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LIST OF ABBREVIATIONS

A/E	Attaching and Effacing
Aw	Water activity
CFSPH,	The Center of Food Secury and Public Health
CFU	Colony forming units,
DAEC	Diffuse-adherent
DALYs	Disability Adjusted life years
DNA	Deoxyribonucleic Acid,
EAggEC	Enteroaggregative *E. coli*
EHEC	Enterohemorrhagic *E. coli*
EIEC	Enteroinvasive *E. coli*
ELISAs	Enzyme-linked Immunosorbent assays
EPEC	Enteropathogenic *E. coli*
ETEC	Eenterotoxigenic *E. coli*
GMPs	Good Manufacturing Practices
HACCP	Hazard Analysis Critical Control Point
HUS	Hemolytic uremic syndrome
IMS	Immunomagnetic separation
LEE	Locus for enterocyte effacement
NSF	Non-Sorbitole fermenting
OIE,	World Organization for Animal Health
STEC	Shiga-toxin–producing *E. coli*
Stx	Shiga toxins
stx1	Shiga toxins 1
stx2	Shiga toxins 2
Tir	*Translocatedintimin receptor*
TTP	Thrombotic Thrombocytopenic Purpura
VT	verotoxins
VTEC	Verocytotoxin producing *E. coli*

ABSTRACT

A cross-sectional study was conducted from December 2017 to May 2018 to investigate the occurrence and antimicrobial susceptibility profile of *E. coli* O157:H7 in cattle fecal samples at Bishoftu abattoir, meat at retail shops and stool of diarrheic patients at Bishoftu hospital. A total of 583 samples consisting 240 feces, 127 meats and 216 stool samples were collected using systematic sampling technique and analyzed according to the recommended standard procedure to isolate and identify the pathogen. In addition, questionnaire survey was conducted to assess the current status of hygienic and handling practices in the abattoir, retail shops and exposure assessments of diarrheic patients. Out of the total 583 samples examined, 31(5.3%) were positive for *E. coli* O157:H7. The prevalence of *E. coli* O157:H7 was 7% (95%CI: 3.81, 10.32), 6.3% (95%CI: 20.16, 10.58) and 2.8 (95%CI: 0.56, 4.99) in feces, meat and stools, respectively. However, statistically no significant difference was observed among the three sample types (X^2 = 4.4969, P = 0.106). Based on univariate logistic regression analysis, all the considered variables at retail meat shops were not statistically associated with the occurrence of the pathogen (P>0.05). Among the risk factors considered the exposure of the diarrheic patients, only four days duration of onset of diarrhea was statistically associated with the occurrence of *E. coli* O157: H7 (OR: 9.51 (95% CI: 1.02, 88.00, P=0.047). The study also revealed that, a varying level of resistance of *E. coli* O157:H7 against the ten commonly used antimicrobials. All the isolates of *E. coli O157:H7* were susceptible to gentamicine and resistant to ampicilin, cefoxitin and nitrofurantoin. Multi-drug resistance was observed in all the isolates. In conclusion, the study showed the occurrence of *E. coli* O157:H7 along beef supply chain with relatively high prevalence in cattle at abattoir and resistant *E. coli* O157:H7 to frequently used antimicrobials suggesting the need for intervention. Eventually, good hygienic practices along the beef supply chain and public education to safeguard the public from the associated risks and further study to identify the sources of the bacteria, establish the clear link between human diarrheal illness and beef consumption and investigating the genetic similarity of the isolates were recommended.

Keywords: *Antimicrobial susceptibility, Beef, Bishoftu, E. coli O157:H7*

1. INTRODUCTION

Food borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and medical and social costs worldwide (Havelaar *et al*., 2015). The risk of the transmission of food borne zoonotic infections is associated with contaminated meat (Nafisa *et al*., 2010). As estimates of WHO on the global burden of Food borne disease due to 31 hazards indicated 600 million foodborne illnesses 420,000 deaths and 33 million Disability Adjusted life years (DALYs).This occur commonly in developing countries particularly in Africa because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory system, lack of financial resources to invest in safer equipment and lack of education for food handlers (Haileselassie *et al*., 2013). 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* (Nouichi and Hamdi, 2009).

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 the host, however, some strains of *E. coli* can be pathogenic and cause fatal disease in humans (Belanger *et al*., 2011). It is gram-negative, facultative anaerobic bacteria which belong to genus Escherichia of family Enterobacteriaceae. It is an enterohemorrhagic *E. coli* (EHEC), is of the best known pathogenic strain and an important emerging zoonotic foodborne pathogen (Farrokh *et al*., 2012; Xia *et al*., 2010b).

Cattle are a major reservoir of *Escherichia coli O157:H7* (Tourret *et al*., 2016; Martorelli *et al*., 2015) and a diversity of foods has been identified as vehicles of illnesses. The microbiological contamination of carcasses occurs mainly during processing and manipulation, such as skinning, evisceration, storage and distribution at slaughter houses and retail establishments (Abdalla *et al*., 2009).

The use of antimicrobials in food cattle to the development of resistance pathogenic *E. coli* O157:H7that can reach humans through the beef food chain (Akbar *et al*., 2014).Antimicrobial resistance is common in *E. coli* O157:H7, include multiple drug resistance (Constable *et al*., 2017; Vijayarani *et al*., 2010; Naik and Desai, 2012).The development of antimicrobial resistance in *E. coli* O157:H7is the matter of increase concern and generate new public health challenge(Newell et al., 2010).

The most frequent mode of transmission for *E. coli* O157:H7 infection is through consumption of contaminated food and water, particularly with consumption of uncooked and contaminated beef product (Sodha *et al*., 2015; Abdalla *et al*., 2009). The habit of consuming raw and/or undercooked meat is one of major source of foodborne *E. coli* O157:H7 (Hubaílek and Rudolf, 2010). In Ethiopia, raw meat is available in open-air local retail shops without appropriate temperature control where consumers either purchase for home consumption or consume at the shop. Minced meat, traditionally named as "Kitfo", is served in restaurants as raw, slightly-cooked or well-cooked throughout the country (Avery, 2004). There is scarcity of information regarding to the occurrence and antimicrobial resistance status of *E. coli* O157 H7 from cattle feces, beef and human stool and its involvement for human diarrhea in and around Bishoftu. Therefore this study was designed with the following objectives.

General objective

- To estimate the prevalence of *E. coli* O157:H7 at abattoir, retail shops and diarrheal illness in humans at Bishoftu municipal abattoir and one private slaughter abattoir, retailer shops and Bishoftu hospital.

Specific objectives

- To isolate and identify *E. coli* O157:H7 from cattle feces and meat and from diarrheic human stool
- To determine antibiotic susceptibility pattern of *E. coli* O157:H7 isolates
- To assess meat handling practice of abattoir and retail workers, beef consumption behavior and risk factor for developing of diarrhea in patient

2. LITERATURE REVIEW

2.1. Meat Production and Consumption Behavior in Ethiopia

Meat is the most valuable livestock product, it defines as the main edible part of domestic mammals; however, recent definition includes, fish, shellfish, poultry and exotic species such as frogs and allegation, that is used as food for human consumption (Bradeeba and Sivakumaar, 2013; Thanigaivel and Anandhan, 2015). Also, meat refers to animal tissue used as food, mostly skeletal muscles and associated fat but it may also refer to organs including the lungs, livers, skin, brains, bone marrow, kidney and a variety of other internal organs as well as blood. Recent increase in the consumption of meat and its products arises from reasons including high protein contents, vitamins, minerals, lipids and savory sensation (Iroha *et al*., 2011).

Meat consumption is often an indicator of the civilization or economic status of a country or that of an individual. People with a higher social or economic status consume a sufficient amount of meat products. Meat supply varies enormously from region to region, and is highest across high-income countries, with the largest meat-eaters in Australia, consuming around 116kgs per person per year. The average European and North American consumes nearly 80 kgs and more than 110 kgs, respectively. In Africa some countries consume as low as 10 kgs per person, around half of the continental average. Higher-income nations such as South Africa consume between 60-70 kilograms per person per year (Hannah and Max, 2018). The average meat consumption globally is 115 grams per day (42 kg per year) (UNEP (2012)

In Ethiopia, the annual contribution of ruminants to meat production is estimated at over 3.2 million tones, representing over 72% of the total meat production. According to (Negassa and Jabbar, 2008), Ethiopia's domestic meat consumption for 2006-2007 has been estimated at 2.4 kg/capita per year for beef, 0.7 kg/ capita per year for sheep meat and 0.4 kg/capita per year for goat meat.

The consumption of animal flesh food in Ethiopia has associated with cultural practices. Meat plays pivotal and vital parts in special occasions and its cultural symbolic weight is markedly

greater than that accorded to most other food. In Ethiopia, a cow or an ox is commonly butchered for the sole purpose of selling within the community. In special occasions, people have a cultural ceremony of slaughtering cow or ox and sharing among the group, called Kircha, which is a very common option of the people in rural area where access of meat is challenging frequently (Seleshe *et al*., 2014). In Ethiopia, beef is a commonly consumed meat and on many occasions may be eaten as raw or undercooked (Kumar and Tadesse, 2011; Avery, 2004). This could increase the occurrence of food borne illness due to EHEC (Ferens and Hovde, 2011).

2.2. Food borne *E. coli* O157: H7

Various new pathogens have emerged due to changing production processes in food industry. Some of these are new pathogens and were unknown previously, others are emerging pathogens for food borne infections, and some others are evolving pathogens that have become more potent (Mor-Mur and Yuste, 2010). Since its first description in 1982, *Escherichia coli* 0157: H7 has emerged as an important global zoonotic food and water-borne pathogen, which produces serious illness in humans such as haemorragic colitis, haemolyticuremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Chekabab *et al*., 2013; Pal and Mahendra, 2016).

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 *et al*., 2007).

2.2.1. Historical Background

Escherichia coli, originally called "*Bacterium coli* commune," was first isolated from the feces of a child in 1885 by German microbiologist Theodor Escherich (Escherich, 1885).In 1982,*Escherichia coli O157:H7* was first identified as a human pathogen after two outbreaks in Oregon and Michigan (Riley, 2014; Sewlikar and D'Souza, 2017). 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 this was among one of the world's worst food borne in terms of morbidity and mortality in humans. Approximately 52% of recorded human disease outbreaks have been associated with cattle products (Griffin and Tauxe, 1991).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 (Constable *et al*., 2017).

2.2.2. Etiology

The infection is caused by *Escherichia coli,* a gram negative, facultative anaerobic, rod-shaped, coliform bacretium in the Phylumsproteobacteria, classgamma Proteobacteria, order Enterobacteriales, family Enterobacteriaceae and genus Escherichia (Tenaillon *et al*., 2010; Farrokh *et al*., 2012; Xia *et al*., 2010b; CDC, 2015). In the genus *E. coli*, there are hundreds of serotypes of *E. coli*which are classified on the bases of various surface antigens referred to as Somatic (O), Capsular (K), Flagellar (H) and Fimbrial (F). Thus, there are approximately 174 O antigens, 56 H antigens, and 103 K antigens that have been identified (Zhang *et al*., 2006).

The pathogenic groups of *E. coli*are divided into six groups on the basis of their virulence properties such as enterotoxigenic(ETEC, causative agent of diarrhea in humans, pigs, sheeps, goats, cattle, dogs and horses), enteropathogenic (EPEC, causative agent of diarrhea in humans, rabbits, dogs, cats and horses), enteroinvasive (EIEC, found only in humans), verotoxigenic (VTEC, found in pigs, cattle, dogs and cats), enterohemorrhagic (EHEC, found in human, cattle, and goats) and enteroaggregative(EAggEC, which found only in human)*E. coli*(Xia *et al*., 2010a; Biswas *et al*., 2006).

STEC species are classified into 2 subtypes: O157 and non-O157, with cases involving O157 strains more frequently associated with more severe diseases, such as the development of hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (Oporto *et al*., 2008). *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 157th O antigen identified and the 7th H antigen(Chapman *et al*., 2001). *Escherichia 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).

2.3. Epidemiology

2.3.1. Geographical Distribution

E. coli O157:H7 infections occur worldwide and this have been reported on every continent except Antarctica (CFSPH, 2009). *Escherichia coli (STEC)* are responsible for gastrointestinal 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). The meta-analysis study on prevalence of *E. coli* O157:H7 globally, estimate that the prevalence of agent in cattle at the global level was 5.68% and also revealed the prevalence of world regions as Table 1

Table 1: 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)

Microbiologically culture proven *E. coli* O157:H7 diarrheal cases have been reported from a number of African countries including South Africa, Swaziland, Central African Republic, Kenya, Ethiopia, Uganda Gabon, Nigeria and Ivory Coast (Raji *et al*., 2006).

In Ethiopia, there were studies conducted by some researchers to determine the occurrence and proportion of *E. coli* O157:H7 in feces, skin swabs and carcasses of cattle, sheep, goat, and human in different areas of the country. Atnafie *et al*. (2017) have been reported a prevalence of *E. coli* O157:H7 from cattle meat, butcher shop, meat handler and knife in Hawassa town. Also, Edget *et al*. (2017)have reported the prevalence from cattle meat in Dire Dawa town. Similarly, Abdissa *et al*. (2017) has been done the studies on prevalence of *E. coli* O157:H7 from cattle fecal, skin swab, carcass swab and human stool in Addis Ababa city and Debre Berhan town. Tables 2 summarize these and others reports on prevalence of *E. coli* O157: H7 regards along beef supply food chain and from humans in Ethiopia.

Table 2: Studies conducted on prevalence of *E. coli* O157:H7 on cattle and human in Ethiopia

Study Area	Sample Unit	Sample Type	Prevalence	References
Hawassa	Cattles Butcher shop Meat handler Knifes	Swab (knife Close to meat transporter),	2.4%	Atnafie *et al*. (2017)
Addis Ababa	Cattle	Fecal	2%	Abdissa *et al.* (2017)
DebreBerhan	Butcher shop	Skin swab	0.5%	
		Intestinal mucosal swabs	0.8%	
		Internal carcass swabs	0.5%	
	Human	Stool	0%	
Dire Dawa	Cattle	Raw meat	2.06%	Edget *et al*. (2017)
Addis Ababa	Human	Stool	4.5%	Ayenew (2017)
Jimma	Cattle	Carcass swab	9.3%	Feleke *et al*. (2017)
		Cecal content	7.3%	
Debre Zeit Addis Ababa		Carcass swab	5.5%	Tassew (2015)
Bahir Dar	Human	Stool	28.9%	Adugna *et al*. (2015)
Addis Ababa	Cattle	Beef	10.2%	Bekele *et al*. (2014)
Addis Ababa	Cattle	Carcass swab	0.72%	Haile (2014)
Mekelle	Cattle	Meat	18%	Balcha e*t al.* (2014)
Haramaya	Cattle	Carcass swab	2.65%	Taye *et al*. (2013)
Modjo	Cattle	Raw meat	4.2%	Hiko *et al*. (2008)

2.3.2. Reservoir 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), and approximately 30% of feedlot cattle shed *E. coli*O157:H7

(Callaway *et al*., 2009). So, ground beef and beef products are identified as major sources of foodborne transmission. All ages of cattle are susceptible to colonization with EHEC, although peak shedding is observed in adult cattle from weaning to 24 months of age(Hussein and Sakuma, 2005; Joris *et al*., 2012). Consequently, cattle are considered to be the major source of *E. coli* O157 causing human disease. 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).

Cattle play an essential role in epidemiology of human *E coli* O157:H7infection and cattle feces considered as primary source which the beef food become contaminated with this pathogen. 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 (CDC, 2017). 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 and Mahendra, 2016).

Regarding beef products, the main contamination step occurs during hide removal, and several studies suggest that initial operation failures are responsible for the contamination of the final product (Brichta-Harhay *et al*., 2008; Brichta-Harhay *et al*., 2008; Santos *et al*., 2017).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 (Callaway *et al*., 2009).

Colonization of *E.coli*O157:H7in adult cattle is asymptomatic (Verstraete *et al*., 2014)because intestinal mucosal cells lack the Stx-specific globotriaosylceramide receptor(Constable *et al*., 2017). One of the factors that contributes to the high prevalence and the main source of STEC in cattle are animals that have the capacity to deposit highly concentrated *E. coli* STEC (levels above 10^4CFU/g) in feces, thus nominated as super-shedding animals (Stephens *et al*., 2009) and this increase the risk of human infection (Chase-Topping *et al*., 2008).

Although there are many unknowns concerning super-shedding animals, it is believed that the main cause is due to the formation of a biofilm in the intestinal epithelium, containing high-density *E. coli* STEC (Munns *et al*., 2015). Biofilms are a community of microorganisms that adhere to a surface through the production of a polymer matrix and a heterogeneous biofilm layer detachment in cattle was first reported in experimental infections (Sadekuzzaman *et al*., 2015). The high concentration of these bacteria in feces would be caused during animal evacuation (Munns *et al*., 2015). In addition, the gallbladder has also been suggested as an O157:H7 reservoir in cattle (Stoffregen, 2004).

2.3.3. 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; Varela-Hernaíndez *et al*., 2007). 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 (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 (Hubaílek and Rudolf, 2010).

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 etc.) (Gordillo *et al*., 2011; Hubaílek and Rudolf, 2010; Su *et al*., 2012; Dontorou *et al*., 2003).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 (Arthur *et al*., 2017; Fink *et al*., 2017). *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 *et al*., 2010). The pathogen is destroyed in pasteurization process, but insufficient heat treatment of ground meat and raw milk forms a potential infection risk (Rahimi and Nayebpour 2012).

2.3.4. Factors affecting survival and growth of *E. coli* O157:H7 in food

A number of factors have a significant influence on the survival and growth of *E. coli*O157:H7in food, including temperature, pH, salt, and water activity. The optimal temperature for growth of *E. coli* O157:H7 is approximately 37^{o}C (98.6^{o}F), and the organism will not grow at temperatures below 8°C to 10°C (46^{o}F to 50^{o}F) or above 44^{o}C to 45^{o}C. *E. coli* O157:H7 survives freezing, with some decline in the concentration of *E. coli*O157:H7 (Buchanan, 1997).

Escherichia coli O157:H7 has been reported to be more acid resistant than other *E. coli*. Acid resistance enhances the survival of *E. coli* O157:H7 in mildly acidic foods and may explain its ability to survive passage through the stomach and cause infection at low doses. The ability to be acid resistant varies among strains and is influenced by growth phase and other environmental

factors. Once induced, acid resistance is maintained for long periods of time during cold storage. Stationary-phase *E. coli* O157:H7 are more resistant than growing cells to acid (Meng and Doyle, 1998). The presence of other environmental stresses, such as temperature or water activity stress, will raise the minimum pH for growth *E. coli* O157:H7 survives in such foods as dry salami, apple cider, and mayonnaise, which were previously considered too acidic to support the survival of food borne pathogens (Buchanan, 1997).

2.4. 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 (Robinson and McKillip, 2010).First, *E. coli* O157:H7 must initially adhere to the microvilli of the host epithelial cells (Mainil and Daube, 2005).

The intimate attachment of the bacterial cell to the host epithelium is attributed to the adhesion intimin and translocatedintiminreceptor (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 adhesion and intimi, is necessary for the production of the A/E lesion and diarrhea(Constable *et al*., 2017).

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 and Daube, 2005). Then, the Stxs can 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 and Kaijser, 2005).

2.5. Disease pattern

The acute disease associated with this organism is named hemorrhagic colitis in humans. The symptoms characteristic to this disease are watery and/or bloody diarrhea, fever, nausea, severe abdominal cramping, and vomiting (Walker *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 and McKillip, 2010)and the illness usually lasts 5–10 days. Life-threatening complications, some victims, particularly the very young, may develop hemolytic uremic syndrome (HUS). 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 (Martorelli *et al*., 2017).

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 and D'Souza, 2017; Chekabab *et al*., 2013). 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 (Bayat *et al*., 2012).

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% (CDC, 2016).

2.6. 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 (CDC, 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). Also, 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 enzyme-linked immunosorbent assays (ELISAs) (Bavaro, 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 and McKillip, 2010). There also Latex Agglutination tests 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 and Baqer, 2014).

2.7. Economic and public health importance

Escherichia coli O157:H7 is an emerging public health concern in most countries of the world (Kiranmayi *et al*., 2010) and it is the most common serotype of shiga toxin producing *E. coli*. Worldwide the Foodborne *E. coli* O157:H7 estimated to cause 2.8 million acute illnesses each year (Majowicz*et al.,* 2014). In United States, the pathogen is estimated to cause over 60,000 illnesses in the each year, resulting in about 2,000 hospitalizations and 20 deaths (Scallan *et al*., 2011) resulting in an economic burden of $607 million (Scharff, 2012), including $370 million for premature deaths, $30 million for medical care, and $5 million in lost productivity (Frenzen *et al*., 2006).

According to CDC, the incidence of EHEC in humans is difficult to determine, because cases of uncomplicated diarrhea may not be tested for these organisms. Patients who develop HUS often require prolonged hospitalization, dialysis, and long term follow up, which are expensive in all directions (CDC, 2005).The cost of *E. coli* O157:H7 to the food industry as a result of recalls, destroyed food, control measures and lost demand associated to loss of consumer confidence is estimated to be in the billions of dollars in the U.S. alone (Frenzen *et al*., 2006).

2.8. Treatment

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, 2009).

2.9. Prevention and Control

Prevention of *E. coli* O157 infection has been difficult because of the broad spectrum of contaminated sources, ranging from food such as beef, milk, produce, and fruits, to non-food origins such as pool water and petting zoo animals (Kassenborg *et al*., 2004). Although could be Prevented 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 programmes 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).

2.10. Antimicrobial resistance

By reason of an increased demand for animal protein, the animal production sectors in low and middle-income countries have been regularly using antimicrobials for therapy, disease prevention and growth (Van Boeckel et al. 2015). This practice could be responsible for antimicrobial resistance among normal flora in the intestinal tracts of food animals, which may subsequently

risk public health due to food animals' weak response to, or loss of response to, drug therapy. The development of antimicrobial resistance in *E. coli* O157:H7 is the issue of *i*ncrease concern and generate new public health challenge (Newell et al., 2010). 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; Vijayarani *et al*., 2010; Naik and Desai, 2012).

In Ethiopia, there are a number of studies have been done on the antimicrobial resistance patterns of E. coli O157:H7 from diverse clinical sources (Gebre-Sealsssie, 2007). For example, researches done by (Taye *et al*., 2013; Hiko *et al*., 2008; Bekele *et al*., 2014; Tassew, 2015) and (Mohammed *et al*., 2017) confirmed that *E. coli* O157:H7 have developed already different degrees of resistant against various commonly used antibiotics including erythromycin Amoxycillin-Clavulanic acid, Sulfonamides, Ampicillin and Tetracycline, some strains also developed multi drug resistant.

3. MATERIALS AND METHODS

3.1. Study area

This cross sectional study was conducted from December 2017 to May 2018 in two abattoirs those, supplying meat for local consumption located in Bishoftu town, meat retailer shop in Bishoftu town and Bishoftu hospital. Bishoftu is located in East Shewa Zone, Oromia regional state, in the central highlands of Ethiopia and is about 47 km distance from Addis Ababa (NMSA, 2008). It has around 104,215 estimated populations according to global population review report 2018. The two abattoirs selected for study purpose are supplying meat for local consumption. One abattoir is private abattoir and one is government/municipal abattoir. The private abattoir is supplying meat for Bishoftu retailer shop and for Addis Ababa city. But the municipal abattoir is supplying meat for meat retailers found in Bishoftu town as well as it also supplies meat up on order when the customers have ceremony and festivals. The municipal abattoir has the capacity of slaughtering up to 40 animals but it slaughters 5-15 animals per day on usual days except on the eve of the Easter. The private slaughter house slaughters from 20-35 animals on usual day and up to 40 animals on the holiday. Both abattoirs are slaughtering animals by hanging upside down. They have stunned animals by sharp knife before bleeding of the animal. The another study area, Bishoftu Hospital, provides service for >10,0400 of the town population and also for the surrounding community.

3.2. Study design and study population

A cross-sectional study design was conducted to determine the occurrence of *E. coli O157: H7* in feces from bovines ready to be slaughtered at one Municipal abattoir and one Private slaughter house and meats from meat retailers in Bishoftu town and case study design was used for isolation of *E. coli O157: H7* from human stool from diarrheic patients at Bishoftu Hospital. The study populations were apparently healthy cattle ready to be slaughtered at both abattoirs

designed for local consumption. In addition, meat from meat retailers and diarrheic patient of Bishoftu Hospital were parts of study population.

3.3. Sample size determination

The required sample size is determined based on the expected prevalence of *E. coli* O157:H7 2.66% in cattle faeces ((Haile, 2014)), and 5.5.% in beef retailer shop (Tassew, 2015) and and13.9% in human stool (Adugna *et al.,* 2015). Therefore, the average expected prevalence was assumed to be 50% for human, with 95% confidence interval at 5% desired precision and using the formula recommended by (Thrusfield, 2005). But if p is below 10% and above 90%, d can be (Naing *et al., 2006).*

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

Where: - n=required sample size, P_{exp}=Expected prevalence and d = desired absolute precision

Accordingly the minimum required samples from cattle feces, meat at retail and humans were 42, 89 and 184 respectively. But to increase the precision of the study the sample size was increased to 240 fecal samples, 127 carcasses and 216 human stools. For questionnaire survey all abattoir and retails shop worker and diarrheic patients from those stools samples taken were interviewed. The parents or the guardian was interviewed for study involving children.

3.4. Sampling method

Systematic random sampling of ready to be slaughtered cattle at Bishoftu Municipal abattoir and one private abattoir those distribute meat for the retailer shops located in Bishoftu town were used. The two abattoirs were visited twice/week for two months and during each visit 10-15 animals were randomly sampled or if the number of animal slaughtered on the same day was less, all animals were sampled. All retailer shops found in the town were included in the study and all

shops those the owner were volenteer to givesample and open during sampling day were sampled. Additionally, purposive sampling technique for diarrheic patients, from individuals those the medical personnel ordered for stool diagnosis at Bishoftu hospital were sampled.

3.5. Sample collection procedure and transportation

Prior to the sample collection, abattoirs, meat retailers shop and Bishoftu Hospital were visited to facilitate research collaboration. Subsequently, cooperation letter was sent to each study site.
In general all samples were sampled aseptically. The meat samples were collected in a sterile polyethylene bags once a week from 127 meat retailer shops found in town which were open during sample collection. The collected carcass sample was put in ice box containing ice pack to create cold chain transportation. The fecal samples were collected from the rectum by using sterile plastic bags from all bovines ready to be slaughtered on the same day. The stool samples were collected from diarrheic patients in collaboration with the laboratory personnel in the hospital. Samples were collected by using universal bottle tube filled with transport media (Tryptone Soya broth) (CONDA, Madird, Spain) which was coded by sample number, date of sampling and age of the patient.

All samples were identified by sample number, date of sampling, source and sample type and collected samples were then transported to the Microbiology Laboratory of the College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu using icebox and kept chilled until microbiological analysis was done.

3.6. Isolation and identification

Up on arrival on the laboratory, 25g collected fecal sample was transferred into a sterile stomacher bag which contains 225 ml of Modified Tryptone soya broth (CM089, Oxoid Basingstoke, UK) supplemented with 20 mg/l novobiocin (mTSB + n) for pre-selective

enrichment. The resulting mixture was homogenized using a stomacher (Seward Stomacher 400, London, UK) at a low speed for 60s and incubated at 41.5^{o}C for 6h.

Isolation and identification of the bacterial strain was then performed as follows: All enriched samples were subjected to immunomagnetic separation (IMS) using Dynabeads^{TM} anti-*E.coli* O157 (Thermo Fisher, Lithuania) as recommended by the manufacturer. Briefly, 1ml of Enrichments received 20 µL of anti-O157 beads (Lithuania) and was mixed for about 30min. The beads was extracted from the enrichment samples and washed three times in phosphate-buffered saline-Tween Sigma,(St. Louis, MO). 20 µL of the final bead-bacteria complexes were spread onto Sorbitol MacConkey agar, (CM0813, Oxoid Basingstoke, England) containing 0.05 mg/l cefixime and 2.5 mg /l potassium tellurite (MAN0009620 Dynal Biotech ASA). All plates were incubated at 37 °C for 24 h. The SMAC-CT agar plates were examined for the presence of non-sorbitol fermenting colonies, suspected colonies; non-sorbitol fermenting colonies (colorless or pale colonies) were picked and confirmed by an *E. coli* O157: H7 latex agglutination test (Oxoid, Hampshire, UK) following the manufacturer's instruction and biochemically screened using the indole, methyl red, Voges–Proskauer and citrate utilization (IMViC) tests.

3.7. Confirmatory test for E. coli O157:H7 by latex agglutination test

Rapid Latex Test kit is a rapid latex agglutination test intended for confirmatory identification of E. coli serogroup O157 (Non-Sorbiotl Fermenting isolates). This test allows the rapid differentiation of E. coli O157 from other E. coli serotypes. The test was conducted by just adding one drop of latex suspension and dispensing near the edge of the circle on the reaction card. Then a portion of a typical colony to be tested was emulsified using a loop in a drop of sterile saline solution near the drop of test latex on the test card. After ensuring a smooth suspension of the bacteria and saline, the test latex was mixed with the suspension and spread to cover the reaction area over the loop. Then, the card was rocked in a circular motion for one minute and examined for agglutination by naked eye. Agglutination of the test latex within one minute was considered as positive result (Biolife, 2010)

3.8. Antimicrobial Susceptibility test

The antimicrobial susceptibility test was performed following the standard agar disk diffusion method according to (CSLI, 2015) using 10 antimicrobial disks (Oxoid Basingstoke, England). The selection criteria of the antibiotics depended on the regular use of the antimicrobials in the ruminants, potential public health importance and recommended from the guideline of antimicrobial susceptibility testing. Pure colonies incubated for 6 hours in Tryptone Soya Broth (Oxid, England) were made to have a turbidity of 0.5 MacFarland standards and inoculated on Muller-Hinton agar (Bacton Dickinson company and Cockeysville USA). Antibiotic discs were then placed and incubated for 24 hours. The result was classified as resistant, intermediate and susceptible after the zone of inhibition of bacterial growth depending on the interpretation for disk diffusin test method set by (CSLI, 2015).

3.9. Questionnaires

A descriptive survey design was used to answer questions concerning the current status of hygiene and sanitation practiced in the abattoir, retail shops and meat eating habit of patients. Hygiene and sanitation were assessed by the use of structured interview and through direct observations of the hygienic status and practices by abattoir and retail shop workers. The target population constituted all retail shops workers in the town, the abattoirs workers and diarrheic patient, those visit hospital on sampling day. The parents or the guardian was interviewed for study involving children. The questions were originally written in English and translated into the Amharic and Afaan Oromo languages when administered. The answer were then translated to English and entered into the original form.

3.10. Data management and statistical analysis

The data collected through questionnaire survey and laboratory results of the samples were entered in to databases using Microsoft Excel computer program and analyzed using STATA

Version 12.0 (STATA corp. College Station, TX, USA).Chi square analysis was done to see the difference in the prevalence rate of the bacteria along the chain. The prevalence of *E. coli* O157:H7 strains from all samples were determined by using descriptive statistics. Logistic regression analysis was done to measure the association of the prevalence of the bacteria with the considered risk factors. The result of the association was reported using Odds ratio and 95% confidence. For human age was classified according to (EAS, 2018) . Effects were reported as statistically significant if p-value was less than 5%.

3.11. Ethical clearance

Ethical clearance was obtained from animal research ethical review committee of Addis Ababa University College of Veterinary Medicine for collecting samples from cattle. For human components ethical clearance was obtained from Federal Democratic Republic of Ethiopia Ministiry of science and Technology. During sample collection all diarrheic patients were informed as they are going to be sampled and also the research purpose was clarified for them, then only volunteers were used for sampling.

4. RESULTS

4.1. Prevalence

Out of the total 583 samples examined, 31(5.3%) were positive for *E. coli* O157:H7. Of which, 7% (17/240) were from feces, 6.3% (8/127) were from meat and 2.8 (6/126) were from stools of diarrheic patient at Bishoftu hospital. However there was statistically no significant difference was observed among the three sample types (X^2= 4.4969, P = 0.106) (Table 3).

Table 3: The prevalence of *E. coli* O157:H7 along beef supply chain in Bishoftu

Sample type	**No. examined**	**No. positive**	**Prevalence (95% CI)**	**X^2**	**p-value**
Feces	240	17	7% (3.81, 10.32)		
Meat	127	8	6.3% (20.16, 10.58)	4.4969	0.106
Stool	216	6	2.8% (0.56, 4.99)		
Total	**583**	**31**	**5.3% (0.035, 0.071)**		

CI; confidence interval, X^2; chi square,

Out of the total 240 fecal sample examined, 17 samples (7%) were positive for *E. coli* O157:H7. Based on univarate logistic regression analysis, all the variables namely duration of meat stay in shop, hand washing practice, using of soap, using of head cover, dirty wall, use of refrigerator and type of cutting board used were not statistically association with occurrence of *E. coli* O157:H7 (P > 0.05)(Table 4).

Table 4: Prevalence of *E. coli* O157:H7 and the associated risk factors in meat at retailer shop in Bishoftu

Risk factors	No examined	No positive (%)	COR (95% CI)	p-value
Duration of meat stay in shop				
One day	96	5 (5.2%)	**	
12 hours	17	2 (11.8%)	2.42 (0.43, 13.7)	0.315
2 days	14	1 (7.1%)	1.4 (0.15, 12.9)	0.767
Hand wash				
Yes	118	8 (0.08%)	**	
No	9	0 (0%) ***		
Using of soap				
Yes	111	8 (7.21%)	**	
No	16	0 (0.0%) ***		
Head cover				
Yes	71	5 (7.04%)	**	
No	56	3 (5.36%)	1.34 (0.31, 5.86)	0.699
Presence of Refrigerator				
Yes	117	8 (6.84%)	**	
No	10	0 (0.0%) ***		
Dirty wall				
Yes	26	1 (3.85%)	**	
No	101	7 (6.93%)	0.54 (0.1, 4.6)	0.569
Cutting Board type				
Marble	42	5 (11.90%)	**	
Wood	33	3 (9.09%)	0.74 (0.163, 3.35)	0.696
Marble and Wood	12	0 (0.0%) ***		
Plastic	40	0 (0.0%) ***		

COR - Crude odds ratio, CI confidence interval; ** Reference variable

***- Variable for which analysis do not compute due to zero value

Table 5 indicated the prevalence of *E. coli O157:H7* in diarrheic patients and the associated risk factors. The relative prevalence of *E. coli* O157: H7 was higher in age group >60 years (10%) when compared with other age groups. Among the risk factors considered, only four days duration of onset of diarrhea was statistically associated with the occurrence of *E. coli* O157:H7 (p=0.047).

Table 5: The prevalence of *E. coli* O157:H7 in diarrheic patients and the associated risk factors

Risk factors	**No Examined**	**No positive (%)**	**COR (95% CI)**	**p-value**
Age				
0-14	41	1(2.44%)	**	
15-29	85	4(4.7%)	1.97 (0.213, 18.25)	0.549
30-44	64	0(0.0%) ***		
45-60	16	0(0.0%) ***		
>60	10	1(10%)	4.44 (0.253, 77.96)	0.307
Sex				
Female	93	1 (1%)	**	
Male	123	5 (4%)	3.89 (0.448, 33.95)	0.218
Religion				
Orthodox	169	3 (0.054%)	**	
Muslim	10	1(10%)	6.14 (0.58, 65.14)	0.132
Protestant	37	2 (5.4%)	3.16 (0.51, 19.63)	0.217
Duration of onset of diarrhea				
Two days	89	1 (1.12%)	**	
Three days	55	0 (0.0%) ***		
Four days	41	4 (9.76)	9.51 (1.02, 88.00)	0.047*
Five days	16	0 (0.0%) ***		
> six days	15	1 (6.66%)	6.28 (0.371, 106.3)	0.203
Consistency of diarrhea				
Watery	94	4 (4.25%)	**	
Bloody	12	0 (0.0%) ***		
Mucoid	26	0 (0.0%) ***		
Mixed	84	2 (2.38)	0.549 (0.09, 3.07)	0.495
Having cattle farming				
No	170	4 (2.35%)		
Yes	46	2 (4.34%)	1.89 (0.334, 10.64)	0.472
Direct contact with cattle feces				
No	174	4 (2.29%)		
Yes	42	2 (4.76%)	2.13 (0.376, 12.0)	0.39

Visiting or contact with cattle feces				
No	172	5 (2.9%)		
Yes	44	1 (2.27%)	0.78 (0.088, 6.823)	0.646
Consumption of raw meat in last two weeks				
No	91	4 (4.34%)		
Yes	125	2 (1.6%)	0.35 (0.063, 1.97)	0.236
Travel status in last two weeks				
No	179	4 (2.23%)		
Yes	33	2 (6.1%)	2.82 **(**0.496, 6.08)	0.242
consumption of food containing beef meat				
No	185	4 (2.16%)		
Yes	31	2 (6.45%)	3.12 (0.54, 17.82)	0.2
Consumption of beef outside home				
No	185	4 (2.16%)		
Yes	31	2 (6.45%)	3.12 (0.54, 17.82)	0.2
Contact with someone with diarrhea				
No	211	4 (1.89%)		
Yes	5	1 (20%)	10.3 (0.969, 10.95**)**	0.053
Attendance of large gathering				
No	208	5 (2.4%)		
Yes	8	1 (12.5%)	5.8 (0.596, 56.44)	0.13

COR odds ratio, CI confidence interval;* Statistical significant; ** Reference variable
***- Variable for which analysis do not compute due to zero value

4.2. Antimicrobial susceptibility

A total of 31 isolates of *E. coli O157:H7* were tested for antimicrobial susceptibility. Table 6 summarized the result of the antimicrobial susceptibility of the ten commercially available antimicrobials. All the isolates of *E. coli O157:H7* were susceptible to Gentamicine. Seventeen (54.8%) isolates were susceptible to Doxycycline, Oxytetracycline and Sulphamethorazole. However, all isolates were resistant to Ampicilin, Cefoxitin and Nitrofurantoin. All the isolates were resistant to at least to three antimicrobials revealing the occurrence of multi-drug resistance.

More specifically, 6/31 (19.3%), 13/31 (42%) and 12/31 (38.7%) of the isolates were resistant to three, four and five or more drug antimicrobials, respectively.

Table 6: Antimicrobial susceptibility test result of *E. coli* O157:H7 isolates

Antimicrobial agent	**Disc concentration**	**Susceptibility and resistance pattern of *E. coli O157:H7*(% of resistant isolate)**		
		S	R	I
Ampicilin	25µg	0(0)	31(100)	0(0)
Cefoxitin	30 µg	0(0)	31(100)	0(0)
Ciprofloxacin	5 µg	27 (87)	0(0)	4(13)
Doxy cycline	30 µg	17 (54.8)	12 (38.7)	2 (6.4)
Gentamicine	10 µg	31 (100)	0(0)	0(0)
Nalidixic acid	30 µg	16 (51.6)	15 (16.1)	0(0)
Nitrofurantoin	50 µg	0 (0)	31(100)	0(0)
Oxytetracycline	30 µg	17 (54.8)	14 (45.2)	0(0)
Streptomycin	10 µg	11 (35.4)	8 (25.8)	12 (38.7)
Sulphamethorazole	100 µg	17(54.8)	6 (19.3)	8 (25.8)

Nb: The number of tested isolate for each antimicrobial were 31

Key: %= Percent, S = Sensitive, I = Intermediate, R = Resistant,

4.3. Questionnaire survey

A total of 28 respondents were interviewed at abattoir. Out of this, 25 (89.3%) were males and 3 (10.71%) were females. From the total respondents 7.14%, 42.86%, 35.71%, and 14%, were take informal education, completed primary education, complete secondary education, and have diploma in animal health respectively. The result also showed that 46.43% of the respondents have more than five years work experience in abattoir work (Table 7).

All the respondents were found having the practice of hand washing before starting and after finishing slaughtering process. The result also indicated that 50% of respondents take medical checkup within every three months and the left take within six months. The observational assessment showed that, all of the workers in the working room put on aprons, boots and hair covering while none of them were wearing hand glove during working time. There was hand washing sink around working area. But, there was no functional hot water bath (sink) for dipping of knife. They use stubbing method for stunning the animal and vertical dressing method for evisceration of animal (Table 7).

Table 7: The meat handling and hygienic practices at abattoir in Bishoftu

Activity	**Performance**	**No. of respondents**	**Percent**
Sex	Male	25	89%
	Female	3	11%
Age	15-24	4	14%
	25-54	24	86%
Educational status	Informal	2	7%
	Primary	12	43%
	Secondary	10	36%
	Diploma	4	14%
Service duration	One yr-five yrs	13	46%
	>five yrs	15	54%
Washing of hand after evisceration	Yes	13	46%
	No	15	54%
Source of water	Bore hole	12	43%
	Municipal	16	57%
Training	Trained	17	61%
	Not trained	11	39%
Medical check up	Three month	12	43%
	Six month	16	57%

Table 8 summarizes response and observational assessment on the handling practices and hygienic status at meat retail shops. The result indicated that majority (65%) of the workers were in a range of 15 up to 24 age group while 35% of the workers were in a range of 25 up to 55 age groups. The majority (44%) of the workers were attended primary education. Ninety one percent of the workers did not practice hand washing before handling or touching the meat.

Table 8 : Response and observational assessment on status and personal hygiene of butchers

Activity	Performance	No. of respondents	Percentage
Age	15-24	82	65%
	25-54	44	35%
	55-64	1	1%
Education level	Informal	41	32%
	Primary	56	44%
	Secondary	30	24%
Protective clothing			
Head cover	Yes	71	56%
	No	56	44%
Frequency to wash white coat	Once a day	115	91%
	Twice a day	12	9%
Hand wash before touching meat	Yes	12	9%
	No	115	91%
Using of soap to hand wash	Yes	123	97%
	No	4	3%
Training	Yes	28	22%
	No	99	78%
Medical checkup	Yes	125	98%
	No	2	2%
Frequency of medical	Once per year	4	3%
	Every three months	93	73%
	Every six months	28	22%

The result of summary response on meat handling practices in Table 9 indicated that all of the abattoirs used closed vehicle for transportation purpose and meat cover on displays. Also, majority (79%) of the retail shops were used plastic wrap material while 6% of the retail shops use newspaper as wrap material. In addition, all of the retail shops were did not use cooling

device at display room. The details of the result on meat handling practices are described in Table 9.

Table 9: Summary of response and observational assessment on meat handling practice

Activity	Performance	No. of respondents	Percentage
Duration of meat stay	One day	96	76%
	12 hours	17	13%
	Two days	14	11%
Meat wrap	Plastic	100	79%
	News paper	7	6%
	Plastic and news paper	20	16%
Storage of offal and meat on same cabinet	Yes	50	39%
	No	77	61%
Use of same knife for offal and meat	Yes	6	5%
	No	121	95%

The result of butcher shop quality and sanitary assessment in Table 10 indicated that majority (71%) butcher shops were constructed from tile floor type. Also, 92% of the butcher shops were having a refrigerator for storage of meat. The result also showed that all of the butcher shops were use municipal water sources. In addition, the entire butcher shops were did not use hot water bath for washing of knifes. As described in Table 10, 79% and 70% of the butcher shops were conduct hygienic practices of knife and cutting boards once per day, respectively. The details of the result on quality and sanitary assessment of butcher shops are described in Table 10.

Table 10: Response of worker and observational assessment on quality and sanitary status of the retailer shop

Activity	Performance	No. of respondents	Percentage
Floor type	Tile	90	71%
	Wood	37	29%
Wall color	White	127	100%
Presence of dirty on wall	Yes	26	20%
	No	101	80%
Having Refrigerator	Yes	117	92%
	No	10	8%
Type of cutting board	Wood	33	26%
	Marble	42	33%
	Metal	0	0%
	Concrete	0	0%
	Plastic	40	31%
Frequency of washing equipment			
Knife	Once per day	100	79%
	Twice per day	4	3%
	More than twice per day	15	12%
	Once in every two days	8	6%
Cutting board	Once per day	89	70%
	Twice per day	5	4%
	More than twice per day	21	17%
	Once in every two days	12	9%
Saw/axes	Once per day	99	78%
	Twice per day	3	2%
	More than twice per day	8	6%
	Once in every two days	17	13%
Display cabinet	Once per day	101	80%

	Twice per day	10	8%
	More than twice per day	15	12%
	Once in every two days	1	1%
Hooks	Once per day	92	72%
	Twice per day	13	10%
	More than twice per day	13	10%
	Once in every two days	9	7%
Floor	Once per day	88	69%
	Twice per day	25	20%
	More than twice per day	11	9%
	Once in every two days	3	2%

5. DISCUSSION

E. coli O157:H7 is one of the most significant food-borne pathogens that have gained increased attention in recent years. The principal cause of disease in human is by eating raw meat. In Ethiopia, raw meat is available in open-air local retail shops without appropriate temperature control where consumers either purchase for home consumption or consume at the shop.

In present study *E. coli* O157:H7 was isolated from feces, meat at retailer shops and stool of diarrheic patient. The overall prevalence of the pathogen was 5.3% (31/583). Interestingly, the prevalence of *E. coli* O157:H7 was highest in feces sample (7%) than meat samples at retailer shop (6.3%) and human stool samples (2.8%). This result is in agreement with the report of Atnafi *et al*., (2017), whose study showed high prevalence rate of *E. coli* O157:H7 in feces samples than meat samples.

The overall prevalence (5.3%) of *E. coli* O157:H7 in this study was higher than 2.4% Atnafie *et al*., (2017) and 4.2% Hiko *et al., (*2008) but greatly lower than 10.2% (Bekele *et al.*, 2014) and 9.3% Feleke *et al*., (2017). This variation may be due to difference in sample size, demography of study area and the techniques used in isolation and identification of the bacteria. Immunomagnetic separation after enriching the sample with Tryptone soya broth enhance the detection of the pathogen (Chapman *et al*., 2001,Ojo *et al*., 2010) as compared to direct plating of test samples on selective agar (Varela-Hernandez *et al*., 2007; Hashemi *et al*., 2010). In the current study the more sensitive technique, Immunomagnetic separation was used.

This study showed the result from fecal sample (7%) was higher than report of Abdissa *et al*., (2017) (1.89%) and Haile, (2014) (2.6%) in Addis Ababa. This result was also in agreement with Omisakin *et al*., (2003) 7.5%) in United Kingdom. In contrast to the current study, a lower prevalence of *E. coli* O157:H7 was reported by Lupind *et al*., (2014) 0.9% in Tanzania. The variation in the prevalence of *E. coli* O157:H7 from cattle feces may be due to time interval between arrival of animal at abattoir and slaughtering, age of animals and nature of feeds (Chapman *et al*., 2001, Reid *et al.*, 2002)

The current findings from meat samples at retailer shop (6.3%) was lower when compared with the reports of Hiko *et al*. (2008) in Modjo town, Bekele *et al*. (2014) in Addis Ababa and Balcha *et al*. (2014) in Mekelle cities who have reported isolation frequency of 8%, 13.3% and18%, respectively. However, the current finding was higher than the reports of Tassew (2015) in Addis Ababa city and Bishoftu town (5.5%), Taye *et al*., (2013) in Haramaya town (2.65%) and Atnafie *et al*. (2017) in Hawassa (2%). The variation observed in the different studies might be due to difference in the sample size, hygienic practices, sample collection procedure sample size and quality of laboratory in isolation was done and geography between the different studies. The different report also revealed 2% Ashgan *et al*., (2015) from Saudi Arabia, 2.3% Hashemi et al., (2010) from Iran and 2.2% Salome and Jacob, (2014) from Nigeria lower than current study. This may be due to the hygienic practices and awareness among the society about the pathogen.

In this study there was 6/216 (2.8%) isolates of *E. coli O157* from patients who visited the hospital with diarrhea, this higher than a report of (Teshale *et al*., 2015) in Jimma town (1.8%) from food handlers. however others similar study in Gondar (Huruy *et al*., (2011) and in Addis Ababa Haile *et al*., (2014) didn't isolate any pathogen using the similar method. Other studies in Ethiopia was conduct on children in Addis Ababa (Ayenew, 2017) and Behir Dar (Adugna *et al*., 2015) were reported (4.5%) and(28.9%) respectively, but in current study the result in children less than 14 year was 1(2.44%). In contrast to the current study, a higher prevalence of *E. coli* O157:H7 was reported in different countries, with 7.5%- in South Africa Atebaa *et al.,* (2008), 7% in Tanzania Raji *et al.* (2008), 7% in Libya (Mohamed *et al*., 2017) and 3.4% in Tunisia Al-Gallas *et al*., (2006). This may be due to small sample size, degree of exposure to animal product and hygienic practice and awareness of the people in the study area. The prevalence of *E. coli* O157: H7 in humans may be linked to the degree of exposure to contaminated animal products. Poor animal hygiene has been identified by others as the contributor to human infection (Dunn *et al.,* 2004).

The development of antimicrobial resistance by the bacteria to these drugs poses a major challenge in both human and animal medicine because these drugs are commonly used in the treatment of human patients and in veterinary practice. Antimicrobial resistance of *E. coli*

O157:H7 isolates from animal and human sources have been reported in Ethiopia by (Bekele *et al.*, 2014), Mersha et al. 2010), and (Adugna *et al*., 2015).

In the current study all of the isolates were 100% susceptible to Gentamicin and 87% to Ciprofloxacine this agreed with the reports of Rahimi and Nayebpour (2012). In contrast to this finding a study conducted in Saudi Arabia revealed that there was resistant strain to Gentamycin (Naser and Wabel, 2007). This variation probably attributed to the expression of resistant gene code by the pathogen which is associated with emerging and re-emerging aspects of the isolates with regards to different agro ecology (Reuben and Owuna, 2013)

In the present result, resistance of the isolates ranges from 19.4% to 100% in which 19.4% to Sulphamethoxazole, 25.8% to Streptomycine 38.7% to Doxycycline, 45.2% to Oxytetracycline 48.4% to Nalidixix acid and 100% resistant to Nitrofurantoin, Ampicilin and Cefoxitin. This, agrement with a study conducted in Saudi Arabia revealed that there was resistant strain to nalidixic acid cefoxitin (Naser and Wabel, 2007) The level of resistance to Oxytetracycline (45.2%) was agrees with (Shitandi and Sternesjö 2001) with 57.9% resistance. The high level of resistance of tetracycline obtained in this study may be as a result of it being the most commonly available antibiotic used as growth promoter, irrational use of antibiotic, incomplete course of therapy and routine chemoprophylaxis among livestock (Olatoye, 2010; Ali *et al* 2010).

Findings from present study indicate that Gentamicin and Ciprofloxacin are the drugs of choice for *E. coli* O157:H7, since all of the isolates were susceptible to Gentamicin and almost all are susceptible to Ciprofloxacin with only 3 isolates having an intermediate resistance.

6. CONCLUSION AND RECOMMENDATIONS

The present study showed a considerable presence of *E. coli* O157:H7 along the beef supply chain, with relatively high prevalence in cattle at abattoir. It also showed the development and occurrence of resistant and multi-drug resistant *E. coli* O157:H7 to frequently used antimicrobials raising serious concern in the study area. The study would provide an insight on the prevalence and antimicrobial susceptibility of *E. coli* O157:H7 in the study area for designing the prevention and control of the disease.

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

- The slaughter houses should be routinely investigated for hygiene practices and corrective measures should be taken accordingly
- Meat handlers and sellers should be educated on the best practices on handling of meat
- Creating public awareness by disseminating the information on the potential risk of developing diarrhea illness due to consumption of meat that contaminated along the beef chain

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8. ANNEXES

Annex 1: Questionnaires (English version)

Table 11: Questionnaire for the abattoir workers (those directly have contact with carcass) on Hygienic Handling Practices at Abattoir

1. Basic information

1.1. Date_____

1.2. Questionnaire Code_____

NO.	Questions	Answers	Skip to
	2. General characteristics of individuals		
2.1	Age	Year completed----------	
2.2	Sex	Male [ ] Female[ ]	
2.3	Ethnicity	Oromo[ ] Amhara [ ] Tigire [] Gurage [ ] others (specify)__	
2.4	Level of Education:	Illiterate [ ] Informal Education [ ] Primary Education [ ] Secondary Education [ ] Other (Specify)……	
2.5	Your role at the abattoir?	Veterinarian/meat inspector [ ] Butchers [ ] Other (specify)	
2.6	Duration of working at the abattoir?	----------	
	3. Possible risk factors for contamination of carcass during slaughter process		
3.1	Stunning before slaughter	Yes[ ] No[ ]	
3.2	If yes, method of stunning	----------	
3.3	How long waited to start flaying after stunning?	Hrs----------	
3.4	Method of carcass dressing?	Vertical (hanging)[ ] Horizontal(on floor)[ ]	

<table>
<tr><td>3.5</td><td colspan="2">Do you use the following protective materials while working in the abattoir?(observe)
<table>
<tr><td>Protective materials</td><td colspan="2">Response</td></tr>
<tr><td></td><td>Yes</td><td>No</td></tr>
<tr><td>Apron</td><td></td><td></td></tr>
<tr><td>white coat</td><td></td><td></td></tr>
<tr><td>Head cover</td><td></td><td></td></tr>
<tr><td>Gloves</td><td></td><td></td></tr>
<tr><td>Boots</td><td></td><td></td></tr>
</table></td><td></td></tr>
<tr><td>3.6</td><td>Do you have sink for washing hands in the abattoir?</td><td>Yes [ ] No [ ]</td><td></td></tr>
<tr><td>3.7</td><td>Do you wash your hands before touching the carcass?</td><td>Yes [ ] No [ ]</td><td></td></tr>
<tr><td>3.8</td><td>Do you wash your hands with soap?</td><td>Yes [ ] No [ ]</td><td></td></tr>
<tr><td>3.9</td><td>Do you use the same knife for flaying and evisceration?</td><td>Yes [ ] No [ ]</td><td></td></tr>
<tr><td>3.10</td><td>Do you wash your hands after evisceration?</td><td>Yes [ ] No [ ]</td><td></td></tr>
<tr><td>3.10</td><td>Do you sink knife in hot water in between flaying and evisceration?</td><td>Yes [ ] No [ ]</td><td></td></tr>
<tr><td>3.12</td><td>Is carcass washed after evisceration?</td><td>Yes [ ] No [ ]</td><td></td></tr>
<tr><td>3.13</td><td>What are the possible sources of contamination of carcass?</td><td>Feces during evisceration [ ] hides during flaying [ ] handlers hand [ ] knife[ ] floor [ ] hanging hook[ ] Others(specify)</td><td></td></tr>
<tr><td>3.14</td><td>What is your source of water for use in the abattoir?</td><td>City/Municipal council [ ] borehole [ ] rain collected water [ ] River [ ] others (specify) [ ]</td><td></td></tr>
<tr><td>3.15</td><td>Have you ever received any training on hygienic handling of carcass?</td><td>Yes [ ] No[ ]</td><td></td></tr>
</table>

3.16	Have you gone for medical checkups to work at the abattoir?	Yes [ ] No [ ]	
3.17	How frequent you go for medical checkup?	Once per year [ ] Every three months [ ] Every six months [ ] others(specify)----------	
3.18	Do you think improvement needed to avoid contamination of carcass at the abattoir?	Yes [ ] No [ ]	

Table 12: Questionnaire for meat handlers on hygienic practices at retail markets

1. Basic information

1.1.Date_____

1.2.Questionnaire Code_____

No	**Questions**	**Response**	**Skip to**
2. General characteristics of individuals			
2.1	Age	Years completed----------	
2.2	Sex	Male [ ] Female[ ]	
2.3	Level of Education:	Illiterate [ ] Informal Education [ ] Primary Education [ ] Secondary Education [ ] Other (Specify)…….	
2.4	Religion	orthodoks [ ] Muslim[ ] Protestant[ ] Catholic[ ] Waaqefataa [ ] others [ ]	
2.5	Duration of selling meat in retail outlet?		
3. Possible risk factors for contamination of meat at retail market			
3.1	What is the means of transporting meat from abattoir to the retail shop?	Open vehicle [ ] Closed vehicle[ ] Animal transport (Cart horse) [ ]	
3.2	Is there any cover on display case?	Yes [ ] No [ ]	3.3

3.3	Is retail shop floor is made of concrete?	(observe) [ ]Tile [ ]wood earthen material[ ] others(specify)	
3.4	Wall and ceiling are clean or free of dust	(observe) Yes [ ] No [ ]	
3.5	Wall painted with white color	Yes [ ] No [ ] →	3.6
3.6	If yes, is there sign of dirty on the wall?	Yes [ ] No [ ]	
3.7	What is the ventilation status of display case and butchery	(observe) Good [ ] Fair [ ] Poor [ ]	
	Good-ventilation allows air flow into the butchery but sieves off dust and other particles [ ] Fair-ventilation allows air flow but do not sieve dust or other particles or allows very little air flow [ ] Poor-ventilation does not allow air flow at all [ ]		
3.8	Is there use of bulbs at the display case	((observe) yes [ ] No [ ]	
3.9	Are there meat cooling facilities at the display cabinet?	(Observe) Yes [ ] No [ ]	
3.10	Do you have a refrigerator for storage of the meat that remains from daily sale?	Yes [ ] No [ ]	
3.11	Do you use the following protective materials while selling or handling meat?(observe)		
3.12	How frequent do you wash the protective (white coat and Apron)?	Once per day in the evening [ ] Twice per day, morning and	

Protective materials	Response	
	Yes	No
Apron/white coat		
Head cover		
Gloves		

		evening [ ] once after every two days [ ] once per week [ ] others [ ]	
3.13	Do you have sink for washing hands	Yes [ ] No [ ]	
3.14	Do you wash your hand before touching the meat?	Yes [ ] No [ ]	
3.15	Do you wash your hand with soap	Yes [ ] No [ ]	
3.13	What is your source of water for use in the butchery?	City/Municipal council [ ] borehole [ ] rain collected water [ ] River [ ] others (specify) [ ]	
3.14	What kind of cutting board you are using?	(Observe) Wood [ ] plastic [ ] Metal [ ] concrete [ ] Marble [ ]	
3.15	How often do you wash the following butchery surfaces and equipments?		

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

3.16	Do you use detergent/disinfectant for cleaning the butchery utensils?	Yes [ ] No [ ] →	3.17
3.17	If "Yes" what types of detergent/ disinfectant do you use		
3.18	Do you sterilize your equipment's	Yes [ ] No [ ] →	3.19

3.19	If "Yes" what are the methods used to sterilize the equipment	_____	
3.20	Do you have any hot water baths for dipping of knives?	Yes [ ] No [ ]	
3.21	Ways of cleaning butchery equipments	Cold water only [ ], cold water with soap [ ] hot water only [ ] hot water with soap [ ] wiping with pieces of fabrics [ ] others (specify)……	
3.22	Do you have routine control of flies in your butcher?	Yes [ ] No [ ] →	3.23
3.23	If "Yes" what are the methods used to control flies?	_____	
3.24	How long does the meat stay in your butchery before it is over?	Less than 12 hours [ ] one day [ ] Two days [ ]	
3.25	Material to wrap meat for sale.	Newspaper[ ] Plastic [ ] Used paper[ ] Others[ ]	
3.26	Do you collect money while handling or selling meat?	Yes [ ] No [ ]	
3.27	Have you ever received any training on hygienic handling of meat?	Yes [ ] No [ ]	
3.28	Do you ever receive complaints from the consumers on the quality of the meat you sell?	Yes [ ] No [ ] →	3.29
3.29	If yes, what kind of complaint?	Abdominal upsets [ ] Tough meat [ ] Dirty meat [ ] others [ ]	
3.30	Have you gone for medical checkups in the last 6 months?	Yes [ ] No [ ]	
3.31	How frequent you go for medical checkup?	Once per year [ ] every three months [ ] every six months [ ]	

3.32	Do you have different storage and display cabinets for offal's and meat?	(observe) Yes [ ] No [ ]	
3.33	Do you use the same equipment while handling meat and the offal's?	Yes [ ] No [ ]	
3.34	Do you believe that the butchery where you work requires some improvement for better handling of meat?	Yes [ ] No [ ] →	3.25
3.35	If yes, what kind of improvement?	_____	

Table 13: Questionnaire for Diarrheic patients

1. **Basic information**

1.1. Date_____

1.2. Questionnaire Code_____

NO.	**Questions**	**Responses**	**Skip to**
2. **Socio demographic characteristics of the patient**			
2.1	Age	Years completed _____	
2.2	Sex	Male [ ] Female[ ]	
2.3	Level of Education:	Illiterate [ ] Informal Education [ ] Primary Education [ ] Secondary Education [ ] Other (Specify)……	
2.4	Religion	Orthodox [ ] Muslim[ ] Protestant[ ] Catholic[ ] Wakefata[ ] Others (specify)…..	
2.5	Occupation	----------	
2.6	Residence	Urban [ ] Rural[ ]	
3. **Clinical information** *(Taking into account the incubation period: E. coli* O157:H7: 2-7 days.			
3.1	Duration of diarrhea since onset	----------	
3.2	Consistency of diarrhea	[ ] watery [ ] mucoid [ ]	

		bloody[ ] mixed[ ]	
3.3	Maximum number of episode of diarrhea in last 24-hour period	_____	
3.4	Episode of diarrhea in the last one to two years one	One [ ] two [ ] three [ ] four and more than four[ ]	
4. Other potential risk factors (Exposure assessment)			
4.1	Do you have cattle for farming?	Yes [ ] No[ ]	
4.2	Have you had visit or contact with cattle in the last two weeks before illness?	Yes [ ] No[ ]	
4.3	Have you had direct contact with cattle feces in the last two weeks before illness?	Yes [ ] No[ ]	
4.4	Did you consume raw meat in the last two weeks before illness?	Yes [ ] No[ ]	
4.5	Beef consumption behavior	Raw [ ] heat treated[ ] semi-heat treated [ ] All forms[ ]	
4.6	Did you travel anywhere during the last two weeks before illness?	Yes [ ] No[ ]	
4.7	If yes(4.7), did you eat food containing beef	Yes [ ] No[ ]	
4.8	Did you eat beef outside home (retail shop, restaurants etc.) during the last two weeks before illness	Yes [ ] No[ ]	
4.9	Had contact with someone with diarrhea illness before becoming ill?	Yes [ ] No[ ]	
4.10	Did you attend a large gathering like wedding ceremony the week before your illness?	Yes [ ] No[ ]	
4.11	If yes, did others develop similar illness?	Yes [ ] No[ ] I don't know [ ]	

Annex 2: Type and preparation of microbiological media used for isolation identification and antimicrobial susceptibility test of *E. coli* O157:H7

1. **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 thoroughlyin universal bottle and sterilize in autoclave at 121 ℃ for 15 minutes. Final PH is 7.0 $\pm$ 0.2 at 25℃.

2. **Novobiocin Solution**

Composition (g/l): Novobiocin 0.45g

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

3. **CefiximeTellurite Sorbitol MacConkey agar(CT-SMAC) (**Oxoid Basingstoke, England)
4. **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.

5. **Nutrient Agar (**CM 0003, OXOID, Basingstoke, Hampshire 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.

6. Tryptone/trptophan medium

Composition (g/l): Sodium chloride (5g),Tryptone (10g) and DL-trptophan (1g)

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℃.

7. Kovac's reagent

Composition (g/l): Para-dimethylaminobenzaldehyde(50 g).Dimethylbutan or peptan(75 ml) and Concentrated pure hydrochloric acid(1.19g/ml) (25 ml)

Preparation: Dissolve the aldehyde in the alcohol by gentle warming in a waterbath, cool and add the acid. Protect from light and store at 4°C temperature.

8. Wash buffer: modified phosphate buffer

Composition (g/l):sodium chloride(8g), potassium chloride (0.2g),Disodium hydrogen phosphate (1.44g),potassium dihydrogen phosphate(0.24g) and polyoxylethylene sorbitanmonnolaurate (0.2g)

Preparation: Dissolve the component in the water. PH: 7.3 + 0.1 at 25℃. Sterilize by autoclaving at 121℃ for 15 minutes. Dispense in bootee or flask in appropriate volume for use

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

Composition (g/l): beef, dehydrated infusion 300.00; 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℃.

10. Sorbitol MacConkey (SMAC) Agar (CM0813, Oxoid Ltd., Basingstoke, Hampshire, England

Composition (g/l): peptone 20 , sorbitol 10, bile salts No.3 31.5, sodium chloride 5, neutral red 0.03, crystal violet 0.001 and agar 15

Preparation: 51.5g of the powder medium was suspended in one liter of distilled water and brought to the boil to dissolve completely. Then it was sterilized by autoclaving at 121°C for 15 minutes. Thereafter, it was allowed to cool to 50 °C and poured into sterile Petri dishes, and lastly

allowed to solidify at room temperature, and stored upside down at 4 to 8°C, refrigerator, for subsequent use and finally adjusted at pH of 7.1±0.2 at 25°C.

11. **Kligler iron agar** (CM0033, Oxoid Ltd., Basingstoke, Hampshire, England)

Composition (g/l): enzymatic digest of casein 10, enzymatic digest of animal tissue 10, lactose 10, dextrose 1, ferric ammonium citrate(0.5, sodium chloride 5, sodium thiosulfate 0.5, phenol red 0.025 and agar 15.

Preparation: 52 g of the powder medium was suspended in one liter of distilled water and brought to the boil to dissolve the medium completely. Then, it was sterilized by autoclaving at 121°C for 15 minutes and cooled to 45-50 ºC before use. Thereafter, the medium was poured in to sterile test tubes, in so doing cooled in slanted position and finally stored in a refrigerator to ensure the shelf life.

Annex 3: Biochemical and serological test procedures

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

Dextrose and lactose fermentation test (KIA test): A sterile straight inoculating needle was used to select an isolated colony from the culture plate and stab needle into the butt of the medium (Oxoid, England). Then, the inoculating needle was withdrawn to the slant and streak back and forth up the slant surface. After that, the tube was cap loosely and incubated aerobically overnight for 18-24 hours at 35±2 °C. After the incubation period, the tube was examined for an acid, gas and hydrogen sulfide production. Thus, the presence of an acid slant-acid butt (yellow/yellow) and cracks, splits or bubbles (gas production) in the medium indicates positive result for *E. coli* (Tiwari *et al.*, 2009).

***E. coli* O157:H7 latex agglutination test:** The *E. coli* O157:H7 latex agglutination assay (DR0621M, Oxoid Ltd., Basingstoke, Hampshire, England) containing latex particles coated with antibodies specific for the *E. coli* O157, and *E. coli* H7antigens. Identification of *E. coli* O157:H7 was carried out following the manufacturer's instructions; hence colonies that agglutinated were considered to be *E. coli* O157:H7. The control latex reagent identifies the non-specific agglutination. The test was done with the following procedures:

1. Suspected colonies (non-sorbitol fermenting colonies) isolated from Sorbitol MacConkey (SMAC) agar (Oxoid, England) which were sub cultured on the nutrient agar (Oxoid, England) was used from 18-24 old culture.
2. For each isolate to be tested dispense one drop of the *E. coli* O157 test latex was dispended into the well of the test slide (reaction card).
3. In like manner, one drop of *E. coli* control latex was dispensed into a separate well of the test slide.
4. Then a drop of sterile saline solution was dispended into each of the test slide.
5. Using a plastic stick (provided), a portion of the colonies was removed from the nutrient agar plate and emulsified in *E. coli* O157 test latex and sterile saline water on the slide as well it was spread over two-thirds of the reaction area. Lastly the plastic stick was discarded properly.
6. Once more using a fresh plastic stick, the process was repeated with the remaining colonies and emulsified in *E. coli* control latex on the slide.
7. Thereafter, the slide was rotated using circular motions for up to 1 minute and observe for the presence of precipitation on the *E. coli* O157 test latex and control latex. If agglutination occurred with the *E. coli* O157test latex and the control latex was negative. Then, it indicates positive result for the *E. coli* O157 serogroup.

Annex 4: Antimicrobial susceptibility test, the disc diffusion method

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

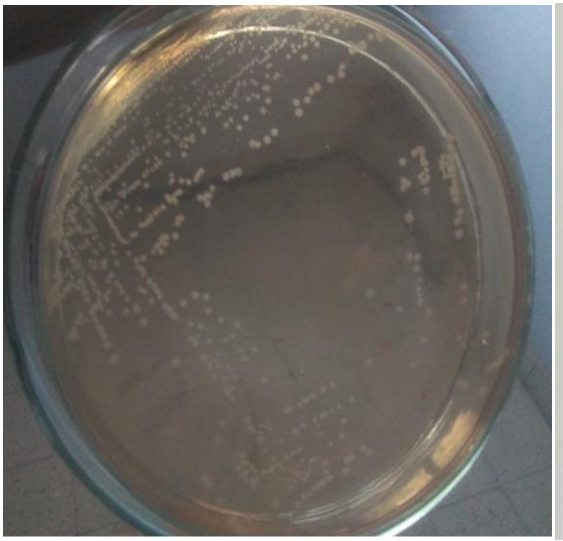
The sizes of the zones of inhibition, to the nearest whole millimeter, were interpreted according to CLSI (2015) criteria as described below (Table 14).

Table 14: Antimicrobial susceptibility test interpretive criteria for *Enterobacteriacea*

Antimicrobial agent	Disk code (concentration)	Zone Diameter: Interpretive Criteria (nearest whole millimeter)			Expired date
		S	I	R	
Ampicillin	AMP25	≥17	14-16	≤ 13	2019
Cefoxitin	FOX30	≥23	20-22	≤19	2019
Ciprofloxacin	CIP[5]	≥21	16-20	≤15	2020
Doxycycline	DO30	≥14	11-13	≤ 10	2020
Gentamicin	GEN[10]	≥15	13-14	≤12	2020
Nalidixic acid	NA30	≥19	14-18	≤13	2019
Nitrofurantoin	F50	≥ 18	14-17	≤13	2020
Oxytetracycline	OT30	≥15	12-14	≤11	2019
Streptomycine	S[10]	≥15	12-14	≤11	2019
Sulphamethoxazole	RL100	≥16	11-15	≤10	2019

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

Annex 5: Pictures




a) *E. coli* O157:H7 colonies on Sorbitol-MacConkey Agar plate

b) When agglutination occur


c) Indole test **d) KIA test**

Annex 6: Ethical clearance certificates

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu/Debre Zeit

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/22/05/10/2018

Name of Applicant: Alemnesh Jufar (DVM, MVSc fellow)

Address: College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Investigating the occurrence of E. coli* O157:H7 *along beef supply chain and in human Diarrhea in Bishoftu town, East Showa, Ethiopia*

Date of application:	15/11/2017
Nature of the project:	non-invasive
Target animal species:	Cattle
Number of animals involved:	240
Study area:	East Ethiopia

Minutes No. and date of review: VM/ERC/05/10/018, 03/01/2018

The above indicated research project is acceptable from ethical perspective, relevance, originality and technical competence points of view. Hence the project is allowed to be executed provided that:

1. All procedures and conditions stipulated in the proposal are respected and any deviation or changes be reported to the committee
2. The project activities be open for occasional supervision by the committee whenever this is deemed necessary
3. Any major work on human subjects require a separate clearance from concerned authority

Dr Getachew Terefe
Chairman

Signature

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

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Please quote Our Ref. No. When replying

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Fax 251-11-4339933 Tel. +251 114338450 P.o.x. Box}34 Bishoftu/Debre Zeit, Ethiopia