OCCURRENCE, ISOLATION AND ANTIMICROBIAL RESISTANCE PATTERN OF *ESCHERICHIA COLI* O157: H7 FROM SLAUGHTERHOUSES AND BUTCHER SHOPS IN BISHOFTU TOWN, CENTRAL ETHIOPIA

MSc THESIS

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LIST OF ABBREVIATIONS

A/E	Attaching and Effacing
CDC	Centers for Disease Control and Prevention
CFSPH,	Center of Food Security and Public Health
CFU	Colony forming units
CI	Confidence Interval
CLSI	Clinical and Laboratory Standard Institute
CSA	Central Statistical agency
CT-SMAC	Sorbitol MacConkey Agar with Cefixime and Tellurite
DALYs	Disability Adjusted life years
DNA	Deoxyribonucleic Acid,
EAggEC	Enteroaggregative E. coli
EHEC	Enterohemorrhagic E. Coli
EIEC	Enteroinvasive E. Coli
EPEC	Enteropathogenic E.coli
ELISA	Enzyme-linked immunosorbent assays
EPEC	Enteropathogenic E. coli
ETEC	Eenterotoxigenic E. Coli
ERIC	Enterobacterial Repetitive Intergenic Consensus
FAO	Food and Agriculture Organization
GMPs	Good Manufacturing Practices
HACCP	Hazard Analysis Critical Control Point
HUS	Hemolytic uremic syndrome
IMS	Immunomagnetic Separation
IMViC	Indole, Methyl Red, Voges Proskauer and Citrate Utilization
ISO	International Organization for Standardization
LEE	Locus for enterocyte effacement
MLST	Multi Locus Sequence Typing

MTSBn	Modified tryptone soya broth containing Novobiocin
NSF	Non-Sorbitole fermenting
OIE,	World Organization for Animal Health
PCR	Polymerase chain reaction
PFGE	Pulsed-Field Gel Electrophoresis
STEC	Shiga-toxin–producing E. coli
Stx	Shiga toxins
Stx1	Shiga toxins 1
Stx2	Shiga toxins 2
Tir	Translocatedintimin receptor
TTP	Thrombotic Thrombocytopenic Purpura
VTEC	Verocytotoxin producing E. coli
WGS	Whole genomic sequence
WHO	World Health Organization

ABSTRACT

Raw beef consumption is time immemorial tradition in Ethiopia. However, unhygienic meat processing and distribution practices are risky to public health. A cross sectional study was carried out from December 2021 to May 2022 to investigate the occurrence and evaluate the antimicrobial resistance pattern of *E. coli* O157:H7 isolated from slaughterhouses and butcher shops in Bishoftu town, Ethiopia. A total of 352 samples (120 fecal, 92 beef cut, and 140 environmental swab) were collected and processed. The isolation and identification process involved the selective enrichment in modified tryptone soya broth supplemented with Novobiocin, plating on MacConkey, Eosin methylene blue and Cefixime-tellurite sorbitol MacConkey (CT-SMAC) agar, biochemical testing (IMViC test), followed by latex agglutination test. The isolates were tested for resistance profile to 13 antimicrobial agents using the standard disk diffusion method. Accordingly, of 352 samples, 14 (3.97%) were found to be positive for *E. coli* O157:H7 serotype; of which, 28.6% (4/14), 21.4% (3/14), and 50% (7/14) were from fecal, beef and environmental swab samples respectively. A significant difference in the occurrences of the pathogen was observed among the sources of sample (p=0.028). Antimicrobial susceptibility test results indicated that 100%, 92.8%, and 64.3% resistance were developed against tetracycline, erythromycin and ampicillin respectively. All *E. coli* O157:H7 isolates were found to be susceptible to azithromycin, cefotaxime, and chloramphenicol. Of the 14 *E. coli* O157:H7 isolates, 12 (85.8%) were resistant to three or more classes of antimicrobials. Unhygienic processing and poor sanitary practices were also noted by observational checklist among personnel in slaughterhouses and butcher shops. In conclusion, the study indicated that the occurrences of multiple antimicrobial resistant *E. coli* O157:H7 in the beef is strongly associated with the current beef processing and distribution practices. Therefore, an appropriate slaughter hygiene, more stringent regulation in the use of antimicrobials in human and animals, and risk based control measures, are recommended to safeguard the public health.

Key words: *Antimicrobials, Beef, Butcher shop, Cross- contamination, E. coli O157:H7*

1. INTRODUCTION

Despite of the improved technology and hygienic practices at all stages of food production, foodborne diseases are continued to be great public health and well-being concerns of individuals and countries across world (Bedasa *et al.,* 2018; Moawad *et al.,* 2017). Especially, developing countries are largely vulnerable to food-borne infections and costs billions of dollars in medical care and social costs (Havelaar et al., 2015). Studies indicated that, each year, 1 out of 10 people get ill from microbial food contamination, resulting in 600 million illnesses, 420 000 deaths and the loss of 33 million healthy years of life globally (Pires *et al.,* 2021). It often follows the consumption of contaminated foodstuffs especially from animal products such as meat from infected animals or carcasses contaminated with pathogenic bacteria including *Escherichia coli*.

Escherichia coli are a normal part of the intestinal micro-flora of many healthy animals and humans. Many E. coli strains are harmless or even beneficial to their host, however, some strains of E. coli can be pathogenic and cause fatal disease in humans (Cosentino *et al.,* 2013). Enterohemorrhagic *E. coli* (EHEC), is of the best known pathogenic strain and an important emerging zoonotic foodborne pathogen (Goldwater & Bettelheim, 2012).

E. *coli O157: H7*, an entero-hemorrhagic *E. coli* (EHEC), is one of the most common causes of foodborne infections in humans. It infects all age groups and the pathogen is noted for its severe consequences following infection, low infective dose and acid resistance (Ferens & Hovde, 2011). Depending on the immune status and the general health of the infected individual, and the dose and virulence of the bacteria, infection with *E. coli* O157: H7 can result in mild diarrhea, severe bloody diarrhea, hemorrhagic colitis, or hemolytic uremic syndrome (HUS) leading to kidney failure (Smith *et al.,* 2014). Globally, EHEC O157:H7 causes 2, 801, 000 acute illnesses annually, with an incidence rate of 43.1 cases per 100,000 persons per year. Among those, a total of 10,200 cases of STEC infections occur in Africa with an incidence rate of 1.4 cases per 100,000 people per year (Majowicz *et al.,* 2014).

Cattle are the primary reservoirs of *E. coli O157:H7* and consumption of beef and beef products are identified as major sources of foodborne transmission (Money *et al.,* 2010). Carcass contamination occurs through skin-to-carcass or fecal-to-carcass transfer of the pathogen during slaughter process at processing plants (Abdissa *et al.,* 2017). Furthermore, microbial cross-contamination can occur during processing and manipulation, such as dehiding, evisceration, storage and distribution at slaughter houses and butcher shops (Zweifel *et al.,* 2014).

To increase the production output, the animal production sector in developing countries have been regularly using antimicrobials for therapy, diseases prevention and growth purpose (Boeckel et al., 2015). Antimicrobials are widely used in cattle for disease prevention and as growth promotion (Herago & Agonafir, 2017). In Ethiopia, different studies have shown that multidrug resistance among *E. coli O157:H7* of animal food origin (Bedasa *et al.,* 2018; Shecho *et al.,* 2017). Tetracycline levels have been found to be especially high in meat and kidney samples from several abattoirs in Ethiopia, exceeding the WHO limits (Darwish *et al.,* 2013). *E. coli O157:H7* can be transmitted to humans through contaminated food and water, directly between persons, and through contact with animals or their environment. The use of antimicrobials in food cattle leads to the development of resistance pathogenic *E. coli O157:H7* that can reach humans through the beef food chain (Ma *et al.,* 2021).

The rising incidence and the potentially serious nature of *E. coli O157* infection are a cause for concern to public health authorities. In line with this, use of sensitive methods to detect *E. coli O157:H7* during surveillance, outbreak investigations, and quality control are recommended (Chapman *et al.,* 2001). *E.coli O157:H7* detection can be done by various methods that include culture based, immunological based, nucleic acid based and biosensors (Valderrama *et al.,* 2016). Molecular techniques (multiplex-PCR, pulsed field gel electrophoresis, multilocus sequence typing, DNA sequencing and many more) have been widely used in surveillance of foodborne pathogens to increase our understanding into the primary source of foodborne pathogens, source of infection and genetic diversity (Adzitey *et al.,* 2012).

In Ethiopia, animals are commonly slaughtered and dressed under unhygienic conditions in the open air or sub-standard slaughterhouses which compromises the microbiological quality and safety of the meat obtained from the animals (Atnafie *et al.*, 2017). Lack of surveillance of food-borne pathogens, lack of education and training among slaughterhouse and butcher shop workers, and poor hygienic practice of food handlers are major factor contributing to the high risk of exposure of Ethiopians to food-borne pathogens such as EHEC (Assefa, 2019). Furthermore, raw meat is available with objectionable hygiene in the open air without adequate temperature control. These may pose a potential risk for the occurrence of foodborne disease because of a widespread tradition of raw meat consumption in the country.

There is a need to investigate the possible sources of STEC O157:H7 in the beef supply chain, quantify risk factors, and evaluate the hygienic performance of slaughterhouses and butcher shops to ensure that, prevention and control strategies are appropriate. However, in Ethiopia, it has not yet been seriously evaluated the establishment level of slaughterhouses and their environment serve as sources of *E. coli O157: H7* particularly to beef meat contamination. Therefore, the general objectives of this study are:

- To investigate the occurrence and antimicrobial resistance profiles of *E. coli O157:H7* from slaughterhouse and butcher shops.
- To seek the extent of meat contamination with respect to the level of slaughterhouse establishment (municipal and private).

Specific objectives:

- To isolate and identify *E. coli O157:H7* from cattle feces, carcasses, and carcass contact surfaces at slaughterhouses and butcher shops.
- To assess the hygienic and sanitary practice in slaughterhouses and butcher shops
- To determine the antimicrobial resistance pattern of the isolates.
- To determine to what extent the slaughterhouse and butcher shops environments serve as sources of *E. coli O175:H7*.

2. LITERATURE REVIEW

2.1. *Escherichia Coli* O157:H7

2.1.1. Historical Background.

In 1884, Theodor Escherich identified a common commensal of the gastrointestinal tract, isolated from the fecal material of neonates and early infants, which he termed *Bacterium coli commune* (Bettelheim, 1988). This bacterium was renamed Escherichia coli after its original discoverer in 1919, thereby establishing Escherichia as a genus, with E. coli as the first species (Henry, 2015).

Since its discovery, *E. coli* has been one of the most studied and well characterized microorganis m, serving as one of the fundamental model systems in microbiology. In 1982, Escherichia coli O157:H7 was first identified as a human pathogen after two outbreaks in Oregon and Michigan (Sewlikar & D'Souza, 2017; Riley, 2014). In this year, three outbreaks of hemorrhagic colitis (HC) caused by *E. coli* serotype O157:H7 occurred in north America, at fast-food (ground beef sandwiches) prepared at restaurants in Oregon and Michigan and a nursing home in Ontario, Canada, Two common-source outbreaks probably food related in nursing homes. In Canada in 1983 (31 cases) and in 1985 (73 cases) accounted for 66 cases of hemorrhagic colitis, 12 cases of hemolytic uremic syndrome (HUS), and 17 deaths (Carter *et al.,* 1987). In central Scotland at the end of 1986 there was a report that 21 people died and more than 500 fell in ill due to an outbreak (Griffin & Tauxe, 1991). In china, in 1999, 177 death with 195 hospitalized patients who had clinically diagnosed of HUS (Xiong *et al.,* 2012). In Africa, the first case of human STEC disease occurred in South Africa in 1990 (Browning *et al.,* 1990) . Since then, *E. coli* O157:H7, and in more recent years also a number of other serotypes, have caused major human illness outbreaks worldwide with considerable morbidity and mortality (Dallman *et al.,* 2015).

2.2. Taxonomy and Characteristics

E. coli are gram-negative, rod shaped and facultative anaerobic bacteria belonging to the genus *Escherichia* within the family Enterobacteriaceae (Ewing, 1986) . *E. coli* belongs to the intestinal microflora of animals and humans. *E. coli* strains can be distinguished by the combination of their O-antigen, H-flagellar antigen, and K-capsular antigen. Serotyping based on O- and H-typing has been considered the standard method (Fratamico *et al.,* 2016). At present, 187 O-groups and 53 H types of *E. coli* species have been identified (Fratamico *et al.,* 2016) and more than 700 sero -types of *E. coli* have been identified (Doyle *et al.,* 2020)

Most *E. coli* strains are harmless, but some serotypes can cause serious diseases in animals and humans, and are occasionally responsible for product recalls due to food contamination(Vogt & Dippold, 2005). The harmless strains are part of the normal flora of the gut and can benefit their hosts by producing vitamin K2 (Bentley & Meganathan, 1982) and preventing colonization of the intestine with pathogenic bacteria (Secher *et al.,* 2016). The pathogenic strains, which cause enteric disease, are grouped into six categories: enterohemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enteroaggregative (EAggEC), and diffuse-adherent (DAEC). These categories differ in their pathogenesis and virulence properties, and each comprises a distinct group of O: H serotypes. *E. coli* O157: H7 is the most predominant and most virulent serotype in a pathogenic subset of EHEC. *E. coli* O157:H7 is so named because it expresses the 157^{th} O antigen identified and the 7^{th} H antigen (P. Chapman, Malo, *et al.,* 2001).

EHEC can grow at optimum conditions of temperature of 37 °C (7 °C to 50 °C), pH of 6-7 (4 to 9) and AW of 0.995 (0.95 to 0.995). For *E. coli* O157:H7 strains the growth is limited to maximum 44.5°C.The ability of STEC to resist acidic pH (close to 2.5) assists the survival in foods with adverse pH and overcomes the acidity barrier of the stomach allowing the entrance and colonization of the intestinal tract (Doyle *et al.,* 2020 ; Castro *et al.,* 2017).

E. coli O157:H7 are non-sorbitol fermenting (NSF), oxidase negative, catalase positive, indole positive, Urease negative, Voges Proskauer negative, and citrate negative (Rosser *et al.,* 2008).

These characteristics are very important in discriminating E. coli O157 from other non O157 STEC strains. Among the non-O157 STEC, serogroups such as O26, O45, O103, O111, O121, and O145 are most frequently accounted for severe human infection (Fan *et al.*, 2019 ; Eichhorn *et al.*, 2015).

2.3. Biochemical properties

Conventional identification of *E. coli* was done using the indole, methyl red, Voges-Proskauer, and citrate utilization (IMViC) test. Approximately 95 % of *E. coli* strains are indole and methyl red positive, but are Voges-Proskauer and citrate negative.

Table 1: Biochemical profile of *E. coli* O157:H7

Test	Reaction	Test	Reaction
Sorbitol	-	Glucose (gas)	+ (98%)*
β-Glucuronidase	-	Indole	+
Salicin	-	Arabinose	+
Esculin	-	Trehalose	+
Arginine dihydrolase	-	Mannitol	+
Adonitol	-	Lactose	+
Inositol	-	Maltose	+
Cellobiose	-	Rhamnose	+
Urease	-	Xylose	+
Citrate	-	Lysine decarboxylase	+
Potassium cyanide	-	Ornithine decarboxylase	+
Sucrose	+ (87%)*	Raffinose	+
Glucose (acid)	+	Dulcitol	+

Source: (Edwards and Fung, 2006) *Few negative strains have been reported

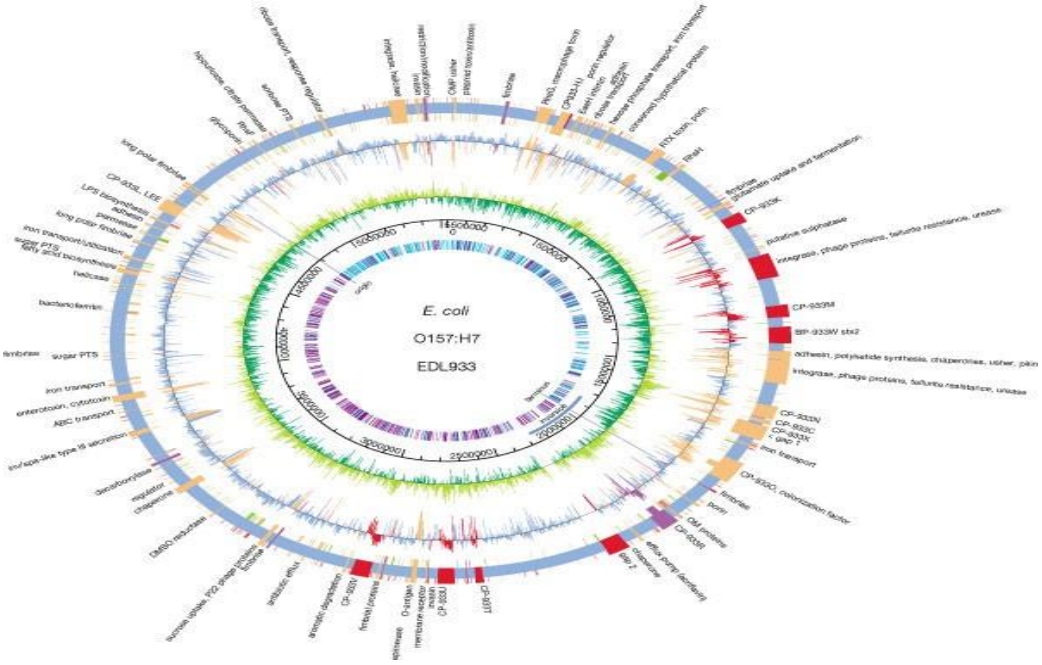
The unique biochemical properties of the *E. coli* O157:H7 are its failure to ferment sorbitol within 24 h and being β-D- glucuronidase negative (Dontorou *et al.*, 2004). These are the best and basic principles used in distinguishing these pathogenic strains from other *E. coli* serotypes.

Even *E. coli* O157:H7 and *E. coli* O157: H- strains are separated by these two biochemical characteristics. SMAC media is used for the detection of *E. coli* O157:H7 using the above principles. Further addition of antimicrobial supplements called cefixime and tellurite on SMAC can reduce the number of false-positive isolates (Leclercq *et al.*, 2002)

2.4. Genomic Organization

E. coli O157:H7 has 5.5 Mb chromosomal size. This genome includes 4.1Mb backbone sequence conserved in all E. coli strains. The remaining are specific to *E. coli* O157:H7 (Perna *et al.,* 2001). Additionally, genome comparison of *E. coli* O157:H7 with nonpathogenic E. coli K12 shows that 0.53 Mb of DNA is missing for E. coli O157:H7, suggesting genomic reduction has also played a role in *E. coli* O157:H7 evolution (Dobrindt *et al.,* 2003). The majority of *E. coli* O157:H7-specific DNA sequences (1.4Mb) are horizontally transferred foreign DNAs such as prophage and prophage-like elements. E. coli O157:H7 contains 463 phage-associated genes compared with only 29 in E. coli K-12 (Wick *et al.,* 2005) .A change in G+C contents is one of the indications that a genomic region has been acquired by horizontal transfer, and estimated that at least 53 different species have contributed to these unique sequences in *E. coli* O157:H7 (Putonti *et al.,* 2006). Virulence-associated genes between two sequenced E. coli O157:H7 strains are nearly identical (99%). Clearly, both the acquisition and loss of DNA have played an important role in the evolution of pathogenesis of *E. coli* O157:H7.

Figure 1: Circular genome map of *E. coli* O157:H7



Source : (Perna et al., 2001)

2.5. Foodborne *E. coli* O157: H7

Various new pathogens have emerged due to changing production processes in food industry. *Escherichia coli* 0157: H7 has emerged as an important global zoonotic food and water-borne pathogen that causes haemorragic colitis, haemolyticuraemic syndrome (HUS) and thrombotic thrombocytopenic purpura in human (Pal & Mahendra, 2016 ; Chekabab *et al.*, 2013).

The new emerging foodborne *E. coli* O157:H7 infections are related to food handling practices with processing and packaging of food, or the importation of certain food from a new geographical area. Its foodborne outbreaks occurred most commonly in communities such as restaurants and schools with ground beef being the most common vehicle among outbreak (Robinson & McKillip, 2010).

E. coli O157: H7 has become a major, worldwide food-borne pathogen known to result in life-threatening conditions. Globally, the Foodborne *E. coli* O157:H7 estimated to cause 2.8 million acute illnesses each year (MA *et al.*, 2018). In United States during 2003–2012, the pathogen is estimated to causes 390 outbreaks, which included 4,928 illnesses, 1,272 hospitalizations, and 33 deaths. The study also indicate that outbreaks attributed to foods consumed raw caused higher

hospitalization rates than those attributed to foods generally consumed cooked (Heiman *et al.*, 2015).

Several reports have documented a significant increase of antibiotic resistance in *E. coli* O157:H7 is the matter of increase concern and generate new public health challenge (Obaidat, 2020 ; Amézquita-López *et al.*, 2016). Humans develop resistance against specific antimicrobials due to consumption of animal products carrying antibiotic-resistant bacteria (Akbar *et al.*, 2014). Antimicrobial resistance is common in E. coli O157:H7, include multiple drug resistance to ampicillin, amoxicillin, ceftriaxone, chloramphenicol, ciprofloxacin, cotrimoxazole, methicillin, tetracycline and vancomycin (Constable *et al*., 2017).

In Ethiopia, studies have indicated that E. coli O157:H7 has been isolated from various foods of animal origin such as meat and raw milk (Asfaw Geresu & Regassa, 2021), poultry *(Shecho et al.*, 2017), and fish (Tilahun & Engdawork, 2020).

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2.6. Major virulence factors of *E. coli* O157:H7

2.6.1. Shiga Toxins (Stxs)

Shiga toxin is a potent cytotoxin and is bacteriophage encoded. Stxs are expanded from a single transcriptional unit and causes damage to a variety of cell types (Jacewicz *et al.*, 1999). Stxs can be divided into two groups called Stx1 and Stx2 but do not generate cross-reactive antibodies that are 56% homologous in amino acid sequences. Stx1 is identical to Stx from Shigella dysenteriae I, but for a single amino acid difference. Virulent isolates of E. coli O157:H7 can express Stx1 only, Stx2 only, or both toxins. Stx2 is known to be more toxic and is more often associated with HC or HUS in human infections than are Stx1 strains (Brashears *et al.*, 2003).

2.6.2. Locus of Enterocyte Effacement Effectors

E. coli O157:H7 colonizes the intestinal mucosa and induces a characteristic histopathological lesion referred to as attaching and effacing (A/E) lesions. The A/E lesion is characterized by effacement of microvilli and bacterial adherence to the epithelial cell membrane. Attached

bacteria

stimulate host cell actin polymerization accumulation, resulting in a raised attachment pedestal (Caprioli *et al.,* 2005). Genetic studies have shown that the genes responsible for A/E lesions map to a 13 region, which has been designated the locus of enterocyte effacement (LEE). The LEE of *E. coli* O157:H7 is composed of at least 41 different genes organized into three major regions; (i) a type III secretion system (TTSS) that exports effecter molecules; (ii) an adhesion called intimin and its translocated receptor, Tir, which is translocated into the host cell membrane by the TTSS; and (iii) several secreted proteins (Esp) as a part of TTSS, which are important in modification of host cell signal transduction during the formation of A/E lesions (Delahay *et al.,* 2001).

2.6.3. Plasmid O157 (pO157)

E. coli O157:H7 possess a putative virulence plasmid called pO157. The pO157 shows a dynamic structure and includes different mobile genetic elements such as transposons, prophages, insertion sequences (IS), and parts of other plasmids. The heterogeneous composition of pO157 can delimit the co-responses to functional regions of pO157. The complete sequence of pO157 reveals 100 open reading frames (ORFs) (Venkatesan *et al.,* 2021). Among them, thirty-five proteins are presumably involved in the pathogenesis of *E. coli O157:H7* infections, but of which only 19 genes have been previously characterized including a hemolysin (*ehxA*), catalase peroxidase (*kat P*), a type II secretion system apparatus (*etp*) and some others (Lim *et al.,* 2010). Nevertheless, the biological importance of pO157 in pathogenesis is not fully understood.

2.7. Pathogenesis

The virulence factors of *E.coli* O157:H7 are its ability to attach and efface the intestinal epithelium and its production of the cytolethal shiga toxin Stx1 and Sxt2. After the victim ingest the food contaminated with *E. coli* O157:H7, the organisms withstands the acidic environment of the human stomach and begins the process of infection (McKillip, 2010).

First, *E. coli* O157:H7 must initially adhere to the microvilli of the host epithelial cells (Mainil & Daube, 2005). The intimate attachment of the bacterial cell to the host epithelium is attributed to the adhesion intimin and translocated intimin receptor (Tir), a bacterial protein, which is inserted

into the host membrane and serves as the response for intimin and mediate adhesion between mammalian cells and attaching and effacing (A/E) pathogens. The bacterial outer membrane adhesin, intimin, is necessary for the production of the A/E lesion and diarrhea .The exact means by which *E. coli* O157:H7 establishes and sustains colonization in the host remains elusive. Once it has successfully colonized and established itself within the host, *E. coli* O157:H7 produces and releases its Stxs in the intestinal lumen. Shiga toxins act to inhibit protein synthesis within target cells (Mainil & Daube, 2005).

In humans, unlike cattle/ruminant that lack vascular expression of Gb3, the Stxs can endocytosed and translocate from intestinal epithelial cells into the bloodstream. Here, the Stxs bind to the Gb3 receptors on glomerular endothelial cells. The Stxs injure the glomerular cells and cause platelets and fibrin to deposit within the glomeruli. Eventually, the deposits decrease renal filtration and lead to the acute kidney damage characteristic of HUS (Welinder-Olsson *et al.*, 2005)

2.8. Epidemiology of *Escherichia coli* O157:H7

2.8.1. Geographical Distribution

E. coli O157:H7 infections occur worldwide and this have been reported on every continent except

Antarctica (Chase Topping *et al.*, 2008). Escherichia coli (EHEC) are responsible for gastrointest inal diseases reported in numerous outbreaks around the world (Parsons et al., 2016). Since its recognition in 1982, it has become an important concern in North America, Europe, South Africa, Japan, South America, and Australia. Particularly, in North America, Japan, and the UK. *E. coli* O157:H7 is the serotype most commonly associated with clinical disease in people. High rates are present in regions of South America, especially Argentina, where HUS is endemic (Constable *et al.*, 2017).

Table 2: Estimated pooled prevalence of *E. coli* O157:H7 in cattle by world region

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

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

2.8.2. Status of E. coli O157:H7 in Ethiopia

In Ethiopia, like other developing countries, it is difficult to evaluate the situation and effect of *E. coli* O175: H7. This is mainly because of the limited scope of studies, lack of coordinated epidemiological surveillance system and inadequacy of laboratory facilities for culture. In addition, under reporting of cases and the presence of other diseases considered to be of high priority may have over shadowed the problem of this important foodborne pathogen in some countries, including Ethiopia.

Several studies were conducted by some researchers to determine the occurrence and proportion of *E. coli* O157:H7 in faeces, skin swabs and carcasses of sheep, goat cattle in different areas of the country. Tables 2 summarize the prevalence of *E. coli* O175: H7 regards along beef supply food chain.

Table 3: Studies conducted on of *E. coli* O157:H7 from cattle in Ethiopia

Study area	Sample unit	Sample type	Prevalence	Reference

Bishoftu	Cattle	Fecal Carcass swab	7.1% 6.3%	Gutema *et al.,* 2021
Bahir Dar	Cattle	Carcass swab	8.9 %	Ayenew *et al.,* 2021
Arsi Zone	Cattle	Beef	2.1%	Asfaw Geresu and Regassa, 2021
Ambo	Butcher shop	Beef	9.1%	Tadese *et al.,* 2021
Addis Ababa	Cattle	Fecal Carcass	6.4% 12%	Hamid *et al.,* 2018
Hawassa	Cattles Butcher shop	Fecal Beef	4.7% 2.7%	Atnafie. *et al.,* 2017
Debre Berhan	Butcher shop	Beef	1.89%	Abdissa *et al.,* 2017
Dire Dawa	Cattle	Raw meat	2.06%	Haile *et al.,* 2017
Jimma	Cattle	Carcass swab Ceca content	9.3% 7.3%	Haile *et al.,* 2017
Addis Ababa	Cattle	Beef	10.2%	Tassew, 2015
Haramaya	Cattle	Carcass swab	2.65%	Taye *et al.,* 2013

2.8.3. Reservoirs of E.coli O157: H7

Livestock are the most important reservoir of *E. coli* O157:H7 with cattle being the principal sources (Tourret *et al.,* 2016), so, ground beef and beef products are identified as major sources of foodborne transmission. Cattle are now considered to be the major source of E. coli O157 causing human disease and transmission may occur through a variety of routes. In addition to the contamination of meat and dairy products, bovine feces can contaminate drinking water and crops intended for human consumption. Various outbreaks have been associated with vegetable products, such as radish and apple cider, presumably following contamination with animal wastes (CFSPH, 2009).

2.8.4. Source of infection

The predominant carriers and shedders of EHEC are healthy domesticated ruminants, cattle in particular, and to a lesser extent sheep and possibly goats (Su *et al.,* 2012) . Cattle food products and fresh products contaminated with cattle faces waste are the most common sources for infections (Callaway *et al.,* 2009). Beef carcass contamination is a direct result of pathogen transfer from cattle hides harboring enterohemorrhagic Escherichia coli. Hide contamination occurs from direct and indirect fecal contamination in cattle production and lairage environments. In each of these environments, individual animals shedding the pathogens at high levels (>104 CFU per gram of feces (Arthur *et al.,* 2010).

Transmission is via the fecal-oral route. The most frequent mode of transmission for E. coli O157:H7 infection is through consumption of contaminated food and water (Sodha *et al.,* 2015). This primarily has been linked to undercooked meat. Human infections have been mostly associated with the consumption of contaminated and improperly cooked minced beef (Catford *et al.,* 2014). However, acquisition of disease by direct contact with animals and manure at petting zoos and dairy farms are of increasing concern. (Constable *et al.,* 2017).It can also transmit direct from person to person or from infected animals. Birds Flies can also transmit mechanically as vectors. The habit of consuming raw and/or undercooked meat is one of the factors that exacerbate the transmission of foodborne *E. coli* O157:H7 (Chekabab *et al.,* 2013).

Cattle feces are the most important source of *E. coli* O157:H7. However, it also present in the feces of other animal species (goat, sheep, horse)(Gordillo *et al.,* 2011). Carcass contamination occurs through skin-to-carcass or fecal-to-carcass transfer of the pathogen during slaughter process at processing plants and this is the major risk factor for human infection. Butcher houses and restaurants are frequently incriminated as sources of *E. coli* O157:H7 for human infections (Fink et al., 2018). *E. coli* O157:H7 is highly virulent , with a low infection dose: an inoculation of fewer than 10 to 100 CFU of *E. coli* O157:H7 is sufficient to cause infection, compared to over one-million CFU for other pathogenic E. coli strains (Greig, 2010).

2.8.5. Disease pattern

Hemorrhagic colitis is an acute disease associated with *E.coli* O157:H7 in human. The symptoms characteristic to this disease are watery and/or bloody diarrhea, fever, nausea, severe abdominal cramping, and vomiting (Walker F *et al.*, 2012). From the point of ingestion, the incubation period of *E. coli* O157:H7 ranges from 8 hours to 16 days, but the typical incubation period is three to four days (Robinson AL, 2010). Life threatening complications, some victims, particularly the very young, may develop hemolytic uremic syndrome (HUS) (Martorelli *et al.*, 2015). HUS, which is characterized by renal failure and hemolytic anemia, occurs in up to 15% of hemorrhagic colitis victims and can lead to permanent loss of kidney function. People of all ages are susceptible to infection with STEC. However, the young and the elderly are more susceptible and are more likely to develop more serious symptoms (FDA, 2012). In the elderly, the combination of HUS with fever and neurologic dysfunction is characteristic of thrombotic thrombocytopenic purpura (TTP) (Sewlikar & D'Souza, 2017b). Haemolyticuraemic syndrome (HUS) consists of the triad micro-angio-pathichaemolytic anemia, acute uraemia and thrombocytopenia. HUS leads to significant morbidity and mortality during the acute phase and it is the most common cause of acute renal failure in children (Karpman *et al.*, 2017). In clinical cases, in human the mortality rate varies with the syndrome. Hemorrhagic colitis alone is usually self– limiting, although deaths can occur. Complications and fatalities are particularly common among children, the elderly, and those who are immunosuppressed or have debilitating illnesses. Infection associated HUS is estimated to be fatal in 1-10% of children and up to 50% of the elderly. In European surveillance, the case fatality rate in all reported EHEC infections was < 0.5% (CFSPH, 2016).

2.8.6. The role of cattle in human E. coli O157:H7 infection

Cattle play an essential role in epidemiology of human *E coli* O157:H7 infection and cattle feces considered as primary source which the beef food become contaminated with this pathogen (Niyonzima *et al.*, 2015).

The first identified human outbreaks of *E. coli* O157:H7 in 1982 was associated with consumption of ground beef, and the importance of cattle as a reservoir for *E. coli* O157:H7 became evident as more outbreaks were associated with undercooked beef and other bovine products such as unpasteurized milk (Laine *et al.*, 2005). The association of *E. coli* O157:H7

with undercooked ground beef and raw rice led to investigations of the role of cattle as a reservoir of the pathogens (Pal & Mahendra, 2016).

Beef, particularly ground beef, continues to be the major source of *E. coli* O157:H7 outbreaks, likely because cattle are the main reservoir for *E. coli* O157:H7. The study conducted in United Stated during 2003–2012, state that there were 353 outbreaks, from those 20% transmission was through consumption of beef and beef product and studies have about 75% of the human E. coli O157:H7 outbreaks to food products of bovine origin (Callaway *et al.,* 2009).

Carriage of clinical *E. coli* O157:H7 isolates by cattle may simply reflect a high probability of pathogen transmission from cattle to people as a consequence of the predominance of beef and dairy cattle among domesticated animals, and the voluminous output of bovine manure. The incidence of human cases of *E. coli* O157:H7 is positively related to cattle density and the cattle to human ration (Heiman *et al.,* 2015) and there is a clear association of cattle density and the occurrence of all STEC-related gastroenteritis in humans; in Ontario, there was a correlation with cattle density, but not with presence of sheep or goats and in Germany the risk increased by 68% per additional 100 cattle/km2 (Callaway *et al.,* 2009).

Colonization of *E. coli* O157:H7 in adult cattle is asymptomatic (Verstraete *et al.,* 2014) because intestinal mucosal cells lack the Stx-specific globotriaosylceramide receptor. Some cattle shed 10^4 CFU *E. coli* O157:H7 per one gram of feces, are called "Super Shedders". Super shedders have prominent outcome for distribution of EHEC in cattle as it is the main reservoir and therefore increase the risk of human infection (Margo Chase-Topping *et al.,* 2008).

2.8.7. Detection of E. coli O157: H7

Clinical cases can be diagnosed by finding the organisms in fecal samples, Food and environmental samples may also be tested to determine the source of the infection. Many diagnostic laboratories can detect identify *E. coli* O157:H7. There is no single technique that can be used to isolate all EHEC serotypes (Spickler, 2016). Infection with this agent is associated with a broad spectrum of illness ranging from mild diarrhea and hemorrhagic colitis to the potentially fatal hemolytic uremic syndrome (HUS). These clinical symptoms used as one diagnoses technique (Rahal *et al.,* 2012).

Common sample are diarrheic feces in animals, predictable food item in both animal and human food, stool of infected individual in human with hemolytic-uremic syndrome and from foodborne outbreaks (Elhadidy *et al.,* 2015). The most sensitive sampling method from animal for STEC O157:H7, is the rectal swab, because STEC specifically colonize the recto-anal junction of the intestinal mucosa that is directly sampled with the swab approach (Constable *et al.,* 2017).

The conventional or cultural standard methods appear to have been used since the inception of microbiological sampling. These methods mainly involve enrichment followed by plating onto selective agar or by plating directly onto selective agar without enrichment, and confirmation of presumptive bacteria colonies by biochemical tests. They are widely used and have the advantage that, they are cheaper, detect only viable bacteria, and yield isolates that can further be characterized and studied (Law *et al.,* 2014) .

Immunoassays and polymerase chain reaction technology have led to more rapid detection of this E. coli in stools, food, and water. Techniques included in this category are PCR and DNA-based techniques, immunomagnetic separation, and ELISAs (Bavaro. M, 2009).

Molecular-based techniques are distinctly advantageous because of their sensitivity, selectivity, and their rapid results. However, molecular-based techniques are appreciably more expensive than traditional plating techniques and are also more novel and unfamiliar. Therefore, the integration of molecular-based approaches into quality control procedures depends on the overall needs and resources of the food processing plant (Robinson AL, 2010). There also Latex Agglutination Test for the rapid identification of *E. coli* O157:H7. The test is best used in conjunction with Sorbitol MacConkey Agar. A positive result is indicated by agglutination with the test reagent, whilst the control reagent should appear milky and smooth (Al-Dragy & Baqer, 2017).

2.8.8. Treatment, Prevention and Control

Treating *E. coli* O157:H7 infection with antimicrobial agents is associated with an increased risk of severe sequel such as HUS (Rahal *et al.,* 2012) and may exacerbates the patient's condition by increasing either the release of preformed Shiga toxins (Stx) upon cell lysis. However, early administration using some antimicrobials is effective (Nassar *et al.,* 2013).

Certain management practices optimize the likelihood of good outcomes, such as avoidance of antibiotics during the pre-hemolytic uremic syndrome phase, admission to hospital, (Davis *et al.,* 2013) and the patients with complications may require in rigorous care including dialysis, transfusion and/ or platelet infusion besides kidney transplant (CFSPH, 2009b).

Prevention of *E. coli* O157:H7 by frequently washing of hands after using the bathroom, before preparing or eating food, and contact with animals. Adequate sanitation and proper processing of foods is seriously important, cook meats thoroughly at a temperature of at least 160°F/70˚C and avoid raw meat, milk, unpasteurized dairy products (Mathusa *et al.,* 2010). Keeping cattle away from water supply, proper disposal of infected faces, good kitchen hygiene may reduce the incidence of *E. coli* O157:H7 human infection. And implementation of *E. coli* O157:H7.Testing contaminated material for and withholding that material, before releasing it to the market is one way of preventing human infection and illness (CFSPH, 2009).

One Health approaches is the opportunity to implement control programs that reduce the multiple impacts of zoonoses in both human and animal populations. Interventions that may control zoonotic infection in animal populations or prevent disease transmission from animals to people may offer more effective and economically viable approaches to disease management than those focusing on the human population alone (Halliday *et al.,* 2015).Vaccines against EHEC O157:H7 for cattle may reduce shedding, and have received full or conditional approval in some countries including the U.S. and Canada, but are not in wide use but there is no human vaccine against enterohemorrhagic Escherichia coli (EHEC) infections(Smith, 2014).

2.8.9. Antimicrobial resistance

Over the past decade, studies have documented an increase in antimicrobial resistance in STEC O157:H7 and non-O157:H7 strains, isolated from domestic animal reservoirs that potentially could impact food and environmental sources (Iweriebor *et al.,* 2015; Kasumigaseki *et al.,* 2012). In agricultural based countries , the misusage of antibiotics for treating either human or plant diseases and for promoting food-animal growth are believed to contribute to the continued

increase in antimicrobial resistance as well as to the emergence of multidrug resistance profiles (Boeckel *et al.,* 2015). The use of antimicrobials to treat STEC infections is highly controversial since these agents can induce Stx production and thus promoting the onset of HUS in humans. For example, sub-inhibitory doses of fluoroquinolones sulfonamides, and quinolones which target DNA synthesis have resulted in an increased production of Stx (McGannon *et al.*, 2010). Resistance to tetracycline was the most common observations found in bovine and human isolates followed by resistance to streptomycin and ampicillin (Fratamico and Smith, 2006).

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted in Bishoftu town, located at 9°N latitude and 40°E longitudes at an altitude of 1850 m above sea level in central high lands of Ethiopia. It has an annual rainfall of 866 mm of which 84% is in the long rainy season (June to September). The mean annual

maximum and minimum temperatures are 26 °C and 14 °C respectively, with mean relative humidity of 61.3% (ADARDO 2007). The livestock production system in the area is both intensive and extensive type (CSA, 2015). In Bishoftu town, there are three export abattoirs, two small cattle slaughterhouses (one municipal and one private) and 131 officially registered butcher shops serving the local community.

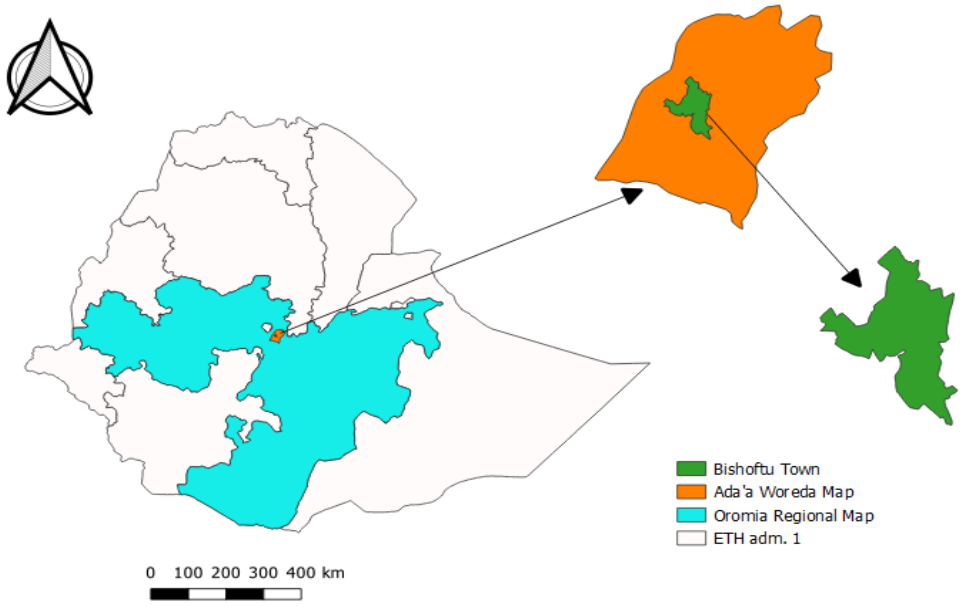

Figure 2: Map of the study area

3.1.1. Slaughterhouses

The two cattle slaughterhouses (one municipal and one private) serve the local community and were small in processing capacity where by the municipal slaughterhouse and the private slaughterhouse usually slaughtered 10–15 and 30-40 cattle per day, respectively. The slaughter process at both slaughterhouses was rather similar. Briefly, the slaughtering process started by

stunning cattle with a sharp knife, bleeding, removal of head and feet and de-hiding the upper part of the hind legs on the floor followed by hanging of the carcass, manual dehiding, evisceration, carcass washing, post-mortem inspection (not regular in the municipal slaughterhouse), carcass labeling and storage at environmental temperature until distribution to butcher shops. In few occasions, however, the municipal slaughterhouse workers were conducted the whole slaughter process on the floor. Beside, municipal slaughterhouses did not have a stand-by pressurized water supply and hot water for hand and equipment, including knives, washing. Moreover, both slaughterhouse workers were free to move and involve in different slaughter steps. The slaughterhouses were located in the community residence. Irrespective of high demand of meat in the weekend, the number of employees in the slaughterhouses were constant to accommodate the demand. At the time of this study, none of the slaughterhouses has HACCP/ISO verification.

3.2. Study design and study population

A cross-sectional study type was carried out from December 2021 to May 2022 at two slaughterhouses (Bishoftu Municipal and privately owned), and 92 butcher shops in Bishoftu town.

Sampling was carried usually once a week, where; 10 cattle were selected by using systematic random sampling method. Fecal contents of the cattle, carcass swabs, and swabs samples from the environment (knives, hook, hand swab, water and wastewater) were collected from the slaughterhouses. Fecal samples were collected from each selected animals directly from the rectum using rectal gloves in the lairage before the slaughter. Representative carcass swab samples were taken after de-hiding and post-evisceration. All swab samples were collected as a pool sample during sample collection.

A simple random sampling technique was employed to select (n=92) butcher shops from a total of 131 butcher shops registered in the town. From each butcher shop, sample of beef cuts (at least 25 g) and a parallel pooled swab sample of butcher's knife, hand, and cutting board were collected. On an average about 15 beef and beef in contact surface samples were collected per week.

The study populations were apparently healthy cattle ready to be slaughtered at aforementioned slaughterhouses. Animals are originated from different areas of the countries, of which the majority were from the nearby town. Most of them were transported by an open air vehicles and the study considers these animals starting from the lairage.

3.3. Sample size determination

The sample size was determined by the following formula given by Thrusfield, (2005).

$$n = \frac{1.96^2 \; X \; Pexp*(1-Pexp)}{(d)^2}$$

Where:

1.96 = the value of Z at 95% confidence interval

d =desired absolute precision

n=required sample size, and

P^{exp}= expected prevalence

According to Gutema *et al*., (2021) the prevalence of *E.coli* O157: H7 in cattle feces and beef carcass was 7.1% and 6.3% respectively. Therefore, using the 7.1% and 6.3% expected prevalence, 95% confidence interval and 5% type I error, the number of cattle and butcher shops estimated to be at least 102 and 91 respectively. But to increase the precision of the study more samples of fecal, carcass swab, and environmental swabs were added.

3.4. Sampling method and transportation

3.4.1. Fecal sample collection

A total of 120 fecal samples, 60 from each slaughterhouse, were collected using sterile, wide mouthed and leak proof universal tube immediately before slaughter. Rectal penetration of cattle was made using sterile arm length glove and fecal material was collected at the rectum and tried to obtain a representative sample of the fecal content. For each cattle a sterile arm length glove was used to avoid cross contamination.

3.4.2. Meat sample collection

During the visit, a total of 92 beef cut samples, from 92 butcher shops, were collected according to the International Organization for Standardization (ISO, 2001). At least 25g of beef was taken from the exterior of the carcass (fat tissue) and surface of lean beef using sterile scalpels and forceps and put into a sterile, separately labeled plastic bags. Scalpel and forceps were cleaned with pieces of gauze dipped in 70% ethanol after each sampling to minimize cross-contaminations.

Pooled carcass swabs (n =12), 6 form each slaughterhouse, were collected from four different sites of the carcass (thorax, brisket, flank and crutch) using the method described by McEvoy *et al.,* (2003), one site covering 100 cm^2 by placing sterile template (10 x 10 cm) on a carcass. For each sampling area, a sterile cotton tipped swab (2 X 3 cm) fitted with shaft was moistened in an approximately 10 ml of buffered peptone water (Oxoid , Hampshire, England), was rubbed first horizontally and then vertically several times across the carcass surface. On the completion of the rubbing process, the shaft was broken by pressing it against the inner wall of the test tube and disposed leaving the cotton swab in the test tube. The four swabs were put into one screw cupped test tube containing 10 ml of sterile buffered peptone water and transported to the microbiology laboratory of the college of veterinary medicine and agriculture of Addis Ababa University.

3.4.3. Environmental Sample Collection

A total of 128 swab samples (36 from the two slaughterhouses and 92 from butcher shops) were collected. Swabs collected from the slaughterhouse environments were, knife, rasp, axe and hook (n= 12) and hands swab (n= 12), and tap water used to wash the carcass (n=12). Similarly swabs from butcher's hand, knives, and chopping boards, were collected during the operation at the butcher shops. Environmental swab samples were taken from meat contact surfaces of 15-20cm^2 using sterile cotton swab moistened in buffered peptone water. Then swabs were immersed into a test tube containing sterile buffer peptone water. In addition, slaughterhouse wastewater samples (50 ml) in the drainage line were collected at every single visit of the slaughterhouses.

All samples were labeled legibly with permanent marker identifying type/source of sample, date of sampling and code of the slaughterhouses / butcher shop. The samples were transported in an ice box containing ice packs to the microbiology laboratory of the college of veterinary medicine and agriculture, Addis Ababa University.

3.5. Assessment of hygienic practices at slaughterhouses and butcher shops

The assessment of the hygienic practices of slaughterhouses and the butcher shop workers were assessed using observational checklists adapted from previous studies (Nurye & Demlie, 2021; Dulo *et al.*, 2015) . At each visit, at least 2 slaughterhouse workers were observed for over 30 minutes during slaughter operation. Similarly, butcher shop workers were checked for hygienic practice at the time of sample collection. During the visits, the workers were checked whether their meat processing and handling practices were safe or not by close personal observations. Data related to lairage establishment, availability of clean water, regular hand washing and disinfection practice of hands, whether they used clean protective cloths, whether they process red and green offal in separate room, the availability of washable chopping boards and knives for processing of abdominal organs and other parts of meat, whether hot water were used for disinfecting knives and rasps, and whether fisting and evisceration were done with most care to avoid carrying dirt were checked.

3.6. Isolation of *E. coli* O157:H7

The protocol described by ISO 16654, (2001) was followed for isolation of *E.coli* O157:H7. Twenty five grams of each fecal and beef cut sample was aseptically transferred into a stomacher bag containing 225mL of modified tryptone soya broth supplemented with 20mg/L Novobiocin (mTSBn; Oxoid, England), homogenized using a stomacher blender (Stomacher 400, Seward Medical, England) for 1 minute at normal speed (200 rpm) and incubated at 41.5 °C for 6 hours. Similarly, all swab samples from the slaughterhouses and butcher shops were homogenized in 9 mL of mTSBn and incubated at 37°C for 24 hrs.

The enriched samples were streaked onto MacConkey agar (Oxoid, England) plates for primary isolation and incubated at 37°C for 24 hrs. Following incubation, the plates were observed for the growth of pink colonies (lactose fermenter). A single, isolated colony was then picked and sub-cultured on Eosin Methylene Blue (EMB) agar (Oxoid, England) for 24hrs at 37°C for formation of metallic green sheen. Suspected colonies of *E. coli* (pinkish color appearance on MacConkey agar and metallic sheen on EMB) (Appendix-3) were then sub-cultured onto nutrient agar (Oxoid, England) at 37°C for 24 hrs. From nutrient agar relevant biochemical tests that included indole, methyl red, Voges-Proskauer reaction and citrate utilization (IMViC) tests and H_2S production test were performed. Indole and H_2S productions were done using tryptone soya broth and triple sugar iron agar (Oxoid, England) respectively, while methyl red and Voges-Proskauer reactions tests were done using MR-VP broth (HiMedia, India). Using Simon's citrate agar (HiMedia, India) citrate utilization test was done. The test reagents used were Kovac's reagent for indole test, methyl red for methyl red test, and alpha-naphthol and 40% KOH chemicals for Voges-Proskauer reaction tests. The H_2S production and citrate utilization tests results were observed and interpreted according to Quinn *et al.*, (2002).

Then the bacterium that was confirmed as *E. coli* was sub cultured onto Sorbitol MacConkey agar (Oxoid, England) supplemented with 0.05 mg/l Cefixime- 2.5 mg/l potassium tellurite and plates were incubated at 35 °C for 20 to 22 hrs. *E. coli* O157:H7 does not ferment sorbitol and, thus, produces slightly transparent colorless colonies (Appendix-3). Then, up to six colorless colonies (non- Sorbitol fermenters) on Sorbitol MacConkey agar were picked and sub-cultured onto nutrient agar slants and incubated at 37°C for 24 hrs. for further confirmatory test.

3.7. Identification of *E. coli* O157:H7

Identification and confirmation of non-sorbitol fermenting *E. coli* O157:H7 was done by latex agglutination test using latex kit (Oxoid, DR0620). The latex kit consists of four components: latex test reagent, latex control reagent, positive controls, and the negative controls. The test reagent contain blue latex particles sensitized with specific antibody against O157 antigen and the control reagent consists of latex particles sensitized with rabbit globulin. The positive controls are suspension of inactivated *E. coli* O157:H7 cells, whereas the negative controls are suspension of inactivated non-specific *E. coli* cells.

The test was performed according to the manufacturer instructions (Oxoid, Hampshire, England). The latex kit was first checked for its performance by using the control suspensions in the kit, the test was continued after the positive control reacts with the test latex showing positive result. Briefly, one drop of 0.85% saline water and latex test were dispensed in to the reaction card separately. Using a sterile wire loop, a few presumptive colonies of *E. coli* O157 were taken and emulsified in to the saline water on the latex card, then slowly mixed with the test latex and checked for agglutination within 1 minute. A result was positive if agglutination of the latex particles occurred within 1 minute (appendix -3). Negative result was obtained if no agglutination occurred and a smooth blue suspension remained after 60 seconds in the test area. Test positive isolates were stoked in glycerol using cryovials for further antimicrobial resistance determination.

3.8. Antimicrobial susceptibility testing

The antimicrobial susceptibility tests were done for all confirmed *E. coli* O157:H7 isolates by using standard agar disc diffusion technique (CSLI, 2014) for the following 13 antimicrobial agents (Oxoid, Hampshire, England, Exp. Date- 2023): ampicillin (AMP, 10 µg), azithromycin (AZM, 15µg), cefotaxime (CTX, 30 µg), ceftazidime (CTZ, 30µg), chloramphenicol (CHL, 30 µg), ciprofloxacin (CIP, 5 µg), colistin sulphate (CT, 10 µg), erythromycin (ERY, 15 µg), gentamicin (GEN,10 µg), kanamycin (KAN, 30 µg), nalidixic acid (NA, 30 µg), sulfamethoxazole (SXT, 25 µg), and tetracycline (TET,30 µg). The selection criteria of the antibiotics depended on the availability of antimicrobials, regular use of the antimicrobials in the ruminants, potential public health importance and recommended from the guideline of antimicrobial susceptibility testing.

Escherichia coli O157:H7 isolates from pure fresh culture was transferred into a test tube of 5 ml tryptone soya broth (Oxoid, England) and incubated at 37°C for 6 hours. The turbidity of the cultured broth was adjusted using sterile saline solution, or more isolated colonies were added to obtain turbidity that is usually comparable with that of 0.5 McFarland standards. A sterile cotton swab was immersed into the suspension and rotated against the side of the tube to remove the excess fluid and then swabbed uniformly on the surface of already prepared Mueller-Hinton agar (Oxoid, England) plates. As soon as the plates dried, antimicrobial discs were placed on the inoculated plates using sterile forceps, and incubated at 37°C for 24 hrs. The results were interpreted as resistance, intermediate or susceptible after the zone of inhibition of the strain was appreciated, hence a standardized table is given by the clinical laboratory standard institute (CLSI, 2014).

3.9. Ethical clearance

Ethical clearance was obtained from animal research ethical review committee of the college of veterinary medicine and agriculture, Addis Ababa University. (Ref. No: VM/ERC/14/02/14/2022). Additionally, human samples were collected following prior explanation of the objectives of the study to the slaughterhouse and butcher shop workers, and sampled based on their consent.

3.10. Data management and statistical analysis

The data generated from the study was arranged, coded and entered to Excel spread sheet (Microsoft® office excel 2013). Descriptive analysis was used to describe the result of proportion analysis. The prevalence of *E. coli O157* in fecal samples, carcass swab and environmental samples was determined by dividing the number of positive samples by the total number of samples examined. The significance of association between *E. coli O157* isolates and sample source and type of sample was assessed using the univariate logistic regression in R statistical software (R.4.0.4). Odds ratio and 95% confidence intervals were used to measure the strength of associations. *P* value was set significant at $P < 0.05$.

4. RESULTS

4.2. Prevalence of *E.coli* O157:H7

Of 352 samples, 69 (19.6%) were positive for *E. coli* and 14 (3.97%) confirmed as *E. coli* O157: H7 strains. With regard to sample sources, 10/168 (5.95%) and 4/184 (2.2%) of *E. coli* O157: H7 were detected from slaughterhouse and butcher shop samples, respectively. Accordingly, from the slaughterhouse samples, *E. coli* O157: H7 was detected in 6 (12.5 %) carcass, carcass contact surface, and wastewater samples. Moreover, four (3.33%) *E. coli O157: H7* isolates were detected from fecal samples. Similarly, from butcher shops collected samples, 3(3.3%) beef cut samples and 1(1.1%) carcass contact surfaces sample were positive for *E. coli* O157:H7. Higher occurrence of *E. coli* O157: H7 was observed in the municipal slaughterhouse (8.33 %, 7/84) than at the private slaughterhouse (3.57%, 3/84).

Table 4: The occurrence of *E. coli* O157: H7 by sample sources and sample type

Sample source	**Sample type (p* =pooled sample)**	**No. of samples Tested N (%)**	**No. Positive *E.coli* O157: H7 *(%)***
Municipal slaughterhouse	Fecal	60	3 (5)
	Carcass swab (p*)	6	2 (33.3)
	Knife swabs (p*)	6	0
	Hand swabs (p*)	6	1 (16.7)
	Water / wastewater	6	1 (16.7)
	Sub-total	84	7 (8.3)
	Fecal	60	1 (1.7)

Private slaughterhouse	Carcass swab (p^*)	6	1 (16.7)
	Knife swabs (p^*)	6	1 (16.7)
	Hand swabs (p^*)	6	0
	Water/wastewater	6	0
	Sub-total	84	3 (3.57)
	Sub-total of slaughterhouses	168	10 (5.95)
Butcher shops	Beef /meat	92	3 (3.3)
	Butcher's hand, knife, cutting board (p^*)	92	1 (1.1)
	Sub-total	184	4 (2.2)
	Total N (%)	352	14 (3.97)

A univariate logistic regression test was used to assess the association of the occurrence of *E. coli* O157: H7 with sample source and sample type separately. Hence, a statistically significant association was found between *E. coli* O157: H7 and source of the samples (municipal slaughter house) (*P = 0.028*) with OR= *4.09 [1.20-16.0]*). The odds of the detection of *E. coli* O157: H7 from municipal slaughterhouse was 4.09 times than from butcher shops. However, no significant association was observed (P>0.05) between *E. coli* O157:H7 and the private slaughterhouse. (Table 4).

Although there was no significant association with sample type, a higher prevalence of *E. coli* O157:H7 was observed from the swab samples (12.5 %) than fecal samples (3.3%)

Table 5: Association of *E. coli* O157:H7 with respected sample source and sample type

Categories		***E. coli* O157: H7**		
		No. of positive	Odd Ratio [95% CI]	P ~value
Sample source	Butcher house	4	Ref	Ref.
	Municipal	7	4.09 [1.20-16.0]	0.028
	Private	3	1.67 [0.32-7.73]	0.510
	Fecal	4	Ref	Ref.

Sample type	Beef /meat	3	0.98 [0.19-4.54]	0.977
	Swab samples	6	1.43 [0.40-5.70]	0.590
	Waste water	1	2.64 [0.13-19.89]	0.404

4.2. Assessment of the hygienic practices in slaughterhouses and butcher shops

The observational survey revealed that 'lairage to stunning' area was dirty. Cattle were stunned stressfully and dragged on the floor after bleeding. There were no means to sterilize equipments. Carcass were quartered manually on the floor using axe in the municipal slaughterhouse. None of the slaughterhouse and butcher shop workers were washing and disinfecting their hands, the processing tools and the floor after each working interval. The same cutting boards, knives, and rasps were used for cutting of meat and abdominal organs. Moreover, risky practices such as dehiding and evisceration were done without taking care to avoid cross contaminations.

Table 6: Summary of Observational Checklist Results on Hygienic Practice in the slaughterhouses and Butcher Shops in Bishoftu town, Ethiopia.

Activities observed and cheeked	**Municipal Slaughterhouse**	**Private slaughterhouse**	**Butcher shops**
Is the lairage wide, cleaned and compartmented?	No	No	
Use of protective clean clothing?	None of them	None of them	Few
Is there clear demarcation between clean and dirty area in slaughterhouse?	No	yes	
Is there adequate supply of clean water?	No	No	Not cheeked
Regular disinfection of hand, the floor and processing tools?	None of them	None of them	None of them
Is slaughtered animals washed before hanging?	No	No	
Is fisting and evisceration is done with most care	No	No	

to avoid carrying dirt?			
Processing rumen and intestine in separate room?	No	Yes	
Use of separate knives for cutting of meat and abdominal contents?	No	No	No
Use of hot water to disinfect knives, rasp and other equipment?	No	No	No
Regular washing of chopping tables and chopping boards?	No	No	No
Use of the separate buckets of water for cleaning knives, washing hands?	No	No	No

4.3. Antimicrobial resistance profile of *E. coli* O157: H7

Antimicrobial susceptibility testing was done for all confirmed isolates against 13 different antimicrobial agents. Accordingly, *E.coli* O157:H7 isolates were found to be resistant to tetracycline (100%), erythromycin (92.8%), and to ampicillin (64.3%). Furthermore, a resistance of 14.3% was observed to ceftazidime, colistin sulphate, kanamycin, nalidixic acid, and sulfamethoxazole. However, none of the isolates were resistant to azithromycin, cefotaxime, and chloramphenicol.

Table 7: Antimicrobial Resistance Patterns of *E. coli* O157: H7 Isolates

Antimicrobial Disc	**Resistance pattern** ***E. coli O157: H7 (n=14)***		
	S (%)	**I (%)**	**R (%)**
Ampicillin (AMP)	3 (21.4)	2 (14.3)	9 (64.3)
Azithromycin (AZM)	14 (100)	0	0
Cefotaxime (CTX)	14 (100)	0	0
Ceftazidime (CTZ)	11 (78.6)	1 (7.1)	2 (14.3)
Chloramphenicol (C)	14 (100)	0	0
Ciprofloxacin (CIP)	12 (85.8)	1 (7.1)	1 (7.1)
Colistin sulphate (CT)	12 (85.8)	0	2 (14.3)
Erythromycin (ERY)	0	1 (7.1)	13 (89.9)
Gentamicin (GEN)	12 (85.8)	1 (7.1)	1 (7.1)
Kanamycin (KAN)	11 (78.6)	1 (7.1)	2 (14.3)

Nalidixic acid (NA)	12 (85.7)	0	2 (14.3)
Sulfamethoxazole (SXT)	12 (85.7)	0	2 (14.3)
Tetracycline (TTC)	0	0	14 (100)

Source: (CLSI, 2014) S=Susceptible, I=Intermediate, R=Resistant

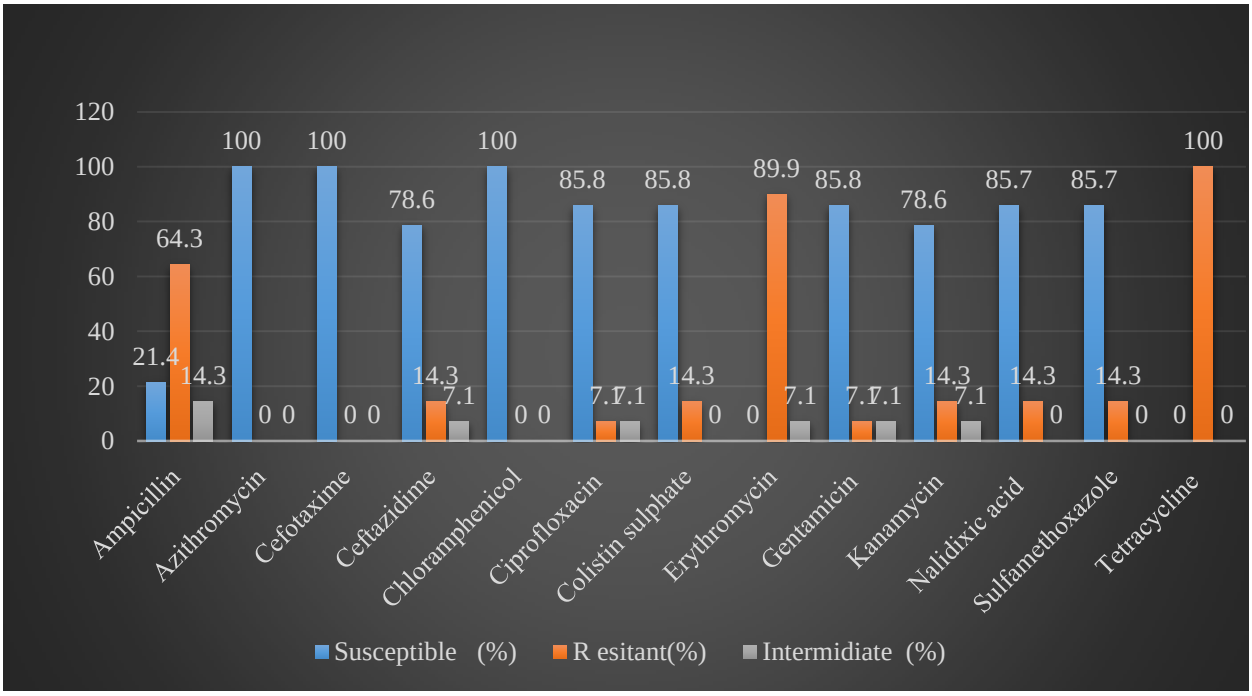

Figure 3: Antimicrobial resistance pattern of *E. coli* O157: H7 isolates

4.3.1. Multi-drug resistance profiles of E. coli O157: H7

This study showed that 12 (85.8%) *E. coli* O157: H7 isolates were resistant to three or more classes of antibiotics. Multi-drug resistance profiles of the isolated 5/14 (35.7%), 5/14 (35.7%) and 1 (7.1%), against three, four and five antimicrobials respectively were registered.

A multidrug resistance pattern consisting of six drugs was also documented in 1/14 (7.1%) of the fecal isolates. The frequency of resistance phenotype was more common for tetracycline, erythromycin, and ampicillin. Statistically significant association was observed between multiple antimicrobial resistant *E. coli* O157: H7 and source of the samples (Table 9).

Table 8: Multidrug resistance (MDR) pattern of *E. coli* O157:H7.

			Sources of MDR				
Source of Isolates	**Resistance pattern**	**Antibiotic Classes**	**Beef n=3**	**Fecal n=4**	**Swab n=6**	**Water n=1**	**Total MDR**
	CT, ERY, TTC	R3		1			1
Municipal	AMP, ERY, TTC	R3			1	1	2
slaughter	AMP, ERY, NA, TTC	R4		1			1
House	AMP,ERY, GEN,TTC	R4			1		1
	AMP,CTX,CTZ,ERY NA, TTC	R6		1			1
	Sub-total			3	2	1	6
Private	ERY, KAN, TTC	R3			1		1
Slaughter	AMP,ERY,TTC	R3			1		1
House	AMP, CIP, ERY,TTC	R4		1			1
	Sub-total			1	2		3
	ERY, KAN, TTC	R3	1				1
Butcher	AMP, ERY, SXT, TTC	R4			1		1

House	AMP, CTZ, ERY, SXT, TTC	R5	1				1
	Sub-total		2		1		3
	Over all MDR (%)		2 (14.3)	4 (28.6)	5 (35.7)	1 (7.1)	12 (85.7)

Abbreviations: R3-R6; resistance to three, four, five, and six classes of antibiotics

Table 9: Analysis of MDR profiles based on samples Source and Samples type

Categories		**No. of antibiotic classes**				MDR/ Total	Prop. of MDR [95% CI]	p-value
		R3	R4	R5	R6			
Sample source	Butcher shop (n=4)	1	1	1		3/4	Ref	Ref
	Municipal (n=6)	4	1		1	6/6	5.48 [1.48-25.96]	0.016
	Private (n=3)	2	1			3/3	1.47 [0.19-9.04]	0.675
Sample type	Fecal (n=4)	3	2		1	3/4	Ref	Ref
	Beef (n=3)	1		1		2/3	0.64 [0.09-3.38]	0.617
	Swabs (n=5)	4	1			2/5	1.18 [0.30-4.86]	0.810
	Wastewater (n=1)	1				1/1	2.64 [0.13-19.89]	0.404

Abbreviations: R3-R6, resistance to three, four, five, and six classes of antibiotics

5. DISCUSSION

5.1. Prevalence of *E.coli O157:H7*

Foodborne infections related to contaminated foods of animal origin are major health concerns in developing countries including Ethiopia. Raw beef consumption is becoming an increasing trend in every corner of the country, mainly at butcher shops. Given that cattle are natural reservoirs of Shiga toxin-producing Escherichia coli strains, higher occurrence of *E.coli* O157:H7 is common in areas where fecal contamination continues such as lairage, slaughter hall, transportation trucks, carcass and carcass in contact surfaces.

The overall prevalence of *E. coli* O157:H7 was (3.97%) in almost exact agreement with the national prevalence estimate (4%) in Ethiopia (Assefa, 2019). This finding was slightly higher than 2.4% by (Atnafie *et al.,* 2017), and lower than 5.4% (Sebsibe & Asfaw, 2020).

Regarding to sample source, the prevalence of *E. coli* O157:H7 at the slaughterhouse level was 5.95% which is in line with the findings of Bekele et al., (2014) which is 5.7%. A lower prevalence than the present finding was reported from Ethiopia (Atnafie *et al.,* 2017), United Kingdom (De Boer & Heuvelink, 2000) and Ireland (Carney *et al.,* 2006) which reported 2.7%, 3.2% and 3.0%, respectively. The variation in the prevalence could be attributed to the fact that there is a difference in slaughterhouse standard, hygienic practice of workers, sampling and isolation methodology, season, geographical origins and number of cattle (Varela-Hernández *et al.,* 2007).

In previous study by Bekele *et al.*, (2014) the occurrence of *E. coli* O157:H7 in butcher shops was observed to be higher, which was 13.3% (17/ 128) collected from butcher shops in central Ethiopia. Likewise, Tadese *et al.*,(2021) and Gutema *et al.,* (2021) found a prevalence of 19.1% and 6.3% in Ambo and Bishoftu butcher shops respectively. The variation in the prevalence of *E. coli* O157:H7 in butcher shops could be due to the status of hygiene and sanitation practices of the butcher shops, sample size and sampling technique. For example, Sebsibe & Asfaw, (2020) recover positive isolates from knife,(1/30), cutting board (3/30),and protective clothes (1/30). However, Pooled sampling method, which was used in this study, recovers only one (1/92), showing sampling methods can be big factor.

The occurrence of *E. coli* O157:H7 was significantly higher at the municipal slaughterhouse (8.3%) than at the private slaughterhouse (3.5%). The slaughterhouse facility, origin of the cattle, worker's hygiene, and transportation of cattle from origin to the slaughterhouse might have contributed to this difference. A survey by Yusuf & Cottington, (2015), discussed that public slaughterhouses have poor management and facilities than private abattoirs in Ethiopia.

Regarding to sample type, carcass and carcass contact surface swabs (12.5 %,) had higher proportion of *E. coli* O157:H7 followed by fecal, content (3.3 %,) and beef samples (3.3 %,). The occurrence of *E. coli O157:H7* in fecal, swab and beef samples were also reported in different studies (Gutema *et al.*, 2021; Sebsibe & Asfaw, 2020; Atnafie *et al.*, 2017). In this study, even though the prevalence of *E. coli* O157: H7 was low, it was observed that there was no declining pattern of prevalence from fecal (3.3%), carcass and carcass in contact surface with wastewater (12.5%), and beef sample (3.3%), demonstrating that cross- contaminations of carcass occur during slaughtering process which in overall reflect the general unhygienic conditions in employees, utensils and environmental sanitation of the slaughter house under study. It also indicate that the sanitary and hygienic measures at the slaughterhouses were ineffective against *E. coli* O157:H7.

Similarly to what is reported by Beyi *et al.,* (2017), which detected (3.6%, 4/110) *E. coli* O157:H7 on the surface of wooden cutting boards, this survey also isolated (1.1%, 1/92) *E. coli* O157:H7 from a pooled swab sample of butcher's hand, knife and cutting board. However, in

this study *E. coli* O157:H7 was not detected at the same time from the beef cut and beef contact surface samples swabbed from the same butcher shop.

In the present study, *E. coli* O157:H7 was isolated from slaughterhouse wastewater (1/12, 8.3%), which is alarming. This finding was lower than a previous study conducted in other countries. Barel *et al.*,(2022) reported a relatively higher 11% prevalence of from slaughterhouses in Kayseri, Turkey. Similar higher prevalence 16%, in Nigeria (Oluwawemimo *et al.,* 2016) and 20.8% in Turkey (Ayaz *et al.,* 2014) were also reported. This finding notes the importance of ensuring establishment of water treatment facilities to slaughterhouse wastewater efflux by governmental authorities.

In general, the result of the present study was lower compared to previous studies conducted in Ethiopia and other countries. Considering the isolation methods employed, Chapman *et al.,* (1994), demonstrated the use of enrichment culture (EC) in modified buffered peptone water followed by immunomagnetic separation (IMS) for the isolation of *E. coli* O157:H7 from bovine faeces. Accordingly, of 1024 bovine rectal swabs, *E. coli* O157 was isolated from 23 by direct culture, and 84 by IMS including the 23 that were isolated by direct culture. Likewise, other researchers have also reported that IMS resulted in a greater detection rate for *E. coli* O157 in beef and bovine feces compared to enrichment plating (Fedio *et al.,* 2011; Tutenel *et al.,* 2003). Thus, absence of immunomagnetic separation (IMS) in the isolation procedure could be one reason to the low overall prevalence of this pathogen in the present study.

The recto-anal junction (RAJ) of cattle is the principal site of colonization for *E. coli* O157: H7 (Cobbold *et al.,* 2007) and it was argued that *E. coli* O157: H7 detected in higher proportion from the intestinal mucosa proximal to RAJ than in the feces (Greenquist *et al.,* 2005). Therefore, in the present study, the low prevalence of *E. coli* O157: H7 in the fecal sample observed may be associated with low shedding pattern of the pathogen by beef cattle included in this study.

The present study was also supported by other studies for the absence of the association between occurrences of *E. coli* O157: H7 and sample type (Abdissa *et al.,* 2017 ; Bekele *et al.,* 2014). In contrast, other researchers have reported that sample type and *E. coli* O157: H7 have a significant association. (Sebsibe & Asfaw, 2020; Abreham *et al.,* 2019).

5.2. Assessment of Hygienic practice in slaughterhouses and butcher shops

This study has also considered observational survey so as to assess the hygiene and sanitary practices of slaughterhouses and butcher shops and the overall slaughtering operations. Hygienic practices during beef production, processing and distribution is essential to formulate a preventive measures to mitigate the contribution of meat to foodborne diseases (Havelaar *et al.,* 2013).

Unfortunately, this study was not able to collect samples from the truck, lairage and hide. However, upon visual observation, the lairage was dirty and there were no compartments which increase fecal contamination of hide due to close contact of animals. Previous works has revealed that the major source of beef carcass contamination was hide of cattle entering to the processing facilities (Fegan *et al.,* 2009; Brichta-Harhay *et al.,* 2008). In a study done by Mersha *et al.*, (2010), a significant association was found between carcass swabs and skin swabs. Arthur *et al.,* (2007) support these findings by demonstrating that antimicrobial interventions targeting cattle hides lead to drastic reductions in the rates of carcass contamination with *E. coli* O157:H7.

Stressful stunning and dragging of cattle on the floor, lack of clear demarcation between dirty and clean area (municipal slaughterhouse), careless fisting and evisceration, sharing of knife, axe, and rasps, uncovered drainage line, lack of sequential decontamination at various stages, infrequent postmortem examination (municipal slaughterhouse), lack of hot water baths for hand

washing and dipping of knives, uncleaned protective cloths (except in few butcher shops), infrequent hand washing were bad practices observed at the slaughterhouses and butcher shops.

At slaughterhouses, workers transported the carcass from the hook bar to the vehicle on their shoulders. They used plastics gown which covered their back. However, the hygienic status of these protective clothes was not up to the standard required for abattoir workers. Personnel working at the slaughterhouses did not wear clean aprons, boots, and hair caps during meat processing. This might be the reason for the occurrence of *E. coli* O157:H7 in the beef sold at butcher shops. Hence, slaughterhouse workers should wear clean protective cloths, and also ensure their hands are always clean so as to produce high quality beef.

Microbial contamination of carcasses is most likely occurred during evisceration (Wambui *et al.*, 2018 ;Wheatley *et al.*, 2014). However, in the slaughterhouses under study, evisceration was conducted without considering the spillage of fecal material in to the carcass from the gut.

With regard to hygiene and sanitation, it is a documented fact that lack of education and training on food safety can contribute to unhygienic handling, processing and display of meat at the slaughtering places and at butcher shops (Jeffer *et al.*, 2021; Tegegne & Phyo, 2017; Dulo, 2014).

The slaughterhouses and butcher shops were not equipped with the necessary equipments which might enable them to maintain the general hygienic practice. For instance, there were no hot water for hand washing and knife dipping, clean towels, foot bath, separate rooms to process rumen and intestine (municipal slaughter house). Above all, inadequate supply of tap water was one of the greatest challenge to maintain hygiene.

Thus, the relative higher prevalence of *E. coli* O157:H7 on carcass swab and beef in the current study might be due to the contamination of carcass with fecal material during the slaughter operation or from different contaminated materials and hands of meat handlers. Hence, because of an increasing trend of raw meat consumption throughout the country, this is an alarming situation. Studies clearly documented that, raw beef can harbor shiga toxin producing E.coli (STEC) that causes diarrhea, hemorrhagic colitis, hemolytic uremic syndrome (HUS) in human (Panahee & Pourtaghi, 2017; Sethulekshmi *et al.*, 2016) .

5.3. Antimicrobial Resistance of *E. coli* O157:H7

Antimicrobial resistance has come recently a challenge for 'One Health' due to the rapid emergence and spread of resistant bacteria among animals, humans, and the environment (McEwen & Collignon, 2018). Antimicrobial resistance may developed spontaneously either by selective pressure or due to misuse by humans or overuse in feeding or treatment of beef cattle by owners (Schroeder *et al.,* 2002). Resistance development may also associated with exchange of resistance factors between related bacteria (Tenover, 2006).

The present study indicated that all *E. coli* O157:H7 isolates were susceptible to three antimicrobials namely azithromycin, Cefotaxime, and Chloramphenicol which was in agreement with a local Ethiopian study isolates from goat (Dulo *et al.,* 2015). Likewise, ceftazidime, ciprofloxacin, gentamycin, kanamycin, nalidixic acid, and sulfamethoxazole showed greater activities against the isolates. In contrast, all *E. coli* O157:H7 isolates were resistant to tetracycline which again comes in parallel with the results of (Chinwe *et al.,* 2017). This is not surprising since tetracycline is often used as a first-choice antimicrobial for disease prevention and treatment in food animals and its widespread use has likely contributed to high rates of resistance (Mayrhofer *et al.,* 2007). Closely related tetracycline resistance to this finding was also reported by Disassa *et al.,* (2017) and (Ababu *et al.,* 2020), from raw milk, in Ethiopia. Higher resistance to Erythromycin (92.8%) and Ampicillin (64.3%) was also observed among *E. coli* O157:H7 isolates. The current finding for tetracycline resistance disagree with other study

on *E. coli* O157:H7 isolated from feces and carcass of cattle slaughtered in the abattoir (Osaili *et al.,* 2013).

Multiple antimicrobial resistance is becoming a common phenomenon among *E. coli* O157:H7 isolates (Ahmed & Shimamoto, 2015) . It may arise from the spread of genetic materials such as plasmids, integrons, and transposons drawn from different sources (Zhao *et al.,* 2001). In this study, multidrug resistance to three or more classes of antimicrobial classes was detected in 12 (85.7%) of the isolates. The most frequently observed resistance pattern in the isolates was resistance to tetracycline in combination with erythromycin, and ampicillin. Among the *E. coli O157:H7* isolates, 42.8%, 28.6%, 7.1%, and 7.1% developed resistance to three, four, five and six antimicrobials classes respectively.

Similar finding of multiple antimicrobial resistance on STEC strains has been documented from Ethiopia (Haile *et al.,* 2022 ; Bedasa *et al.,* 2018; Shecho *et al.,* 2017), and other part of the world (Amézquita-López *et al.,* 2016 ; Maal-bared *et al.,* 2013 ; Govaris *et al.,* 2011) .

Statistically significant association was observed between the sample source (municipal slaughterhouse), and multiple antimicrobial resistance (Table 9). Accordingly, isolates from municipal slaughterhouse were 5.84 time more likely to get multiple antimicrobial resistance than isolates from butcher shops. However, no statistical significance was established between sample type and multiple antimicrobial resistance which was in contrast with a report by (Sebsibe & Asfaw, 2020). Based on sample type, from fecal 28.6% (4/14), carcass and carcass contact swabs 35.7% (5/14), beef 14.3% (2/14), and from water and wastewater 7.1% (1/14) multiple antimicrobial resistance pattern was observed (Table 9). The higher multiple antimicrobial resistance seen in this study might be due to presence of only few isolates tested for susceptibility compared to overall study sample, difference in sample source, variability of resistant gene within isolates, and type of antimicrobials used in the study. This result, multi drug resistant wastewater isolates particularly, suggests slaughterhouse efflux would become source of resistance pathogen to the environment.

6. CONCLUSION AND RECOMMENDATIONS

E. coli O157: H7 was detected from the feces, carcass swab, and environmental samples with slightly higher occurrence in carcass swab, possibly suggesting that hide and intestinal content during evisceration are the key source of microbial contamination. Moreover, the pathogen was isolated from butcher's hand, knife and cutting board (pooled sample) indicating that the possibility of carcass contamination during slaughtering operations. *E. coli* O157:H7 which were isolated from raw beef were found to be resistant to multiple antimicrobials highlights the potential threat to public health. Moreover, the assessment of the hygienic and sanitary practice in the slaughterhouses and butcher shops, indicated that there were poor personal and general hygiene measures.

A significant association was also observed between the occurrences of *E. coli* O157:H7 and the source of the samples i.e. Municipal slaughterhouse. The occurrence of multidrug resistant *E. coli* O157:H7 isolates in slaughterhouse wastewater is a serious point of concern as resistant genes can easily be transferable into the environment as well as to the human and animal populations.

Generally, this study showed that the occurrences *E. coli* O157:H7 in raw beef with multiple antimicrobial resistance pattern was associated with unhygienic meat processing and the low

standard establishment of slaughterhouses. Therefore, consumption of raw beef may be an important source for *E. coli* O157:H7 infections in the country.

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

- Control measures to reduce the public health risk in the beef supply chain needs to be addressed at slaughterhouses by reducing carcass contamination.

- Personnel working in the slaughterhouses and butcher shops should be given basic meat handling and processing training. There should be also awareness on microbial meat contamination to the public. Hence, the people push to demand high quality beef.

- Implementing more stringent regulation on the use of veterinary antimicrobials.

- Slaughterhouses should be equipped with basic facilities and necessary infrastructures. In that regard, wastewater treatment facilities should also be stablished in slaughter houses to reduce the transmission of antimicrobial resistant organisms and genes to the environment.

- Slaughterhouses need to adopt good hygienic practice, good manufacturing practices and HACCP principle, for the best control of pathogens such as *E. coli* O157:H7.

- Further detailed research involving different production type, season, ecology, different types of food animals and their products should be undertaken to determine the molecular epidemiology of *E. coli* O157:H7 so that appropriate and effective control and preventive measures could be designed.

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8. APPENDICES

Appendix 1: Type and preparation of microbiological media used for isolation, identification and antimicrobial susceptibility test of E. coli O157:H7

1. Buffered Peptone Water (Cat. 1402.00, CONDA, Madrid, Spain)

Composition (g/l): Enzymatic digest of casein 10.0g; Sodium chloride 5.0g; disodium phosphate dodecahydrate 9.0g and potassium dihydrogen phosphate 9.0g

Preparation: suspend 20 grams of components in 1000ml of distilled water. Mix well and distribute into universal bottle of suitable capacity to obtain the portions necessary for the test and sterilize in autoclave at 121 ℃ for 12 minutes. Final PH is 7.0 $\pm$ 0.2 at 25℃.

2. Modified Tryptone Soya Broth (TSB) (CM089, Oxoid Basingstoke, UK)

Composition (g/l):: Enzymatic digest of casein (17.0 g), Enzymatic digest of soya (3.0 g), sodium chloride (5.0 g), Bile salt no.3(1.5g) (Di-Base potassium phosphate(k_2 HPO_4 (4.0 g), Glucose (2.5 g)

Preparation: suspend 33g grams of components in 1 litter of distilled water. Mix thoroughly in universal bottle and sterilize in autoclave at 121 ℃ for 15 minutes. Final PH is 7.0 $\pm$ 0.2 at 25℃.

3. Novobiocin Solution

Composition (g/l): Novobiocin 0.45g

Preparation: dissolve Novobiocin in 100ml of water and sterilized by filter paper

4. MacConkey Agar (CM0007B,Oxoid, England)

Composition (g/l): Peptone 20.0 Lactose 10.0, Bile salts, 5.0, Sodium chloride, 5.0,Neutral red, 0.075,Agar, 12.0, pH 7.4 ± 0.2

Preparation: Suspend 52g in 1 liter of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Dry the surface of the gel before inoculation.

5. Eosin Methylene Blue Agar (Oxoid, England)

Composition (g/l): Peptone (10.0g), Lactose (10.0g), Dipotassium hydrogen phosphate (2.0g), Eosin (0.4g), Methylene blue, (0.065g), Agar, (15.0g), pH 6.8 ± 0.2

Preparation: Suspend 37.5g in 1 liter of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 60°C and shake the medium in order to oxidise the methylene blue (i.e. restore its blue color) and to suspend the precipitate which is an essential part of the medium.

6. Cefixime Tellurite Sorbitol MacConkey agar(CT-SMAC) (Oxoid Basingstoke, England)

Composition (g/l):: Enzymatic digest of casein (17.0 g), Enzymatic digest of animal tissue (3g), sorbitol (1g), Bile salts no.3 (1.5g), Sodium chloride (5.0g), (Neutral red 0.03g), crystal violet(0.001g).

Preparation: 50 g of the powder was suspended in 1 liter of distilled water. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121^{o}C for 15

minutes. Then Potassium tellurite (2.5 mg/l) and Cefixime (0.05mg/l) were added on the prepared base media tempered at 50-55^{O}C. gently shacked and poured into Petri dishes.

7. Nutrient Agar (CM 0003, OXOID, Basingstoke, England)

Composition (g/l): peptic digest of animal tissue 5.00; sodium chloride 5.00; beef extract 1.5; yeast extract 1.5; agar 15.

Preparation: suspend 28 grams in 100ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure (121℃) for 15 minutes. Mix well and pour in to sterile petridishes. Final PH (at 25℃): 7.4 $\pm$ 0.2.

8. Triple sugar agar (CM 0277, OXOID, Basingstoke, England)

Preparation: suspend 65 grams in 1000ml of distilled water. Bring to boil to dissolve completely. Mix well and distribute in to containers. Sterilize by autoclaving at 121℃ for 25 minutes. Allow the set as slope with 2.5 cm butts. PH: 7.4 + 0.2 at 25℃.

Composition (g/l): 'lab-lemco' powder 3.0; peptone 20.0; sodium chloride 5.0; lactose 10.0; sucrose 10.0; glucose 1.0; ferric citrate 0.3; sodium thiosulfate 0.3; phenol red 0.025; agar 12.0

9. Simmons Citrate Agar (M 099-500g, HIMEDIA, Mumbai, India)

Preparation: suspend 24.28 grams in 1000ml distilled water. Heat to boiling to dissolve the medium completely. Dispense as desired in tubes of flasks sterilize by autoclaving at 15 Ibs pressure (121℃) for 15 minutes.

Composition (g/l): magnesium sulphate0.20; ammonium dihydrogen phosphate 1.0; dipotasium phosphate 1.00; sodium citrate 2.00; sodium chloride 5.00; bromothymol blue 0.08; agar 15.00.

10. MR-VP Medium (M 070-500g, HIMEDIA, Mumbai, India)

Preparation: suspend 17.0 gram in 1000ml distilled water. Heat if necessary to dissolve the medium completely. Distribute in to test tubes 10ml amounts and sterilize by autoclaving at 15 Ibs pressure (121℃) for 15 minutes.

Composition (g/l): buffered peptone 7.00; dextrose 5.00; dipotassium phosphate 5.00

11. Tryptone soya agar (Oxoid, England)

Composition (g/l): Pancreatic digest of casein (15.0g) Enzymatic* digest of soya bean (5.0g), Sodium chloride (5.0g) Agar, (15.0g) PH: 7.3 ± 0.2 @ 25°C

Preparation: Dissolve the component in the water by boiling if necessary. Sterilize by autoclaving at 121℃ for 15 minutes. PH: 7.3 + 0.1 at 25℃

12. Mueller-Hinton Agar (CM 0337, OXOID, Basingstoke, England)

Composition (g/l): beef, dehydrated infusion 300; casein hydrolysate 17.5; starch 1.5; agar 17.00

Preparation: suspend 38 grams in 1000ml of distilled water. Bring to boil to dissolve the medium completely. Sterilize by autoclaving at 121℃for 15 minutes. PH: 7.3 + 0.1 at 25℃.

13. 0.5 McFarland standards

Composition: 1.17% BaCl.2H2O solution and 0.36N of 1% sulfuric acid (H2SO4).

Preparation: Add approximately 85Ml of 1% H2SO4 to a 100ml of volumetric flask, using a 0.5ml pipette add 0.5ml of 1.17% BaCl.2H2O drop wise to the H2SO4 while constantly swirling the flask. Bring to 100ml with 1% H2SO4.place a magnetic stirring in the flask and place on the magnetic stirrer for approximately three to five minutes. Examine solution visually to make

certain it appears homogeneous and free of visible clumps. Dispense three to seven ml, cub tube tightly and seal with paraffin and keep at dark and room temperature.

Appendix 2: Chemical and reagents used for E.coli o157: H7 biochemical test

A. Kovac's reagent

Preparation: Dissolve 10g P-dimethylamino benzaldehyde (Sigma, Steinheim, Germany) in 150 ml ethanol alcohol, and slowly add 50ml concentrated hydrochloric acid while constantly stirring the mixture. Finally, pale color was formed and stored in brown bottle at refrigerator.

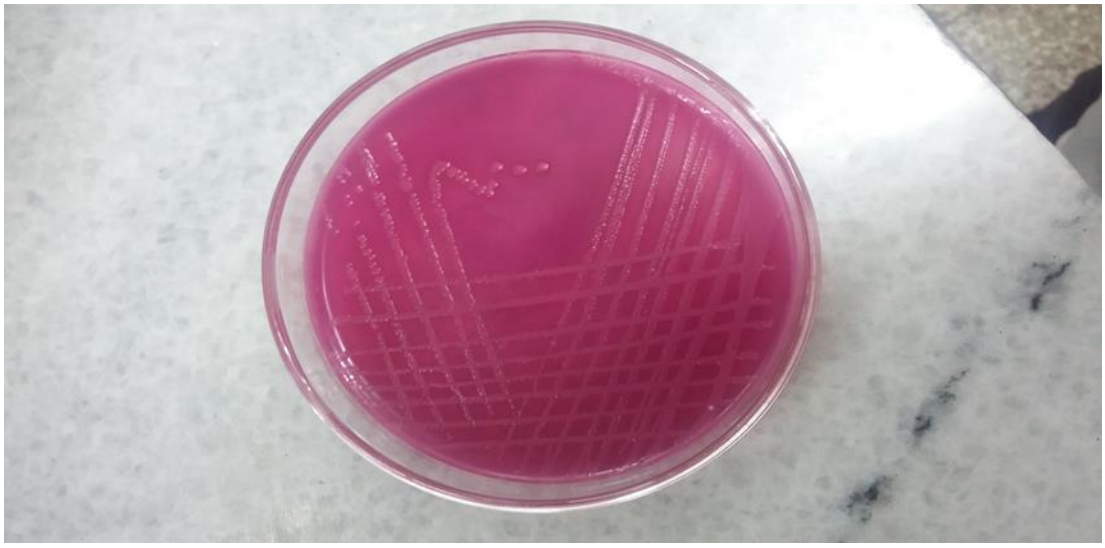
B. Methyl Red reagents

Preparation: Dissolve (0.1g) methyl red in 300ml alcohol and finally add 200ml-distilled water. Finally, red color was formed and stored in brown bottle at refrigerator.

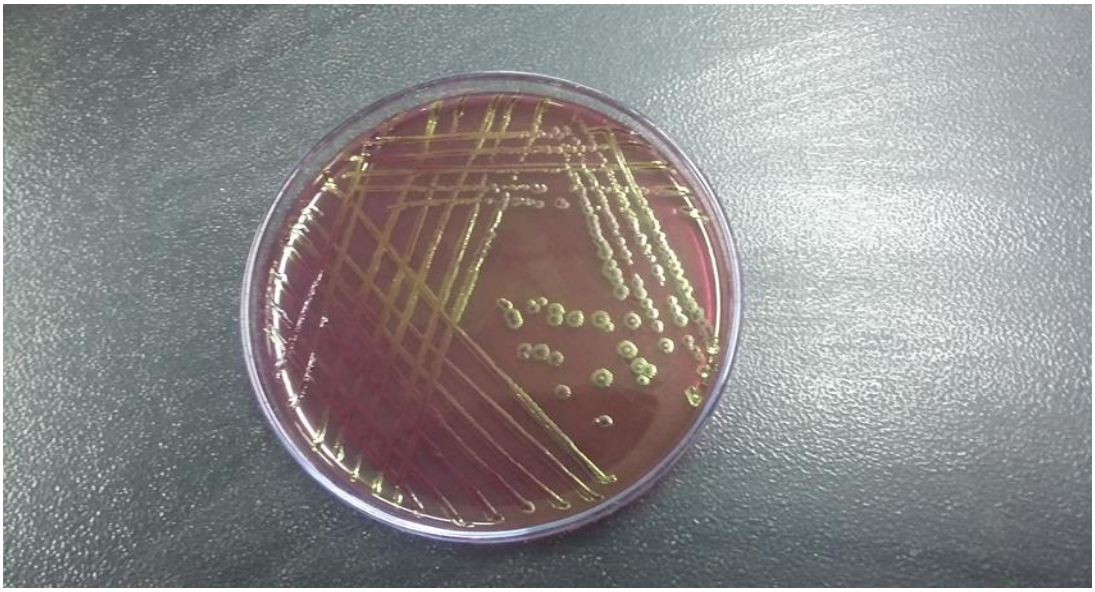
C. Voges-Proskauer (VP) reagent

Preparation: a) VP-reagent-1: Dissolve 5g α-Naphthol (Sigma, Steinheim, Germany) in small amount of ethyl alcohol and bring to 100ml in flask. Alcohol should be color less. Then store in Brown bottle and in refrigerator. b) VP-reagent-2: Add less than 100ml distilled water to 40g pellets of KOH in cold water bath to prevent overheating and bring to 100ml. Finally 40% solution that store in polyethylene bottle at refrigerator.

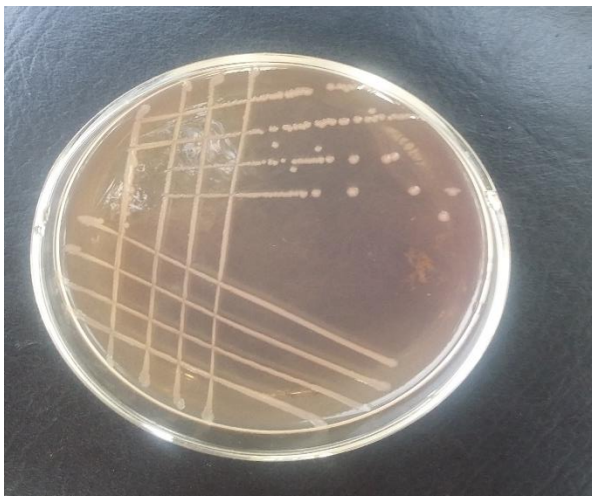
Appendix 3: Pictures showing various biochemical test results and material for isolation of E.coli O157:H7

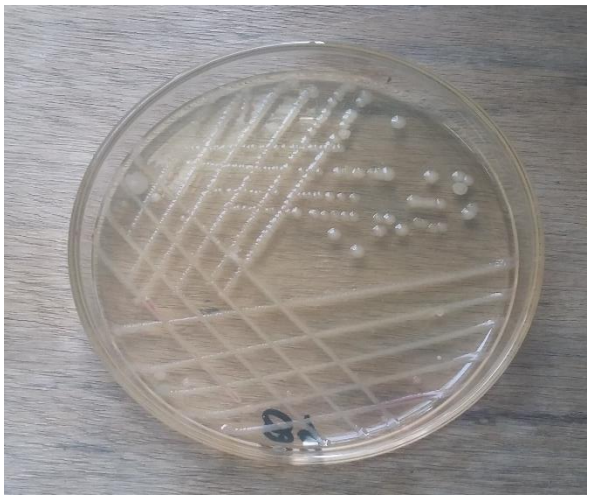


Lactose fermenting pink colonies on MacConkey agar

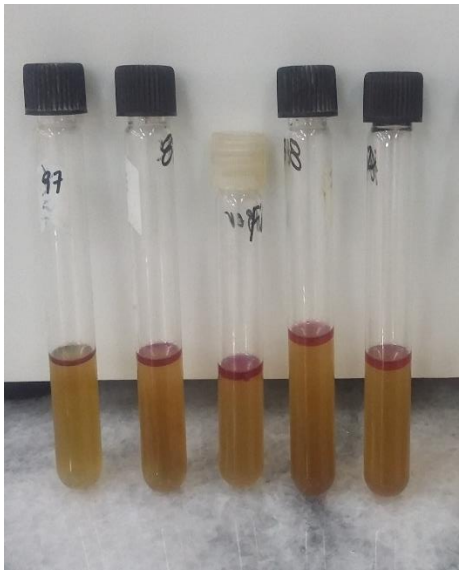


Metallic green sheen colonies on Eosin Methylene Blue (EMB) Agar

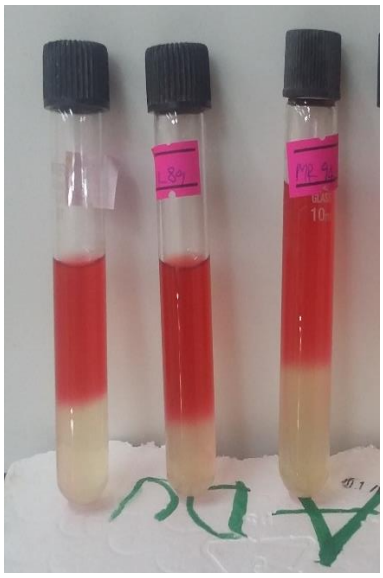




Colorless colonies on Sorbitol MacConkey Agar (CT-SMAC)

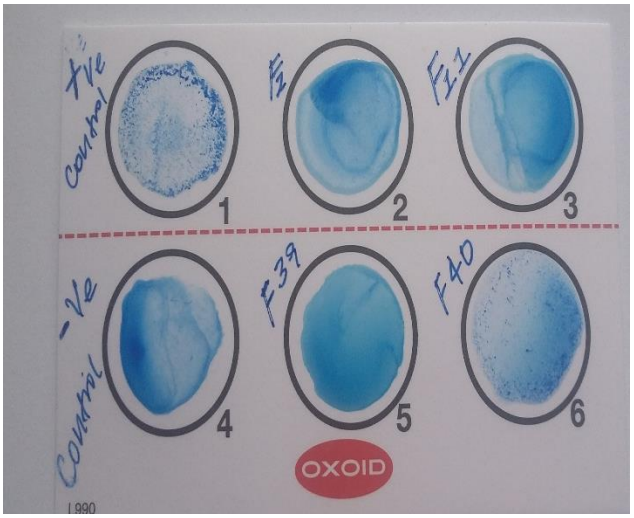


Indole positive (red ring)

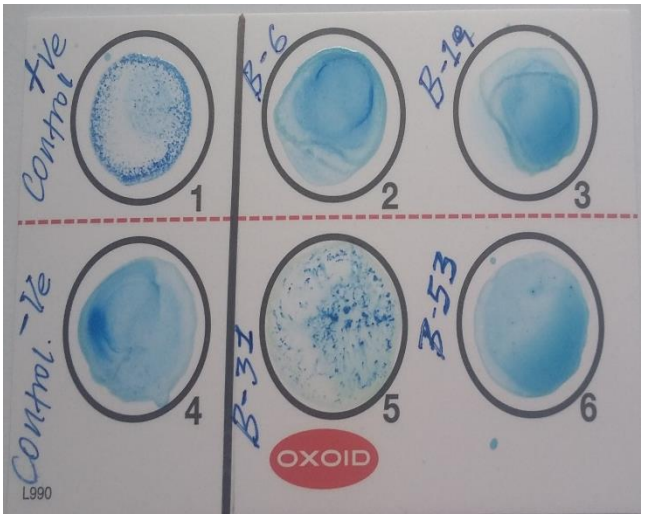


Methyl Red positive

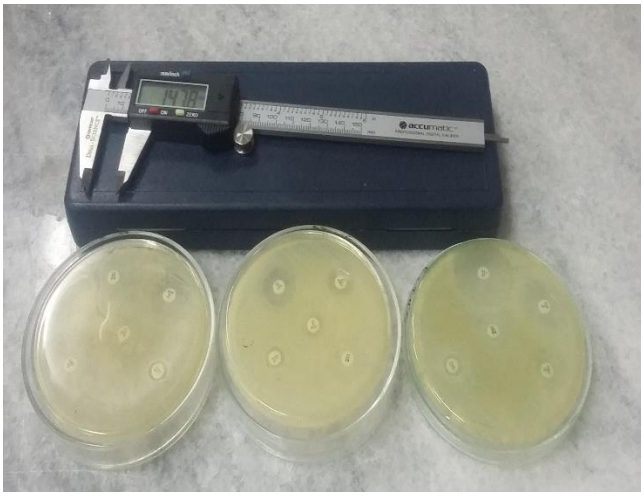
H_2S negative on TSI test



Latex positive (F-40, Card No.-6)



Latex positive (B-31, Card No. 5)



Antimicrobial susceptibility test

Carcass swab sample collection