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OCCURRENCE AND ANTIMICROBIAL RESISTANCE OF *E.COLI* O157:H7 IN CATTLE, BEEF AND DIARRHEIC PATIENTS IN SEBETA TOWN, ETHIOPIA.

MVSc Thesis

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OCCURRENCE AND ANTIMICROBIAL RESISTANCE OF *E.COLI* O157:H7 IN CATTLE, BEEF AND DIARRHEIC PATIENTS IN SEBETA TOWN, ETHIOPIA.

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

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STATEMENT OF AUTHOR

First, I declare that this thesis is my genuine work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for MVSc. degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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LIST OF ABBREVIATIONS

A.A	Addis Ababa
A/E	Attaching and Effacing lesions
ATCC	American Type Culture Collection
CLSI	Clinical and Laboratory Standards Institute
DAEC	Diffuse adherent *Escherichia Coli*
E. COLI	*Escherichia Coli*
EAEC	Enteroaggregative *Escherichia Coli*
EHEC	Enterohaemorrhagic *Escherichia Coli*
EIEC	Enteroinvasive *Escherichia Coli*
ELISA	Enzyme Linked Immuno Sorbent Assay
EPEC	Enteropathogenic *Escherichia Coli*
EspA	*Escherichia Coli* Secreted Protein A
ETEC	Enterotoxigenic *Escherichia Coli*
ExPEC	Extra intestinal *E.coli*
FAO	Food and Agriculture Organization
FBI	Food Borne Illness
Gb3	Globotriaosylceramide
HC	Haemorrhagic Colitis
HUS	Haemolytic Uraemic Syndrom
IMS	Immunomagnetic Separation
IMVIC	Indole, Methyl red, Voges proskauer and Citrate
LEE	Locus of Enterocyte Effacement
MAb	Monoclonal Antibody
MUG	Methyl Umbelliferyl Glucoronide
PCR	Polymerase Chain Reaction
PH	Power of Hydrogen
rRNA	Ribosomal Ribonucleic acid
SMAC	Sorbitol Mac-Conkey Agar

STEC	Shigatoxin producing *Escherichia Coli*
Stx	Shigatoxin
Tir	Translocated Intimin Receptors
TTP	Thrombocytic Thrombocytopaenic Purpura
UK	United Kingdom
USA	United States of America
VT	Verotoxin
VTEC	Verotoxigenic *Escherichia Coli*
WHO	World Health Organization

SUMMARY

A cross- sectional study was conducted from November 2019 to May 2020 on apparently healthy cattle slaughtered at Sebeta Municipal Abattoir, on meat at retail shops in Sebeta and stool samples from diarrheic patients admitted at Sebeta health center, central Ethiopia. The study was conducted to determine the prevalence of *E.coli* O157:H7 and to assess the anti-microbial resistance profile of the isolates. A total of 267 animal source samples (120 faecal, 120 carcass swab and 27 meat) and 125 human diarrheic stool samples were collected during the study period. *E.coli* O157:H7 was isolated and confirmed using bacteriological culture, biochemical tests and Latex- agglutination test. All isolates were then subjected to susceptibility testing with selected anti-microbials and their susceptability pattern was checked. Out of 267 animal source samples examined, *E.coli* O157:H7 was detected in 9(3.4%) (95% CI= 0.02-0.06) of the samples. When separated by the source of the samples, *E.coli* O157:H7 was detected in 4(3.3%) (95% CI = 0.01-0.08) of faeces, 3(2.5%) (95% CI= 0.02-0.23) of carcass swab and 2(7.4%) (95% CI = 0.01-0.07) of meat sample. Out of 125 diarrheic stool samples that were examined, 4(3.2%) (95% CI= 0.01-0.08) of the samples were positive for *E.coli* O157:H7. The study showed no statistically significant difference in the prevalence of *E.coli* O157:H7 among sample sources and between considered risk factors. All isolates from animal as well as human sample showed 100% susceptibility to Ciprofloxacin, Meropenem, Sulfamethaxazole+ Trimetoprim, Nalidixic acid, Ceftraxone and Gentamicin. On the other hand, few isolates showed resistance to Amoxicillin-clavulanate, Tetracycline and Ampicillin. In conclusion, this study indicates the occurrence of *E.coli* O157:H7 in raw meat and the resistance of the isolates to one or more antimicrobial agents. This might point out raw meat as a potential risk to public health associated with the habit of raw meat consumption at the study area, so control measures and routine inspection on the hygienic status of slaughter houses and retail shops is crucial. Furthermore, anti-microbial use at the animal population mainly in food animals should be under practice to ensure consumer safety.

Key words: *E.coli O157:H7, Cattle, Beef meat, Slaughter house, Prevalence, Sebeta, Human*

1. INTRODUCTION

The term food-borne diseases or food-borne illnesses are used to denote gastrointestinal complications that occur following recent consumption of a particular food or drink (Dhama *et al.,* 2013). Food-producing animals (e.g., cattle, chickens, pigs, and turkeys) are the major reservoirs for many food-borne pathogens such as Campylobacter species, non-Typhi serotypes of *Salmonella enterica*, Shiga toxin-producing strains of *Escherichia coli*, and *Listeria monocytogenes*. The zoonotic potential of foodborne pathogens and their ability to produce toxins causing diseases or even death are sufficient to recognize the seriousness of the situation (Heredia and García, 2018).

Food-borne disease is divided into three types: 'Infection', 'intoxication', and toxin-mediated infection (Nyenje and Ndip, 2013). 'Infection' is caused by the ingestion of viable microorganisms. Intoxication is caused by the ingestion of preformed toxin in food which is produced by the pathogen and toxin- mediated infection is caused by bacteria that produce enterotoxins during their colonization and growth in the intestinal tract (Teplitski *et al.,* 2009).

Mitigation against food-borne diseases is facing new challenges due to the globalization of the food market, climate change and changing in human food consumption pattern since fresh and minimally processed foods are currently preferred (Schelin *et al.,* 2011).

Even if viruses are more responsible for more than 50% of all food-borne illnesses; most hospitalizations and deaths due to food borne diseases occur due to bacterial pathogens. The infections range from mild gastroenteritis to life-threatening neurologic, hepatic, and renal syndromes resulting from the "disease-causing" microbe, or by the human body's reaction to the microbe itself (Teplitski *et al.,* 2009). Other common symptoms include fever, bloody diarrhea, abdominal cramping, headache, dehydration, myalgia, and arthralgias (Timothy *et al.,* 2015).

Microbial food-borne illness still remains a global concern despite the extensive scientific progress and technological developments achieved in recent years in developed countries (Mersha *et al.,* 2009). Developing countries including Ethiopia are mostly affected because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment, and lack of education for food-handlers (Disassa *et al.,* 2017).

Currently, more than 2 million deaths occur every year in developing countries due to foodborne diseases, which are among more than 13 zoonoses implicated in over 2 billion illnesses worldwide (Odeyemi, 2016). Over the last 20 years, the emergence of major foodborne pathogens such as Salmonella and *E. coli* have persisted as a major public health concerns and provide clear examples of the persistence of foodborne pathogens despite considerable efforts aimed at prevention and control (Mengistu *et al.,* 2017).

Escherichia coli (*E. coli*), a Gram negative bacilli belongs to the family enterobacteriaceae and the genus Escherichia are a group of bacteria that are commonly found in the lower intestinal tract of healthy humans and animals. However, there are many types of *E.coli*, a few of which are potentially pathogenic by a variety of infective and toxin-producing mechanisms. Shiga toxigenic *E.coli* (STEC) are not pathogenic to ruminants, but they commonly cause serious diseases in humans worldwide where consumption of undercooked meat, raw dairy products and under cooked vegetables are common means of transmission to humans (Farrokh *et al.,* 2012).

Escherichia coli O157: H7, an enterohemorrhagic *E. coli* (EHEC), is the most common zoonotic pathogen responsible for the majority of severe cases of human diseases. It infects all age groups and the pathogen is known for its pathogenicity, low infectious dose and ability to survive in extra-intestinal environments (Ferens and Hovde, 2011). The pathogen causes hemorrhagic colitis and is often associated with devastating or life-threatening systemic manifestations. The most severe sequelae, the hemolytic uremic syndrome (HUS), results from Shiga toxins (Stxs) produced by the bacteria in the intestine and act systemically on sensitive cells in the kidneys, brain, and other organs (Gyles, 2007).

The first outbreak occurred in 1982 in which ground beef was implicated. *E.coli* O157:H7 has since been a steadily increasing cause of foodborne illness worldwide (FAO/WHO, 2006). A large outbreak occurred in Japan associated with contaminated radish sprouts in which more than 12,000 persons were affected (Michino *et al.,* 1999). A recent outbreak was occurred in Canada associated with contaminated pork products (Honish *et al.,* 2017). An outbreak has also occurred among children visiting farms where they came into direct contact with animal carrying *E. coli* O157:H7 and its environment (Grif *et al.,* 2005).

Globally STEC causes 2,801,000 acute illnesses annually, with an incidence rate of 43.1 cases per 100,000 person-years. This burden leads to 3,890 cases of HUS and 230 deaths. 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 person-years (Majowicz *et al.,* 2014). STEC O157:H7 contributes 10% to this burden (Majowicz *et al.,* 2014 by Lupindu 2018). Ethiopia is located in a sub-region that experiences the second highest foodborne disease burden in the world where STEC *E.coli* is one of the leading causes of foodborne disease disability adjusted life years (Havelaar *et al.,* 2015).

There is a risk potential of exposure to Verotoxigenic *Eschericia coli* in Ethiopia and the misuse of medical and veterinary drugs creates a suitable condition for the development of resistant strains (Dulo *et al.,* 2015). The habit of consuming raw and/or undercooked meat is one of the factors that exacerbate the transmission in the country since most people prefer to eat raw or undercooked beef (Beyi *et al.,* 2017).

Considering the high exposure in Ethiopia, a number of studies have been conducted in different areas of the country to determine the carriage rate in different livestock populations (Atnafie *et al.,* 2017, Abdissa *et al.,* 2017, Dulo, 2014, Dulo *et al.,* 2015 and Shecho *et al.,* 2017). However, there is no previous study done in cattle as well as in human in Sebeta town, central Ethiopia. In addition, even if a number of studies conducted in different areas of the country, the zoonotic potential of *E.coli* 0157:H7 directly relating to the habit of raw meat consumption which indirectly indicates carcass contamination at the value chains has never been assessed well.

Therefore, the objectives of this paper are the following.

General objective

- To assess the prevalence of cattle carcass contamination and public health significance of *E.coli* O157:H7 in Sebeta town, central Ethiopia.

Specific objectives

- To estimate the prevalence of *E.coli* O157:H7 in cattle faeces, carcass swab, beef and diarrheic patients in Sebeta town.
- To determine the antimicrobial resistance profile of *E.coli* O157:H7 isolated from cattle and human.
- To investigate the potential risk factors for zoonotic transmission of *E.coli* O157:H7 from cattle to human.

2. LITERATURE REVIEW

2.1. *Escherichia Coli* and its pathotypes

Escherichia coli (*E. coli*) are Gram-negative, rod-shaped, facultative anaerobic bacteria. Most *E. coli* strains harmlessly colonize the gastrointestinal tract of humans and animals as a normal flora. Despite the fact that *Escherichia coli* as a commensal bacteria can be found in intestinal microflora of a variety of animals including man, not all the strains are harmless, and some can cause debilitating and sometimes fatal diseases in humans as well as mammals and birds(Jafari *et al.,* 2012). They are bile-tolerant, non fastidious organisms that are easily cultured on routine laboratory media. They ferment lactose and grow best under mesophilic temperatures with an optimum at 37^0C and most *E. coli* have the b-glucoronidase enzyme that breaks down complex carbohydrates (Adamu *et al.,* 2014).

Escherichia coli is divided in to two types, pathogenic *E. coli* and non-pathogenic *E. coli*. The non-pathogenic strains of *E. coli* described as commensal *E. coli* are present in the normal microflora of intestine. They are harmless, hinder the growth of harmful bacteria and produce vitamins. In addition play several important roles in humans, such as performing specific metabolic functions which are absent in humans, modulating the morphology and physiology in the gut as well as assisting in development of the immune system(Shah *et al.,* 2018). The pathogenic *E. coli* strains can be further classified into intestinal diarrheagenic *E. coli* which causes diarrhea and extra intestinal *E. coli* (ExPEC) which causes wide range of illnesses in humans such as meningitis and chronic urinary tract infections (Jafari *et al.,* 2012).

Based on the mechanism of the disease and presence of virulence factors, at least seven classes of diarrheagenic *E. coli* are identified, namely, enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic *E.coli*(EHEC),enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and the recently emerged, adherent invasive *E. coli* (AIEC). Of all diarrheagenic *E. coli* identified, Shiga-toxin or Verotoxin producing (STEC/VTEC) EHEC is the most important pathotype in human diseases (Shah *et al.,* 2018).

Enterotoxigenic *E.coli* (ETEC) is the most common cause of travellers' diarrhoea, infecting all age group, with mild to severe watery diarrhoea, usually without blood, mucus or pus, sometimes nausea may occur in certain patients, abdominal cramping and mild fever. Severe cases of diarrhoea in children especially under the age of five years may lead to mortality. Besides humans, it is also an important *E. coli* strain which cause diarrheal disease in piglets as well as other newborn animals(Shah *et al.,* 2018).

EPEC was the first pathotype of *E. coli* to be described. It is associated with large outbreaks of infant diarrhoea. Although large outbreaks of infant diarrhoea due to EPEC have largely disappeared from industrialized countries, EPEC remains an important cause of potentially fatal infant diarrhoea in developing countries (Kaper *et al.,* 2004). Colonic mucosa is the infection site for EIEC where invasion of M cells, macrophages and epithelial cells occur resulting in a watery diarrhea, which in severe cases may be followed by the onset of scanty dysenteric stools containing blood and mucus (Jafari *et al.,* 2012).

Enteroaggregative *Escherichia coli* (EAEC) represent a heterogeneous group of *E. coli* strains. Manifestations associated with EAEC infection include watery diarrhea, mucoid diarrhea, low-grade fever, nausea, tenesmus, and borborygmi. In early studies, EAEC was considered to be an opportunistic pathogen associated with diarrhea in HIV patients and in malnourished children in developing countries. In recent studies, associations with traveler's diarrhea, the occurrence of diarrhea cases in industrialized countries and outbreaks of diarrhea in Europe and Asia have been reported (Jensen *et al.,* 2014). The pathogenicity of DAEC strains is inadequately understood but it has been associated with diarrhea in young children under 12 months which is typically mild without blood in the feces (Moshtagian *et al.,* 2016).

Adherent-invasive *Escherichia coli* (AIEC) are commonly found in ileal lesions of Crohn's Disease (CD) patients, where they adhere to intestinal epithelial cells and invade into and survive in epithelial cells and macrophages, thereby gaining access to a typically restricted host niche. Colonization leads to strong inflammatory responses in the gut suggesting that AIEC could play a role in CD immunopathology (Nash *et al.,* 2010).

2.2. EHEC with emphasis on *E.coli* 0157:H7

Enterohaemorrhagic *Escherichia coli* (EHEC) is an emerging pathogen that has stimulated worldwide interest in several large food-borne outbreaks. The recognition of EHEC (enterohemorrhagic *Escherichia coli*) as an aetiological agent of diarrhea with life-threatening complications has made this kind of infection a public health problem of serious concern. Enterohaemorrhagic (EHEC) has been known to cause severe and bloody diarrhoea as well as haemorrhagic colitis (HC) and haemorrhagic uraemic syndrome (HUS) especially among children and older patients. The production of the verocytotoxins VT1 and VT2 are the main features of EHEC (Welinder-Olssoni and Kaijser, 2005).

Escherichia coli O157:H7, the major Shiga toxin producing *E. coli* serotype has been recognized as the primary cause of various human diseases, including hemorrhagic colitis, thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome (Kilonzo *et al.,* 2011). The first public health significance of *E. coli* O157.H7 traced back to 1982, after reporting two unfamiliar enteric outbreaks connected to contaminated undercooked hamburger patties in U.S. Pathogenic *E. coli* O157:H7 is frequently found in various environment including, food, livestock, soil and manure (Premarathne *et al.,* 2017).

Cattle serve as the primary and natural reservoir of *E. coli* O157:H7, although other animals such as goats and sheep may be carriers as well. In cattle, *E. coli* O157:H7 colonizes the large intestine and rectum (Hussein and Sakuma, 2005). Cattle are asymptomatically colonized by *E. coli* O157:H7, but in rare instances, young calves may experience initial diarrhea and subsequently become asymptomatic. This asymptomatic state and lack of complications in cattle can be best explained by the lack of a critical receptor on the surface of their vascular endothelial cells (Mainil and Daube, 2005; Moxley, 2004). Although cattle appear to be the main reservoir of *E. coli* O157:H7, infected persons, animals including sheep, goats, pigs and poultry also transmit the disease (Engdaw and Temesgen, 2016).

Zoonotic transmission of *E. coli* 0157:H7 occurs after consumption of undercooked meat or deficiently pasteurized dairy products or contact with contaminated fomites. Other causal factors

include exposure to contaminated water from potable drinking sources, swimming pools and lakes, contaminated food such as insufficiently cooked meats, inadequately washed leafy greens and fruits, unpasteurized drinks including apple juice, and direct contact with contaminated animals in petting farms (Money *et al.,* 2010). *E. coli* O157:H7 infection can arise through person-to-person transmission and may be transmitted through the surrounding environment as the microorganism is able to survive in the environment for an extended period of time. (Steinmuller *et al.,* 2006).

People from any age group can be affected by *E. coli* O157:H7 infection; while old people and young children can be more susceptible. Low infective dose as 10-100 cells, stress resistance mechanisms, and production of toxins contribute to the severity of the *E. coli* O157:H7 infection. In addition, efficient acid resistant methods facilitate survival and colonization of the organism under the acidic conditions in the gastrointestinal tract and food with low pH value. Therefore, *E. coli* O157:H7 is considered as a serious threat to consumers (Premarathne *et al.,* 2017).

2.3. Characteristics of *E. coli* O157:H7

2.3.1. General Characteristics

E. coli O157:H7 expresses somatic (O) antigen 157 and flagella (H) antigen 7. *E. coli* O157:H7 has unique features of delayed D-sorbitol fermentation (>24 h) and inability of producing β-glucuronidase, which can hydrolyze a synthetic molecule, 4-methylumbelliferyl-D-glucuronide (MUG). Thus, Sorbitol MacConkey (SMAC) agar supplemented with MUG should be used for the detection of *E. coli* O157:H7. *E.coli* O157:H7 can be further confirmed by a commercially available latex agglutination assay (Lim *et al.,* 2010).

The antigenic composition of outer cell membrane is the most important characteristic that leads to identification of this serotype. *E. coli* O157:H7 have a cell envelope structure typical of Gram-negative cells, and thus, possess an outer membrane with a lipopolysaccharide component that is distinct from the cytoplasmic membrane. The O157 antigen is defined by the carbohydrate

composition and structure within the lipopolysaccharide. The H7 antigen is determined by the unique polypeptide composition of the flagella (Beneduce *et al.,* 2003). *E. coli* O157: H7 is capable of growing over the pH range of 4.4-9.0 and the minimum water activity level for its growth is 0.95(Zelalem *et al.,* 2005).

2.3.2. Growth and survival in food

The optimum temperature for *E. coli* O157:H7 is 37 °C, the same as that of other *E.coli*. Other faecal coliforms are able to grow well at 44 °C while growth of *E. coli* O157:H7 may be slowed or inhibited. The resistance to heat of *E. coli* O157:H7 is not noteworthy and usual pasteurization temperatures are sufficient to kill more than 10^4 cells per ml. *Escherichia coli* O157:H7 seems to have no particular tolerance to salt and low aw levels. Bacterial growth has been documented with NaCl concentration ranging from 2.5 to 6.5%, when other growth affecting factors were favourable. Its acidic resistance plays a key role in food-borne illness linked to this pathogen (Beneduce *et al.,* 2003).

2.3.3. Environmental survival

E. coli O157:H7 can survive and persist in numerous environments such as soil, water and in animal reservoirs. *E. coli* O157:H7 has been shown to survive for a year in manure-treated soil and for 21 months in raw manure that had not been composted (Jiang *et al.,* 2002). *E. coli* O157:H7 can survive for a long time in water, especially at cold temperatures. Water trough sediments contaminated with bovine feces can serve as a long-term (>8 months) reservoir of *E. coli* O157:H7 and the surviving bacteria in contaminated troughs is a source of infection. *E. coli* O157:H7 also survives and replicates in Acanthamoeba polyphaga, a common environmental protozoan that is widely distributed in soil, water, and fecal slurry (Lim *et al.,* 2010).

2.4. Mechanisms of bacterial pathogenicity

The pathogenicity of *E.coli* 0157:H7 relies on its virulence factors. The major virulence factors associated with its pathogenicity are the Locus of Enterocyte Effacement (LEE) encoded factors, toxins, plasmid (p0157) and adhesins. Stx is the outstanding virulence factor for developing HUS. Another significant process for bacterial survival and colonization is adhesion. Adhesive factors may lead to bacterial colonization, proliferation and pathogenesis. The *E. coli* O157:H7 can adhere on intestinal mucosal tissues and secreting several proteins, enzymes and toxins (Svennerholm and Steele, 2004). Another factor that contributes to infect a new host is disruption of tight junctions, which is regulated by *E. coli* secreted protein F (UEspF) (Saeedi *et al.,* 2017).

2.4.1. Shiga – like toxin

In response to stress, Stx binds to its receptor, Gb3 on Paneth cells of the intestinal mucosa. Stx with N-glycosidase activity inactivates the eukaryotic ribosome by cleavage of a specific adenine base from 28S rRNA within the large (60S) ribosomal subunit, then stop protein synthesis and leading to cell death. Two different Shiga-like toxins involving Shiga-like toxin 1 and 2 are recognized in *E. coli* O157:H7 that both are produced via encoding genes in association with prophages. Shiga- like toxin 1 is very similar to the type 1 toxin of Shigella dysenteriae while Shiga- like toxin 2 is genetically and immunologically distinct with 55–60% similarity in genetic and amino acid sequences. Shiga-like toxins are responsible for bloody diarrheal symptom and hemolytic uremic syndrome (Fig.1) (Saeedi *et al.,* 2017). The toxins are produced by the pathogen in the colon and cause local damage. The ability to pass through the bloodstream to the kidney plays a role in causing HC and HUS (Adamu *et al.,* 2014). The classification of shiga-toxin producing *Escherichia coli* (STEC) found in animals is summarized in Table 1.

Table 1: Classification of shiga-toxin producing *Escherichia coli* (STEC) found in animals

Type	**STEC subsets: Common designation**	**Common serotypes/ Serogroups**	**Geographical Distribution**	**Animal Reservoir**	**Site of isolation in Animals and Derived products**
Zoonotic	O157 EHEC	O157:H7	Worldwide, more common common in industrialised countries	Cattle, sheep, goats, pigs[c]	Intestine, faeces,meat, milk, cheese
	Non-O157 EHEC	O26[b],O111[b], O103,O113, O145	World wide	Cattle, goats, pigs, chickens, sheep	Intestine, faeces,meat, milk, cheese
Potentially zoonotic(a)	None	O17,O56, O87, O108,O109,O130, O136, O149	World wide	Cattle, sheep, goats, pigs	Intestine, faeces, meat
Animal pathogenic	EDEC	O138, O139, O141	World wide	Pigs	Intestine

(a): not as yet associated with disease in animals or humans **(b):** strains of some serotypes also cause haemorrhagic enteritis in cattle. **c)** Probably an accidental host.

Source: (Gyles, 2007).

2.4.2. Adherence factors

There are different adhesive structures pertaining to bacterial cells of *E. coli* O157:H7 that have contribution in the process of attachment to surfaces such as mucosal and epithelial cells. The adhesive structures may be fimbrial or non-fimbrial, which would mediate the process of binding and colonization. Fimbriae and fimbrial adhesins are thread-like with long structures, found on the bacterial surface. The first adhesins identified in *E. coli* are type one fimbriae, which are the most usual adhesins produced. These adhesins are associated with attachment of bacteria to the mannose containing glycoproteins on the surface of eukaryotic cells (Law, 2000).

2.4.3. The Locus of Enterocyte Effacement

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. 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) (Caprioli *et al.,* 2005).

The pathogenicity island relating to the locus of enterocyte effacement gene is the virulence factor recognized in *E. coli* O157:H7 which can lead to adhesion upon intestinal epithelial cells and the appearance of intestinal lesions (Iyoda *et al.,* 2011).

2.4.4. Plasmid O157 (pO157)

E. coli O157:H7 contains a highly conserved plasmid, named pO157. The pO157 is a non conjugative F-like plasmid with a range size from 92 to 104 kb. The plasmid provide various host beneficial traits, such as resistance to antibiotics and heavy metals, production of toxins and other virulence factors, biotransformation of hydrocarbons and symbiotic nitrogen fixation(Frost *et al.,* 2005). All isolates of *E. coli* O157:H7 harbor the 60 MDa pO157 plasmid that contains the hly

operon encoding an enterohemolysin. This hemolysin, with the aid of specialized transport systems, may allow the bacterium to utilize the blood released into the intestine as a source of iron (Rahal *et al.,* 2012).

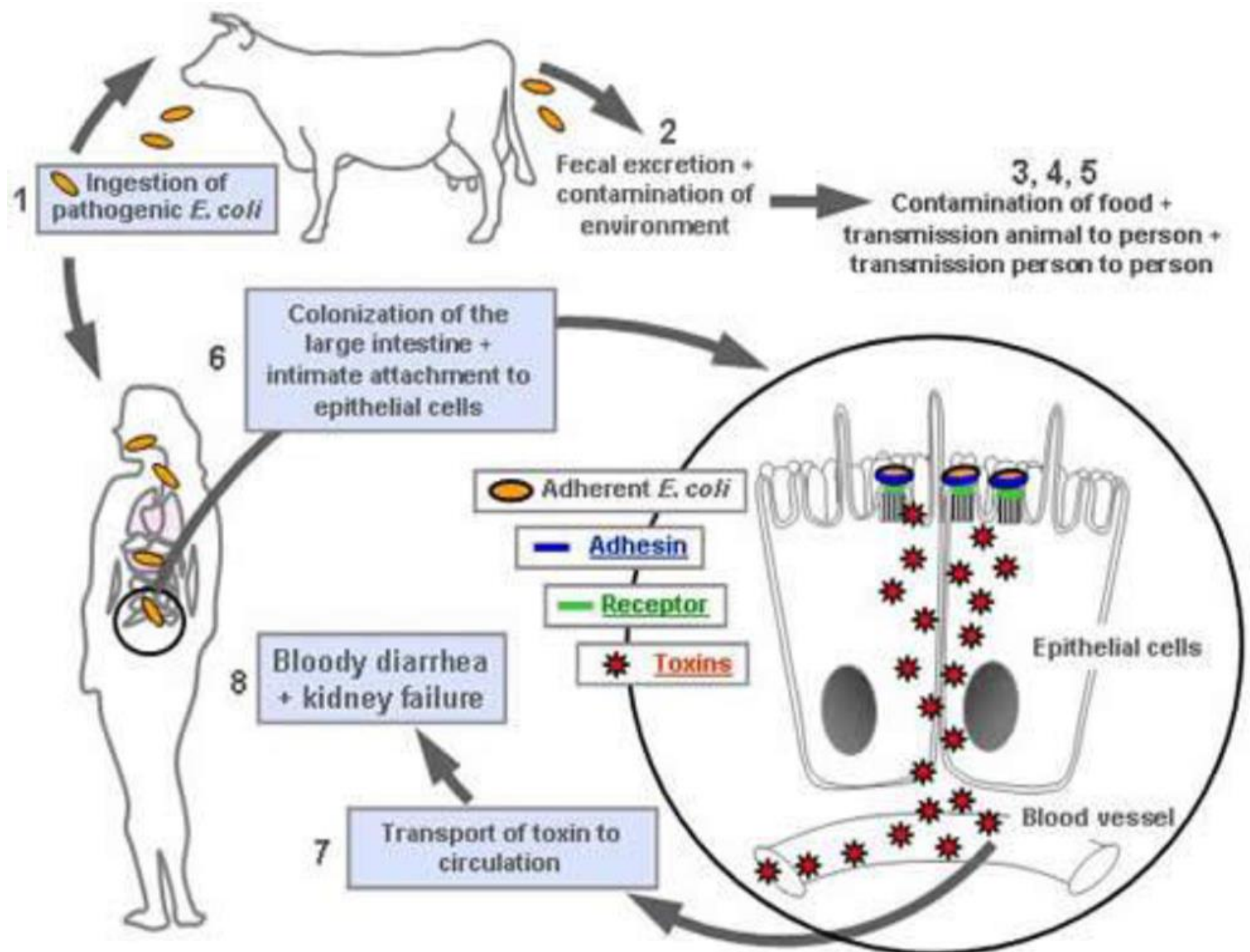

Figure 1: The mechanism by which shiga toxin-producing Escherichia coli (STEC) cause bloody diarrhoea and haemolytic uraemic syndrome in humans

Source: http://www.ecl-lab.ca/en/ecoli/pathogenesis.asp

2.5. Antimicrobial resistance

Food animals, in particular mature cattle, which may be asymptomatic carriers of *E. coli* O157, when exposed to antimicrobial agents in the animal production environment, may serve as a reservoir of antimicrobial-resistant bacteria. In a study a total of 361 *Escherichia coli* O157 isolates, recovered from humans, cattle, swine, and food during the years 1985 to 2000, were examined to better understand the prevalence of antimicrobial resistance and it was found that 27% of the isolates being resistant to tetracycline, 26% to sulfamethoxazole, 17% to cephalothin, and 13% resistant to ampicillin in which highest frequencies of resistance occurred among swine isolates (Schroeder *et al.,* 2002).

In another study by Disassa et al (2017) antimicrobial susceptibility of *E. coli* O157:H7 isolated from 380 raw milk was done and the antimicrobial susceptibility profile showed that *E.coli* O157:H7 were resistant to tetracycline (81.8%), streptomycin (81.8%), and kanamycin (63.6%). Multidrug resistant strains of *E. coli* from food, animal and humans are increasingly being encountered. The most frequently reported resistance phenotype of *E.coli* 0157:H7 isolates are found to resist streptomycin, sulfixoxazole and tetracycline (White *et al.,* 2002).

2.6. Epidemiology of *E.coli* 0157:H7

Understanding how the organism live and grow in nature or having a clue about its reservoirs and how human get contact with them is necessary in order to understand the epidemiology of the organism(Caprioli *et al.,* 2005).

2.6.1. Reservoirs of E. coli O157:H7

Ruminants have been identified as the major reservoir of *E. coli* O157:H7, with cattle as the most important source of human infections. Other ruminants known to harbor these bacteria include sheep, goats, and deer. STEC bacteria are occasionally isolated from other animals but it is

believed that the bacteria are present as transients and that the animals acquired these bacteria from meat, foods, or water contaminated by fecal material from ruminants (Caprioli *et al.,* 2005).

VTEC have been detected in calves, dairy cows and beef cattle worldwide. Prevalence of these bacteria in cattle and their excreta appears to vary seasonally as well as with the age of the animals and other factors but is generally <10%. Examination of naturally and experimentally infected calves and cattle demonstrated that most *E. coli* O157:H7 adhere to mucosal epithelium in a short 5 cm long region just proximal from the recto-anal junction. As a result, *E. coli* O157:H7 is present predominantly on the surface of the cow pats (Low *et al.,* 2005; Naylor *et al.,* 2003). The presence of *E. coli* O157:H7 in cattle illustrates the complex, interrelated nature of the environment, livestock production practices, food safety, and the science of microbiology, particularly microbial ecology (Fig. 2) (Rasmussen and Casey, 2001).

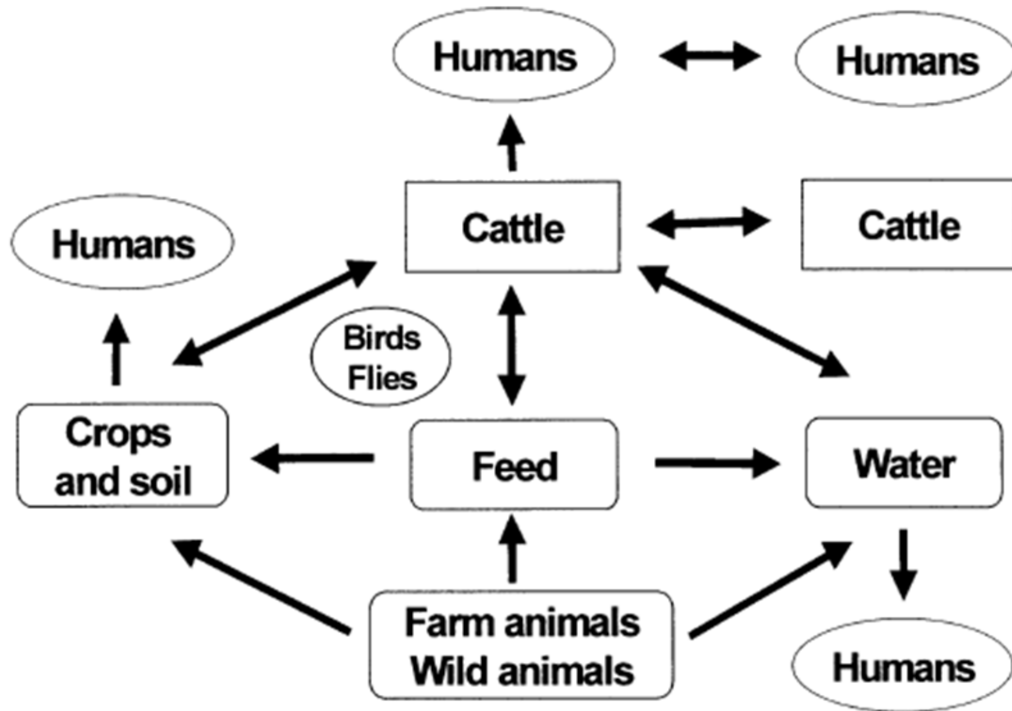

Figure 2: Transmission of *E. coli* O157:H7 in the environment giving rise to a cycle of infection that may enable maintenance of the organism in cattle herds

Source: (Bach *et al.,* 2002).

2.6.2. Transport hosts

Birds are thought to be possible transport hosts for *E. coli* O157:H7. Some wild birds harbor these bacteria, and pigeons might also spread these bacteria around a farm environment. Flies and beetles, including houseflies and filth flies of several species and dung beetles, collected on farms with animals shedding *E. coli* O157:H7, contain detectable levels of these bacteria. These insects frequent fecal deposits and may be able to transfer these bacteria to foods, feed and water. Fruit flies could contribute to widespread contamination of wounded apples that may be processed into cider. Slugs are known vegetarian pests that frequently traverse leafy vegetables and may be present on these foods when harvested (Pal and Ayele, 2017).

2.6.3. Occurrence in Animals

In studies on the prevalence of *E. coli* O157:H7 in dairy and beef cattle, researchers generally estimated that fewer than 10% of cattle carry this pathogen; many estimates were lower than 2%. More sensitive culture techniques have been developed to detect these bacteria, and several studies have reported increased prevalence in cattle during warmer months of the year (Gansheroff and O'Brien, 2000). Thus, estimates of the prevalence of O157:H7 in cattle that average isolation rates over warm and cold seasons or that only include samplings from cooler months dilute the impact of high shedding in the warmer months. For example, during a year-long study in England in 1997, the monthly prevalence of *E. coli* O157 in cattle was 4.8-36.8% and was at its highest in spring and late summer (Chapman *et al.,* 1997).

E. coli O157:H7 may also colonize poultry. According to a study by Dincoglu, and Gonulalan (2016) the presence of *E. coli* O157:H7 in chicken meat samples was determined. For this purpose, 155 chicken meat samples were analyzed and *E. coli* O157:H7 were found in 3 (1.94%) of the total samples examined. In another study, the prevalence of *E.coli* 0157:H7 was determined form meat and meat products and the prevalence was found to be 2.5% and 5% in raw chicken and ground chicken respectively (Hessain *et al.,* 2015).

2.6.4. Occurrence in human

E. coli O157:H7 was known to be a human pathogen since 1982. In 1993, the Council of State and Territorial Epidemiologists recommended that *E. coli* O157:H7 be a nationally reportable disease and that clinical laboratories screen at least all bloody stools for this pathogen (Kiranmayi *et al.,* 2010).

Experts estimate that 76 million cases of human diseases, 325,000 hospital admissions and 5,000 mortalities are caused annually in USA by consumption of contaminated food. Nearly 74,000 cases and 61 deaths annually are attributable to this pathogen in USA due to consumption of contaminated beef (Kiranmayi *et al.,* 2010). During July–October 2014, an outbreak of 119 cases

of *E. coli* O157:H7 infections associated with exposure to contaminated pork products occurred in Alberta, Canada (Honish *et al.,* 2017).

Butcher-associated outbreaks occur more commonly in UK than in any other country in which 30 outbreaks were recorded between 1995 and 2004. About 40% of outbreaks in Scotland between 1994 and 2003 were foodborne (accounting for 83% of cases), 54% were environmental, and 6% had both transmission routes (Pennington, 2010).

Number of human infections peaks during the summer months which is may be due to more frequent consumption of ground beef and frequent contact with domestic animals. In addition, houseflies and blow flies can carry relatively high concentrations of potentially virulent *Escherichia coli* during summer (Keen *et al.,* 2006).

2.6.5. Routes of Human infection

E. coli O157:H7 in ruminant feces may be directly ingested by persons in close contact with animals. Fecal contamination of meat may occur during slaughter, may enter lakes or drinking water sources by action of rain or wind, and may be deposited on fruits and vegetables inadvertently or by use of manure for fertilization. In addition, some animals may transport these bacteria from a fecal source to drinking water or foods (Pennington, 2000).

Direct contact

E. coli O157:H7 shed by infected animals may be spread to many surfaces in enclosures where ruminants are kept including the hides of other animals (Childs *et al.,* 2006). Depending on moisture and humidity, these bacteria may persist on gates, stiles and other farm surfaces for more than four weeks (Williams *et al.,* 2005). *E. coli* O157:H7 survives in cattle feces for up to 18 weeks at 15ºC. That’s why a number of people residing on dairy farms acquired the infection (Pal and Ayele, 2017).

Person-to-person spread of *E. coli* O157:H7 has been the primary mode of infection in many outbreaks especially in day care facilities particularly where there have been lapses in hygiene (Lim *et al.,* 2010). During July–October 2014 in Canada, Alberta, person-to-person spread was identified as the likely vehicle in which 4 (3%) of the patients acquired the infection through household contact with an outbreak-associated patient (Honish *et al.,* 2017).

Contaminated food

Beef, lamb, and mutton can be contaminated during slaughter and processing by exposure to feces or hides containing *E. coli* O157:H7 (Elder *et al.,* 2000). Milk from dairy cows, sheep, and goats may be contaminated with *E. coli* and other bacteria from the environment. Outbreaks of *E. coli* O157:H7 due to contaminated dairy products are usually associated with unpasteurized milk, but there have been some cases of post-pasteurization contamination. Manure is a valuable fertilizer for crops but manure containing *E. coli* O157:H7 may be a source of contamination for vegetables or fruits that are not normally cooked before eating (Fremaux *et al.,* 2007). Foods can also be contaminated with *E. coli* O157:H7 by cross-contamination during food preparation and by infected workers who don't practice good hygiene (Jackson *et al.,* 1998).

In most developing countries, continued use of untreated waste water and manure as fertilizers for the production of fruits and vegetables is a major contributing factor to contamination (Uzeh and Adepoju, 2013). Several outbreaks of gastroenteritis have been linked to the consumption of contaminated fresh vegetables and fruits and the use of manure has led to concern for the potential of contamination of minimally processed vegetables such as salad vegetables (Ingham *et al.,* 2004).

Contaminated water

Since 1985, waterborne transmission of EHEC strains has been identified and certain Microbiological, epidemiological, and environmental studies have associated different uses of water with human outbreaks including recreational, drinking, irrigation, and wastewater. The source of contamination of recreational water could related to the bathers themselves, but also to

sewage discharges and wild or domestic animals. The use of contaminated irrigation water in agriculture has also been reported as transmission routes for *E. coli* O157:H7 to the vegetables grown in these fields. Other studies showed that *E. coli* O157 is commonly present in animal and human wastewaters (Muniesa *et al.,* 2006).

Drinking water is also a source of large outbreaks. In an outbreak which was occurred in Cabool, Missouri (USA), 243 people were affected and the main source of the outbreak was city- supplied water which was contaminated by deer faeces (Acha and Szyfress, 2001).

2.7. Public health significance of *E.coli* 0157:H7

2.7.1. High risk groups

The highest risk of infection occurs among children, older and immunocompromised patients. In one study stool specimens were obtained from diarrheic patients and according to the result of the study, the highest rate of isolation of *E.coli* 0157:H7 was from fecal specimens from children and elderly patients (Musa *et al.,* 2010).

2.7.2. Transmission of Disease

Transmission usually occurs through consumption of undercooked or contaminated foods of bovine origin, faecal contamination of other food products or direct contact with infected animals. Cattle and sheep are usually recognized as the principal reservoirs responsible for the proliferation of *E. coli* O157:H7. In addition, infection could occur via faecal-oral route, so the numbers shed in faeces and susceptibility of the host could possibly determine transmission of the organism (Kiranmayi *et al.,* 2010). Many foods and dairy products have acted as vectors for the transmission of *E.coli* 0157:H7 (Fig. 3). Among those ground beef hamburgers; steak tenderised by injection; steak tartare; kebabs; ready-to-eat cold meats including poultry, pork, and beef products; salami and other fermented meat products; venison jerky; cheese; milk; butter; yoghurt;

ice cream; apple juice; grapes; coleslaw; lettuce; spinach; radishes; alfalfa sprouts; and melons could be mentioned(Pennington, 2010).

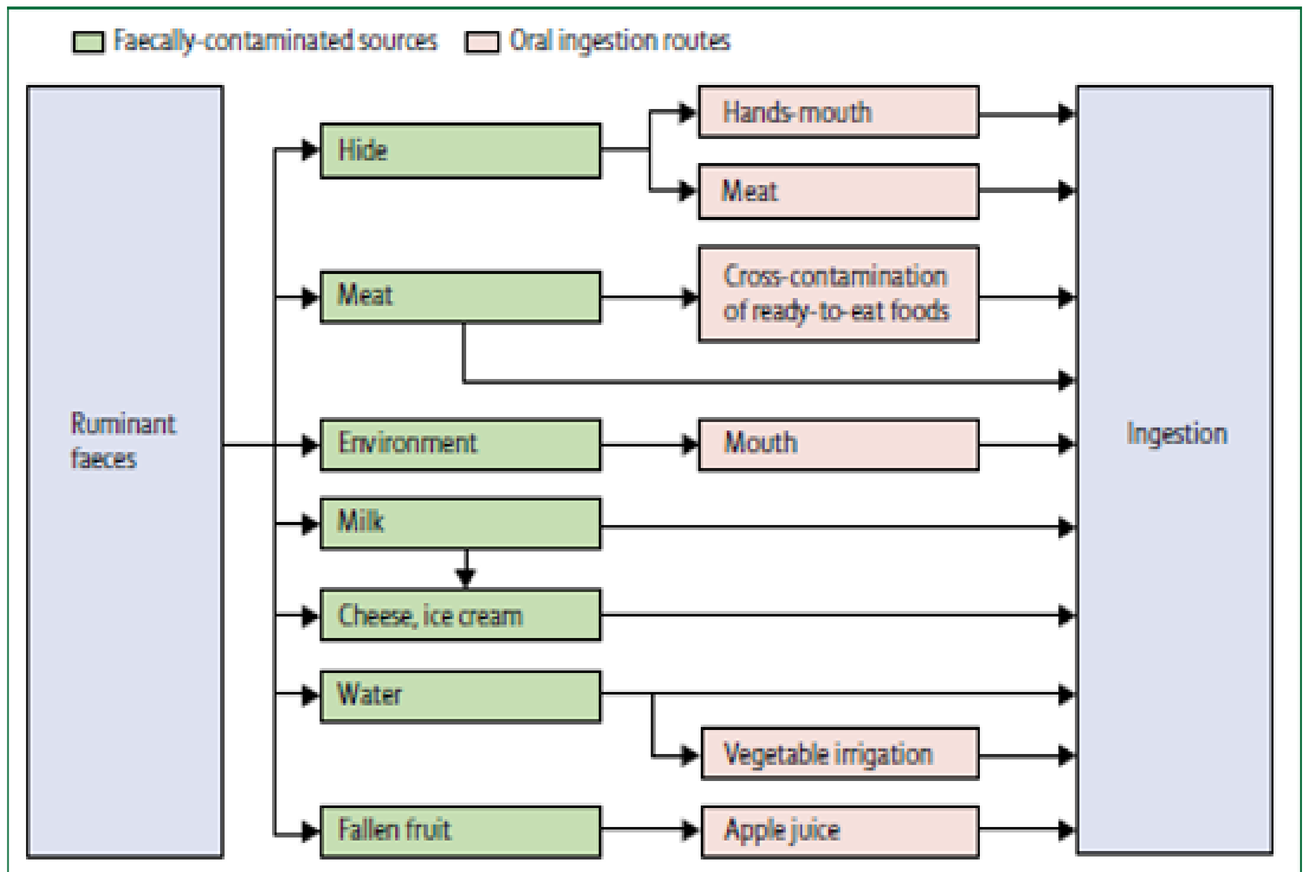

Figure 3: Routes of transmission of *E.coli* O157:H7

Source: (Pennington, 2010)

2.7.3. Clinical presentation in humans

The incubation period of *E. coil* infection lasts form 2-9days (average 3-4 days). *E.coli* 0157:H7 represents a number of signs in humans, but it does not produce a clinical disease in cattle and other animals, except diarrhea, so these animals act as carriers for *E.coli* 0157:H7. In human a wide spectrum of illness ranging from mild diarrhea to hemorrhagic colitis, haemolytic uraemic syndrome and Thrombocytic Thrombocytopaenic Purpura can be caused by the O157:H7

serotype of Shiga toxin producing *E.coli* (STEC), especially among children, the elderly and others with under developed immunity (Engdaw and Temesgen, 2016).

Most cases initiate with non-bloody diarrhea and self-resolve without further complication. However, some patients progress to bloody diarrhea or HC in 1–3 days. In 5–10% of HC patients, the disease can progress to the life threatening sequelae, HUS or Thrombocytic Thrombocytopenic purpura (TTP) (Banatvala *et al.,* 2001).

Haemorrhagic Colitis (HC) is characterized by severe abdominal cramps accompanied by a non bloody diarrhea. In most patients the watery diarrhea becomes grossly bloody after two or three days. Fever may be totally absent or may be of the low-grade type and its presence is more common in patients with severe illness (Rahal *et al.,* 2012).

Hemolytic uremic syndrome (HUS) is characterized by acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia. HUS typically develops after a prodromal diarrheal illness, but may occur without diarrhea and is the major cause of acute renal failure in children (Banatvala *et al.,* 2001). Thrombocytic Thrombocytopaenic Purpura (TTP) is characterized by thrombocytopenia, hemolytic anemia, azotemia, fever, thrombosis in the terminal arterioles and capillaries and neurological symptoms that dominate clinical picture. Blood clot in the brain may occur, frequently resulting in death (Acha and Szyfress, 2001).

2.8. Diagnosis of *E.coli* O157:H7

Culture isolation of *E. coli* O157:H7 is gold standard for identification. For reducing the analysis time, rapid immunological detection systems have been employed based on antigen antibody interaction for the detection of bacterial cells, toxins and spores. PCR is a rapid and reliable tool for the molecular-based diagnosis of the pathogen (Saeedi *et al.,* 2017).

2.8.1. Culture and isolation techniques

The three most important phenotypic differences between *E. coli* O157:H7 and other *E. coli* are inability to ferment sorbitol, lack of β–glucuronidase enzyme and weak or no growth at temperatures above 44 °C (Meng *et al.,* 1997). The culture isolation techniques used to verify the suspect presence of *E. coli* O157:H7 in food are based upon these characteristics, and can help to distinguish between non-pathogenic *E. coli* and pathogenic *E. coli* O157:H7 strains (Beneduce *et al.,* 2003).

The best selective media for presumptive identification of *E. coli* O157:H7 was found to be Sorbitol Mac- Conkey Agar (SMAC). Detection is carried out by the use of this selective media, which consists of bile salts, a carbohydrate source, sorbitol and an indicator. Under normal laboratory conditions, *E.coli* O157:H7 does not ferment sorbitol. If *E.coli* O157:H7 is present, colourless colonies will appear while other Enterobacteriaceae will show up as pink colonies. In addition several biochemical and other tests (the IMViC tests) can be used for further confirmation (Deisingh and Thompson, 2004).

2.8.2. Latex Agglutination Test

Sorbitol negative colonies selected from S-MAC will be tested with *E. coli* 0157 antiserum or latex reagents (O157 antibody-coated latex and control latex) for the isolation and identification of *E. coil* O157:H7 (Zelalem *et al.,* 2005). However, this method requires precaution to control false-positive identifications of the pathogen (Jay, 2000).

2.8.3. Immunological methods

These methods rely on the use of monoclonal or polyclonal anti-O157 antibodies to capture O157 cells or anti-Stxs antibodies. The methods are based on the detection of Stx in samples where the number of Stx-producing strains is expected to be high. Their application in the environment, where the numbers of Stx bacteria are usually low, may be limited and needs to be evaluated (Muniesa *et al.,* 2006).

Enzyme linked Immunosorbent assay (ELISA)

ELISA is a rapid and reliable test which significantly reduces the time required to screen meats for the presence of *E. coli* O157. Primary enrichment cultures grown for 8-16 hours can be tested in less than one hour, allowing ELISA-negative product to be released within 24 hours. Enrichment broths presumptively positive for *E. coli* 0157on the basis of positive ELISA tests can be cultured further for confirmation by standard methods (Wu *et al.,* 2004).

A sandwich ELISA could be capable of detecting all known subtypes of Stx1 and Stx2 by incorporating a pool of newly developed antibodies with high affinity. This type of assay is highly sensitive with a limit of detection for the different subtypes of Stx1a and Stx2a between 10 and 50 pg/mL in phosphate buffered saline (He *et al.,* 2016).

Immunomagnetic separation (IMS)

Immunomagnetic separation is important in enhancing the sensitivity of detection of *E. coli* O157:H7 in food. This method consists of the use of magnetic beads coated with anti-O157 antibodies to capture O157 cells which can then be separated and concentrated with a magnet. Concentrated antigen is then assayed by other method (Muniesa *et al.,* 2006).

2.8.4 .Molecular Methods

Provide extremely sensitive techniques that are able to detect and quantify pathogenic bacteria with a sensitivity and specificity not achievable by culture techniques and biochemical tests. Genotypic identification of bacteria avoids all inconvenience of phenotypic assays, like variation in enzymatic activity when bacteria are cultured in different media, emergence of biochemical mutants and presence of strain of different species that are very closely related and possess the same phenotype but different genotype (Beneduce *et al.,* 2003).

Polymerase chain reaction (PCR)

The suitability, time saving and relatively low cost of PCR techniques makes possible to develop sensible and specific assay to detect *E.coli* 0157:H7 and other STEC, amenable to the requirements of most health surveillance and food control laboratories (Beneduce *et al.,* 2003).

In a study, Real- time PCR was used to confirm diagnosis of E. coli O157:H7 by using two genes (rfbO157 and flic H7) and the results of PCR assay coincide with those of bacterial culturing and latex agglutination test, which indicated that PCR assay was more sensitive and trusted method for detecting of this organism(Hasan *et al.,* 2018).

2.9. Treatment

Treatment of infection with EHEC strains, including *E. coli* O157:H7 is mainly based on supportive therapy, particularly rehydration. The use of anti-motility agents, which inhibit peristalsis and delay clearance of the organism, poses a risk factor for progression to HUS (Rahal *et al.,* 2012).

The usefulness of antimicrobial therapy for STEC infections is not recommended, but it is still a debatable issue. Because antimicrobials may lyse bacterial cell walls, thereby liberating Shiga toxins, and/or cause increased expression of Shiga toxin genes in vivo, they are not recommended for treating STEC O157 infections. As a result, therapy is primarily based on fluid and electrolyte replacement. However, recent studies suggest that some antimicrobials, if administered early in the course of infection, may prevent disease progression to HUS (Schroeder *et al.,* 2002).

In light of the difficulties in treating this agent, alternate treatment approaches were investigated. One study illustrates the use of natural products for the treatment and prevention of *E. coli* O157:H7. The study was performed on infant rabbits and it reveals that the administration of Lactobacillus casei, commonly known for its probiotic efficiency, had a protective effect against the toxins of *E. coli* O157:H7 (Ogawa *et al.,* 2001).

2.10. Control and Prevention

Prevention and control measures for *E. coli* O157:H7 infection should be applied at all stages of the food chain, from farm to fork and should include all the necessary hygienic measures. Since the infection primarily occurs via faecal-oral route, the preventive measures include food hygiene measures like proper cooking of meat, consumption of pasteurized milk, washing fruits and vegetables especially those to be eaten raw and drinking treated water and personnel hygiene measures like washing hands after toilet visits (Kiranmayi *et al.,* 2010).

Control of *E. coli* O157:H7 in cattle is possible through vaccination. The goal of vaccination is either to reduce the susceptibility of cattle to colonization by *E. coli* O157:H7, or to decrease the duration of such colonization. Researchers believe that *E. coli* O157:H7 adheres to the wall of the large intestine by secreting virulence factors directly into host cells. A vaccine that induces production of antibodies against these virulence factors could prevent adherence of the organism and result in its elimination from the gastrointestinal tract (Bach *et al.,* 2002).

In preliminary studies, reported that vaccinating cattle with two recombinant antigens, Tir and EspA, reduced fecal shedding of *E coli* O157:H7 compared with unvaccinated control animals (Potter and Finlay, 2000 by Bach *et al.,* 2002). Li et al. (2002) suggested that Tir would make a promising vaccine candidate due to its highly immunogenic response in human patients and its essential role in adherence in both humans and cattle.

2.11. Status of *E.coli O157:H7* in Ethiopia

In Ethiopia, there are studies conducted by researchers to determine the occurrence and proportion of *E. coli* O157:H7 in cattle, beef and human in different areas of the country. A study was performed with the aim of determining the prevalence of *E. coli* O157: H7 form beef and an overall prevalence of 13.3% (17/28) was found (Bekele *et al.,* 2014).

In a study, the prevalence of *E.coli* O157:H7 was estimated from cattle faeces and *E.coli* O157:H7 was found in 7% of the samples examined (Jufare, 2018). A study was conducted by Tassew (2015) at Debrezeit Elfora export abattoir and Addis Ababa abattoir enterprise in order to isolate *E.coli* O157:H7 from cattle slaughterd at the abattoirs. Based on the study, 251 cattle carcass swab samples were collected and processed. According to the result, 9 (3.6 %) of the samples were found to be positive for *E. coli* O157:H7.

Studies on human patients were also conducted at different regions of the country in order to assess the public health importance of the pathogen (Kahsay *et al.,* 2008, Adugna *et al.,* 2015, Abdissa *et al.,* 2017, Jufare, 2018). Detailed information on the prevalence of *E.coli* 0157:H7 form distinct sample types at different parts of Ethiopia are summarized in (Table. 2).

Table 2: Summary on the prevalence of *E.coli* O157:H7 in Ethiopia

Sample source		Author and Year	Site	Prevalence rate
	Cattle	Atnafie *et al.,* 2017	Hawassa	4.7%
Animal	Catle	Jufare, 2018	Bishoftu	7%
	Cattle	Abdissa *et al.,* 2017	A.A & Debre Berhan	1.89%
	Cattle	Haile *et al.,* 2017	Jimma	7.3%
	Beef	Abdissa *et al.,* 2017	A.A & Debre Berhan	0.54%
	Beef	Tassew, 2015	Bishoftu, A.A	3.6%
Food source	Beef	Atnafie *et al.,* 2017	Hawassa	2.7%, 2%
	Beef	Mengistu *et al.,* 2017	Eastern Ethiopia	2.06%
	Beef	Bekele *et al.,* 2014	Addis Ababa	13.3%
	Beef	Beyi *et al.,* 2017	Central Ethiopia	4.5%
	Beef	Bedasa *et al.,* 2018	Bishoftu	3.07%
Human		Kahsay *et al.,* 2008; by Engdaw and Temesgen, 2016	Gondar	0.0%
	Stool	Adugna *et al.,* 2015	Bahirdar	28.9%
		Abdissa *et al.,* 2017	A.A & Debre Berhan	0.0%
		Jufare, 2018	Bishoftu	2.8%

3. MATERIAL AND METHODS

3.1. Study area

This study was conducted in Sebeta town which is located in special zone of Oromia regional state in central highlands of Ethiopia at 24 km west of Addis Ababa on the main road to Jimma. The area receives an average annual rainfall of around 1100 mm, more than 85% of which falls in the main rainy season (June to September). The altitude of the area ranges from 2200–2600 meter above sea level, and the average annual temperature ranges from 6–21°C.(MoARD, 2010).

There is only one municipal abattoir in Sebeta town. The abattoir is located 7 km away from the city center. Only male cattle are slaughtered and the main source of the cattle to be slaughtered is from Jimma, Sebeta, Teji and Butajira towns. On average, 20-30 cattle can be slaughtered per day mainly on Wednesday and Friday. The abattoir provides slaughtering service to 27 meat retail shops in Sebeta town.

Sebeta health center is a large health center found a little bit far away from the city center. The center has a laboratory with different diagnostic kits. In addition to other health problems, many people with diarrhea case visited the center each day.

3.2. Study Samples

The study comprised of apparently healthy cattle brought from different areas of the country and slaughtered at Sebeta municipal abattoir and 27 meat retail shops in Sebeta town. Male cattle including all age groups were included in the study. The study also comprised of diarrheic patients admitted at Sebeta health center in Sebeta. Diarrhea was defined as the passage of three or more loose or liquid stools in a 24 hour period or more frequent passage than is normal for the individual (WHO, 2017). Human patients of both sex and age of 15 years and above with recent history of raw meat consumption and those without history of consumption were included in the

study. Age definition for human patients was done based on a survey made on diarrheic patients visiting the health center.

3.3. Study design and sample size determination

A cross-sectional study was conducted to estimate the prevalence of *E. coli* O157: H7 in cattle, beef and human and to determine the antimicrobial resistance profile of the isolates. A systematic random sampling method was used to sample cattle from the abattoir. For the study in human, purposive sampling method was used in which diarrheic patients those ordered for stool diagnosis at Sebeta health center were sampled. Twenty-seven beef meat retail shops in Sebeta town obtaining meat from the Municipal abattoir were included in the study based on their permission for sample taking. Rectal faecal sample was taken from systematically selected cattle at the abattoir and then the animal was followed through the slaughtering line to examine its carcass for possible contamination during the slaughtering process. Patients at the age of 15 and above were included in the study. The patients were included in the study after obtaining informed consent from adult participants (age > 18 years) or their guardians for participants below the age of 18 years. A questionnaire was provided for the patients or their care takers to confirm if they had the habit of consuming raw meat and the incubation period was taken under consideration. In addition, patients with no history of raw meat consumption was also included in the study for comparing the significance with that of other sources. Sample size was determined by using the formula given by Thrusfield (2005).

$$n=\frac{Z^2\ Pexp\ (1-Pexp)}{d^2}$$

Where, n= required sample size

Z= statistic for level of confidence = 1.96

d=desired absolute precision of 0.05

Pexp= expected prevalence.

An expected prevalence of 4.7% which was found from cattle carcass (beef) at Addis Ababa was used for the study (Bekele *et al.,* 2014). Based on this prevalence the calculated required sample size was 69, but for the purpose of increasing precision additional samples were collected. 120 carcass swab samples and 120 faecal samples were collected, so a total of 240 abattoir samples were collected during the study period. For the study on human, an expected prevalence of 2.9% (calculated prevalence for the age 15 and above) was taken from a study by Jufare (2018) from Bishoftu. The calculated sample size was then became 43, but for the sake of increasing precision, a total of 125 diarrhea samples were collected. The whole 27 meat retail shops were included and a total of 392 samples were collected during the study period.

3.4. Sample collection and transportation

3.4.1. Faeces and carcass swab sampling

Fecal and carcass swab samples were collected from cattle population slaughtered at Sebeta Municipal abattoir using systematic random sampling technique. The technique was based on dividing the total number of cattle to be slaughtered to the desired sample size for calculating the interval and selecting the animals based on the calculated interval. Faecal samples were collected directly from the rectum using rectal glove, kept in a sterile universal bottle and were labeled. Carcass swab samples were collected based on the method described in ISO17604 (2005) by placing sterile template (10 x 10 cm) on specific sites of a carcass. Three different sites including the abdomen (flank), thorax (lateral) and breast (lateral); which are considered to be with a high rate of contamination were chosen for sampling. Sterile cotton and forceps was used for carcass swab sampling. Cotton was held with a forceps, soaked in an approximately 10 ml of buffered peptone water and was rubbed horizontally and then vertically several times on the preferred carcass sites. On completion of the rubbing process, the cotton was placed in the test tube with 10 ml of buffered peptone water and labeled. Finally, both the fecal and carcass swab samples were transported using ice box in cold chain to National Animal Health Diagnostic and Investigation Center (NAHDIC), Bacteriology laboratory, for microbiological analysis.

3.4.2. Raw meat sampling from retail shops

Twenty five gram of whole cuts of raw meat (beef) samples were collected from retail shops following aseptic techniques. The samples were put in a sterile universal bottle and were transported using ice box in cold chain to National Animal Health Diagnostic and Investigation Center (NAHDIC), Bacteriology laboratory, for microbiological analysis.

3.4.3. Stool sampling from diarrheic patients

Stool samples from diarrheic patients were collected using sterile universal bottles by experienced laboratory technicians and were transported using ice box in cold chain to Addis Ababa University, College of Veterinary Medicine and Agriculture, for microbiological analysis.

Inclusion criteria

Diarrheic Patients aged 15 and above, with or without history of raw meat consumption and who were willing to take part in the study were included.

Exclusion criteria

Patients under 15 years and those who refused for sample taking were excluded from the study.

3.5. Sample preparation

Sample preparation and enrichment was conducted according to the general guidelines of (ISO 16654: (2001) and OIE (2018). .Approximately 1g of faeces/stool sample was suspended into 9 ml of Tryptone soya broth, vortexed and incubated for overnight at 37°C. The carcass bacterial swabs were also suspended in to tryptone soya broth and incubated overnight at 37 ^{0}C. 25 g of raw whole cuts of meat sample collected from each meat retail shops were taken out from the universal bottle, weighed for accuracy and chopped aseptically. The chopped sample was then

placed in a universal bottle with 225 ml of buffered peptone water. Finally, the mixture was homogenized using a homogenizer for 2 minutes and the resulting suspension was incubated for 24 hour at 37^0c.

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

Each prepared sample was inoculated on MacConkey agar and incubated at 37^0c for 24 hours. Typical pink colonies on MacConkey agar (Lactose fermenters) were transferred to EMB (eosin methylene-blue agar). Colonies with Metallic sheen character on EMB which is the typical feature of *E.coli* were transferred to CT- SMAC (Sorbitol MacConkey with cefixime and tellurite) which is selective for *E.coli* 0157:H7, to check the presence of the bacteria. Pale colonies on CT-SMAC (non- sorbitol fermenters) which were suspected as *E.coli* 0157:H7 were transferred to Nutrient agar. Colonies from Nutrient agar were stained using gram stain to observe their staining and morphological characteristics. Then Biochemical (IMViC) tests were performed for gram negative isolates (pink rods) for the sake of further identification (Quinn *et al.,* 2004, Beneduce *et al.,* 2003).

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

This test was performed according to the Wellcolex *E. coli* O157:H7 latex test by adding 40µl of sterile saline in a circle on a reaction card. Then a portion of colony to be tested was emulsified using a sterile single use plastic loop in a drop of sterile saline solution on the test circle. After ensuring a smooth suspension of the bacteria and saline, a drop of test Latex was placed on the emulsified test sample. The contents on the circle were then mixed carefully for spreading the latex over the entire area of the circle. Finally, the card was rocked in a circular motion for thirty seconds and examined for agglutination by naked eye. Agglutination of the test latex within thirty seconds was considered as positive result. Confirmation for H7 antigen was done after culturing O157 positive isolates for 24 hours at 37^oc in Tryptone soya broth (TSB). The procedure was the

same with that of the O157 antigen and was done by applying the H7 test latex on the TSB-grown culture.

3.8. Antimicrobial susceptibility test

The antimicrobial susceptibility test was performed using the Kirby-Bauer Disk Diffusion Susceptibility Test Protocol (Hudzicki, 2009). The antibiotic discs were selected based on their availability, potential public health importance and recommendation from the CLSI guideline (CLSI, 2017).

Four to five fresh isolated colonies of the test organism was taken using a sterile inoculating loop, suspended in a test tube with 2 ml of sterile normal saline and the saline tube was vortexed to create a smooth suspension. The turbidity of the suspension was adjusted at 0.5 McFarland standard by adding more organism when the suspension was too light or diluting with sterile saline when the suspension was too heavy in which McFarland Densitometer was used as a standard measurement for checking the turbidity. A sterile swab was dipped in to the inoculum tube and was rotated against the side of the tube to remove excess fluid. The dried and well prepared Muller-Hinton agar plates were inoculated by streaking the swab three times over the entire agar surface and were rotated approximately 60 degrees each time to ensure an even distribution of the inoculum. At last the plates were rimmed with the swab in order to pick up any excess fluid and allowed to dry for 5 minutes. After the plates were dried, the antimicrobial disks were placed on the surface of the agar using sterile forceps and the disks were gently pressed with the forceps to ensure complete contact with the agar surface. Finally, once all disks were in place, the plates were incubated at 37^0C for 18 hours. After 18 hours of incubation, the diameter of inhibition zone formed around each disk was measured using digital caliper and results were classified as sensitive, intermediately resistant, and resistant based on the standardized table supplied by the manufacturer (CLSI, 2017). A standard reference strain of *E. coli* (ATCC 25922), was used as a positive control (Hudzicki, 2009).

Table 3: Antibiotic disks used along with their respective concentration and zone diameter break points in millimeter.

Antimicrobial agent	Symbol	Disk content	Interpretive Categories and Zone Diameter Breakpoints(nearest whole mm)		
			S	**I**	**R**
Ciprofloxacin	CIP	5µg	≥ 21	16-20	≤ 15
Chloramphenicol	C	30 µg	≥ 18	13-17	≤ 12
Cefotaxime	CTX	30 µg	≥ 26	23- 25	≤ 22
Meropenem	MEM	10 µg	≥ 23	20- 22	≤ 19
Sulfamethaxazole + Trimetoprim	SXT	25 µg	≥ 16	11- 15	≤ 10
Nalidixic acid	NA	30 µg	≥ 19	14- 18	≤ 13
Amoxicillin-clavulanate	AMC	30 µg	≥ 18	14- 17	≤ 13
Tetracycline	TE	30 µg	≥ 15	12- 14	≤ 11
Ampicillin	AMP	10 µg	≥ 17	14- 16	≤ 13
Ceftraxone	CRO	30 µg	≥ 23	20-22	≤ 19
Streptomycin	S	10 µg	≥ 15	12-14	≤ 11
Gentamicin	CN	10 µg	≥ 15	13- 14	≤ 12

Key: S= Susceptible, I= Intermediate, R= Resistant

Source: CLSI (2017)

3.9. Questionnaire Survey

A questionnaire survey was used to assess the potential risk factors associated with carcass contamination both at abattoir and meat retail shops. Questions concerning the current status of slaughtering and personal hygiene practiced in the abattoir were provided. Hygiene and sanitation was determined by the use of structured questionnaire and through indirect observation. The target population constituted all abattoir workers involved in slaughter process. Questions concerning hygienic handling of meat and sanitation at meat retail shops were provided and the general hygienic and sanitary conditions were assessed through informal observation. A questionnaire was also provided for diarrheic patients to identify the major risk factors associated with the disease.

3.10. Ethical Clearance

Ethical clearance was obtained from animal research ethical review committee of Addis Ababa University College of Veterinary Medicine (Certificate Ref. No: VM/ERC/12/02/12/2020, Date: 28/01/2020) and Ethical clearance for human subjects was obtained from Oromia Health Bureau (BEFO/HBTFH/1-16/313, Date: 07/02/2012).

3.11. Data Management and Analysis

Data obtained was arranged, coded and entered to Excel spread sheet (Microsoft® office excel 2010). The data was then exported to R-Statistical software (version R-3.5.1) for appropriate statistical analysis. Chi-square test was done to study the association between sample sources on the prevalence of *E.coli* O157:H7 and logistic regression was used to study the association between the prevalence of *E.coli* O157:H7 with considered risk factors in which the magnitude of the risk factors for the occurrence of the pathogen was compared using an odds ratio. Descriptive statistics was used to determine the proportion of *E.coli* O157:H7 isolates in which number positives were divided by the total number of samples and multiplied by 100. Descriptive

statistics such as frequencies were used to present the findings of the questionnaire. A p-value ($p < 0.05$) was used as indicative of a statistical significant difference.

4. RESULTS

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

A total of 240, 27 and 125 samples were collected from abattoir, meat retail shops and health center at Sebeta respectively. Samples were examined for the presence of *E.coli* O157:H7 and from those examined, the pathogen was detected in 4(3.3%) of faeces, 3(2.5%) of carcass swab sample, 2(7.4%) of meat sample and 4(3.2%) of stool sample. Result showing the prevalence of *E.coli* O157:H7 from different animal source samples is indicated in (Table 4).

The test statistics showed that there was no statistically significance difference (P>0.05) in the prevalence of *E.coli* O157:H7 among animal source samples that were examined.

Table 4: Prevalence of *E.coli* O157:H7 from different animal source samples.

Sample source and type	No examined	No positive (%)	95% CI	$\chi 2$	p-value
Abattoir					
Faeces	120	4(3.3%)	0.01-0.08		
Carcass swab	120	3(2.5%)	0.02-0.23	1.63	0.44
Retail shop					
Beef	27	2(7.4%)	0.01-0.07		
Total	**267**	**9(3.4%)**	0.02-0.06		

4.2. Prevalence of *E.coli* O157:H7 associated with different risk factors

4.2.1. Prevalence at abattoir

Prevalence of *E.coli* O157:H7 was assessed from cattle faeces and carcass swab along with the associated risk factors (Table 5 and 6). There was no statistically significant difference (p>0.05) in the prevalence of *E. coli* O157:H7 between all the risk factors that were considered.

Table 5: Prevalence of *E.coli* O157:H7 form cattle faeces

Variable	No of animal examined	No positive (%)	OR(95% CI)	p- value
Age				
Young	44	1(0.83)	0.57(0.03-4.58)	0.627
Adult	76	3(2.5%)		
Breed				
Local	82	3(2.5%)	1.41(0.17-28.9)	0.771
Cross	38	1(0.83)		
Origin				
Butajira	20	0(0)		
Jimma	24	2(1.7%)	1.09(0.98-1.21)	0.130
Sebeta	52	1(0.83%)	1.02(0.93-1.12)	0.686
Teji	24	1(0.83%)	1.04(0.94-1.16)	0.447

Table 6: Prevalence of *E.coli* O157:H7 form carcass swab

Variable	No of animal examined	No positive (%)	OR(95% CI)	p- value
Age				
Young	44	2(1.7%)	3.57(0.33-78.2)	0.305
Adult	76	1(0.83%)		
Breed				
Local	82	3(2.5%)	1.04(0.98-1.1)	0.236
Cross	38	0(0)		
Origin				
Butajira	20	1(0.83%)		
Jimma	24	1(0.83%)	0.99(0.9-1.09)	0.862
Sebeta	52	1(0.83%)	0.97(0.89-1.05)	0.460
Teji	24	0(0)	0.95(0.87-1.04)	0.298

4.2.2. Prevalence at retail shops

Prevalence of *E.coli* O157:H7 was assessed at different retail shops considering factors such as hygienic practice of butchers and meat retail shops sanitation as risk factors for the occurrence of the bacteria (Table 7). There was no statistically significant difference ($p>0.05$) in the prevalence of *E. coli* O157:H7 between the considered risk factors.

Table 7: Prevalence of *E.coli* O157:H7 at meat retail shops associated with different risk factors

Risk factors	No examined	Prevalence (%)	OR(95% CI)	p-value
Hand wash with soap				
Yes	13	1(3.7%)	1.0(0.82-1.23)	0.959
No	14	1(3.7%)		
Dirty wall and floor				
Yes	15	2(7.4%)	1.14(0.94-1.39)	0.203
No	12	0(0)		
Dirty cutting board				
Yes	10	2(7.4%)	1.22(1.0-1.49)	0.058
No	17	0(0)		
Dirty gown and head cover				
Yes	11	1(3.7%)	1.03(0.84-1.27)	0.792
No	16	1(3.7%)		
Have refrigerator				
Yes	19	1(3.7%)	0.93(0.74-1.16)	0.531
No	8	1(3.7%)		
Meat wrapping material				
News paper	15	2(7.4%)		
Plastic	12	0(0)	0.88(0.72-1.07)	0.203
Collect money while working				
Yes	17	1(3.7%)	0.96(0.78-1.19)	0.707
No	10	1(3.7%)		
Means of cleaning				
Water & detergent	11	0(0)		
Water only	16	2(7.4%)	1.13(0.92-1.39)	0.239

4.2.3. Prevalence in Human

Public health significance of *E.coli* O157:H7 was assessed from diarrheic patients with different risk factors considered (Table 8). There was no statistically significant difference ($p>0.05$) in the prevalence of *E. coli* O157:H7 between all the risk factors considered.

Table 8: *E.coli* O157:H7 prevalence in human: With different risk factors considered

Variable	No examined	No positive(%)	OR (95% CI)	p- value
Age				
15-30	55	1(0.8%)		
31-45	40	2(1.6%)	1.03(0.96-1.1)	0.392
46-59	22	1(0.8%)	1.03(0.94-1.12)	0.545
≥ 60	8	0(0)	0.98(0.86-1.12)	0.788
Sex				
Male	75	3(2.4%)	1.02(0.96-1.09)	0.537
Female	50	1(0.8%)		
Food as a cause				
Raw beef	65	3(2.4%)	1.05(0.89-1.23)	0.577
Fruit juice	5	0(0)		
Raw tomato	19	0(0)	1.0(0.84-1.19)	1.0
Salad	36	1(0.8%)	1.03(0.87-1.21)	0.744
Direct contact with cattle faeces				
Yes	30	2(1.6%)	1.05(0.97-1.13)	0.219
No	95	2(1.6%)		

Direct contact with diarrheic patients				
Yes	4	0(0)	0.97(0.81-1.15)	0.714
No	121	4(3.2%)		
On set of diarrhea				
One day before	40	1(0.8%)		
Two days before	65	3(2.4%)	1.02(0.95-1.09)	0.554
Three days before	20	0(0)	0.98(0.89-1.07)	0.608
Consistency of diarrhea				
Mucoid	11	0(0)	1.0(0.85-1.18)	1.0
Watery	106	4(3.2%)	1.04(0.91-1.18)	0.563
Bloody	8	0(0)		

4.3. Antimicrobial susceptibility pattern

The resulting *E.coli* O157:H7 isolates from animal (n=9) and those from human (n=4) were subjected to 12 different antimicrobials. The test result revealed a varying degree of susceptibility profile. All isolates from animal and human showed 100% susceptibility to Ciprofloxacin (CIP), Meropenem (MEM), Sulfamethaxazole + Trimetoprim (SXT), Nalidixic acid (NA), Ceftriaxone (CRO) and Gentamicn (CN). Isolates from animal showed a decreased susceptibility pattern to antimicrobials such as Amoxicillin-Clavulanate (AMC) and Tetracycline (TE) whereas human isolates showed a decreased susceptibility pattern to Tetracycline (TE), Ampicillin (AMP) and Streptomycin(S). Animal isolates showed resistance to antimicrobials including Amoxicillin-Clavulanate (AMC), Tetracycline (TE), Ampicillin (AMP) and Streptomycin(S). Human isolates also showed resistance to these respective antimicrobials except streptomycin. Detail for the susceptibility and resistance pattern of animal and human isolates is illustrated in (Table 9) and (Table 10) respectively.

Table 9: Antimicrobial susceptibility pattern of *E. coli* O157:H7 isolates from animal (n=9) to12 selected antimicrobial agents:

Antimicrobial used	*E.coli* 0157:H7 isolates		
	S	I	R
Ciprofloxacin (CIP)	9(100%)	0(0)	0(0)
Chloramphenicol (C)	7(77.8%)	2(22.2%)	0(0)
Cefotaxime (CTX)	8(88.9%)	1(11.1%)	0(0)
Meropenem (MEM)	9(100%)	0(0)	0(0)
Sulfamethaxazole + Trimetoprim (SXT)	9(100%)	0(0)	0(0)
Nalidixic acid (NA)	9(100%)	0(0)	0(0)
Amoxicillin-clavulanate (AMC)	4(44.4%)	4(44.4)	1(11.1%)
Tetracycline (TE)	5 (55.6%)	1(11.1%)	3(33.3%)
Ampicillin (AMP)	8(88.9%)	0(0)	1(11.1%)
Ceftriaxone (CRO)	9(100%)	0(0)	0(0)
Streptomycin (S)	7(77.8%)	1(11.1%)	1(11.1%)
Gentamicin (CN)	9(100%)	0(0)	0(0)

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

Table 10: Antimicrobial susceptibility pattern of *E. coli* O157:H7 isolates from human (n=4) to12 selected antimicrobial agents:

Antimicrobial used	*E.coli* 0157:H7 isolates		
	S	I	R
Ciprofloxacin (CIP)	4(100%)	0(0)	0(0)
Chloramphenicol (C)	3(75%)	1(25%)	0(0)
Cefotaxime (CTX)	2(50%)	2(50%)	0(0)
Meropenem (MEM)	4(100%)	0(0)	0(0)
Sulfamethaxazole + Trimetoprim (SXT)	4(100%)	0(0)	0(0)
Nalidixic acid (NA)	4(100%)	0(0)	0(0)
Amoxicillin-clavulanate (AMC)	0(0)	2(50%)	2(50%)
Tetracycline (TE)	1(25%)	1(25%)	2(50%)
Ampicillin (AMP)	1(25%)	0(0)	3(75%)
Ceftraxone (CRO)	4(100%)	0(0)	0(0)
Streptomycin (S)	1(25%)	3(75%)	0(0)
Gentamicin (CN)	4(100%)	0(0)	0(0)

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

4.4. Questionnaire survey

4.4.1. Employees information at Sebeta Municipal abattoir

There were a total of 20 slaughter house workers in which all of them were male. From the total respondents 10 (50%), 6(30%) and 4(20%) had 4- 5 month, 1 & 1\2 year and 2 years of service duration at the abattoir respectively. 12(60%) of them have completed primary education and the rest 8(40%) of them were those that complete secondary education . 15 (75%) and 5 (25%) of the slaughter house workers were in the age group of 18-29 and 30-45 respectively.

4.4.2. Slaughter house workers awareness on food-borne disease, cattle carcass contamination and associated public health impact

Majority (60%) of respondents were aware about food borne disease whereas 8 (40%) of them did not have any awareness about the disease. 12 (60%) of them answered that contaminated food is the route for the transmission of the disease to people , 3(15%) and 5 (25%) of them believed that canned food and contaminated water could be the possible route of transmission respectively. 13 (65%) of respondents were aware about carcass contamination and 14(70%) of them believed that contamination could bring serious health problem to consumers. More information concerning slaughter house workers awareness on food borne disease, cattle carcass contamination and associated public health impact is summarized in (Table 11).

Table 11: Summary on slaughter house workers awareness about food-borne disease, cattle carcass contamination and associated public health impact.

Knowledge	Response	Frequency	P (%)
Awareness about food borne disease	Yes	12	60%
	No	8	40%
Possible ways that people could acquire food borne disease	Through contaminated food	12	60%
	Through canned food	3	15%
	Through contaminated water	5	25%
Awareness about carcass contamination	Yes	13	65%
	No	7	35%
Cause for cattle carcass contamination	Dirty working area	12	17%
	Dirty utensils	13	19%
	Workers dirty hands and clothing	13	19%
	Cattle hide during flaying	9	13%
	Faeces due to accidental GIT puncture	11	16%
	Dirty(contaminated water)	11	16%
Carcass contamination could bring serious health problem to consumers	Yes	14	70%
	No	6	30%

4.4.3. Response on Personal hygiene of slaughter house workers and general hygienic practice.

From the total respondents 13 (65%) of them always take caution to avoid carcass contamination. 18 (90%) of respondents wash their hands and the carcass after evisceration. All of (100%) of slaughter house workers wash their working clothes and boots regularly. Based on their response majority of them (90%) took work related trainings and most of them believed that it was helpful, but as they complained despite hygiene, trainings given were more inclined to technical aspects which mainly focuses on stunning, restraining and bleeding techniques. Trainings concerning slaughtering and personal hygiene as well as carcass contamination were not given more emphasis. Due to this there was hygiene gap among slaughter house workers. The entire slaughter house workers took medical test before being employed at the abattoir, but no one was taking regular medical checkups. Results concerning slaughter house workers personal hygiene and general hygienic practice are summarized in (Table 12).

Table 12: Summary on results concerning personal hygiene and general hygienic practice.

Activity	Response	Frequency	P (%)
Always take caution to avoid carcass contamination	Yes	13	65%
	No	7	35%
Wash hands and carcass after evisceration	Yes	18	90%
	No	2	10%
Regularly wash working clothes and boots	Yes	20	100%
	No	0	0%
Frequency of washing clothes and boots	Every day	0	0%
	Once per week	10	50%
	Twice a week	10	50%
Source of water for use in the abattoir	City supplied	20	100%
Took work related medical test to work at the abattoir	Yes	20	100%
	No	0	0%
Take regular medical check up	Yes	0	0%
	No	20	100%
Took work related training	Yes	18	90%
	No	2	10%

4.4.4. Observational assessment on the hygienic practice at the abattoir

As per the observation made almost all slaughter house workers did not have hand washing practice before touching the carcass. Their dressing materials such as knives as well as working clothes including overalls and boots were not clean all the time. There was no strict separation between carcass and GIT contents and since they use floor type carcass dressing method the rate of carcass contamination is higher. Other attributes indicating hygienic practice at the abattoir are summarized below in (Table 13).

Table 13: Summary on observational assessment concerning hygienic practice at abattoir

Observation	Remark	
Hand washing before touching the carcass	No	
All knives are completely clean and free from contamination	No	
Method of carcass dressing	Mixed(Hanging and on the floor)	
Carcass and GIT content kept separately	No	
Finger nails short and clean	No	
Use the following protective materials while working	Apron	No
	Head cover	Yes
	Glove	No
	Boots	Yes
	Over all	Yes
Eating while working	Yes	

Strict separation between clean and dirty areas	No
Clothes free from faecal contamination	No
Latrine available	Yes
Latrine has adequate water supply and soap for hand washing	No
Veterinary inspectors present to examine and pass carcass for human consumption	Yes
Use vehicle to deliver carcass to butcher house	Yes

4.4.5. Basic information on retail shops under study

Twenty seven retail shops that get slaughter service from the abattoir were included in the study. From respondents at butchery houses (all male), 31 (77.5%), 5 (12.5%) and 4 (10%) were at the age group of 18-30, 31-35 and 36-45 respectively. 24 (60%) and 10 (25%) of them had completed secondary and primary education respectively. 6 (15%) of them were illiterate.

4.4.6. Butchers/meat handlers status and general hygienic practice at retail shops

Based on hygienic assessment made at retail shops, after slaughter/delivery, 19 (70%) of the retail shops directly placed the meat on the display cabinet at the butcher shop, but in 8 (30%) of them another temporary meat storage area was used which could be a great factor for contamination. 18 (67%) of retail shops placed meat covered despite in 9 (33%) of them in which meat was left open. This is obvious that it could be a major risk factor for meat contamination. All (100%) of retail shops practiced regular cleaning of butchery house, display cabinet, hooks, equipment and surfaces in the house, but 16 (59%) of them used only water and 11(41%) of them used detergent along with water. In 10 (37%) of the retail shops money was collected by a cashier, but in 17 (63%) of them the butchers (meat handlers) collect money by themselves which is inappropriate in terms of hygienic practice. In 5 (19%) of the retail shops the same equipment was used for

handling of meat and offal where as in 22 (81%) of the shops separate equipment was there. This is appreciable in terms of its importance in avoiding contamination. Additional information on hygienic practice at retail shops is summarized in (Table 14).

Table 14: Summary on Butchers status and general hygienic practice at retail shops

Activity	Response	Frequency	P (%)
After being received from abattoir/after slaughter the meat placed directly on the display cabinet at the butcher shop	Yes	19	70%
	No	8	30%
The meat left open/covered	Covered	18	67%
	Open	9	33%
Covering material	Plastic	18	67%
	Leaf	9	33%
Regular cleaning of butcher house display cabinet and hooks	Yes	27	100%
	No	0	0%
Means of cleaning	Water only	16	59%
	Water and detergent	11	41%
Regularly wash working gown and head cover	Yes	27	100%
	No	0	0%
Frequency of washing	Daily	5	19%
	Once a week	7	26%
	Twice a week	15	55%

Collect money while working	Yes	17	63%
	No	10	37%
Always wash hands with soap before touching meat	Yes	13	48%
	No	14	52%
Regularly clean equipment such as knife, axe and cutting board	Yes	27	100%
	No	0	0%
Frequency of cleaning	Once a day	27	100%
	Twice a day	0	0%
	Three times a day	0	0%
Means of cleaning	Water only	16	59%
	Water and detergent	11	41%
Source of water for use	City supplied	27	100%
Have refrigerator for storage of meat	Yes	19	70%
	No	8	30%
Protect meat from flies	Yes	27	100%
	No	0	0%
Means of protection	Using leaf	5	19%
	Thorough cleaning	15	55%
	Smoking	7	26%
Material used for meat wrapping	Plastic	12	44%

	Newspaper	15	56%
Make sure that the wrapping material is clean enough	Yes	16	59%
	No	11	41%
Use the same equipment for handling meat and offal	Yes	5	19%
	No	22	81%
Received training on food borne disease	Yes	9	33%
	No	18	67%
Received training on hygienic handling of meat	Yes	15	56%
	No	12	44%
Took medical test before being employed at the butchery house	Yes	20	74%
	No	7	26%
Take regular medical checkup	Yes	0	0%
	No	27	100%

5. DISCUSSION

In the present study, *E.coli* O157:H7 was detected in 4(3.3%) of faeces, 3(2.5%) of carcass swab sample, 2(7.4%) of meat sample and 4(3.2%) of stool sample. Inspite of these findings, there was no any statistically significant difference ($p > 0.05$) on the occurrence of the pathogen among the different risk factors considered in both animal as well as human sample. This could be due to few positive sample results.

In this study, faecal prevalence of *E.coli* O157:H7 is 3.3% which is in line with a report by Atnafie et al. (2017), who reported a prevalence of 4.7%. The present study revealed a prevalence which is lower than previous studies by Jufare (2018), Haile et al. (2017) and Hamid et al. (2018) from Ethiopia and Musa et al. (2010) from Sudan who reported a prevalence of 7%, 7.3%, 6.4% and 8% respectively. Very high and low *E.coli* O157:H7 prevalence was also reported by authors from different countries including Al-Rudha et al. (2016) from Iraq and Mailafia et al. (2017) from Nigeria, who reported a prevalence of 91.25% and 0.2% respectively. These disagreements in prevalence among different studies could be attributed to difference in geographical area or may be due to divergence in husbandry practices leading to distinguishable rate of exposure of the animals to the pathogen.

The present study revealed a prevalence of 2.5% for carcass swab samples examined for the presence of *E.coli* O157:H7. This result is in agreement with a report of 2.7% (Atnafie *et al.,* 2017) and 3.6% (Tassew, 2015), higher than a previous report of 0.54% (Abdissa *et al.,* 2017), slightly lower than reports of 4.7% and 4.5% by Bekele et al. (2014) and Beyi et al. (2017) form Ethiopia and 4% by Kiranmayi and Krishnaiah (2010) from India respectively. On the other hand the prevalence of the present study is significantly lower than a prevalence of 9.3% and 12% reported by Haile et al. (2017) and Hamid et al. (2018) respectively. This particular variation in prevalence among studies could be associated with a difference in Abattoir setting, facility and hygienic status including personal hygiene which significantly could influence faecal- carcass contamination.

In the present study, the prevalence of *E.coli* O157:H7 from raw beef at meat retail shop is 7.4%. This result agreed with the result of Hiko et al. (2008) and Jufare (2018) who reported a prevalence of 8% and 6.3% respectively. The result of this study is slightly higher than a report of 5.71% (Mashak, 2018) from Iran and higher than previous reports of 2% (Kiranmayi and Krishnaiah, 2010) from India, 2.06% (Mengistu *et al.,* 2017), 2% (Atnafie *et al.,* 2017) and 3.07% (Bedasa *et al.,* 2018) from Ethiopia. The present result is significantly lower than reports of 21.9%, 25.46% and 53% by Bekele et al. (2014), Vijayan et al. (2017) and Dahiru et al. (2008) from Ethiopia, India and Nigeria respectively. In contrary, significantly lower prevalence (0.29%) was reported by Itelima and Agina (2011) from Nigeria. A difference in the prevalence of the above studies could be due to a varying degree of hygiene of the retail markets and hygienic handling of butchers on the other hand.

The prevalence of *E. coli* O157:H7 in feces of the slaughtered animals is higher when compared with carcass swab samples in the present study. This finding is in line with the findings by Atnafie et al. (2017) and Abdissa et al. (2017) at Hawassa municipal abattoir and plants located in Addis Ababa and Debre berhan respectively. The higher prevalence in faeces may be due to the pathogen residence in the intestine (Al-Rudha *et al.,* 2016). In addition prevalence in carcass is determined by the rate of faecal- carcass contamination.

Prevalence of *E. coli* O157:H7 at abattoir level (2.9%) which is lower than retail shops (7.4%) is in line with a report by Bekele et al. (2014) of which the prevalence in abattoir is less than that of retail shops which were 5.7% and 14.6% respectively. The higher prevalence in retail shops may be as a result of increased contamination of the carcass during transport due to uncleaned vehicle or unhygienic handling and storage at butcher shops. In other way, the finding in abattoir is in line with Atnafie et al. (2017) who reported a prevalence of 2.8%.

Considering stool samples examined, 3.2% of the samples were positive for *E.coli* O157:H7. This result is in agreement with previous report of 2.9% and 3.6% by Jufare (2018) from Ethiopia and Raji et al. (2008) from Tanzania. The results of this study is significantly higher than a report of 0% (Abdissa *et al.,* 2017, Kahsay *et al.,* 2008; by Engdaw and Temesgen, 2016) from Ethiopia and lower than a report of 5% by Musa et al. (2010) and Itelima et al. (2014) from Sudan and

Nigeria respectively. In the present study, the prevalence of *E.coli* O157:H7 is higher in male (2.4%) than their female counterparts (0.8%). This result is in agreement with a study by Itelima et al. (2014) who reported a prevalence rate of 3.43% and 1.57% in male and female subjects respectively. The higher prevalence of the pathogen in males rather than females in this study could be attributed to male social life like eating and drinking outside home in restaurants and cafes in groups which could increase their vulnerability to the pathogen. In addition, males are occupationally more exposed to the pathogen since male are more involved in occupations that has more exposure like animal husbandry practice, abattoir practice, meat butchering and rendering(Itelima *et al.,* 2014).

Due to their therapeutic use in human and veterinary medicine, antimicrobials are routinely used for disease prevention and growth promotion in animal production (Schroeder *et al.,* 2002). The use of antimicrobials in the treatment of farm animals has been linked to the development of multidrug-resistant microorganisms which is a threat to public health (Adzitey, 2020).

In the present study, all isolates from animal (n=9) were subjected to 12 different antimicrobial agents. According to the test result, all isolates were found to be Susceptible to SXT (Sulfamethazole + Trimetoprim), NA (Nalidixic acid), CRO (Ceftraxone) and CN (Gntamicin). This result is in agreement with different studies from Ethiopia. The susceptibility of the isolates to SXT is in line with a study by Hiko et al. (2008), Bekele et al. (2014) and Beyi et al. (2017). Susceptibility to NA is in line with Atnafie et al. (2017), Hiko et al. (2008), Bekele et al. (2014) and Beyi et al. (2017). Suceptability to CRO is in agreement with studies by Atnafie et al. (2017 and Bekele et al. (2014) and isolates susceptibility to CN is supported by Hiko et al. (2008) and Atnafie et al. (2017).

The resistance of the isolates to TE (Tetracycline) and AMP (Ampicillin) in the present study is in line with a study by Fuh et al. (2018) from Nigeria. Even though, studies from Ethiopia reported 100% susceptibility of isolates to TE (Bekele *et al.,* 2014, Haile *et al.,* 2017 and Beyi *et al.,* 2017). This variation may be due to the development of resistance to antimicrobials through stable genetic change heritable from generation to generation through specific mechanisms including mutation, transduction, transformation and or conjugation (Reuben and Owuna, 2013).

In this study, higher resistance of the isolates was observed to TE which is in line with a study by Reuben and Owuna (2013) from Nigeria who reported a higher resistance to TE among the isolates that were tested. This study reported 33.3% resistance of the isolates to TE. This is in agreement with Musa et al. (2010) from Sudan who reported 30% resistance of the isolates to this antibiotic. The high level of resistance of tetracycline obtained in this study may be as a result of the inappropriate or over use of the antibiotic for certain therapeutic purpose. On the other side, this study revealed 100% susceptibility of isolates to CIP (Ciprofloxacin) which is in agreement with a study from Sudan (Musa *et al.,* 2010) and USA (Galland *et al.,* 2001).

In the present study, all isolates from human (n=4) were also subjected to the same 12 different antimicrobial agents like animal isolates. Based on the test result, all isolates were found to be Susceptible to CIP (Ciprofloxacin). This result is in agreement with Musa et al. (2010) from Sudan. 100% susceptibility of the isolates to NA, CN and CRO in this study is in agreement with a study by Raji et al. (2008) from Tanzania. In this study isolates showed resistance to antimicrobials including TE and AMP which is in line with Fuh et al. (2018) from Nigeria and Musa et al. (2010) from Sudan. The present study revealed 100% susceptibility of the isolates to SXT which is in disagreement with a study by Raji et al. (2008) who reported 60% of isolates being resistant to this antimicrobial agent.

The questionnaire indicated that most workers at the abattoir were aware about food borne disease, ways of transmission and public health consequences, but based on their response, trainings on hygiene and hygienic practice at the abattoir were not sufficient enough since trainings given were focused on general stunning and slaughtering procedures.

Even if most of slaughter house workers responded as if they were following good hygienic practice, indirect observations made revealed that none of them were engaged in the practice of hand washing while attempting to touch carcass and had poor experience in keeping working equipment clean. The abattoir followed both hanging and floor type method of carcass dressing procedure. The later one could obviously aggravate the contamination rate since it increases carcass contact with the floor.

More importantly, there was no strict separation between clean and dirty areas and also carcass and GIT were never kept apart which could be another factor that could devastate faecal- carcass contamination. In addition, possibility of faecal- carcass contamination is high at the abattoir since the animals excrete at the time of stunning and later the excreta spreaded to other free areas through workers boots. The animals also contaminate carcass of other animal while struggling.

The slaughter house workers were also not good in keeping personal hygiene. As simple observation indicated that workers were eating while slaughter, had dirty fingers, nails and working clothes and boots.

As hygienic handling of processing equipment and working clothes like an overall and boots, the structure of slaughter house plays an important role in preventing direct and indirect contamination of sterile carcass from disease causing micro-organisms like *E.coli* 0157:H7. In the present study, the abattoir was built simple with corrugated iron and there were no separate processing rooms like, stunning, bleeding, evisceration, inspection and chilling room which contributes a lot for contamination since all activities are performed in one circle. Slaughter house wastes were not also handled in a good manner in which all wastes were lying in a gutter surrounding the slaughter house.

In addition, there was no any mechanism for the prevention of rodents and dogs from entering the abattoir since the fence was not protective enough, so a large number of stray dogs were found aggregated at the entrance of the slaughter house in each day of slaughter which is not safe.

Meat retail shops and butchers working at the shops should be in a good hygiene for the safety supply of meat for consumers. As per butchers response in the present study, most of the retail shops experienced good hygiene including personal hygiene, but informal observations indicated that there was poor hygienic practice in activities including equipment handling and surface cleaning. More importantly, problem of hand washing practice and performing dual activities like cutting meat and collecting money at the same time which could contribute a lot for higher contamination and means for the occurrence of food- borne disease.

Other devastating factor for the occurrence of food- borne disease at retail shops is the failure of strict separation of carcass from offal and the use of the same knife for handling both. In addition, most butchers were not engaged in professionally associated trainings and had poor practice in hygienic handling of meat which could leads to the delivery of unsafe food for the consumer.

6. CONCLUSION AND RECOMMENDATIONS

The present study revealed the occurrence of *E.coli* O157:H7 in cattle, beef and humans in Sebeta. The study could reveal faeces as a major source of contamination for cattle carcass in addition to poor hygienic practice and personal hygiene at slaughter house and unsafe meat handling at retail shops. It might also indicate that raw meat could be a significant factor for the occurrence of the pathogen in human associated with the habit of consumption at the study area. The study point out the presence of resistant isolates to one or more antimicrobials that may lead to a potential public health threat. Generally, this study could give an insight on the prevalence of *E.coli* O157:H7 and its public health significance associated with the consumption of unsafe food at the study area.

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

- Awareness on food- borne pathogens and the associated public health consequences should be provided to slaughter house workers and meat handlers at retail shops.
- Training should be given to slaughter house workers on the basis of personal hygiene and hygienic practices during slaughter.
- Meat handlers at retail shops should be trained on basic hygienic and safe meat handling practice.
- Nominees at slaughter house and owners of retail shops should work in strong collaboration with food safety organizations for the production and supply of safe food for the consumer.
- Veterinarians and medical professionals should work together for the possibility of awareness creation to the public about severe health consequences associated with raw meat consumption.
- Rigorous monitoring of Antimicrobial use in the animal population must be in place to prevent Anti-microbial resistance by pathogens and to further avoid potential public health threat.

- Further studies on the prevalence of *E.coli* O157:H7 and its public health significance mainly associated with raw meat consumption must be undertaken at the study area and also molecular characterization should be undertaken to detect virulence genes and similarity between isolates to see if there is transmission from cattle to human via beef consumption.

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8. ANNEXES

Annex 1: Composition and preparation of medias used for transportation, isolation and identification

1. Buffered Peptone Water (BPW) (HIMEDIA, India)

1.1. Composition

Ingredients	**g/liter**
Proteose peptone	10.00
Sodium chloride	5.00
Disodium hydrogen phosphate	3.5
Potassium dihydrogen phosphate	1.5

1.2. Preparation

Suspend 20 gram in 1000ml of distilled water. Heat if necessary to dissolve the medium completely. Dispense in 50 ml amount in to tubes or flasks as desired and sterilize in autoclave at 121^0c for 15 minutes.

2. Enrichment medium: Trypton Soya broth (TSB) (HIMEDIA, INDIA)

2.1. Composition

Ingredients	**g/liter**
Tryptone	17.00
Soya peptone	3.00
Sodium chloride	5.00
Dipotassium hydrogen phosphate(K_2HP0_4)	2.5
Dextrose(Glucose)	2.5

2.2. Preparation

Suspend 30 gram in 1 liter of distilled water. Heat if necessary to dissolve the medium completely. Mix well, dispense in tubes or flasks as desired and sterilize for 15 minutes in the autoclave set at 121^0c.

3. Medias for primary isolation

3.1. MacConkey agar (BBLTM, USA)

3.1.1. Composition

Ingredients	**g/liter**
Pancreatic digest of gelatin	17.0
Peptones (Meat and Casein)	3.0
Lactose	10.0
Bile salts	1.5
Sodium chloride	5.0
Agar	13.5
Neutral red	0.03
Crystal violet	0.001

3.1.2. Preparation

Suspend 50 gram in 1 liter of distilled water. Mix thoroughly, boil for 1 minute to dissolve the media completely and sterilize at 121^0C for 15 minutes.

3.2. Eosin Methylene Blue (EMB) (OXOID, ® Hampshire, England)

3.2.1. Composition

Ingredients	**g/liter**
Peptone	10.0
Lactose	10.0
Di-potassium hydrogen phosphate	2.0
Eosin Y	0.4
Methylene –blue	0.06
Agar	15.0

3.2.2. Preparation

Suspend 37.5 gram in 1 liter of distilled water. Mix thoroughly, boil to dissolve the media completely and sterilize at 121^0C for 15 minutes.

4. Selective isolation medium: Cefixime tellurite Sorbitol Macconkey agar (CT- SMAC)

4.1. Base Medium (SMAC agar)(CRITERION, USA)

4.1.1. Composition

Ingredients	**g/liter**
Agar	13.5
Gelatin peptone	17.0
Sorbitol	10.0
Sodium chloride	5.0
Proteose peptone	3.0
Bile salt # 3	1.5
Neutral red	30.0
Crystal violet	1.

4.1.2. Preparation

Dissolve 58.0 gram of the medium with 1000 ml of deionized water, stir to mix thoroughly and boil to dissolve completely. Then sterilize for 15 minutes in the autoclave set at 121^0c.

4.2. Potassium tellurite solution

4.2.1. Composition

Ingredients	**g/liter**
Potassium tellurite	0.35g
Water	1ml

4.2.2. Preparation

The refrigerated, concentrated Potassium Tellurite Solution was warmed to 45-50 °C. The 3.5% solution was prepared aseptically by adding 18 ml of warm, deionized sterile water and was mixed thoroughly.

4.3. Cefixime solution

4.3.1. Preparation

0.05 Mg of the powder was dissolved in 1000 ml of deionized sterilized water and was mixed thoroughly.

4.4. Complete medium (CT- SMAC)

4.4.1. Composition

Ingredients	**Per ml**
Base media	1000ml
Potassium tellurite solution	1.0ml
Cefixime	1.0ml

4.4.2. Preparation

Cool the freshly sterilized base medium to between 44^0c and 47^0c . Add 1 ml of the tellurite solution and 1 ml of the cefixime solution to 1000ml of the base medium. Then mix and pour about 15 ml amounts in to sterile petridish and allow it to solidify.

4.4.3. Principle and interpretation

This medium is recommended for the isolation of enteropathogenic *Escherichia coli* O157:H7, which ferments lactose but does not ferment sorbitol, hence produces colourless colonies. Despite most *E. coli* strains, *E. coli* O157:H7 ferments sorbitol slowly or not at all. The growth of *E. coli* O157:H7 on MacConkey Agar with Sorbitol shows colourless colonies unlike most of the fecal flora that ferment sorbitol and appear pink. Therefore, MacConkey Agar with Sorbitol is relevant for the isolation of *E. coli* O157:H7.

5. Nutrient agar (HIMEDIA, INDIA)

5.1. Composition

Ingredients	**g/liter**
Peptone	5.0
HM Peptone B#	1.5
Yeast extract	1.5
Sodium chloride	5.0
Agar	15.0

5.2. Preparation

Suspend 28 gram in 1 liter of distilled water, mix thoroughly, boil to dissolve completely. Then sterilize for 15 minutes in the autoclave set at 121^0c.

6. Mueller-Hinton Agar (OXOID, UK)

6.1. Composition

Ingredients	g/liter
Beef, dehydrated infusion	300
Casein hydrolysate	17.5
Starch	1.5
Agar	17.0

6.2. Preparation

Suspend 38 gram in 1000ml of distilled water. Mix thoroughly, boil to dissolve the medium completely and sterilize by autoclaving at 121^0c for 15 minutes.

7. Simmons Citrate Agar (OXOID, UK)

7.1. Composition

Ingredients	g/l
Magnesium sulphate;;;;;	0.2
Ammonium dihydrogen phosphate	0.2
Sodium ammonium phosphate;	0.8
Sodium citrate, tribasic	2.0
Sodium chloride	5.0
Bromothymol blue	0.08
agar	15.0

7.2. Preparation

Suspend 23 gram in 1 liter of distilled water. Bring to the boli to dissolve completely and sterilize by autoclaving at 121^0c for 15 minutes.

8. MR-VP Medium (OXOID, UK)

8.1. Composition

Ingredients	**g/l**
Peptone;	7.0
Glucose	5.0
Phosphate buffer	5.0

7.2. Preparation

Suspend 17.0 gram in 1000ml distilled water. Mix well and distribute in to final containers. Then sterilize by autoclaving at 121 ^{0}C for 15 minutes**.**

Annex 2: IMViC tests and Latex agglutination test principle

1. Indole test

1.1. Test principle

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

1.2. Test procedure

5 ml of the TSB medium was inoculated with the test organism and was incubated at 37^0c for 24 hours. Then after 24 hours of incubation, 3 drops (0.2ml) of kovac's reagent was added and then the tube was gently shake, kept for a minute and was observed for any red color formation at the surface of the media.

2. Methyl red(MR) test

2.1.Test principle

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

2.2.Test procedure

A bacterial colony was picked up and inoculated into MR-VP broth medium. It was incubated at 37^0c for 48 hours and then 5 drops of Methyl red was added. Finally the result was observed for the development of a stable red color in the surface of the medium.

3. Voges -Proskauer (VP) test

3.1.Test principle

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

3.2.Test procedure

The MR-VP broth was inoculated with a pure culture of the test organism and was incubated at 37^0c for 48 hours. Then two reagents, 3 ml of alpha-naphthol followed by 1 ml of 40% KOH were added. Then the tube was shaked gently and allowed to remain undistributed for 10-15 minutes.

4. Citrate utilization test

4.1. Test principle

Citrate agar slants contain sodium citrate, bromthymol blue (pH indicator), sodium and water. If an organism is able to utilize citrate as a sole carbon source for their energy needs, an enzyme called citrase will catabolize the citrate into oxaloacetic acid and acetic acid. The oxaloacetic acid is then broken down into pyruvate and the acetic acid is converted to CO2. The CO2 reacts with the water and sodium in the media to produce alkaline sodium carbonate. Under alkaline conditions Bromthymol blue indicator turns from green to blue.

4.2.Test procedure

A well isolated colony was taken and inoculated as a single streak on the slant surface of the citrate agar tube and then the tube was incubated at 37^0c for 48 hours.

5. *E. coli* O157:H7 Latex Test Kit as a confirmatory for *E. coli* O157:H7

5.1. Test Principle

The polystyrene latex particles provided in the kit are coupled to antibodies against *E. coli* serotype O157:H7. When the latex particles are mixed on a test card with fresh colonies of *E. coli* O157, the bacteria will bind to the antibody causing the latex particles to agglutinate (positive reaction). Bacteria that are not *E. coli* O157:H7 will not bind to the antibody and will not agglutinate the latex particles (negative reaction).

Annex 3: Questionnaire

Verbal consent for slaughter house workers and butchers at retail shops

Hello, my name is Dr. Beruktayet Wondu. I am from Addis Ababa University. College of Veterinary Medicine and Agriculture. I am currently running a research on a food borne pathogen which has public health significance. I would like to provide you a few questions about the sanitary condition of your slaughter house/butcher shop including your personal hygiene during work. The objective of this study is to assess practices involving slaughter hygiene/meat handling hygiene which is important to improve the sanitary status so as to safeguard the safety of beef meat reaching to the consumer since lack of hygiene and high rate of contamination is a great factor for the occurrence of the pathogen in human. Your cooperation and willingness is important in identifying risk factors responsible for the occurrence of. the pathogen and for further control. Your name will not be written in this form. Your participation is voluntary and information that you give will be kept secret. In addition, you are not obliged to answer any question that you don't want. Are you volunteer?

Verbal consent for diarrheic patients

Hello, my name is Dr. Beruktayet Wondu. I am from Addis Ababa University. College of Veterinary Medicine and Agriculture. I am currently running a research on a food borne pathogen which could be transmitted to human through several ways in which contaminated food is one of the causes. I want to ask you some questions which are important in identifying the source of the pathogen. It will take only few minutes. Your participation is voluntary and I would like to tell you that your privacy and confidentiality is respected. Are you volunteer?

Section I. Questionnaire for slaughter house workers to assess their knowledge and hygienic Practices at the Abattoir

Date_________________________

Questionnaire code _______________________

Respondent Name: _______________ Sex _______________ Age ________________

Educational Status 1) Illiterate 2) Primary education 3) Secondary education 4) Informal education 5) Other (specify) __________________________________

Role at the abattoir? 1) Veterinarian /meat inspector 2) Butcher 3) Other (specify)

Duration of working in the abattoir ____________________

S. No	Question	Response	Skip to
1.	Do you have any awareness about food borne disease?	1. Yes [ ] 2. No [ ] If No skip	/______/
2.	What could be the possible ways that people could acquire food borne disease?	1. Through contaminated food 2. Through canned food 3. Through contaminated water 4. If other, specify	/______/
3.	Do you have any awareness about carcass contamination?	1. Yes [ ] 2. No [ ] If No skip	/______/
4.	If Yes, What do you think is the cause for cattle carcass contamination?	1. Dirty working area 2. Dirty utensils 3.Workers dirty hands and clothing 4. Cattle hide during flaying 5. Faeces due to accidental GIT puncture 6. Dirty(contaminated water) 7. If other, specify	

5.	Do you think carcass contamination could bring serious health problem to consumers?	1. Yes [ ] 2. No [ ]	
6.	Do you always take caution to avoid carcass contamination?	1. Yes [ ] 2. No [ ]	/______/
7.	Do you wash your hands and the carcass after evisceration?	1. Yes [ ] 2. No [ ]	
8.	Do you regularly wash your working cloth and boots?	1. Yes [ ] 2. No [ ]	
9.	If yes, how frequently you wash your working cloth and boots?	1. Every day 2. Once per week 3. Twice a week	/______/
10.	What is the source of water for use in the abattoir?	City supplied [ ] borehole [ ] rain collected water [ ] River [ ] others (specify)_____________	/______/
11.	Did you take any work related medical tests to work at the abattoir?	1. Yes [ ] 2. No [ ]	/______/
12.	Do you regularly take work related medical checkups?	1. Yes [ ] 2. No [ ]	
13.	Have you ever engaged in any work related training?	1. Yes [ ] 2. No [ ]	

Section II. Practices (slaughter house observation checklist).

Hand washing before touching the carcass?	1. Yes [ ] 2. No [ ]
How washed?	1. Running water [ ] 2. Bucket [ ]
All knives are completely clean and free from contamination	1. Yes [ ] 2. No [ ]
Method of carcass dressing?	1. Hanging [ ] 2. On the floor [ ] 3. Mixed [ ]
Carcass and GIT content kept separately	1. Yes [ ] 2. No [ ]
Finger nails short and clean	1. Yes [ ] 2. No [ ]
Eating while working	1. Yes [ ] 2. No [ ]
Strict separation between clean and dirty areas	1. Yes [ ] 2. No [ ]
Clothes free from faecal contamination	1. Yes [ ] 2. No [ ]
Latrine available?	1. Yes [ ] 2. No [ ]
Latrine has adequate water supply and soap for hand washing?	1. Yes [ ] 2. No [ ]
Veterinary inspectors present to examine and pass carcass for consumption	1. Yes [ ] 2. No [ ]
Use vehicle to deliver carcass to butcher houses	1. Yes [ ] 2. No [ ]

Section III. Questionnaire for meat handlers on hygienic practices at retail shops

Date__________________________

Questionnaire code ________________________

Respondent Name: ______________ Sex________________ Age __________________

Educational Status 1) Illiterate 2) Primary education 3) Secondary education 4) Informal education 5) Other (specify) ___________________________________

S. No	Question	Response
1.	After being received from the abattoir/after slaughter, will the meat be put directly at the display cabinet in the butcher shop?	
2.	Do the meat left open or covered?	1. Open 2. Covered
3.	If covered, what is the material that you use for covering?	1. Plastic 2. Leaf 3. Other
4.	Is there regular cleaning of the butcher house, display cabinet and hooks?	1. Yes [ ] 2. No [ ]
5.	If yes, what do you use for cleaning?	1. Only water 2. Water and soap
6.	Do you regularly wash your working gown and head cover?	1. Yes [ ] 2. No [ ]
7.	If Yes, how often?	A. Daily B. Once a week C. Twice a week
8.	Do you collect money while working?	1. Yes [ ] 2. No [ ]
9.	Do you always wash your hands with soap before touching meat?	1. Yes [ ] 2. No [ ]
10.	Do you regularly clean butcher house equipment's and surfaces such as knife, axe and	1. Yes [ ] 2. No [ ]

	cutting board?	
11.	If Yes how frequently?	A. Once per day in the morning B. Twice per day, in the morning and afternoon C. Three times a day, in the morning, afternoon and evening D. Other(specify)
12.	What do you use for cleaning of the equipment's and surfaces in the butcher house?	1. Water only 2. Water and soap
13.	What is the source of the water that you use in the butcher house?	A. City supplied B. Borehole C. Rain collected water D. Others (specify)
14.	Do you have a refrigerator for storage of the meat that has not been sold for the day?	1. Yes [ ] 2. No [ ]
15.	Do you protect the meat from flies?	1. Yes [ ] 2. No [ ]
16.	If Yes, what method do you use for protection?	1. Leaf 2. Thorough cleaning 3. Smoking
17.	What material do you use for wrapping of meat during sale?	A.Newspaper B.Plastic C.Other(specify)
18.	Do you make sure that the	1. Yes [ ] 2. No [ ]

	wrapping material you use is clean enough?	
19.	Do you use the same equipment for handing meat and offal?	1. Yes [ ] 2. No [ ]
20.	Have you ever received training on food borne disease?	1. Yes [ ] 2. No [ ]
21.	Have you ever received training on hygienic handling of meat?	1. Yes [ ] 2. No [ ]
22.	Did you take medical test before being employed at the butchery house?	1. Yes [ ] 2. No [ ]
23.	Do you regularly take medical checkups?	1. Yes [ ] 2. No [ ]
24.	If Yes, how frequently?	A.Once/year B. Every 6 month C. Every 3 month

Section IV. Questionnaire designed for diarrheic patients.

Date ______________________ Questionnaire code____________________

1. Age_____________________

2. Sex _____________________

3. Address _________________

4. Did you eat raw meat for the last one week? 1. Yes [ ] 2. No [ ]

5. If No, what other food did you consume for the last one week?

A. Raw vegetable B. Raw milk C. Other (specify)

6. If yes, from where you got it?

A. Butcheries of Sebeta town B. backyard slaughter C. Other (specify)

7. What type of meat is it?

A. Beef B. Mutton C. Chevon D. Other (specify)

8. Duration of onset of diarrhea? A. A day before B. 2 days before C. 3 days before D. Other (specify)

9. Consistency of diarrhea? A. Watery B. Mucoid C. Bloody

9. Do you have an additional symptom? 1. Yes [ ] 2. No [ ]

10. If yes state the symptom? ____

Assessment of other possible risk factors

1. Do you have cattle? 1. Yes [ ] 2. No [ ]

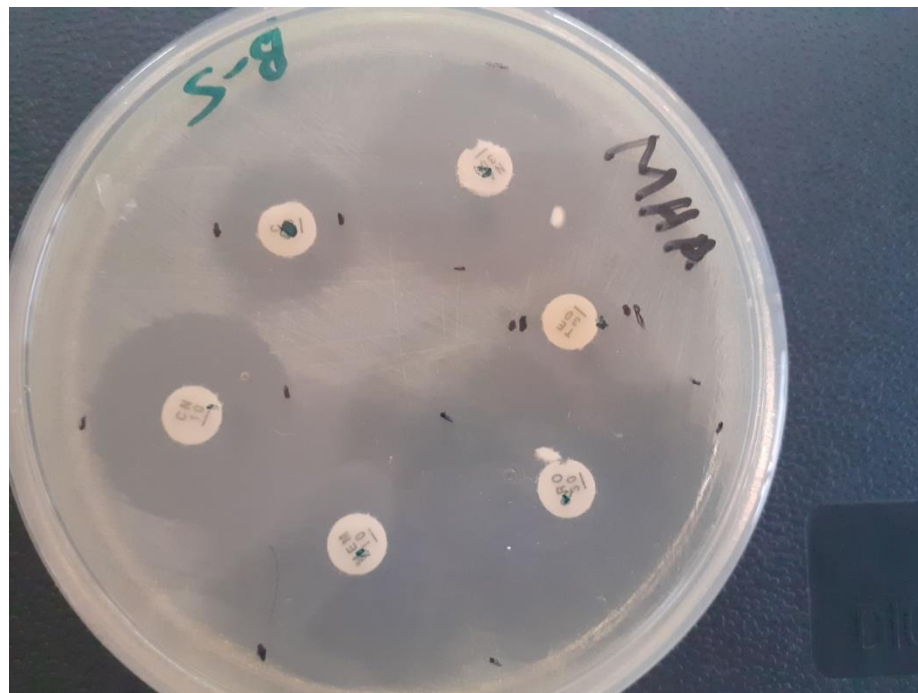
2. If Yes, Do you manage their barn and did you have any contact with their faeces recently?

2. Did you have direct contact with a person with diarrhea for the last one week? Yes [ ] 2. No [ ]

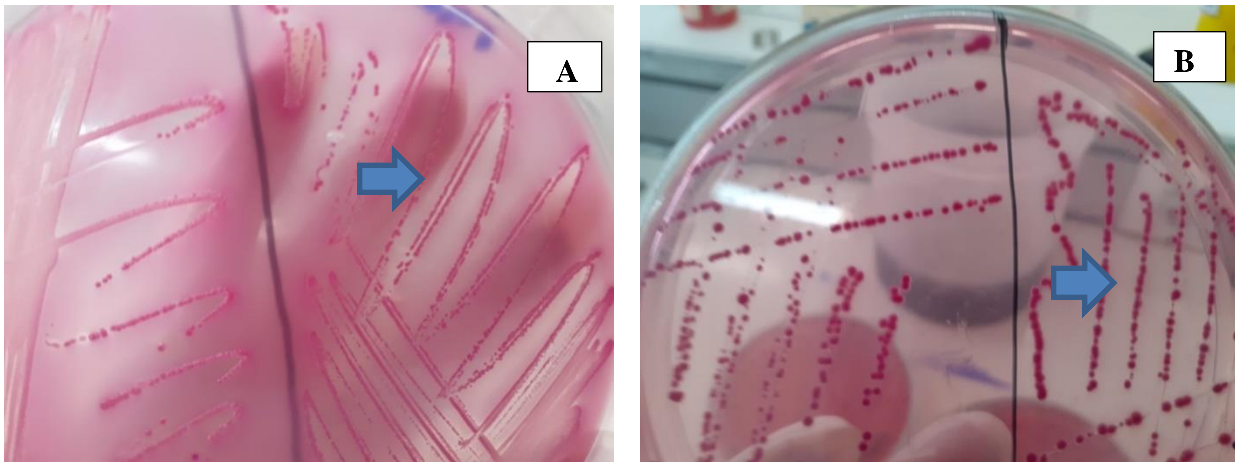
3. If your answer is Yes, state how?____

Annex 4: Pictures of Laboratory equipment, sample collection, laboratory work and results

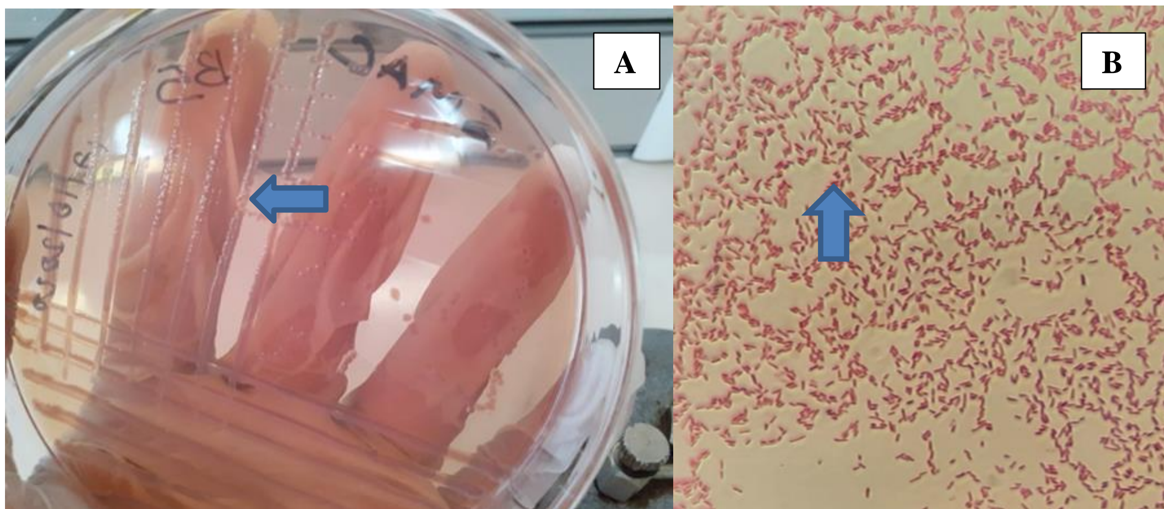
McFarland Densitometer used for checking turbidity during antimicrobial testing



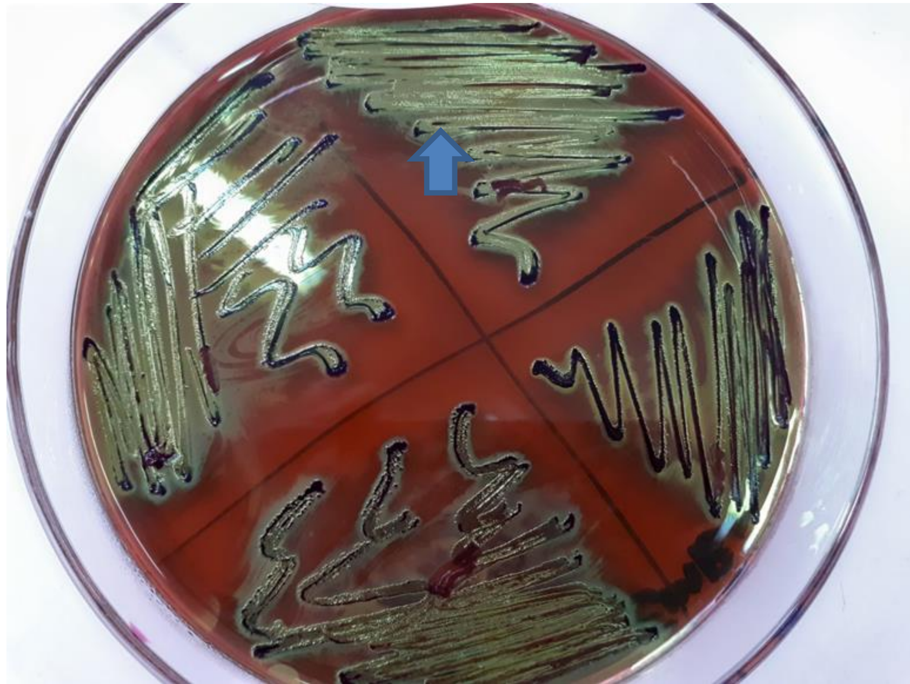
Anti- microbial susceptibility test results



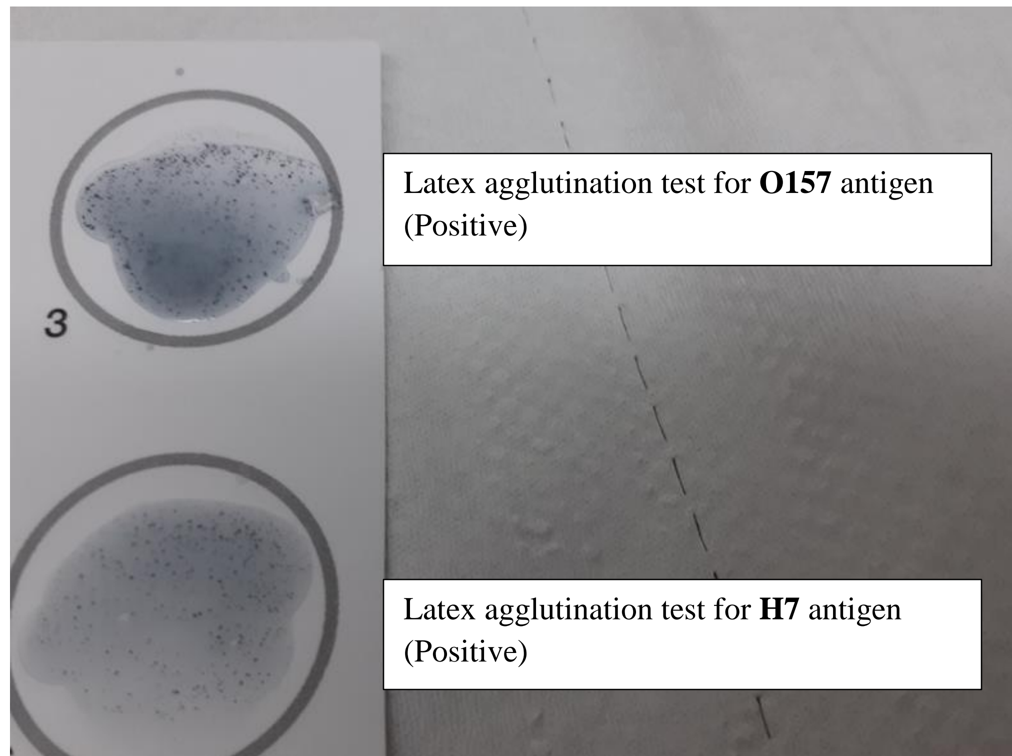
E.coli on MacConkey and SMAC agar (MacConkey (**A**), SMAC (**B**))



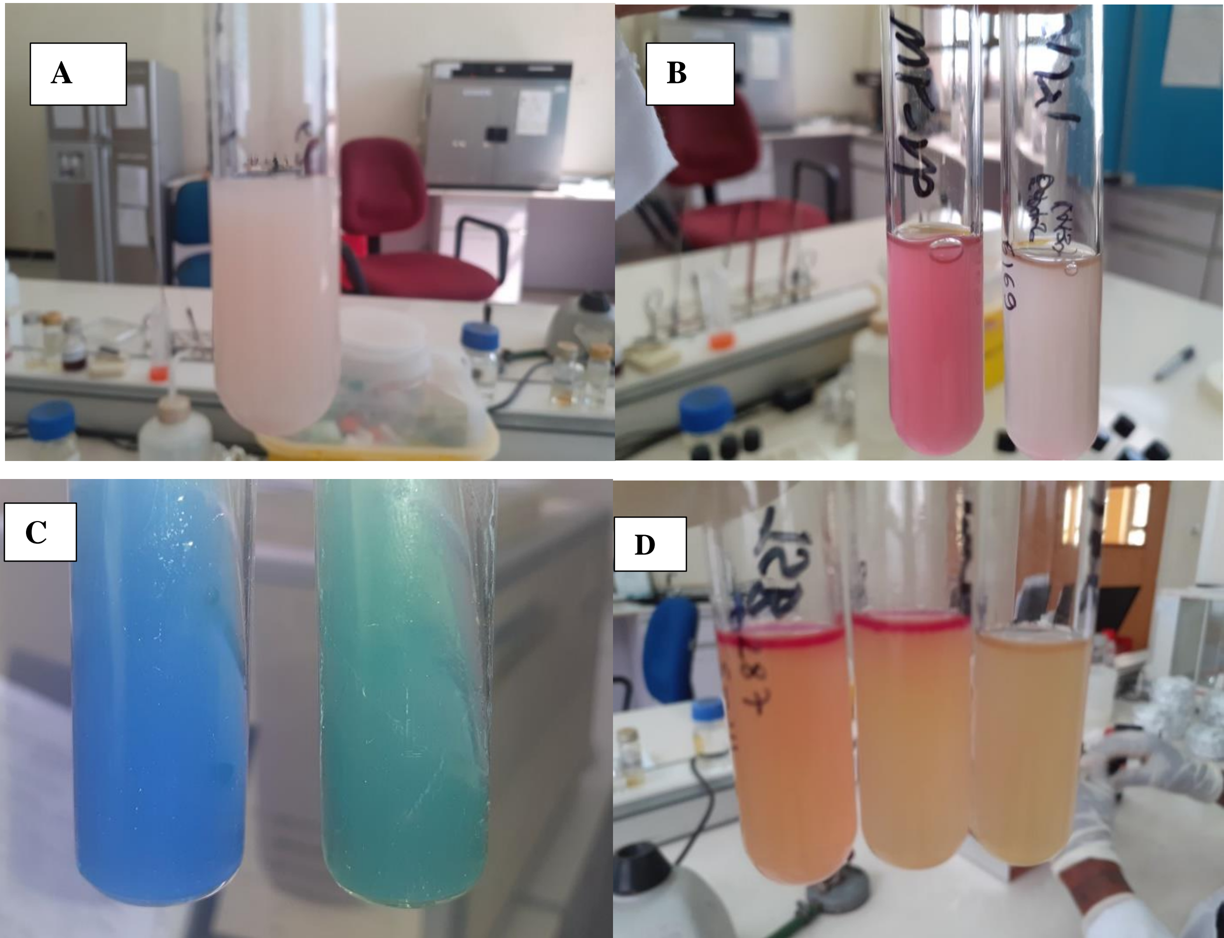
E.coli on SMAC agar (Pale colonies) (**A**) and Gram stain for *E.coli* (Pink, short rod) (**B**)



E.coli growth characteristics on EMB agar



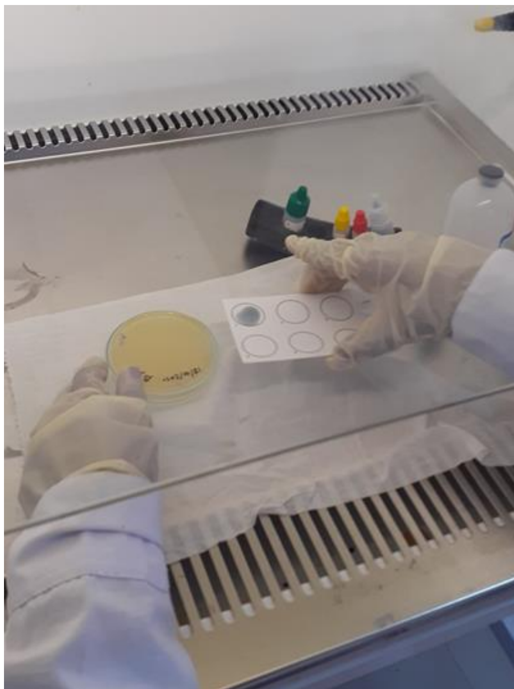
Latex agglutination test for *E.coli* 0157 and H7 antigens



IMViC test for *E.coli* (Negative VP- test (A), MR- test (B: cherry red color**(positive), Citrate test (C:** blue color **(Positive), Indole test (D:** Dark pink color**(positive).**

Antimicrobial Susceptibility testing

Culturing of bacteria



Latex agglutination test

Measuring zone of inhibition

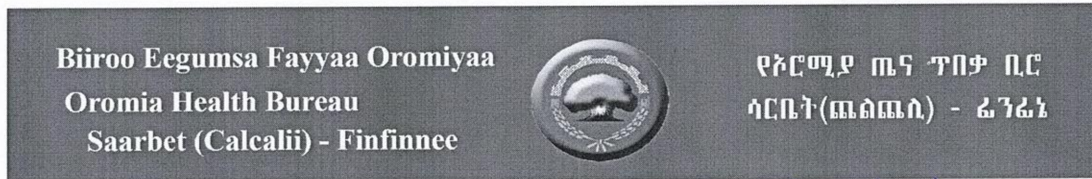
A. Rectal faecal sample collection

B. Carcass swab sampling

C. Raw beef meat collection

D. Collection of stool sample

Annex 5: Ethical clearance



Lakk/Ref.NO BEFO/HBTFH/1-16/313
Guyyaa/Date 7/02/2012

Wa/Eg/Fa/Bu/Mag/Sabataatiif

Sabata

Dhimmi: **Xalayya Deggersa Ilaala.**

Akkuma beekamu Biiroon Keenya Ogeyyii, dhabbile akkasumas namoota qorannoo geggessuuf propozaala dhiyeeffatan propoozaala isaanii madaaluun akkasumas iddo biratti ilaalchisani fudhatama argatan (approved) dhiyeffatan, propoozaala isaanii ilaaluudhaan waraqaa deggersa ni kenna. Haaluma kanaan mata dure **''Prevalence Antimicrobial Resistance Profile and Public Health Significance of E.Coli 0157 : H7 from Cattle Slaughtered at Sebeta Municipal Abattoir and Beef from Retail Shops, In Sebeta Ethiopia"** Jedhamu irratti magaalaa keessan kessatti qorannoo geggessuuf propoozaala isaanii koree "Health Research Ethical Review Commite" Biiroo keenyatti dhiyeffatani jiru. Haaluma kanaan koreen "Health Research Ethical Review Committee" Biiroo keenyaa piropoozaal kana ilaaluun mirkaneessee qorannoon kun akka hojii irra oolu murteessee jira.

Kanaafuu, hojji qorannoo kana irratti deggersa barbaachisa ta`e akka isin gaafachaa qorannoon kun qacceffamee eerga xumuramee booda firii isaa koppii tokko BEFO tiif akka galii godhan galagalcha xalayaa kanaan isaan beeksifna.

Haaluma kanaan anis''Dr.Bruktaaayit Wonduu'' wayitti qorannoon kun qaaceffame xumuramu firii isaa koppii tokko BEFO tiif galii gochuuf mallattoo kootiin nimirkanessa.

Nagaa wajjin!

Gammachuu Shuumii
Daayirektoori Daayirektooreettii Balaa Tasaa Fayyaa Hawaasaa Qorannoo Fi Qorannoo Fayyaa

Maqaa ''Dr.Bruktaaayit Wonduu''

Mallattoo___________________

Bilbila_0911823099

G/G

''Dr.Bruktaaayit Wonduu'' tiif

B/J

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

Animal Research Ethics Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/12/02/12/2020

Name of Applicant: Beruktayet Wondu (DVM, MVSc fellow)

Address: . College of Veterinary Medicine and Agriculture (Addis Ababa University)

Title of the project: *Prevalence, antimicrobial resistance profile and public health significance of E. coli O157:H7 from cattle slaughtered at Sebeta municipal abattoir and beef from retail shops in Sebeta, Ethiopia*

Date of application: **28/01/2020**

Nature of the project: non-**invasive**
Target animal species: **cattle**
Number of animals involved: **146**
Study area: **Sebeta, Ethiopia**

Minutes No. and date of review: **VM/ERC/01/12/020, 03/02/2020**

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

1. All procedures and conditions stipulated in the proposal are respected, minor comments are corrected and any deviation or changes be reported to the committee
2. The project activities be open for occasional supervision by the committee when this is deemed necessary
3. A separate clearance is required for any work (except questionnaire) on human subjects

Dr Getachew Terefe
Chairman

Signature

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Please quote Our Ref. No. When replying

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