 2012), which includes $370 million for preventable deaths, thirty million dollars for health services, and $5 million for decreased productivity.

2.4. Global Epidemiology

Except for Antarctica, every continent has recorded cases of *E. coli* O157:H7 infections (CFSPH, 2009). According to Parsons *et al.* (2016), Escherichia coli (STEC) is the cause of many outbreaks of gastrointestinal illnesses that have been recorded worldwide. Since its official acknowledgment in 1982, it has grown to be a significant issue in Australia, North America, Europe, South Africa, and Japan. In North America, Japan, and the UK in particular, O157:H7 is the *E. coli* serotype that is most frequently connected to clinical disease in people. High rates are present in regions of South America where HUS is an endemic illness, such as Argentina (Constable and colleagues, 2017). The meta-analysis study on the incidence of *E. coli* O157:H7 found regional variations in prevalence and calculated that the pathogen was prevalent in cattle with a prevalence of 5.68% over the world.

Table 1: *E. coli* O157:H7 prevalence estimates per area of the world in cattle

World	Sample number	Positive cattle number	Total study	Pooled Estimated (%)
World estimate	220,427	12,683	140	5.68
Africa	626	118	4	31.20
Asia	14,916	937	22	4.69
Europe	88,643	5,425	53	5.15
Latin America and Caribbean	4313	73	11	1.65
Northern America	11,0641	6,059	46	7.35
Oceania	1,288	71	4	6.85

Source: - Islam and colleagues (2014).

Several African countries, such as South Africa, Swaziland and the Central African Republic, Ethiopia, Kenya, Uganda, Gabon, Nigeria, and the Ivory Coast have reported cases of *E. coli* O157: H7 diarrhea (Raji and collaborators, 2006

2.4.1. Hosts of E. coli O157 reservoirs

According to Tourret *et al.* (2016), cattle are the main source of *E. coli* O157:H7 in livestock, making ground beef and beef products significant sources of foodborne transmission. Currently, cattle are thought to be a significant source of the human disease-causing *E. coli* O157, and there are many different ways that it can spread. Bovine excrement can pollute drinking water and crops meant for human use, in addition to meat and dairy products. Because of contamination with animal feces, several outbreaks have been linked to vegetable products like radish and apple cider (CFSPH, 2009).

2.4.2 The source of disease

Domesticated healthy ruminants, primarily cattle but also sheep and possibly goats, are the primary shedders and carriers of EHEC (Su, 2012). According to Callaway *et al.* (2009), the most prevalent causes of infection are fresh goods contaminated with cow dung and dietary items made from cattle. Enter hemorrhagic pathogens are transferred from cattle's hides to carcasses, infecting them. Direct and indirect fecal contamination causes hide pollution in areas where cattle are raised and butchered (Arthur *et al.*, 2010).

The oral-fecal route serves as the mode of transmission. According to Sodha and colleagues (2015), consuming contaminated food or drink is the most common way that *E. coli* O157:H7 diseases are spread. The consumption of undercooked meat has frequently been associated with this. According to Catford *et al.* (2014), the consumption of polluted and wrongly cooked beef mince is associated with a great deal of human illness.

On the other hand, there is rising worry about the risk of catching a disease from being in close proximity to animals and waste at dairy farms and petting zoos (Constable *et al.*, 2017). Disease transmission, however, is increasingly a problem at dairy farms and petting zoos because of direct contact with animals and waste (Constable *et al.*, 2017). It can also spread from person to person or from a sick animal to a human. In addition, the disease can be spread through mechanical vectors like birds and insects. Consuming meat that has been undercooked or raw is one of the causes of food-borne *E. coli* O157:H7, as reported by Hubalek and Rudolf (2010).

E. coli O157:H7 primarily found in cattle feces. Studies (Gordillo *et al.,* 2011; Hubalek and Rudolf, 2010; Su *et al*., 2012; Dontorou *et al*., 2003) have also found it in the feces of other animals, including goats, lambs, and horses. The main risk factor for human infection during the process of slaughtering at processing plants is carcass contamination, which takes place when the disease passes from skin to carcass or feces to carcass. In contrast to *E. coli* O157:H7, which needs inoculation of 10 to 100 CFU to trigger disease, butcher shops and restaurants are frequently mentioned as sources of *E. coli* O157:H7 that cause illnesses in humans, according to Arthur *et al.* (2017) and Fink *et al.* (2017).

2.4.3. Pathogenesis

E. coli O157:H7 produces the cytolethal Shiga toxins Stx1 and Sxt2, which make it virulent due to its ability to adhere to and escape the intestinal epithelium, after the affected person eats the contaminated food, *E. coli* O157: H7 lives in the stomach's acidic environment and begins to infect the body (Robinson and McKillip, 2010).

E. coli O157:H7 must initially attach to the microvilli of the host epithelial cells (Mainil and Daube, 2005). It is believed that the bacterial proteins intimin and translocated intimin receptor (Tir), which are inserted into the host membrane and function as the intimin response and mediate adhesion between cells of mammals and affixing and effacing (A/E) pathogens, are in charge of the bacterium's close attachment to the cell. The A/E lesion and diarrhea must have timi, a bacterial outer membrane adhesin, to develop (Constable *et al.*, 2017). How *E. coli* O157:H7 grows and sustains colonization in its host is still a mystery. *E. coli* O157:H7 produces and releases toxins once it has successfully established itself and grown inside the host.

From intestinal epithelial cells, Stxs can move into the blood. The Gb3receptors on glomerular endothelial cells are where the Stxs bind in this instance. The Stxs damage the glomerular cells and lead to the deposition of platelets and fibrin within the glomeruli. According to Welinder-Olsson and Kaijser (2005), the deposits eventually reduced renal filtration and caused acute kidney damage indicative of HUS.

2.4.4. Disease pattern

Humans with this organism suffer from hemorrhagic colitis, an acute illness. Watery and/or bloody diarrhea, fever, nausea, excruciating stomach cramping, and vomiting are symptoms that are typical of this illness (Walker *et al.*, 2012). *E. coli* O157: H7 *can* infect people 8 hours to 16 days after consumption, although the median incubation time is 3 to 4 days (Robinson and McKillip, 2010), and the sickness typically lasts 5 to 10 days. Some victims, especially the very young, may develop hemolytic uremic syndrome (HUS), which can have life-threatening complications (Martorelli *et al.*, 2017).

Up to 15% of people who experience hemorrhagic colitis develop HUS, which is characterized by hemolytic anemia and renal failure. HUS can result in a permanent loss of kidney function. STEC infection can affect people of any age. However, younger people and older people are more prone to symptoms, which can become more severe. (FDA, 2012) According to Sewlikar and D'Souza (2017) and Chekabab *et al.* (2013), thrombotic thrombocytopenic purpura (TTP) is most common in older patients who also have neurologic impairment and HUS.

The three components of hemolytic-uraemic syndrome (HUS) include micro-angio-pathichaemolytic anemia, acute uraemia, and thrombocytopenia. HUS is the most frequent cause of acute renal failure in children and causes high morbidity and death during the acute phase (Bayat *et al.*, 2012). The death rate varies with the syndrome in human clinical cases. Although deaths can happen, hemorrhagic colitis alone typically has a self-limiting course. Children, the elderly, immunosuppressed patients, and those with life-threatening conditions are at an increased risk of complications and mortality. According to estimates, 1%–10% of youngsters and up to 50% of older people dies from infection-associated HUS. For all known EHEC infections under European surveillance, the case fatality rate was 0.5% (CDC, 2016).

2.4.5. The contribution of cattle to human E. coli O157:H7 disease

Cattle play a significant role in the epidemiology of E coli O157:H7 infections in humans and feces from cattle are the primary source of the disease's contamination of beef products. When the first *E. coli* O157: H7 outbreaks in humans were linked to the consumption of ground beef in 1982, it became clear that cattle were an important reservoir for the illness as more outbreaks were linked to undercooked beef and other bovine products, including unpasteurized milk (CDC, 2017). The association between *E. coli* O157 stimulated studies into the role of cattle as a reservoir for infections: H7 and underdone ground beef and raw rice (Pal and Mahendra, 2016).

The majority of these disease outbreaks related with beef, particularly ground beef, which makes sense given that cattle are the key cause of *E. coli* O157: H7. A research done in the US between 2003 and 2012 found that there were 353 outbreaks, 20% of which spread by consuming beef and beef products. According to studies, food products having a bovine origin are responsible for over 75% of human *E. coli* O157:H7 epidemics (Callaway *et al.*, 2009).

The prevalence of beef and dairy cattle among domesticated animals, as well as the sizeable output of bovine feces, may be all that is necessary to indicate that there is a high likelihood of transmission of pathogens from cattle to humans in the presence of the *E. coli* stains O157:H7 isolate in cattle. According to Heiman *et al.* (2015), cow density directly correlated with both the frequency of *E. coli* O157: H7 infections in humans and the proportion of cattle to people. According to Callaway *et al.* (2009), the risk rose by 68% in Germany for every additional 100 cattle per square kilometer. In Ontario, the relationship held regardless of the presence of sheep or goats. For all human cases of gastroenteritis caused by STEC, this correlation still holds true.

Due to the lack of a Stx-specific globotriaosylceramide receptor in intestinal mucosal cells, adult cattle exhibit symptoms of *E. coli* O157:H7 colonization (Verstraete *et al*., 2014). When cows shed 104 CFU of *E. coli* O157:H7 per gram of waste, they are known as "Super Shedders." Super shedders have a substantial impact on the disease's spread in cattle, which serve as the primary reservoir for EHEC, raising the chance that people may become infected (Chase-Topping *et al*., 2008).

2.4.6. E. coli O157: H7 detection

Analyzing food and environmental samples can help identify the infection's source. Clinical conditions can be determined by identifying the bacteria present in fecal samples. *E. coli* O157:H7 diagnosed in a wide range of diagnostic methods. According to the CDC (2016), no single technique can successfully isolate every EHEC serotype. From diarrhea and hemorrhagic colitis to the potentially fatal hemolytic uremic syndrome (HUS), infection with this toxin linked to a wide range of illnesses (Rahal *et al*., (2012).

Stools from individuals with human hemolytic-uremic syndrome, predictable dietary elements in both animal and human food, and samples from foodborne outbreaks are a few examples of typical sample types (Elhadidy *et al*., 2015). Rectal swabbing, which takes a direct sample of the intestinal mucosa at the recto-anal junction of the animal, is the most precise method of animal sampling for STEC O157:H7 (Constable *et al*., 2017).The detection of *E. coli* in feces, food, and water has become simpler thanks to immunoassays and polymerase chain reaction technology. Additionally included in this group are PCR and DNA-based techniques, ELISAs and immune-magnetic separation (Bavaro, 2009).

Molecular-based techniques offer a substantial advantage because of their, sensitivity selectivity, and short turnaround times. However, molecular-based plating technologies are substantially more costly, unique, and unknown when compared to traditional plating processes. As a result, the food processing facility's overall demands and resources (Robinson and McKillip, 2010) influence the use of molecular-based techniques in quality control procedures. The Latex Agglutination Test is another option for the rapid detection of *E. coli* O157: H7. When coupled with Sorbitol MacConkey Agar, the test yields the best results. The test reagent should produce agglutination, while the control reagent should appear milky and smooth, according to Al-Dragy and Baqer (2014).

2.4.7. E. coli O157: H7 situation in Ethiopia

In Ethiopia, research was done to find out how frequently and how much *E. coli* O157: H7 was found in feces, skin swabs, and carcasses of sheep, goats, and cattle in different parts of the country. Table 2 summarizes the frequency of *E. coli* O157:H7 in the cattle supply chain.

Table 2: Research on *E .coli* O157: H7 in beef samples and related environmental samples in Ethiopia

Study Area	Sample Unit	Sample Type	Prevalence	References
Bedelle	Bovine Carcass Worker hand knife	feacal samples, carcass swabs, butcher hand swabs knife swabs were	4.7%, 3.3%, 1.1% 1.1%	Fikadu *et al.*, 2023
Hawassa	Cattles Butcher shop Meat hander Knifes	Swab (knife Cloth of meat transporter)	2.4%	Atnafie *et al.*, 2017
Addis Ababa Debre Berhan	Cattle Butcher shop	Fecal Skin swab Intestinal mucosal swabs Internal carcass swabs	2% 0.5% 0.8% 0.5%	Abdissa*et al* ., 2017
Dire Dawa	Cattle	Raw meat	2.06%	Edget *et al.*, 2017
Jimma	Cattle	Carcass swab Cecal content	9.3% 7.3%	Feleke *et al.*, 2017
Debre Zeit Addis Ababa		Carcass swab	5.5%	Tassew, 2015
Addis Ababa	Cattle	Beef	10.2%	Bekele *et al.*, 2014
Addis Ababa	Cattle	Carcass swab	0.72%	Haile, 2014
Haramaya	Cattle	Carcass swab	2.65%	Taye *et al.*, 2013
Modjo	Cattle	Raw meat	4.2%	Hiko *et al.*, 2008

2.4.8 Treatment, Prevention and Control

Antibiotics used to treat *E. coli* O157:H7 infections are linked to a higher risk of serious complications including HUS (Rahal *et al.*, 2012), and they may make the patient's condition worse by causing more preformed Shiga toxins (Stx) to be released after cell lysis. However, certain antibiotics can be successful when given early (Nassar *et al.*, 2013). It has been demonstrated that two therapy strategies—avoiding the use of antibiotics during the pre-hemolytic uremic syndrome phase and hospitalizing patients—increase the chance of successful results (Davis *et al.*, 2013). The requirement for intensive care, such as dialysis, blood transfusions, and/or platelet infusions, in addition to kidney transplantation may be necessary for patients who have problems (CFSPH, 2009).

It will be possible to stop the spread of *E. coli* O157: H7 by often washing your hands after using the toilet, before preparing or eating food, and after coming into contact with animals. It is vital that food be handled properly and that areas are kept clean. Eat only pasteurized dairy products, stay away from consuming raw meat, and fully cook all meats to a temperature of at least 160°F/70°C (Mathusa *et al*., 2010). The potential to establish control measures that lessen the numerous effects of zoonoses in both human and animal populations is one health strategy. More efficient and financially feasible approaches to disease management may be provided by interventions that limit zoonotic infection in animal populations or stop illness from spreading from animals to people (Halliday *et al.*, 2015). Cattle EHEC O157: H7 vaccines may reduce shedding and have obtained full or provisional approval in several nations, including the U.S. and Canada, although they are not widely used. A vaccination cannot protect against human enter hemorrhagic Escherichia coli (EHEC) infections (Smith, 2014).

2.5. *Escherichia coli* O157:H7 antimicrobial resistance (AMR)

E. coli O157:H7 antibiotic resistance is a rising issue that poses significant issues for public health. (Newell and others, 2010) It is brought on by widespread overuse or abuse of antibiotics in both human and veterinary medicine, among other situations. According to Akbar *et al.* (2014), the use of antibiotics in food cattle promotes the growth of *E. coli* O157 H7 that is resistant-pathogenic and can infect people through the beef food chain.

E. coli O157:H7 frequently exhibits multidrug resistance to ciprofloxacin, ampicillin, chloramphenicol, amoxicillin, ceftriaxone, vancomycin, and methicillin (Constable *et al.*, 2017; Vijayarani *et al.*, 2010; Naik and Desai, 2012). In cattle, antibiotics are used to treat clinical sickness, stop and control the spread of common diseases, and advance animal growth. However, due to human antibiotic use and exposure to resistant bacteria found in the intestines of food animals, rising antibiotic usage has promoted the establishment and survival of resistant bacteria in people (McEwen *et al.*, 2002).

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in Addis Ababa in between November 2022 and June 2023 on samples collected from the main municipal abattoir and retail butcher shops. Addis Ababa is a grassland biome at an elevation of 2,355 meters (7,726 feet), and its coordinates are 9°1′48″N 38°44′24″E. The city is a part of the Awash watershed and is located at the base of Mountain Entoto. At its lowest point, the area around Bole International Airport, Addis Ababa rises to a height of 2,326 meters (7,631 feet), approximately 3,000 meters (9,800 feet), in the Entoto Mountains to the north. According to the most current population count, which was carried out in 2007 by the Ethiopian National Statistics Agency (CSA), Addis Ababa has a total population of 2,739,551 individuals in both urban and rural areas.

The Addis Ababa municipal enterprise has two slaughter stations: the main abattoir is located in Addis Ababa sub-city at specific place called 'Kera' and the branch abattoir is located in Addis Ababa sub-city Akaki kality, Akaki'. The investigation was conducted at the main abattoir, where 900–1200 cattle and 400–550 sheep are slaughtered each day. During Christian and Muslim holiday seasons, this number may rise to 2000 or even higher for cattle alone.

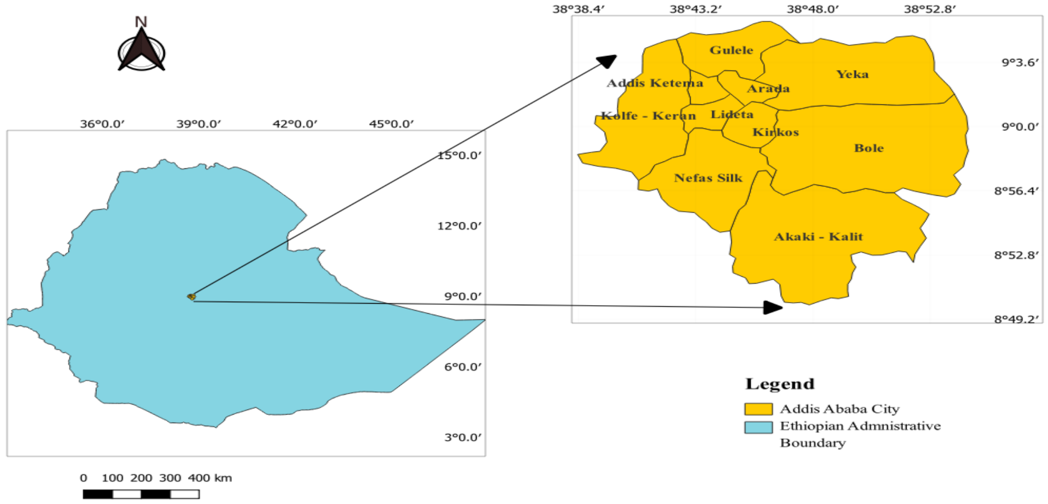

Figure 1:- Map of study Area

3.2. Study Design

A cross-sectional study was conducted to assess Epidemiology of *Escherichia coli* O157: H7 and Its Antimicrobials Susceptibility Profile in Beef at Addis Ababa, Ethiopia by using the samples sourced from the main Addis Ababa Municipal Abattoir Enterprise (AAAE) and the retail butcher shops in Addis Ababa city. A descriptive questionnaire surveys were also conducted to identify knowledge gaps and evaluate the current hygienic status at both sample sources.

3.3. Sample Size Calculations

The sample size was estimated by using a statistical technique provided by Thrusfield (2005) under the assumption of a 95% confidence interval and 5% precision.

$$n = \frac{(z)^2 * p^{exp}(1-p^{exp})}{d^2}$$

Where n is the required sample size.

Z is the value from the standard normal distribution that corresponds to the desired confidence level (Z = 1.96 for the 95% CI).

P^{exp} = expected prevalence

D = required absolute precision

Sample sizes were determined using previous studies. Haile *et al.* (2022) found 3.6% *E. coli* O157:H7 in samples from butcher shops in Addis Ababa city, while Hamid *et al.* (2018) found 6.4% *E. coli* O157:H7 in samples from abattoirs in the same city.

The previous studies had calculated 146 samples for the abattoir and butcher shops, with 92 for the abattoir and 54 for the butcher shops. In this study, 285 samples were used, 135 samples from 60 butcher shops and the 150 samples from one abattoir (the main abattoir) in Addis Ababa for microbiological samples analysis.

3.3. Sample Types and Study Population

At an abattoir, 60 cattle were selected by using systematic random sampling method in the slaughter hall, and their carcasses swabs were sampled after slaughter to assess the association between the animals' related risk factors (sex, age, breed, and body score condition) and *E. coli* O157:H7 in their carcasses after laboratory analysis. Ages of animals were determined according (Ensminger, 1983) and the Body Condition Score (BCS) of cattle w categorized according to (Phil, 2017). In addition to these at an abattoir, 90 environmental pooled swab samples from three sample types (workers hands, n = 30; knives, n = 30; vehicles or cars swabs). At retail butcher shops, 135 samples (carcass samples, n = 60; workers hands' swabs, n = 25; cutting board swabs, n= 25; and knife swabs, n = 25) were collected. Totally, 120 people, 60 from butcher shops and 60 peoples from the abattoir chosen at randomly, and asked if they would be open to taking part in the questionnaire survey study.

3.4. Sample collection Techniques, and transportation

For sample collection, the Buffer Peptone Water samples transport media were prepared by dissolving 25.5g of 3M BPW ISO powder in 1000 ml of purified water and incubating at 37 °C for 24 hours. After 24 hours, the media were checked for sterility or the absence of microorganisms. To collect a sample, 10 ml of sterile prepared media was poured into a sterilized 100-ml sample collection bottle.

On each sampling day, which is normally twice a week, around eight (8) to ten (10) animals were randomly systematically selected from the total animals ready to slaughter that day in the slaughter hall using their owners' codes to identify risk factors for *E. coli* O*15*7:H7 related to their sex, age, breed, and body condition score recorded.

The neck, brisket, fore rib, flank, and rump areas that are believed to have a high rate of contamination were all selected and butchered animals that were submitted to a carcass swab sampling of roughly 100 cm2 of the washed beef carcass surface in the slaughterhouse. Following the procedure outlined in ISO 17604 (2012), carcass swab samples were taken by applying a sterile template (10 × 10 cm) to certain locations on a corpse. For the carcass swab sampling, sterile cotton, and forceps were utilized. The Cotton held in place with forceps, then saturated in roughly 10 ml of buffered peptone water, and then repeatedly rubbed on the desired carcass areas both horizontally and vertically. After the cotton had been scrubbed 10 ml of buffered peptone water was added, and the test tube was labeled. Additionally, sterile gauze swabs were used in accordance with regular operating protocols to swab the blades, workers' hands, and vehicle samples from the abattoir's ambient pool.

The Butcher shops were selected by using simple randomly sampling method from those legally registered and get service from selected abattoir, after having their list from logbook of abattoir administrative office. 250g of raw beef samples from retail butcher shops were taken using aseptic methods from each chosen retail butcher shop for bacteriological laboratory analysis. The samples collected in sterile smasher bag and placed in an icebox and an Environmental swab samples were simultaneously taken from workers' hands swab, knives swabs, and cutting boards swabs in the retail butcher shops using the same 10% buffered peptone water transport media as in the abattoir.

All necessary information, including the sample type, sampling the date of, and the code of the abattoir or butcher shop, was clearly written on the labels of every sample using a permanent marker. Each sample was aseptically sealed in a polyethylene bag and transported in the icebox containing to the Ethiopian Agricultural Authority's (EAA's) regulatory lab, the Animal Products and Inputs Quality Testing Centre (API-QTC), in Addis Ababa, where all bacteriological analyses were carried out.

3.6. Questionnaire Survey

Semi-structured questionnaires were developed in English and pilot tested on a small number of participants. Before beginning the interview, respondents were informed of the survey's goals and provided with a consent form. Participants were asked their socio-demographic information and risk factors related to *E. coli* O157:H7 by translating English-prepared questions to their own language.

The sanitary handling practices at the slaughterhouse and beef retail shops studied using in-person interviews and direct personal observation. The list of items and questionnaires, which were divided into sections for questions for in-person interviews, a checklist for personal observations, and information about the respondents' socio-demographic characteristics, was modified from a similar earlier study carried out in Ethiopia.

A total of 120 people were chosen at random and asked whether they would be prepared to participate in the questionnaire survey study; 60 of these people worked in butcher shops and 60 in abattoirs. Using a descriptive survey approach, the present levels of meat handling, storage conditions, and use of safety equipment at the researched abattoir and butcher shops were investigated. The target group consisted of all workers at butcher shops and slaughterhouses. At the butcher shops, when the workers are two or above, only one person is selected based on their interest.

Through interviews, demographic information on the staff members of the slaughterhouse and butcher shop was gathered, including gender, age, educational level, employment status, work history, and position within the abattoir. Additionally, information was gathered about the abattoir's infrastructure and risk factors for *E. coli* O157:H7, including hygiene (hand washing, use of knives, and equipment), employee knowledge of the food borne illness, receiving training relevant to their current jobs in the job area, and employees' attitudes toward safely handling and storing meat as well as food borne illness. In the 60 chosen butcher shops, 60 people in total were included for the conditions noted, such as the percentage of people wearing white clothing and head cover

3.7. Sample Analysis in the Laboratory

3.7.1. Sample preparation and enrichment

From 250 g of meat samples collected at butcher shops, 25 g of each was weighed and enriched into an Erlenmeyer flask containing 90 mL of 3M Molecular Buffer Peptone Water (3M BPW) and incubated at 37°C for 24 hours. Sample preparation and enrichment were conducted according to the general guidelines of ISO 16654:2001

3.7.2. Detection of E. coli O157: H7 by 3M ™ Molecular Detection Assay method

3M's Molecular detection technology is an accurate and simple technique for identifying pathogens such as Salmonella, *E. coli* O157 (including H7), and Listeria in food types. It generates highly sensitive results by targeting and amplifying nucleic acids in enriched samples, and positive samples can be identified in as little as 15 minutes. The 3M^{TM} Molecular Identification Assay for *E. coli* O157 (including H7) was created to test 96 samples simultaneously for *E. coli* O157 in one hour, and classifies the findings as positive or negative on the linked computer loaded with the machine's (Loff *et al*., 2014) . Software Screening for *E. coli* O157: H7 was conducted on all samples collected at abattoirs (carcass swabs, knives, workers hand hides, and carcasses) and retailers' butchers. The following procedures were used for all samples. In the present studies, the 3M^{TM} Molecular Detection Assay *E. coli* O157 (including H7) methods were used to detect *E. coli* O157 in all sample types collected from both sources by using the 3MDS protocol guide as follows:

Following enrichment, 20 micro liters of the enriched samples were put into separate lysis (LS) tubes, and 20 micro liters of sterile buffer peptone water were deposited into one LS tube as a negative control (NC). A lid was placed over a rack of LS tubes, and the mixture was inverted three times. The LS tube rack was heated for 15 minutes at a temperature of 100 °C. The top was lifted, revealing the rack of LS tubes placed on a chill block for ten minutes, after which the rack was taken off and mixed three times. 20 micro liters of each sample's lysate were transferred into reagent tubes after the rack was tapped three times on the bench and kept at room temperature for 5 minutes. Pipette the mixture five times, gently up and down, and then cap the tubes.

The 20 µL of sample lysate transferred into matrix control tubes, mixed by pipetting up, and down five times in tubes sealed with caps. Then 20-µl negative control lysate transferred into one reagent tube, and mixed by gently pipetting up and down five times in a sealed tube with a cap. After that, 20-µL Negative Control (NC) lysate was transferred into one Reagent Control (RC) tube. Mix with a gentle pipette five times up and down, and then cap the tube. The speed loader tray received the closed tubes.

To begin the assay, the speed loader tray placed into the instrument, and the lid was shut. The outcome displayed with a negative and positive sign on a computer screen attached to a 3M molecular tool, and the matrix value of the software analysis displayed.

3.7.3. Identification and Isolation

The 3M TM Molecular Detection Assay Method was used to detect *E. coli* O157:H7 positive results in samples collected and analyzed. The positive *E. coli* O157:H7 results in samples identified by The Biolog Gen III Microplate system (Biolog Universal Guideline, 2008). On the day of detection, CT-SMAC (Sorbitol MacConkey with Cefixime and Tellurite) agar was prepared and incubated at 37 °C for 24 hours. The media were checked for sterility, and one ml of sample was picked from each positive sample and streaked on the prepared sterile CT-SMAC media, which was incubated at 37°C for 24 hours to allow the growth of *E. coli* O157:H7. On the next day, the formation of pale-colored colonies (non-sorbitol fermenters), which were suspected to be *E. coli* O15:H7 was observed on CT-SMAC media. Non-sorbitol fermenters (pure pale-colored colonies of *E. coli* O157:H7) were transferred to nutrient agar (oxoid) and incubated at 37 °C for 24 hours to get pure colonies. Then pure isolated colonies were transferred into BUG (Biolog Universal Growth Medium), incubated at 33°C for 24 hours. Before OmniLog's identification process, subcultures were created using the same culture media to produce pure culture colonies as follows: A bacterial suspension was first created with the appropriate bacterial density by the instrument's instructions. The GEN III micro plates were then aseptically inoculated with the bacterial suspension. The micro plates were covered with a lid and incubated in an Omni log incubator at 37° C for 22 hours. The instrument using the in-built database then identified the bacterial suspension.

3.8. Antimicrobials Susceptibility Test Methods

The disk diffusion method used to evaluate the antibiotic susceptibility profiles of the verified isolates *E. coli* O157:H7 according to the "Clinical Laboratory Standard Institute's (CLSI) recommendation. In this research, eight (8) antimicrobial disk types were used to evaluate the antimicrobial susceptibility test of isolated *E. coli* O157:H7 from the samples. The criteria for selecting antimicrobials were decided by the availability of discs in the local market, the usage of antimicrobials in ruminants, and the possible public health importance of the Clinical and Laboratory Standards Institute's guidelines (CLSI, 2012).

Selected pure colonies of the identified isolates were mixed with saline solution to achieve the 0.5 McFarland standard. The test antibiotics were then applied to Mueller-Hinton agar (Merck, SA), which had been equally swabbed with the combination. Following that, each plate was incubated for 16 to 24 hours at 37°C. The CLSI-recommended method was followed, and the cleared zones were measured in millimeters with the values translated as "resistant (R)," "intermediate (I)," or "susceptible (S)." The microorganism was considered sensitive to the antibiotic if the observed zone of inhibition was bigger than or equal to the size of the standard zone. The bacterium is termed resistant if the measured zone of inhibition is smaller than the usual size (Table 3).

Table 3: The Kirby-Bauer antibiotic susceptibility test's zones of inhibition Interpretation

S.No	**Antibiotic disk With concentration**	**Codeof antibiotics**	**Diameter of zone of inhibition in mm**		
			Resistance ≤	**Intermediate**	**Susceptible ≥**
1	Ampicillin (10 μg)	AM10	13	14-16	17
2	Amoxicillin (10 μg)	AX2	13	14-16	17
3	Gentamicin (10 μg)	CN10	12	13-14	15
4	Penicillin G(10 μg)	P10	11	12-21	22
5	Streptomycin(10 μg)	S10	11	12-14	15
6	Sulfamethoxazole +Trimethoprim(25μg)	STX25	10	11-15	16
7	Tetracycline (30μg)	TE30	11	12-14	15
8	Vancomycin (30 μg)	VA30	9	10-14	15

3.9. Data Management and Analysis

The Microsoft Excel® version 2010 program was used to enter, code, and filter data produced in the field and laboratory. Stata 14 was used to analyze the data. Descriptive statistics were used, such as proportions, standard deviations, and 95% confidence intervals. When the p-value fell below 0.05, a difference that was statistically significant was taken into account. Prevalence was determined by dividing the number of positive samples by the total number of samples examined multiplied by 100. Pearson's chi-square (X^2) and Fisher's exact tests were used to determine the relationship between various risk factors and the occurrence of *E. coli* O157:H7.

3.10. Ethical Consideration

The College of Veterinary Medicine's Animal Research Ethical Committee at Addis Ababa University must approve the procedure before any samples are taken. VM/ER C/02/15/022, December 23, 2022, is their authorization code.

4. RESULTS

4.1. Prevalence *E. coli* O157: H7

Over all, the prevalence of *E. coli* O157:H7 in the abattoir and butcher shops was 6.32% at 95% CI [4.0–9.83]. Regarding the contribution to the total prevalence of *E.* coli O157:H7, the abattoir carcass swabs were 10 (3.51%), the abattoir worker hands swab sample was 1 (0.35%), and the swab of the knife used in the abattoir was 1 (0.35%). The butcher shop cutting board swabs were 4 (1.4%), the butcher shop knife swab was 1 (0.35%), and the carcass sample was 1 (0.35%).

The test statistic association showed that there was a statistically significant difference (*df* =7) (P =0.04) in the prevalence of *E. coli* O157:H7 among sample types that were examined from sample sources (the abattoir and the butcher shops). Result showing the prevalence of *E. coli* O157:H7 from an abattoir and butcher shops' with different types of samples indicated in Table 4.

Table 4: *E. coli* O157:H7 prevalence and its statistical associations among sample types by sources

Sample source	Sample Type	No of sample examined	No of positive (%: CI)	Prevalence	Chi-Square (X^2)	Fishers exact p-value
Abattoir	Carcass swabs	60	10(16.7:9.1 -28.5)	(6.67%)		
	Workers hand swab	30	1(3.33: 0.4-20.98)	(0.67%)		
	Knife swabs	30	1(3.33:0.4-20.98)	(0.67%)	21.86	0.004
	Vehicle swabs	30	0	(0%)		
	Subtotal	150	12(8:4.56-13.63)	8%		
Butchers shop	Carcass sample	60	1(1.67:0.23-11.15)	0.74%		
	Cutting board swabs	25	4(16:5.99-36.30	2.96%		
	Workers hands swabs	25	0	(0%)		
	Butcher Knife swabs	25	1(4:0.53-24.45)	(0.74%)		
	Subtotal	135	6(4.44:1.99-9.63)	(4.44%)		
	TOTAL	285	18(6.32:4.0- 9.82)	(6.32%)		

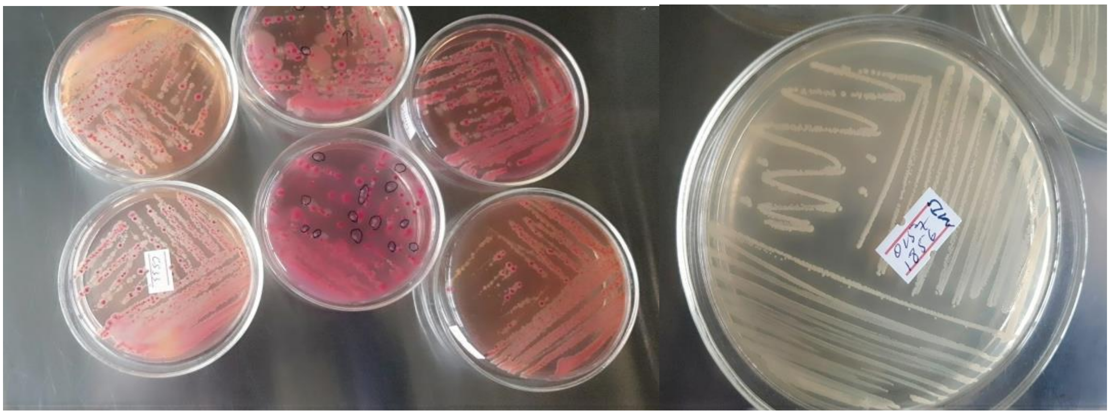

Figure 2:- *E. coli* O157:H7 colonies color on Sorbitol-MacConkey (colorless) and Nutrient Agar (Greyish white) media, respectively

4.2. Prevalence of *E. coli* O157:H7 in the Abattoir

E. coli O157:H7 strains were isolated on 12 (8%) at 95% CI [4.6, 13.6] samples out of 150 samples collected in the abattoir for bacteriological laboratory analysis from beef carcass swabs (n = 60) and 90 swabs samples from abattoir environmental (workers hands swabs = 30, knives swabs = 30, and vehicles swabs = 30). Of the 12 (8%) samples that tested positive for *E. coli* O157:H7, 10 (6.67%) were taken from carcass swabs, 1 (0.67%) from an abattoir worker's hands swabs, and one (0.67%) from a knife swab. No positive result was detected from swab samples from meat transportation vehicles.

4.2.1. E. coli O157:H7 *prevalence and risk factors associated with cattle*

E. coli O157:H7 strains were found in 10 (16.67%) of the carcass swab samples from the 60 cattle whose carcass samples were examined for the presence of *E. coli* O157:H7 after slaughter. According to the category of animal-related risk factors, the prevalence of *E. coli* O157:H7 was 8/50 (16%) and 2/10 (20%) in males and females, while 3/18 (16.7%), 6/37 (16.2%), and 1/5 (20%) were from animals aged under 5 years, 5-8 years, and above 8 years, respectively.

Based on the animal's Body Condition Score-related risk factor category, the prevalence of *E. coli* O157:H7 was detected in 6/33 (18.2%) who had a good body condition, 2/18 (11.1%) who had a very fat body condition and 2/9 (22.2%) who had an extremely fat body condition. *E. coli* O157:H7 was prevalent in 7/53 (13.2%) local breeds of cattle, and 3/7 (42.86%) were crossbreed cattle between their breeds, according to the cattle's breed-related risk factor category.

Cattle-related risk factors (Age, sex, breed, and body condition score) were tested statistically for their relationship to the prevalence of *E. coli* O157:H7 in carcass swabs. However, none of the associations were found to be statistically significant (Fischer's p-values for Sex = 0.668; Age = 1.0; Body condition score = 0.713; Breed = 0.083) during this study, as shown in the following Table.

Table 5: Cattle-related risk factors associated with the prevalence of *E. coli* O157:H7

Variable	**Sub Category**	**Number of examined**	**Positive (%:CI)**	**Chi-square (X^2)**	**Fisher's exact -value**
Sex	Male	50	8(16 : 8.0 -29.3)	0.096	0.668
	Female	10	2(20:4.5-57)		
Age	<5years	18	3(16.7: 5.2-42.4)	0.045	1.000
	5-8 years	37	6 (16.2: 7.3-32.4)		
	>8 years	5	1 (20: 2.0-75.3)		
Body Condition Score	Good	33	6 (18.2:8.2-37.2)	0.654	0.713
	Very fat	18	2(11.1:2.6-39.9)		
	Extremely fat	9	2(22.2:4.96-61.0)		
Breed	Local	53	7(13.2:6.3-25.7)	3.914	0.083
	Cross	7	3(42.8:12.6-79.62)		
Total		**60**	**10**		

4.2.2. E. coli O157:H7 *prevalence and statistical correlation with samples from an abattoir*

The statistical relationship between the prevalence of *E. coli* O157:H7 and the sample types (carcass swabs, knife swabs, and workers hands swabs) from an abattoir was also examined. The test results showed that there were statistically significant relationships (Fisher's exact P = 0019, χ^2 = 10.51) between the prevalence of *E. coli* O157:H7 and sample types from abattoir sources, as shown in Table 6.

Table 6: Prevalence of *E. coli* O157:H7 in carcass and environmental pooled swab samples in the abattoir

Sample type	No of Samples	No of Positive(%:CI)	Chi- square (χ^2)	Fisher P-Value
Carcass swabs	60	10(16.7% : 9.1- 28.5)		
Worker hands swabs	30	1(3.3:0.4- 21)		
Knife swabs	30	1 (3.3:0.4- 21)	10.51	0.019
Vehicles swabs	30	0		
Total	**150**	**12(8:4.56-13.63)**		

4.3. Prevalence of *E. coli* O157: H7 Butcher shops

At butcher shops, the prevalence of *E. coli* O157:H7 was 6/135 (4.44%) at 95% CI [1.99–9.63]. From the total positive samples, 4/25 (16%) were from the cutting board swabs, 1/60 (1.67%) was from the carcass sample, and 1/25 (4%) was from the knife swab sample they used to cut the carcass. No positive result was detected from swab samples from butcher shop workers' hands.

4.3.1 Associations' of E. coli O157:H7 *with sample types in the butcher shops*

From total positive samples 6/135 (4.44%), 60 carcass samples collected 1/60 (1.67%) were positive, from 25 cutting board swabs samples tested 4/25 (16%) were positive, and from total 25 butcher shops knife swabs sampled 1/25 (4%) were positive. There was a statistical association between positive results for *E. coli* O157: H7 in samples and sample types collected in butcher shops with a Fisher's P-value (p = 0.040, *df = 3)* during the study period. The presence of *E. coli* O157: H7 in different samples collected was directly associated with the cumulative problems related to hygienic practices, especially washing cutting boards and knives, and the meat handling and storage in Addis Ababa butchers' shops, as shown in Table 7

Table 7: Association of *E. coli* O157: H7 and samples types from butcher shops

Sample type	Number of sample	No of positive (%: CI)	Chi-Square (X^2)	Fishers p-value
Carcass samples	60	1(1.67:0.23-11.24)		
Cutting-board swabs	25	4(16: 5.96-36.42)		
Workers hands swabs	25	0	**10.125**	**0.040**
Knife swabs	25	1(4:0.528- 24.64)		
Total	**135**	**6(4.44:1.99-9.63)**		

4.4. *E. coli* O157:H7 multidrug resistance in the samples

In this study, antimicrobial resistance testing was done against eight (8) different antimicrobial agents; accordingly, the isolated *E. coli* O157:H7 showed 100% (18) resistance against three antibiotics: amoxicillin (10 *µ*g*)*, penicillin G *(10 g)*, vancomycin (30 *µg)*, and 94% resistance to ampicillin *(10 µg))*. In contrast, both the streptomycin (10 *µg*) and the sulfamethoxazole-trimethoprim *(25 µg)* showed 100% (18/18) susceptibility to *E. coli* O157: H7 isolated from different sample types. Additionally, gentamicin (10*µg)*, and tetracycline (30 *µg*) were susceptible by 17/18 (94.44%) and 16/18 (88.9%) to isolate *E. coli* O157:H7, respectively. From the total 18 *E. coli* O157:H7 strain isolates from samples during the study period, 1/18 (5.56%), 1/18 (5.56%), and 2/18 (11.11%) showed intermediate resistance against Ampicillin

(10 μg), Gentamicin (10 μg), and Tetracycline (30μg), respectively. as shown in Figure 3 and Table 8.

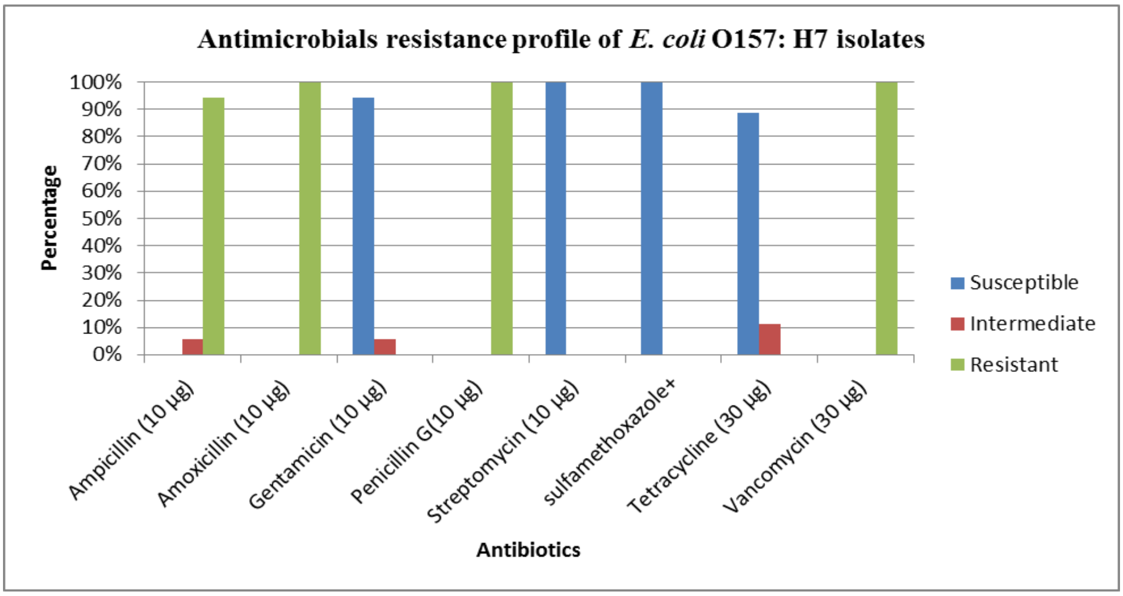

Figure 3: Antimicrobial susceptibility test results for *E. coli* O157:H7

Table 8: Antimicrobial resistance profile of *E. coli* O157:H7 isolated in the samples

Drug concentration by μg)	**Disk code**	**Frequency (%) of resistance and susceptible *E. coli* O157: H7 isolates (n = 18)**			**Total**
		Susceptible	Intermediate	Resistant	
Ampicillin (10 μg)	AM10	-	1(5.56%)	17(94.4%)	18
Amoxicillin (10 μg)	AX2	-	-	18(100%)	18
Gentamicin (10 μg)	CN10	17(94.44%)	1(5.56%)	-	18
Penicillin G(10 μg)	P10	-	-	18(100%)	18
Streptomycin (10 μg)	S10	18(100%)	-	-	
Sulfamethoxazole+ trimethoprim (25 μg)	STX25	18(100%)	-	-	18
Tetracycline (30 μg)	TE30	16(88.9%)	2(11.11%)	-	18
Vancomycin (30 μg)	VA30	-	-	18(100%)	18

4.5. Assessment of Sanitation Practices in the Abattoir and Butcher Shops

4.5.1. The Socio-demographics of participants and the Assessment of sanitation practices in the Abattoir

Socio-demographics of participants and the observed condition in the abattoir

Of the 60 workers in the abattoir who participated, 60 (100%) were male, and no females participated. Based on the age category, 16/60 (26.7%), 38/60 (63%), and 6/60 (10%) participants were below 35 years old, between 35 and 50 years old, and above 50 years old, respectively. Based on this questionnaire's survey results, more than half of the employees (63%) in Addis Ababa's abattoir were between 35 and 50 years old. By their educational level, 29 (48.3%), which is almost half of them, have not reached secondary school. From the total 60 people who participated in the interview, only 17 (28.3%) and 14 (28.3%) had joined educational levels of secondary school and tertiary (diploma above), respectively. When we compared by employment status, 42 (70%) and 18 (30%) employees in the abattoir worked on a daily basis (temporary) and full-time (permanent), respectively. Out of the total number of people interviewed by their roles in the abattoir, 56 (93.3%) were those who worked on butchering (skinning, evisceration, flaying) and those who had direct contact with the carcass of butchered animals. Only four (6.7%) meat inspectors got the chance to interview during the study period. In the municipal abattoir during the study period, observational studies were conducted concurrently. Workers were observed for which and how they use the hygienic-related safety equipment and ethical garments that have the probability of cross-contamination of carcasses and the environments of the abattoir.

The observational assessment showed that all of the workers in the working room put on boots, and from the total 60 persons, 44 (73.3%) used head covers, 16(26.7%) did not use Head-covers, 42 (70%) used Aprons, and 18 (30%) never used aprons. In a surprising way, none of them was wearing mouth masks or gloves during working time out of the total 60 people involved, as shown in Table 9.

Table 9: Socio-demographics of participants and the observed condition in the abattoir

Variables	**Sub Variables**	**Variables Sub-category**	**Frequency (%)**
Socio demographics information's	Sex	Male	60(100%)
		Female	0(0%)
	Age	Below 35 years	16(26.7%)
		B/n 35 to 50 Years	38(63.3%)
		Above 50 years	6(10.0%)
	Educational status	Primary	29(48.3%)
		Secondary school	17(28.3%)
		Tertiary	14(23.3%)
	Employment status	Temporary	42(70.0%)
		Permanent	18(30.0%)
	Employees role in abattoir	Meat inspector only	4(6.7%)
		Butchering	56(93.3%)
Conditions observed	Use Apron	Yes	42(70.0%)
		No	18(30.0%)
	Use Head-cover	Yes	44(73.3%)
		No	16(26.7%)
	Use Mask	Yes	0(0%)
		No	60(100%)
	Use Glove	Yes	0(0%)
		No	60(100%)
	Boots	Yes	60(100%)
		No	0(0%)

Assessment of sanitation practices in the Addis Ababa municipal abattoir

The study found that 12 (20%) had less than 5 years' experience, 42 (70%) had between 5 and 10 years' experience, and 6 (10%) had more than 10 years' experience. 50 (83.33%) employees work in the abattoir at least three times per week, with 5 (8.33%) joining the work place daily and 5 (8.33%) working once a week.

From the total interviewed, 56 (93.33%) of them do their work based on the abattoir work situation (there is no time limitation) during their shift day, whether they are employed on a permanent or temporary basis for those jobs, and the left person works 8 hours during their shift per day. Only eight (13.3%) of the employees at the abattoir had any knowledge of food-borne illnesses, compared to 52 who had no such knowledge (86.7%). Despite the fact that many workers lack knowledge about food-borne illnesses, more than half of the abattoir employees (51.7%) have received informal on-the-job training for their particular occupations from their seniors. In the Addis Ababa slaughterhouse, employees wash their hands, and as they told me, 56 of them (93.3%) wash their hands before beginning and after finishing their work, and four (6.7%) wash their hands in between each step.

In the study, participants were asked if they wash their hands with soap or not. Of the 4 (6.7%) who said they only wash their hands with soap, 56 (93.3%) employees said they do not wash their hands with soap in the slaughterhouse because there is no soap available there. During the study period, 56 (93.3%) of the questioned people used the same knife for different butchering operations in the abattoir, such as splitting meat, flaying, and evisceration, whereas only four (6.7%) used different knives for different slaughtering processes in the abattoir (Table 10).

Table 10: Questionnaires and survey results on hygienic practices in the abattoir

Variables	**Variables Sub-category**	**Frequency (%)**
How long have you worked in an abattoir?	<5years	12(20%)
	Between 5 and 10 years	42(70%)
	Above 10 years	6(10%)
How many days a week do you work at this abattoir?	Daily	5(8.33%)
	At least three Times per week	50(83.33)
	Once a week	5(8.33%)
How many hours do you work per day?	8hours	4(6.67%)
	Situational	56(97.3%)
Do you have any knowledge of Food-borne diseases?	Yes	8(13.3%)
	No	52(86.7%)
Have you received any training for your specific job?	Yes	29(48.3%
	No	31(51.7%
At what time do you clean your hands while working at an abattoir?	Between each process	4(6.7%)
	Before and after	56(93.3%)
Do you clean your hands with soap at work?	Yes	4(6.7%)
	No	56(93.3%)
Do you use the same knife for butchering procedures, especially flaying and evisceration?	Yes	56(93.3)
	No	4(6.7%)

4.5.2. Assessment of sanitation practices in cattle carcass retail butcher shops

Out of the 60 participants interviewed during the study period in 60-selected retail butcher shops that sell cattle carcasses in Addis Ababa city, 55/60 (91.7%) were males and 5/60 (8.3%) were females by sex. From the total 60 people surveyed, 23/60 (38.5%), 30/60 (50%), and 7/60 (11.7%) were under 35 years old, between 35 and 50 years old, and over 50 years old according to their age category. By educational level status, 27/60 (45%), 26/60 (43.3%), and 7/60 (11.7%) of the participants were learning up to primary, secondary, tertiary, or diploma and above, respectively. Of the 60 people surveyed, 22 (36.7%), 24 (40%), and 7 (23.3%) have worked in butcher shops for up to three years, three to five years, or more than five years, respectively. Out of the 60 people who were interviewed for their roles in the retail butcher shop, 23 (38.8%) were selling meat, 31 (51.7%) were selling meat and working as cashiers, and six (10%) were selling meat and delivering food to customers in the butcher shop's hotel and restaurant rooms, as shown in the following table:

Table 11: Socio-demographic status of employees in selected butcher shops retailing cattle carcasses in Addis Ababa City

Variables	**Sub-variables**	**Variables Sub-category**	**Frequency (%)**
Socio-demographic Information	Sex	Male	55(91.7%)
		Female	5(8.3%)
	Age	Below 35 years	23(38.3%)
		Between 35 to 50 Years	30(50%)
		Above 50 years	7(11.7%)
	Educational Status	Primary	27(45%)
		Secondary school	26(43.3%)
		Tertiary	7(11.7%)
	Work experience	Less than 3years	22(36.7%)
		Between 3 and 5years	24(40%)
		Above 5years	14(23.3%)
	Employees role in butcher shop	Serving as a meat seller only	23(38.3%)
		Serving as both a meat seller and cashier in the shop	31(51.7%)
		Serving as both a meat seller and food delivery for customers	6(10%)

This study found that 20 (33.3%) and 17 (28.3%) of the employees worked between 12–14 and 15–17 hours per day, respectively, while 23 (38.3%) worked based on the situation of the market, and if meat was sold and finished, they went to their homes.

When workers in butcher shops in Addis Ababa city were asked about their knowledge of Food-borne diseases (FBD), it was surprising to learn that only 16 (26.7%) of them did, while 44 (73.3%) did not.

Only 25% (15/60) of the workers in the butcher shops were aware of meat hygiene, handling, and safety, while 75% (45/60) were not. According to the butcher shop employees interviewed during the study period, all of them wash their hands while handling carcasses at work .Of these, 42 (70%) employees do not wash their hands with soap before or after handling meat, while 18 (30%) do.

The most important details are that the 56 (93.3%) butcher shop employees in Addis Ababa city used the same knife to cut meat and that they cleaned the knives 36 (60%) at the beginning and end of their shifts, 35% when they noticed the knives getting dirty, and 3 (5%) when they began their shifts. In the retail butcher shop, 27 (45%) employees washed the cutting board as they started their shifts, 21 (35%) at the start and end of their shifts, 4 (6.7%) after finishing their shifts, and 8 (13.3%) when they noticed it was getting dirty.

Table 12: Assessment of hygienic risk factors related to *E. coli* O157: H7 in Butcher shops

Variables	Variables sub category	Frequency (%)
How many hours do you work per day?	between 12 and 14hrs	20(33.3%)
	between 15 and 17hrs	17(28.3%)
	situational	23(38.3%)
Do you have any information on meat hygiene, handling, and safety?	Yes	15(25%)
	No	45(75%)
Do you have any knowledge of Food Borne Disease?	Yes	16(26.7%)
	No	44(73.3%)
Do you wash your hands while touching carcasses at work?	Yes	60(100)
	No	0(0%)
Do you use soap to wash your hands before and after touching meat?	Yes	18(30%)
	No	42(70%)
Do you use the same knife for cutting meat?	Yes	56(93.3)
	No	4(6.7%)
When you wash your knife?	At the start and end of the job	36(60%)
	At the beginning	3(5%)
	Based on the situation	21(35%)
When do you wash the cutting board?	At the starting job	27(45%)
	at the start and end of the job	21(35%)
	after finishing the job	4(6.7%)
	situational	8(13.3%)
Conditions observed in retail butcher shops		
Use head cover	Yes	36(60%)
	No	24(40%)
Wear white coat	Yes	51(85%)
	No	9(15%)

5. DISCUSSIONS

In the present investigation, the overall prevalence rate of *E. coli* O157: H7 was 6.32% (18/285) among all samples collected at the abattoir and butcher shops. The current E. *coli* O157 occurrence finding of 6.32 closely agrees with the prevalence estimates of 6.3% and 6% by (Gutema *et al.*, 2021) and (Zelalem *et al.*, 2019), respectively. It is also higher than the findings of Shumi *et al.* (2021), which were 4/288 (1.39%) at Jimma, Atnafie *et al.* (2017), which were 2.4% (15/630), and Abunna *et al.* (2023), which were 14/352 (3.97%). However, the *E.coli* O157:H7 prevalence in our findings is lower than the earlier reported prevalence by Tadese *et al.* (2021) in Ambo, which was 9.1%, and the pooled *E. coli* O157: H7 prevalence estimates in cattle for Africa (31.2%), which was indicated in Islam *et al.* (2014). In this study, eight sample types from sample sources (the abattoir and meat-selling shops) were analyzed, and there was statistical significant variance (df = 7, P =0.04) in the frequency of *E. coli* O157:H7 among sample sources. This variation in the prevalence of *E. coli* O157:H7 may be associated to seasonal variations, level of hygienic conditions in abattoirs and retail butcher shops; the different detection methodologies of *E. coli* O157:H7, daily slaughter capacity in abattoirs and lack of knowledge about food borne disease at abattoir and butcher shops (Calle *et al*., 2021).

The current study revealed that among putative risk factors considered in the study none of them statistically associated with occurrence of *E. coli* O157:H7. The Fisher's exact p-values for sex, age, body condition score, and breed of animals were found to be 0.668, 1.000, 0.713, and 0.083, respectively. The current findings is in line with reports made by Ethiopian researchers (Hamid *et al*., 2018) and Atnafie *et al.* (2017), who have found no association between the prevalence of *E. coli* O157: H7 and the sex or breed of cattle. However in contrary to current finding, Fikadu *et al*. (2023) reported that associations of the slaughtered cattle's age groups with occurrence of *E. coli* O157: H7. The discrepancies in prevalence may be attributed to sample sizes, the geographical origin of the animals slaughtered in abattoirs, the time of the study, and sampling methodologies (Abdissa *et al.* 2017).

In terms of the sample source the prevalence of *E. coli* O157: H7 at the abattoir level was 8% (12/150) in the present study, which is consistent with the previous studys done by Hiko *et al*.(2008) and Abunna *et al.* (2023) who reported 8% and 8.3% prevalence at Bishoftu, respectively. However, other reserachers from Ethiopia; Edget *et al.* (2017), have pointed out the low prevalence of *E. coli* O157:H7 in abattoirs. at Haramaya University (HU) slaughterhouses(2.2%), the Dire Dawa administrative city (4%) and Jimma by Sebsibe *et al.* (2020) town, and 6.3% by Bekele *et al.* (2014).These results were low in comparison to our present study findings.

A higher prevalence of *E. coli* O157:H7 than this investigation (8%) in abattoirs was recorded by various researchers around the world. 13.3% (17/128) on cattle by Bekele *et al.* (2014) in Addis Ababa; 50/540 (9.3%) in Egypt (Osaili *et al*., 2013); and 49.4% in two Nigerian abattoirs (Akanbi *et al.,* 2011). The disproportion in incidence, however, is associated with variations in abattoir standards, sample type, worker hygiene conditions, sampling and isolation techniques, geographical origins, and animal numbers (Atnafie *et al.,* 2017).

In the Addis Ababa municipal abattoir during this study period, there was a statistically significant variation in the prevalence of *E. coli* O157:H7 (Fisher's exact P = 0.019) among the various sample types taken from the abattoir. High prevalence was observed in meat swab samples (16.67% (10/60), followed by 3.33% (1/30) from knife swab samples, and 3.33% (1/30) from workers hands' swabs samples. Nevertheless, there was no *E. coli* O157:H7 isolates from swab samples of vehicles at the abattoir.

The present study revealed that the prevalence of *E. coli* O157:H7 from beef carcass swabs at abattoir sources was 10/60 (16.67%). This prevalence was in harmony with previous studies reported by Fufa *et al.* [2023] (16.7%) at a private slaughterhouse in Bishoftu town, Hamid *et al.* [2018] (12%) in Addis Ababa, 11.11%) in an Irish abattoir by McEvoy *et al. (*2003) and in Northern Italy (11%) by Alonso *et al.* (2007). Contrary to the current study, lower prevalences of carcass swabs at abattoirs were reported in Turkey (2%) by Inat and Siriken (2010) and in Ethiopia (2.67% at Hawasa, 2.77% at Jimma, and 4.69% at Addis Ababa) by Atnafie *et al.* (2017), Shumi *et al. (*2021), and Bekele *et al.* (2014), respectively.

The variance in prevalence may be attributed to the research duration, animal stress, season, and the abattoir's sanitary state (Alam and Zurek, 2006). According to Fitzgerald *et al.* (2003), Edrington *et al.* (2004), and Brown-Brandl *et al.* (2009), *E. coli* O157:H7 shedding is more common during warmer months and has been linked to cow heat stress. *E. coli* O157:H7 prevalence has been associated with lairage and transportation to beef processing facilities, but this increase may be due to exposure to and contact with contaminated fecal material present in these environments rather than, or in addition to, increased fecal shedding (Arthur *et al.*, 2007; Dewell *et al*., 2008; Bach *et al*., 2004). In a questionnaire survey study, 56 out of 60 people (93.3%) confirmed that they used the same knife for all butchering operations, including flaring and evisceration. This may raise the risk of cross-contamination between the carcass, the worker's hands, and the knife (Sebsibe & Asfaw, 2020).

Moreover, as stated by Arthur *et al.* (2017) and Fink *et al.* (2017), butcher shops and restaurants are sources of *E. coli* O157:H7 that lead to human infections. The overall occurrence of Escherichia coli O157:H7 in beef meat and meat contact surfaces at Addis Ababa butcher shops was six (4.44%) [95% CI = 1.99–9.63] out of 135 examined samples. This finding is in line with different previous studies done in Ethiopia, such as Hiko *et al.* (2008*)*, who reported 4.2% from Modjo and Bishoftu, and Atnafie *et al.* (2017), who reported 4.2% from Hawassa. A lower prevalence was observed in Addis Ababa at 3.64% (14/384) (Haile *et al*., 2022), 3/150 (2%) in Hawassa, and 1/125 (0.8%) in Addis Ababa and Debre-Berhan. Meanwhile, a higher prevalence of 8/125 (6.3%) was reported in beef cut samples collected at retail shops (Gutema *et al*., 2020).

In the current investigation, *E. coli* Ol57:H7 was detected in 16% (4/25) of the cutting board swabs, 4% (1/25) of the knife swabs, and 4% (1/25) of the cattle carcass samples collected from butcher shops. However, no positive results were found in samples taken from the hands of butcher shop workers in Addis Ababa. Similar to this, there was no positive result from swab samples collected from the workers hands in retail butcher shops. This finding is in agreement with the findings of Abdissa *et al. (2017),* Mengistu *et al. (2017),* Shumi *et al. (2021),* and Sebsibe & Asfaw (2020) from different parts of Ethiopia. However, the current study is inconsistent with Abunna *et al.* (2023), who reported 1.1% (1/93) at Bishoftu town, and Othman *et al. (2022),* who reported 20% from the butcher shop worker's hands in Mosul city, Iraq. The variation in these findings may be due to the geographical location and weather conditions under which the research was conducted and the hygienic conditions among butcher shops in both cities (Beyi *et al.,* 2017).

The *E. coli* O157:H7 prevalence in our investigation was higher in cutting board swabs (4/25) compared to the other sample types obtained from butcher shops in Addis Ababa city. This finding was consistent with that of Amente *et al.* (2022), which were found to be four (16.7%) at Haramaya and slightly closer to that of Sebsibe & Asfaw (2020), which was 10% (3/30) reported at Jimma town. The low prevalence of *E. coli* O157:H7 on the cutting board samples obtained from butchers was also noted, including (0.8%) in Ethiopia by Abdisa *et al.* (2017), (3.3%) by Atnafie *et al.* (2017) at Hawasa, 3.6% (4/110) by Beyi *et al.* (2017) in central Ethiopia, and 7.7% by Othman *et al.* (2022) in Iraq. The butchering environment, sample size, and laboratory procedures are all factors that could affect the results of ruminant meat testing. Additionally, differences in the cleanliness of retail butcher shops, the kind of samples, the sampling method, the culture techniques, and the method of detection could also affect the results. Ruminant meat can contain *E. coli* O157:H7 based on the tools used, personnel's attire, and facilities (Gansheroff and O'Brien, 2000). The results of this study suggest that a lack of sanitation in the abattoir slaughterhouse is the cause of the positive *E. coli* O157:H7 isolates discovered in carcasses, knives, and the hands of abattoir workers. Abattoir employees lack knowledge of food-borne illnesses, but more than half (51.7%) have received informal on-the-job training from their seniors. Despite this, many lack knowledge of food-borne illnesses (Atnafie *et al.,* 2017).

Because of a lack of soap, the study discovered that 56 (93.3%) of the workers at the slaughterhouse in Addis Ababa do not wash their hands. During the study period, 56 (93.3%) of the questioned people used the same knife for different butchering operations, while only four (6.7%) used different knives for different slaughtering processes. The findings of this study indicate that the presence of positive *E. coli* O157:H7 isolates found in carcasses, knives, and abattoir worker hands indicates a lack of sanitation in the abattoir slaughterhouse (Nel *et al.,* 2004).

From a broader perspective, antimicrobial resistance is ranked as the third-biggest threat to public health in the twenty-first century. Antibiotics are the veterinary drugs that are most frequently used to treat animal infections. The widespread use of these drugs could promote the emergence of drug resistance. Two major causes of this problem are the overuse of these medicines and their incorrect prescribing. Antimicrobial resistance can emerge on its own because of selective pressure, human error, or excessive use in the care or feeding of beef cattle by owners (WHO, 2016; Tello *et al*., 2012).

In this study, antimicrobial resistance testing was done against eight different antimicrobial agents, such as ampicillin (10μ *g*), amoxicillin (10μg) (gentamicin (10 g), penicillin G (10 μg), streptomycin (10 μg), sulfamethoxazole + trimethoprim (25 g), tetracycline (30μ *g*), and vancomycin (30 μg). Among this multidrug, four of them showed higher resistance to isolated *E. coli* O157: H7 in the samples with 100% resistance was recorded for Amoxicillin (10 μg), Penicillin G (10 μg, Vancomycin (30 μg) and 94.4% (17/18) was found in Ampicillin (10 μg). Similar to what we discovered, numerous researchers from around the globe have reported finding *E. coli* O157:H7 multidrug resistance in a variety of sample types taken from abattoirs and butcher shops. These resistances included 100% to amoxicillin (10 μg) reported by Abdisa *et al.* (2017) and Tadese *et al.* (2021), 100% to ampicillin and vancomycin reported by Abebe *et al.* (2023), and 100% to penicillin G and vancomycin reported by Shumi *et al.* (2021) and Ajuwon *et al.* (2021).

Sulfamethoxazole trimethoprim (25 μg) and streptomycin (10 μg) both demonstrated 100% susceptibility to *E. coli* O157:H7 in the current investigations. This result has similarities with the previous researches results reported by Abdisa *et al.* (2017), which found 100% susceptibility for the drugs streptomycin, tetracycline, and a combination of sulfuramethoxazole and trimethoprim. Atnafie *et al.* (2017) reported 100% susceptibility for gentamicin and 94.1% for gentamicin and streptomycin. According to Beyi *et al.* (2017), tetracycline and trimethoprim-sulfamethoxazole both have 100% susceptibility. According to Palma *et al*. (2020), the widespread, careless, and imprudent use of antibiotics in veterinary and human medicine results in the genetic modification of bacterial strains for the development of resistance and an increase in the prevalence of resistance among pathogens.

6. CONCLUSION AND RECOMMENDATIONS

A higher prevalence of *E. coli* O157:H7 was detected in an abattoir and butcher shops, particularly in carcass swabs and meat cutting boards. Moreover, a multidrug-resistant strain of *E. coli* O157:H7 was also identified, which could be a public health threat. This finding suggests that the presence of *E. coli* O157:H7 in carcasses, knives, and abattoir workers' hands could be linked to poor abattoir sanitation and poor personnel hygiene. To address these concerns, the following recommendations are proposed:

- Control measures to reduce the public health risk in the beef supply chain need to be address at slaughterhouses by reducing carcass contamination.
- Slaughterhouses should be equipped with basic facilities and necessary infrastructures.
- Provide training to abattoirs and butchers shops workers on proper hygiene practices to reduce the risk of contamination
- Slaughterhouses need to adopt good hygienic practices and the HACCP principle for the best control of pathogens such as E. *coli* O157:H7.
- Implementing more stringent regulations on the use of veterinary antimicrobials
- Increase public awareness about food safety and antimicrobial resistance

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8. ANNEXES

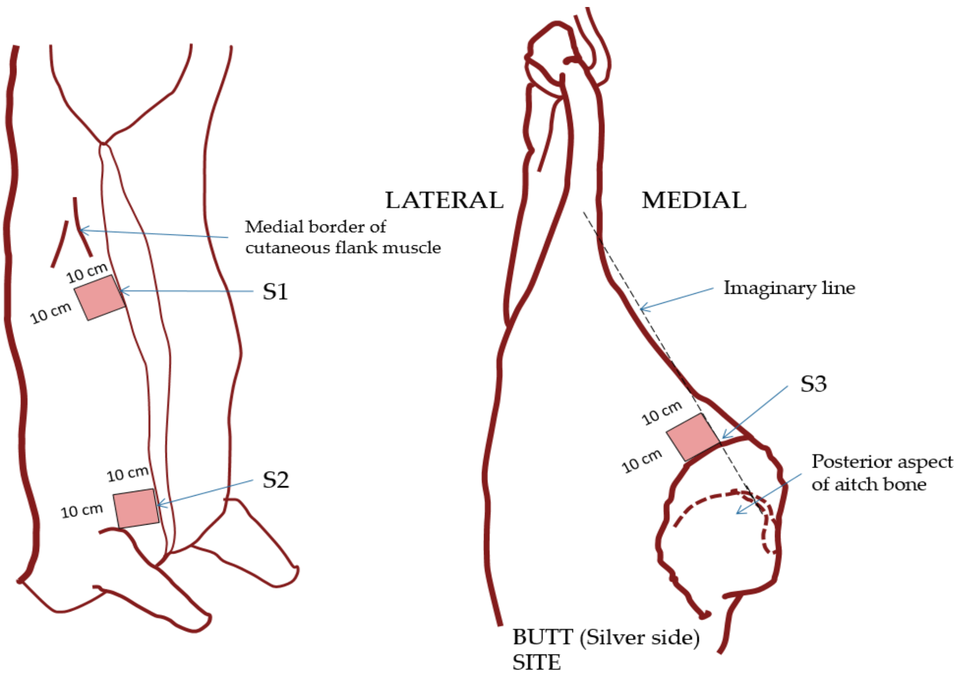
Annex 1: Sample collection Format Abattoir and butcher shops

Date ________________

Abattoir samples									Butcher shop				Result
sample code	Sex	Age	Breed	BCS	Carcass	Hands	Knife	Vehicle	carcass	knife	W/.hand	C/ board	
1													
2													
3													
4													
5													

Annex 2: Informal Consent

INFORMED CONSENT
My name is Gemechis Tegegn. I am working my MSc research at Addis Ababa University College of Veterinary Medicine and Agriculture (AAUCVMA). I would like to interview you a few questions about the sanitary condition of your slaughterhouses and some of the questions require physical observation. Taking swab samples from your hands with objectives of assessing knowledge and practice concerning slaughter safety and hygiene, which is important to improve the sanitary status to safeguard the safety of carcass reaching consumer from slaughterhouses. Your willingness and cooperation are helpful in identifying knowledge and practice related problems related to the Addis Ababa abattoirs enterprise. All information that you give will be kept strictly confidential, your participation is voluntary, and you can drop it any time you want. If it is your permission to continue, please continue to the next section of interview



Annex 3:Meat sample collection area for the laboratory bacteriological analysis

Annex 4: Lists of Medias Used in the laboratory

3M Buffered Peptone Water (3M Peptone Water)

Formula	grams per litre
Enzymatic digest of casein	10.0 g
Disodium hydrogen phosphate dodecahydrate	9.0 g
or anhydrous disodium hydrogen phosphate	3.5 g
Water	1L
Potassium dihydrogen phosphate	1.5 g

PH 7.0 ± 0.2 at 25°C

Sorbitol-Macconkey Agar (SMAC) With BCIG (CM098)

Formula	gm/litre
Peptone	10.
Sorbitol	020.0
Sodium chloride	5.0
Bile Salts No. 3	1.5
Crystal violet	0.001
Neutral red	0.03
Agar	15.0
5-bromo-4-chloro-3-indolyl-b-D-glucuronide (BCIG)	0.1

pH 7.1 ± 0.2 @ 25°C

Directions

Suspended 51.6g of SMAC weighed with BCIG in one litre of distilled water. Mixed well and sterilized by autoclaving at 121°C for 15 minutes. Poured into sterilized Petri dishes.

Nutrient Agar (Oxoid,)

Ingredients	gm/liter5.00
Peptone digest of animal tissue Yeast	gm1.50 gm1.50
extract Beef extract Sodium chloride	gm5.00 gm15.00
Agar PH at 25 OC	gm7.4 ± 0.2

Directions:

Powder: suspended 28g in1liter of distilled water.

Boiled to dissolve completely.

Sterilized by autoclaving at 121 OC for 15 minutes.

Biolog Universal Growth Agar (BUG)

Specifications: (70101, Biolog, Inc, Hayward, CA94545, USA) Final pH 3 +/- 0.1 at 25°C

Directions:

Mix 57 grams of BUGTM agar into one liter of purified water.

Boiled gently to dissolve components, Sterilize by autoclaving at 15 Ibs pressure and 121°C for 15 minutes and dispense as desired

Muller Hinton Agar (Himedia)

Composition	gram/Litre
Beef infusion from acid hydrolysate	300.00
of casein	17.50
Agar	17.00
Starch	1.

Annex 5: Questionnaires format (English Version) At Abattoir

Questionnaire for the abattoir workers on Hygienic Handling Practices at Abattoir

Date____

Questionnaire Code___________________________________

NO	Questions	Answers
	General characteristics of individuals	
1	Age	Year completed-----------
2	Sex	Male [ ] Female[ ]
3	Level of Education:	Illiterate [ ] Informal Education [ ] Primary Education [ ] Secondary Education [ ] Other (Specify)………………
4	Your role at the abattoir?	Veterinarian/meat inspector [ ] Butchers [ ] Other (specify)
5	Duration of working at the abattoir?	-------------------------------
	Possible risk factors for contamination of carcass during slaughter process	
9	Method of carcass dressing?	Vertical (hanging)[ ] Horizontal(on floor)[ ]
10	Do you use the following protective materials while working in the abattoir?(observe) 10.1)Apron yes __________no______ 10.2) white coat Yes________ no________ 10.3)Gloves yes_________no________10.4) Boots Yes_________no ________ 10.5) Head cover Yes________no_______10.6) Mask yes________no_________	
11	Do you have sink for washing hands in the abattoir?	Yes [ ] No [ ]
12	Do you wash your hands	Yes [ ] No [ ]

	before touching the carcass?	
13	Do you wash your hands with soap?	Yes [ ] No [ ]
14	Do you use the same knife for flaying and evisceration?	Yes [ ] No [ ]
15	Do you wash your hands after evisceration?	Yes [ ] No [ ]
16	Do you sink knife in hot water in between flaying and evisceration?	Yes [ ] No [ ]
17	Is carcass washed after evisceration?	Yes [ ] No [ ]
19	What is your source of water for use in the abattoir?	City/Municipal council [ ] borehole [ ] rain collected water [ ] River [ ] others (specify) [ ]
	Do have any information about food born disease?	Yes [ ] No[ ]
20	Have you ever received any training on hygienic handling of carcass?	Yes [ ] No[ ]
21	Have you gone for medical checkups to work at the abattoir?	Yes [ ] No [ ]

Annex 6: Questionnaires (English Version) at butcher shops

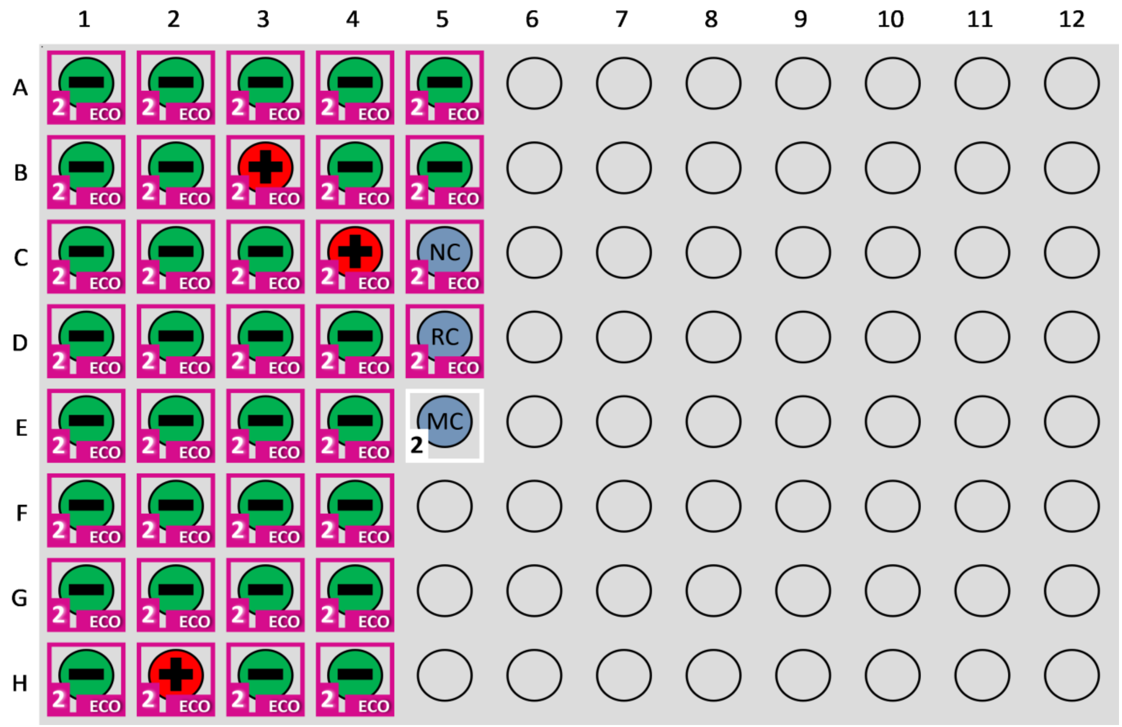
Questionnaire for the butcher shops workers on Hygienic Handling practices

Date____

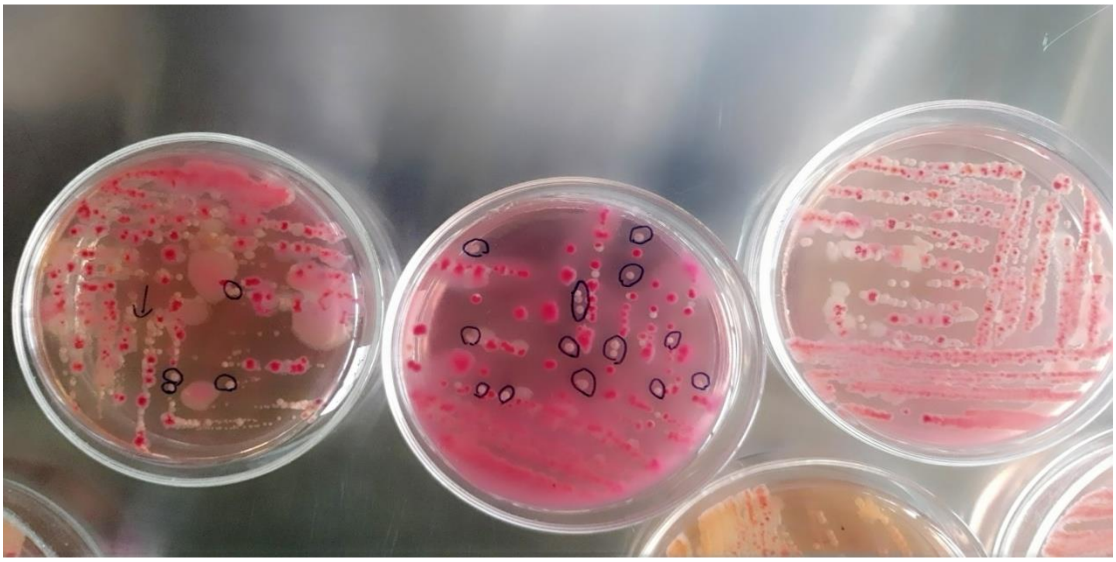
Questionnaire Code__________________________________

NO	Questions	Answers
	General characteristics of individuals	
1	Age	Year completed-----------
2	Sex	Male [ ] Female[ ]
3	Level of Education:	Illiterate [ ] Informal Education [ ] Primary Education [ ] Secondary Education [ ] Other (Specify)………………
4	what is your job in butcher show?	Retailer [ ] Retailer and cashier[ ] Other (specify) [ ]
5	Work experience in this specific job	[ ]years
6	How many hours do you work per day?	[ ] by hours
7	Do you have any information on meat hygiene, handling, and safety?	Yes [ ] No [ ]
	do you have any information about food born disease?	Yes [ ] No [ ]
8	Do you wash your hands while touching carcasses at work?	Yes [ ] No [ ]
9	Do you use soap to wash your hands before and after touching meat?	Yes [ ] No [ ]
10	Do you use the same knife for cutting meat?	Yes [ ] No [ ]

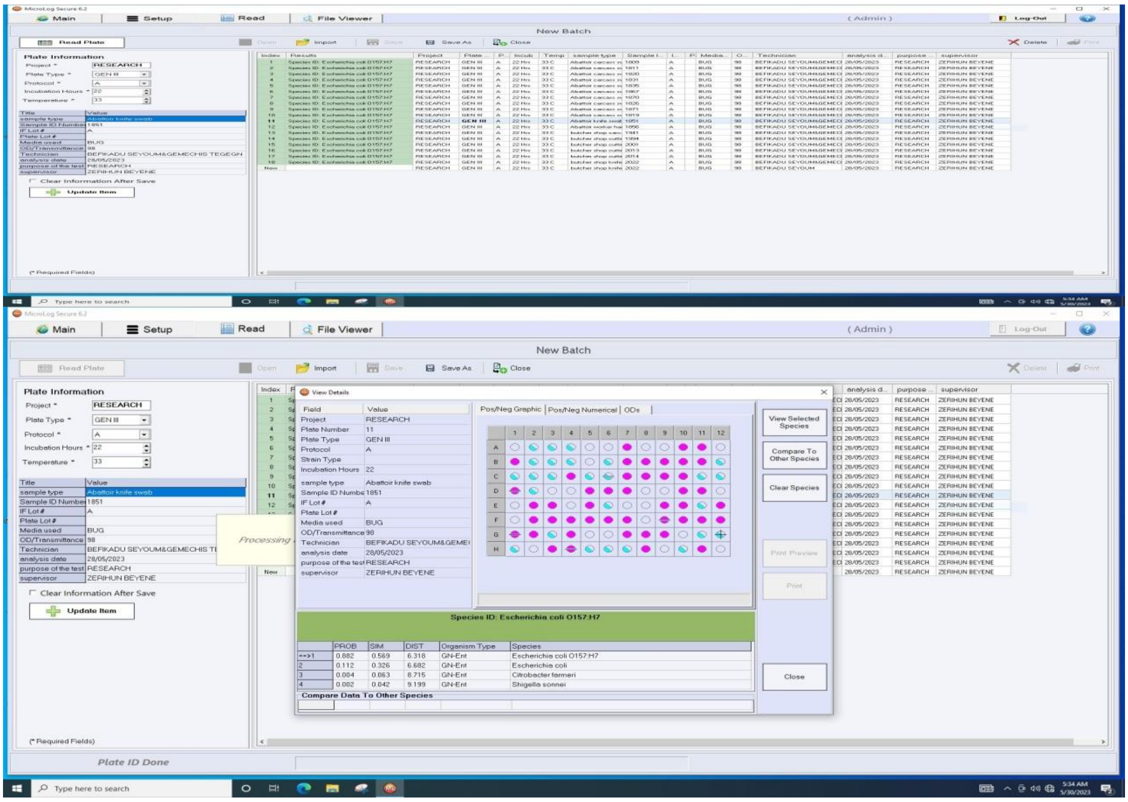
11	When you wash your knife?	Yes [ ] No [ ]
12	Do wash meat cutting /chopping board	Yes [ ] No [ ]
13	When do you wash the cutting board?	__________time / during
	Observed condition at butcher shops during interviews	
14	The number of people working in during observation time	____________by number
15	Use a hair cover or hat	Yes [ ] No [ ]
16	Use a white coat	Yes [ ] No [ ]



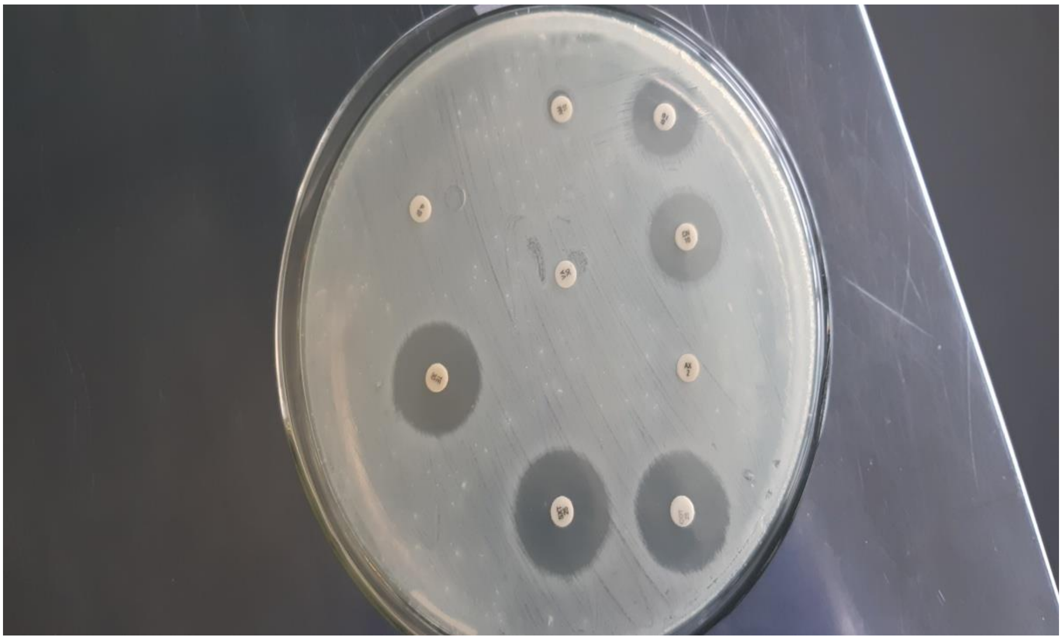
Annex 7: Result of 3M Molecular Detection Assay *E .coli* O157:H7



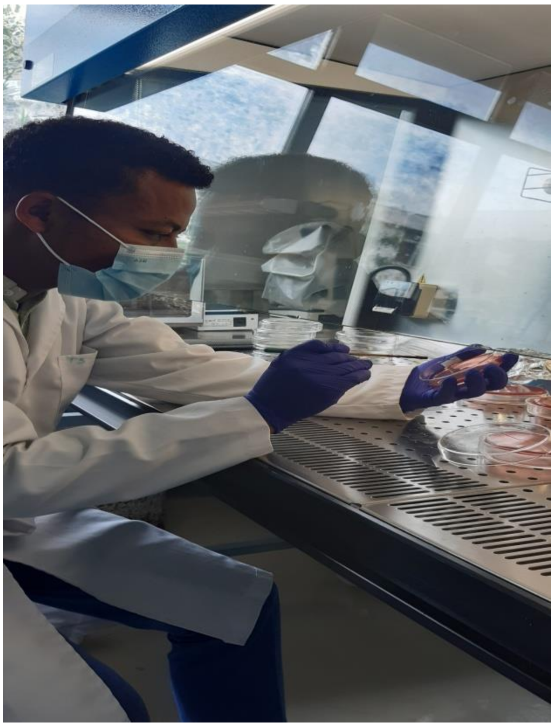
Annex 8: *E. coli* O157:H7 on Sorbitol MacConkey agar (the encircled on picture)



Annex 9: Gen III Micro plate all and individual *E. coli* O157:H7 isolate on computer



Annex 10: Zone of inhibition of antibiotics to the samples isolates *E. coli* O157:H7



Annex 11: Laboratory activities pictures

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical cleara nce certificate

Certificate Ref. No: VM/ERC/08/02/15/2023

Name and affiliation of applicant: **Gemechis Tegegn Tilahun (DVM, MSc student)**
Department of Clinical Studies, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Epidemiology of Escherichia coli O157: H7 and its antimicrobial resistance profile in beef at Addis Ababa municipal Abattoir, Addis Ababa, Ethiopia*

Date of application:	**December, 2022**
Nature of the project:	**Field investigation (questionnaire and meat sampling)**
Target animal species:	**No live animal involved**
Number of animals involved:	**None**
Study area:	**Addis Ababa, 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 **Gemechis Tegegn.**

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

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

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Annex 12: Ethical clearance certificate