ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *ESCHERICHIA COLI* O157:H7 IN RAW BEEF, MUTTON AND CHEVON AT ADDIS ABABA ABATTOIR ENTERPRISE AND SELECTED RETAIL SHOPS, ADDIS ABABA, ETHIOPIA

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JUNE, 2012

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TIZETA BEKELE ATOMA

A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Veterinary Medicine in Tropical Veterinary Public Health

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Board of External Examiners: **Signature**

1. Professor Getachew Tilahun _____

2. Dr. Dereje Gudeta _____

3. Dr. Genene Tefera _____

Academic advisors

Dr. Girma Zewde (DVM, PhD, Associate Professor) _____

Dr. Genene Tefera (DVM, MSc, PhD) _____

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LIST OF ABBREVIATIONS

A/E	Attaching and effacing
A_w	Water activity
ATCC	American type culture collection
CDC	Centers for Disease Control and Prevention
CFSPH	Center for food security and public health
CFU	Colony Forming Units
CSA	Central Statistics Authority
CT-SMAC	Sorbitol MacConkey Agar with Cefixime and Tellurite
CO_2	Carbon dioxide
DAEC	Diffuse-adherent *E. coli*
DNA	Deoxy ribo nucleic acid
EAEC	Enteroaggregative *E. coli*
EHEC	Enterohemorrhagic *E. coli*
EIEC	Enteroinvasive *E. coli*
ELISA	Enzyme linked immunosorbent assay
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. Coli*
Gb	Globotriaosylceramide
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Point
HC	Hemorrhagic colitis
HUS	Hemolytic uremic syndromes
IFT	Institute of Food Technologists
Ig	Immuno globulin
IMViC	Indole, MethylRed,Voges Proskauer and Citrate Utilization
ISO	International Organization for Standardization
LEE	Locus for enterocyte effacement
LPS	Lipo poly sacaride
MoARD	Ministry of Agriculture and Rural Development

MOH	Ministry of health
NCCLS	National Committee for Clinical Laboratory Standards
NPH	National public health
NVI	National Veterinary Institute
OIE	Office for international des epizootics
ORFs	Open reading frames
PCR	Polymerase chain reaction
RNA	Ribosomal nucleic acid
SPSS	Statistical package for the social sciences
STEC	Shiga toxin producing *E. coli*
Stx	Shiga toxin
T	Transmittance
Tir	Translocated intimin receptor
TTP	Thrombotic thrombocytopenic purpura
US	United state
USDA	United states department of agriculture
VTEC	verocytotoxigenic *E. coli*

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ABSTRACT

A study on the prevalence of *Escheriehia coli* O157:H7 and antimicrobial susceptibility test was undertaken on beef, mutton (sheep meat) and chevon (goat's meat) obtained from Addis Ababa abattoir enterprise and retail shops at Addis Ababa from August 2011 to April 2012. A total of 384 raw meat samples consisting of 64 beef, 64 mutton and 64 chevon were collected each from the abattoir and retail shops that mean a total of 128 beef, 128 mutton and 128 chevon were analyzed during the study period. *E. coli* O157:H7 was isolated and identified according to OmniLog identification technique. Out of 384 meat samples examined, 39 (10.2%) were positive to *E. coli* O157:H7. Among the meat samples examined, beef was the most frequently contaminated with *E. coli* O157:H7 with an overall prevalence of 13.3%. Of the examined samples, 9.4% mutton and 7.8% chevon were also positive for *E. coli* O157:H7. With regard to meat source, the prevalence rates of *E. coli* O157:H7 at abattoir and at selected retail shops were recorded to be 5.7% and 14.6%, respectively. No significant variation in prevalence rate among the three types of meat types ($p>0.05$) was observed. However, significant differences in prevalence of *E. coli* O157:H7 was observed among meat sources ($p<0.05$). The antimicrobial susceptibility investigation of 39 *E. coli* O157:H7 isolates using 10 commonly marketable antimicrobial discs revealed that the isolates were susceptible to nine antimicrobials from 69.3% to 100% except streptomycin which showed susceptibility of 48.7%. Results of the present study on antimicrobial sensitivity test indicated that an over all resistance of 28.2% and 30.8% was developed to streptomycin and amikacin. Similarly, 5.1%, 5.1%, 7.7%, 12.8% and 17.9% resistance rates were developed against nalidixic acid, tetracycline, amoxacilin-clavulanic acid, cephalothin and ciprofloxacin, respectively. Multidrug resistance to three or more drugs was detected in 4 (57.1%), 1 (14.3%) and 2 (28.6%) of the isolates from beef, mutton and chevon, respectively. This indicates the possible risk of *E. coli* O157:H7 particularly for the consumers who have the habit of eating raw or undercooked meat, elderly and imunocompromised individuals in Ethiopia. These findings stress the need for implementation of *E. coli* O157:H7 prevention and control strategies from farm production to consumption of meat and meat products.

Keywords: Abattoir, Addis Ababa, Antimicrobials, *Escherichia coli* O157:H7, Prevalence, Retail shops, Raw meat

1. INTRODUCTION

Most gastrointestinal microbial diseases of domestic animals are caused by the bacteria belonging to the family *Enterobactericeae* which includes most important genera: *Escherichia, Shigella, Salmonella, Yersinia* (Baron *et al*., 1994; Leclercq *et al.,* 2002; Quinn *et al.,* 2002). Some of them cause food borne infection in humans (Baron *et al*., 1994; Acha and Szyfres, 2001).

Escherichia coli are a normal part of the intestinal micro-flora of many healthy animals, including humans. Many *E. coli* strains are harmless or even beneficial to the host; however, some strains of *E. coli* can be pathogenic to humans and are harbored in food animals. *E. coli* O157: H7 is the best known pathogenic strain (Acha and Szyfres, 2001; MOH, 2001; IFT, 2003).

E. coli O157: H7 some times referred as verocytotoxigenic *E. coli* (VTEC), whereby more than 100 serotypes produce verocytotoxins. These serotypes are also classified as enterohemorrhagic *E. coli* (EHEC), and are implicated mainly in human diseases. EHEC strains have been described as important and emerging foodborne pathogens (Vernozy- Rozand, 1999). These strains can cause severe diarrhea and kidney damage. Young children, the elderly, and those with weakened immune systems are the most vulnerable for infection. In addition to these, *E. coli* are important causes of bactereamia, surgical infection and respiratory tract disease, mostly in patients whose normal defense mechanisms have been breached (IFT, 2003; Wilkerson *et al.,* 2004).

Cattle have been identified as a major reservoir of *Escherichia coli* O157 (Chapman *et al*., 2001) and consumption of foods of bovine origin have been associated with some of the largest food poisoning outbreaks in which this organism was identified as the etiologic agent (Meng and Doyle, 1998).

E. coli O157: H7 also produces shiga toxin and is called shiga toxin *E. coli* (STEC), and it is an important cause of food borne illness in humans. Ruminants appear to be more frequently colonized by *E. coli* STEC than are other animals, but the reason(s) for this is unknown. The

comparative study of the frequency, magnitude, duration, transmissibility and colonization of sheep by *E. coli* O157: H7 to that of other pathotypes of *E. coli* showed that STEC is better adapted to persist in the alimentary tract of sheep than are other pathotypes of *E. coli*. This indicates that sheep can also act as reservoir and source of infection for humans and other animals (Cornick *et al.,* 2000).

The microbiological contamination of carcasses occurs mainly during processing and manipulation, such as skinning, evisceration, storage and distribution at slaughterhouses and retail establishments. Fecal matter is a major source of contamination and could reach carcasses through direct deposition, as well as by indirect contact through contaminated and clean carcasses, equipment, workers, installations and air (Pal, 2012). Cattle slaughtering operations, such as bleeding, dressing and evisceration expose sterile muscle to microbiological contaminants that were present on the skin, the digestive tract and in the environment (Gill *et al.,* 1996; Bacon *et al*., 2000; Abdalla *et al*., 2009).

The most frequent mode of transmission for *E. coli* O157:H7 infection is through consumption of contaminated food and water. However, it may also spread directly from person to person and occasionally through occupational exposure (Abdalla *et al*., 2009).

E. coli O157:H7 has been found in the intestines of healthy cattle, deer, goats, and sheep (Acha and Szyfres, 2001; MOH, 2001; IFT, 2003). It causes over 73,000 illnesses and approximately 60 deaths in the US each year. Enterohemorrhagic *E. coli* infections are estimated to cost the US economy approximately $1 billion per year (USDA, 2008).

There are a few reports on the prevalence and antibiotic resistance status of *E. coli* O157: H7 from raw meat in Ethiopia and little or no studies have been carried out in Addis Ababa. Thus the current study has been designed to determine the prevalence and assess the sensitivity of the microorganism isolated from beef, sheep and goat's meat at Addis Ababa abattoir and in retail shops in Addis Ababa.

The objectives of this research were:

- To isolate and identify *Escherichia coli* O157: H7 from the carcasses of cattle, sheep and goats slaughtered at Addis Ababa Abattoir Enterprise and meat presented for sale in retail shops in Addis Ababa.
- To determine the prevalence of *E. coli* O157: H7 in beef, mutton and chevon.
- To study the antimicrobial susceptibility pattern of *E. coli* O157: H7 isolates to selected antimicrobials.

2. LITERATURE REVIEW

2.1. Historical background

In 1982, 47 individuals who resided in Michigan and Oregon were afflicted with a severe form of gastroenteritis called hemorrhagic colitis that included intense abdominal cramping in combination with bloody diarrhea (Riley, 1987; Mainil and Daube, 2005). An investigation by the Centers for Disease Control and Prevention (CDC) concluded that the outbreak was caused by the ingestion of undercooked, contaminated hamburgers that originated from a fast-food restaurant chain. Curiously, the hamburgers were determined to be contaminated with a novel strain of *Escherichia coli* that had not been previously considered as human pathogen (Armstrong *et al.,* 1996). The strain expresses O-antigen 157 and H-antigen 7 and deviates from the other recognized strains of pathogenic *E. coli* in both pathology and virulence. Because of its association with hemorrhagic colitis, the O157:H7 strain was categorized into a new class of pathogenic *E. coli* called enterohemorrhagic *E. coli* (EHEC) (Mainil and Daube, 2005). Since 1982 numerous outbreaks have been documented, and it is estimated that *E. coli* O157:H7 is responsible for greater than 73 000 cases of illness and 61 deaths each year in the United States (Mead and Griffin, 1998).

Shortly after *E. coli* O157:H7 was determined to be a human pathogen; Karmali *et al*. (1985) observed that stool sample from children with hemolytic uremic syndrome contained a substance that was toxic to Vero cells. The verocytotoxin was produced by *E. coli* isolates with O157:H7 the prominent serotype causing infection. In recognition of its distinct clinical manifestations, *E coli* O157:H7 became the first of several strains referred to as enterohaemorrhagic *E. coli* (EHEC), which are now believed to account for over 90% of all cases of haemolytic ureamic syndrome (HUS) in industrialized countries (Mead and Griffin, 1998).

2.2. Taxonomy

Escherichia coli is a gram negative bacterium in the phylum P*roteobacteria*, class *gamma Proteobacteria*, order *Enterobacteriales*, family *Enterobacleiaceae* and genus *Escherichia*. The family *Enterobacteriaceae* can be divided in to three groups based on the pathogenecity for animals (Garrity *et al*., 2004). The first group is those with uncertain significance for animals. The second group includes major pathogens of animals such as *Salmonella* species, *E. coli* and three of the *Yersina* species. The third group is composed of opportunistic pathogens that are known occasionally to cause infections in animals, and includes species with in the genera *Citrobacter, Edwardsiella, Enterobacter, Klebsiella, Morganella, Proteus, Serratia and Shigella* (Quinn *et al*., 2002; Schaffner and Smith, 2004).

In the current classification of the genus, *Escherichia* also consists of other species such as *E. hermannii, E. fergusonii, E. vulneris E. albetii, E. adecanioxycate* and *E. blattae* (Scheutz and Strockbine, 2001).

Escherichia coli is classified into different serotypes according to the scheme originally developed by Kauffmann, which is based primarily on the somatic O antigens (polysaccharide and thermostable) that differentiate *E. coli* into more than 170 serogroups (Pal, 2007). The flagellar H antigen, which is thermolabile and proteinic, distinguishes the serotypes (56 to date) of each serogroup (Riemann and Cliver, 2006). The pathogenic strains, which cause enteric disease, are grouped into six categories: enterohemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enteroaggregative (EAggEC), and diffuse-adherent (DAEC). The last two categories are not yet well defined. These categories differ in their pathogenesis and virulence properties, and each comprises a distinct group of O:H serotypes. Their clinical symptoms and epidemiological patterns may also differ. In terms of zoonoses, the most important category is the enterohemorrhagic *E. coli*, which is also the most severe (Riemann and Cliver, 2006).

The term EHEC refers to *E. coli* serogroups including O26, O111, O103, O104, O118 and O145 with various H antigen types. *E. coli* O157: H7 is the most predominant and most virulent serotype in a pathogenic subset of EHEC. *E. coli* O157:H7 is so named because it expresses the 157^{th} O antigen identified and the 7^{th} H antigen (Chapman *et al.*, 2001).

2.3. Major characteristics

2.3.1. Morphology

As other *E. coli* strains, *E. coli* O157: H7 is cylindrically shaped bacteria, which are between 0.3-1.0 µm in diameter and between 1.0-6.0 µm in length. They do not form spores. Thus, they are described as Gram-negative, non acid fast, non-sporing rods (Quinn *et al.,* 2002). Most *E. coli* are motile, especially the O157: H7 strain by means of peritrichous flagellae at opposed to polar, which are strings of protein made in the shape of a corkscrew. They are continuously replenished from inside as they may be broken off. They are attached to a hook-like structure embedded in the cell wall, which rotates around 360º causing the screw-like flagellae to push the bacteria through the water. In changing the direction, it is reversed along the 360º (Baron *et al.,* 1994; Quinn *et al.,* 2002). *E. coli* are normally the most common facultative anaerobe in the large bowel. Most *E. coli* strains adhere to the mucus overlying the surface of the large bowel and distal small bowel (Baron *et al*., 1994).

2.3.2. Growth and survival characteristics

A number of factors have a significant influence on the survival and growth of *E. coli* O157: H7 in food, including temperature, pH, salt, and water activity (Meng and Doyle 1998).

The optimum growth temperature for *E. coli* O157: H7 is 37 ^{o}C, the minimum is 7-8 ^{o}C and its maximum temperature is 41 ^{o}C in selective *E. coli* broth. The bacterium grows only poorly at temperatures greater than this in non-selective media (Chapman *et al.,* 2001; MOH, 2001). The upper growth temperature for *E. coli* O157: H7 is culture medium dependent. The organism survives well in chilled and frozen foods. For example only little change was noted in number of bacteria in hamburgers stored at -20 ^{o}C for 9 months (Chapman *et al.,* 2001). The effect of environmental stress and food production processing on the growth, survival and inactivation of *E. coli* O157: H7 are well recognized. The microorganism is also heat resistant, but influenced by pH,

growth condition and growth phase of the cells, and the method of heating (MOH, 2001). Thermal resistance is higher in foods of high fat content that are packed under low oxygen atmospheres (MOH, 2001; Fratamico and Smith, 2006).

E. coli O157:H7 is relatively acid-tolerant compared to other foodborne pathogens. The pathogen can grow at pH levels ranging from 4.4 to 9.0, and can survive for extended periods in foods at pH levels of 3.5–5.5. It survived for up to 2 months, with only a 100-fold reduction in cell numbers during fermentation, drying and storage of fermented sausage; for 5–7 weeks in mayonnaise at 5°C; and for 10–31 days in apple cider at 8°C (Glass *et al*., 1992; Zhao *et al*., 1994). Application of warm (20°C) and hot (55°C) acetic, citric, and lactic acid sprays did not appreciably reduce the levels of *E. coli* O157:H7 on raw beef (Brackett *et al*., 1994).

Optimum growth of *E. coli* O157: H7 is found to be at water activity (a_w) of 0.995 and its minimum a_w is 0.950 (MOH, 2001). Studies on the effect of a_w on the survival and growth of *E. coli* O157: H7 focused primarily on the effect of sodium chloride, though, presumably, *E. coli* O157: H7 behaves similarly with other *E. coli.* It can survive for many weeks when desiccated, particularly at refrigeration temperature (Boyce *et al.,* 1995; Mutaku *et al.,* 2005).

The bacteria can grow in the presence or absence of oxygen (MOH, 2001). Growth can occur in vacuum- packed meat at 8 and 9 ^{o}C, but not when the meat is packed under 100% CO_2 . Survival on fermented meat was equivalent when packed under air or under vacuum (Boyce *et al.,* 1995). In the absence of organic matter, benzalkonium chloride, chlorhexidine gluconate, ethanol and hot (70 ^{o}C) water are effective sanitizers against *E. coli* O157: H7 (MOH, 2001). Standard water chlorination is effective against these bacteria (MOH, 2001; Basser *et al.,* 2003).

E. coli O157:H7 survived in inoculated tap and bottled spring and mineral water for up to 300 days or more (Warburton *et al*., 1998), and for 14 days at less than 15°C in farm water stored outdoors, demonstrating the potential that farm water might serve as a vehicle for transfer of the organism in a herd (McGee *et al*., 2002). The bacteria survived for 77, 226 and 231 days in manure-amended autoclaved soil stored at 5°C, 15°C and 21°C, respectively (Jiang *et al*., 2002). It persisted for 25-41 days in fallow soils, 47-96 days on rye roots, and 92 days on alfalfa roots (Gagliardi and Karns,

2002). Persistence of the bacteria was not affected by the presence of manure, whereas the presence of clay increased persistence (Riemann and Cliver, 2006).

2.3.3. Biochemical characteristics

Conventional identification of strains is done using indole, methyl red, Voges-Proskauer, Simmon's citrate that show positive, positive and negative, negative results respectively. Other biochemical testes used are β-D-galactosidase, urease, glutamate decarboxylase, sorbitol fermentation and β-glucuronidase test (Leclercq *et al.,* 2002). *E. coli* O157: H7 and Non-O157 EHEC strains can be separated by two biochemical characteristics. *E. coli* O157: H7 serovar are unable to produce acids from sorbitol and possess no β D-galactosidase (Leclercq *et al.,* 2002). These biochemical characteristics have been exploited in isolation and identification of this serovar. Sorbitol- MacConkey agar with cefixime and tellurite (CT-SMCA) is used for detection. By further inhibitors effect, CT-SMCA reduces the number of false positive isolates (Okrend *et al.,* 1990; Chapman *et al.,* 2001; Leclercq *et al.,* 2002). A new well-based identification system, was recently developed by Bio-Merieux Vitek for the identification of Enterobacteriaceae and other non fastidious Gram- negative bacteria, after an incubation period of 22 ± 2 hours at 37 ± 1°C (Leclercq *et al.,* 2002).

2.3.4. Antigenic characteristics

Escherichia coli have some surface antigens that are responsible for the pathogenecity and hence important in serotyping of the strain. The known antigens are the somatic (O), the flagellar (H), the fimbrial (F) and capsular (K) antigens in which each of them has several subtypes and groups (Quinn *et al*., 2002; IFT, 2003). The "O", the somatic antigens, is determined by the side chains ion lipopolysaccharide molecules on the outer membrane, whereas the "H" the flagellar, "K" capsular and "F" the fimbrial antigens are protein. Thus, *E. coli* serotype O157:H7 is designated by its somatic O and flagellar H antigens. These antigens have characteristics of immunogenicity and pathogencity, hence, they help in the diagnosis of the agent (Quinn *et al*., 2002). There are suitable test kits that are used for serotyping based on their antigenic characteristic (NPH, 2006).

2.3.5. Molecular characteristics

Within the bacterial cell, all the structures involved for growth and multiplication are governed by genome. These genomes include the ribosomal genome, the RNA, the genomic DNA and plasmid DNA's. The DNA are present as a super coiled circular strand, which if stretched out would be up to 1.4 mm long in *E. coli* (Holoda *et al.,* 2005). In addition to these, there may be present in some *E. coli* a separate coil of DNA which are derived from bacterial viruses (bacteriophages) or obtained by transfer from other *E. coli*. These separate DNA pieces may confer on *E. coli* resistance to a number of antibiotics, the ability to grow on certain unusual substrates, to produce certain virulence factors, and other characteristics may not known or any combination of these. One of such DNA is plasmid DNA (Vidal *et al.,* 2004). Horizontal acquisition of the locus for the enterocytes effacement (LEE) locus, which is patogenecity island, genetic material by *E. coli* O157:H7 that inserted in to the genome may also be responsible for genetic variation. The LEE has low Guanine + Cytosine base content. Molecular characteristics are important in diagnostic procedures for instance, when Shiga toxin(s) is (are) not demonstrated, so that detection of one or more toxin genes by PCR is used for confirmation (Vidal *et al.,* 2004; Holoda *et al.,* 2005).

2.4. Virulence factors

The infectious dose of *E. coli* O157:H7 is reported to be as few as one to 100 CFU/mL (Welinder-Olsson *et al.,* 2005; Robins-Browne, 2005) which is lower than most other enteric pathogens. The low infectious dose exemplifies the potent virulence of *E. coli* O157:H7, and the virulence of this microorganism stems primarily from the activities of three major virulence factors (Robinson and McKillip, 2010).

The first and by far the most critical virulence factor is the production of one or both phage-encoded Shiga toxins (Stxs) called Stx1 and Stx2 (Welinder-Olsson *et al*., 2005; Orth and Wurzner, 2006), and these Stxs are among the most potent cytotoxins currently known to affect eukaryotic cells. Each Stx is composed of a single A subunit (or active subunit) and 5 identical B (or binding) subunits (Jores *et al.*, 2004) and are therefore, members of the AB5 family of toxins. The B subunit binds to globotriaosylceramide (Gb3) receptors located on the surface of a variety of

host endothelial and epithelial cells (Jores *et al.,* 2004), and the A subunit is internalized through endocytosis where it ultimately inhibits protein synthesis in the host cell by exerting its nglycosidase activity (Robinson and McKillip, 2010).

Stx1 is nearly identical to the Shiga toxin produced by *Shigella dysenteriae* and differs only by a single amino acid. However, Stx2 is by far the more virulent of the two toxins (Jores *et al.,* 2004). To illustrate, *E. coli* O157:H7 can produce either one or both of the Stxs, but the production of Stx2 alone is still more virulent than either the production of Stx1 alone or the production of both Stx1 and Stx2 simultaneously. In addition, there are several variant forms of each Stx including Stx1, Stx1c, and Stx1d as well as Stx2, Stx2c, Stx2d, Stx2d activatable, Stx2e, and Stx2f, and these variants differ in their activities and potencies as well. For example, Stx2e typically affects swine and binds to Gb4 receptors (Bielaszewska and Karch, 2005), while Stx2c and Stx2d more commonly lead to hemorrhagic colitis and HUS in humans and further demonstrates the greater virulence of Stx2 compared to Stx1. Although the type of Stx produced directly influences the severity of disease, the production of Stxs alone is insufficient for *E. coli* O157:H7 to mount a successful toxicoinfection. Instead, the assistance of the locus of enterocyte effacement (LEE) and the pO157 plasmid is required (Welinder-Olsson *et al.,* 2005).

The LEE is a bacterial pathogenicity island, which is a fragment of DNA that encodes a battery of specialized virulence factors. Typically, pathogenicity islands, including the LEE, are transferred between bacteria through lateral gene transfer, insert into the bacterial chromosome, and augment overall virulence. The LEE is present in *E. coli* O157:H7 as well as in other pathogenic *E. coli* and is essential for colonizing eukaryotic cells (Jores *et al.,* 2004). A number of critical components are encoded within the LEE and include the type III secretion system (TTSS), an adhesin protein known as intimin, the translocated intimin receptor (Tir), and a number of effector proteins (Ceponis *et al.,* 2005). The expression of these components is stringently regulated and influenced by ambient conditions, multiple regulators, and quorum sensing. When expressed, the components orchestrate the formation of the very characteristic attaching and effacing (A/E) lesions that develop as a result of *E. coli* O157:H7 infection (Kaper *et al.,* 2004).

Histopathologically, the A/E lesion is distinguished by the destruction (or effacement) of brush border microvilli and by the intimate bacterial attachment to the host epithelial cell. The formation of the A/E lesion is coordinated by the TTSS, which is a complex, multi-subunit organelle encoded within the LEE. Each LEE-encoded subunit of the TTSS is systematically assembled in order to form a complete and functional apparatus (Kaper *et al.*, 2004). The apparatus ultimately forms a pore in the host cell membrane and also serves as a portal, or channel, through which bacterial effector proteins can pass from the bacterial cell into the host cell. Once inside, the effector proteins influence signal transduction and the phosphorylation of numerous proteins that force actin to polymerize, leading to microvilli effacement and to cytoskeletal rearrangements that form the actin pedestals. Finally, following actin rearrangement, the bacteria transfer a protein called the translocated intimin receptor (Tir) through the TTSS, which embeds into the host cell membrane and serves as the receptor for the intimin adhesin protein. Once intimin interacts with the Tir, intimate attachment of the bacterial cell to the host cell occurs and promotes bacterial colonization of the intestinal tract (Frankel and Phillips, 2008).

In addition to the LEE, the pO157 plasmid also plays a role in the virulence and pathogenicity of *E. coli* O157:H7 (Lim *et al.*, 2007). The pO157 is highly prevalent in *E. coli* O157:H7 compared to other plasmids, and it is postulated that nearly all strains contain the pO157. The specific functions of pO157 in relation to *E. coli* O157:H7 pathogenicity remains largely unknown and is often debated in the scientific literature (Yoon and Hovde, 2008). Some have asserted that the pO157 is involved in the expression of fimbriae that promote and strengthen bacterial adherence to host cells. Conversely, other research indicates that the pO157 has little influence on bacterial adhesion. Although many of the pO157 functions are disputed, the sequence of the large plasmid is known as well as a handful of its gene products (Robinson and McKillip, 2010).

The pO157 plasmid is approximately 90kb and contains 100 open reading frames (ORFs) (Yoon and Hovde, 2008). Furthermore, it is known that pO157 encodes a type II secretion system, an enterohemolysin, a serine-protease, a catalase-peroxidase, a lymphocyte inhibitor, potential adhesins, and others. The function and impact of these components is not fully understood, but the pO157 remains a primary area of interest in the scientific domain (Robinson and McKillip, 2010).

2.5. Epidemiology

2.5.1. Distribution

E. coli O157:H7 infections occur worldwide and this have been reported on every continent except Antarctica (CFSPH, 2009). Since its recognition in 1982, it has become an important concern in North America, Europe, South Africa, Japan, South America, and Australia. High rates are present in regions of South America, especially Argentina, where HUS is endemic (Acha and Szyfres, 2001). The highest incidence rates occur in children, in elders and immunocompromised peoples. Cattle feces are the most important source of *E. coli* O157:H7, however, the presence of *E. coli* O157:H7 in the feces of other animal species has been well recognized. Thus, it is distributed globally in the soil, water, vegetation, decaying matter, and the large intestine of most animals and humans (Chapman *et al.*, 2001). *E. coli* O157:H7 has been also reported in ground beef in countries like Ireland, Norway, Canada, Spain, Italy, Egypt (De Boer and Hauvelink, 2001) and Ethiopia (Demisse, 2005; Mutaku *et al*., 2005; Hiko *et al.,* 2008; Mersha *et al.,* 2010)

2.5.2. Reservoir

Livestock are the most important reservoir of *E. coli* O157:H7 with cattle being the principal sources (Chapman *et al.,* 2001; Sargeant and Smith, 2003).The first identified outbreaks of *E. coli* O157:H7 were associated with consumption of ground beef, and the importance of cattle as a reservoir for *E. coli* O157:H7 became evident as more outbreaks were associated with undercooked beef and other bovine products such as unpasteurised milk (JOINT FAO/WHO, 2002). Cattle are now considered to be the major source of *E. coli* O157 causing human disease and transmission may occur through a variety of routes. In addition to the contamination of meat and dairy products, bovine feces can contaminate drinking water and crops intended for human consumption (Akashi *et al.,* 1994; Leclercq *et al.,* 2002). Various outbreaks have been associated with vegetable products, such as radish and apple cider, presumably following contamination with animal wastes (JOINT FAO/WHO, 2002).

In addition to cattle, *E. coli* O157 strains have been isolated from a variety of other species including horses, sheep, goats, elk, deer, opossums, raccoons, dogs, poultry, galliforms (chicken-like birds), wild birds such as the passerines (finches, canaries, songbird), columbiforms (pigeons and doves), raptores (birds of prey), and ratites (emus and ostriches), that have high incidence of normal gut flora providing host protection against colonization by harmful microbes. Even it was also found in houseflies (Radostitis *et al*., 2000; Davis *et al.*, 2005). Animals that are not normal reservoir hosts for *E. coli* O157:H7 may serve as secondary reservoirs after contact with ruminants (CFSPH, 2009).

2.5.3 Mode of transmission

EHEC are transmitted by the fecal–oral route. They can be spread between animals by direct contact or via water troughs, shared feed, contaminated pastures or other environmental sources. Birds and flies are potential vectors. In one experiment, EHEC *E. coli* O157:H7 was transmitted in aerosols when the distance between pigs was at least 10 feet. The organism was thought to have become aerosolized during high pressure washing of pens, but normal feeding and rooting behavior may have also contributed (CFSPH, 2009).

The environments of domestic ruminants, especially cattle, may be an important reservoir for *E. coli* O157:H7 and pose a continued risk for human exposure. These environment acts as source of infection for animals and human (CDC, 2005; Davies *et al.*, 2005). Little work has been done to investigate *E. coli* O157:H7 survival in the environment and studies primarily focused on bacterial survivability in farm animal manure slurry or water that act as a source of infection for itself and/or other animals (Gansheroff and O'Brien, 2005).

The most frequent mode of transmission for *E. coli* O157:H7 infection is through consumption of contaminated food and water. However, it may also spread directly from person to person and occasionally through occupational exposure. Outbreak investigations have provided important information about how this pathogen is transmitted (Richard, 2006) (Figure 1).

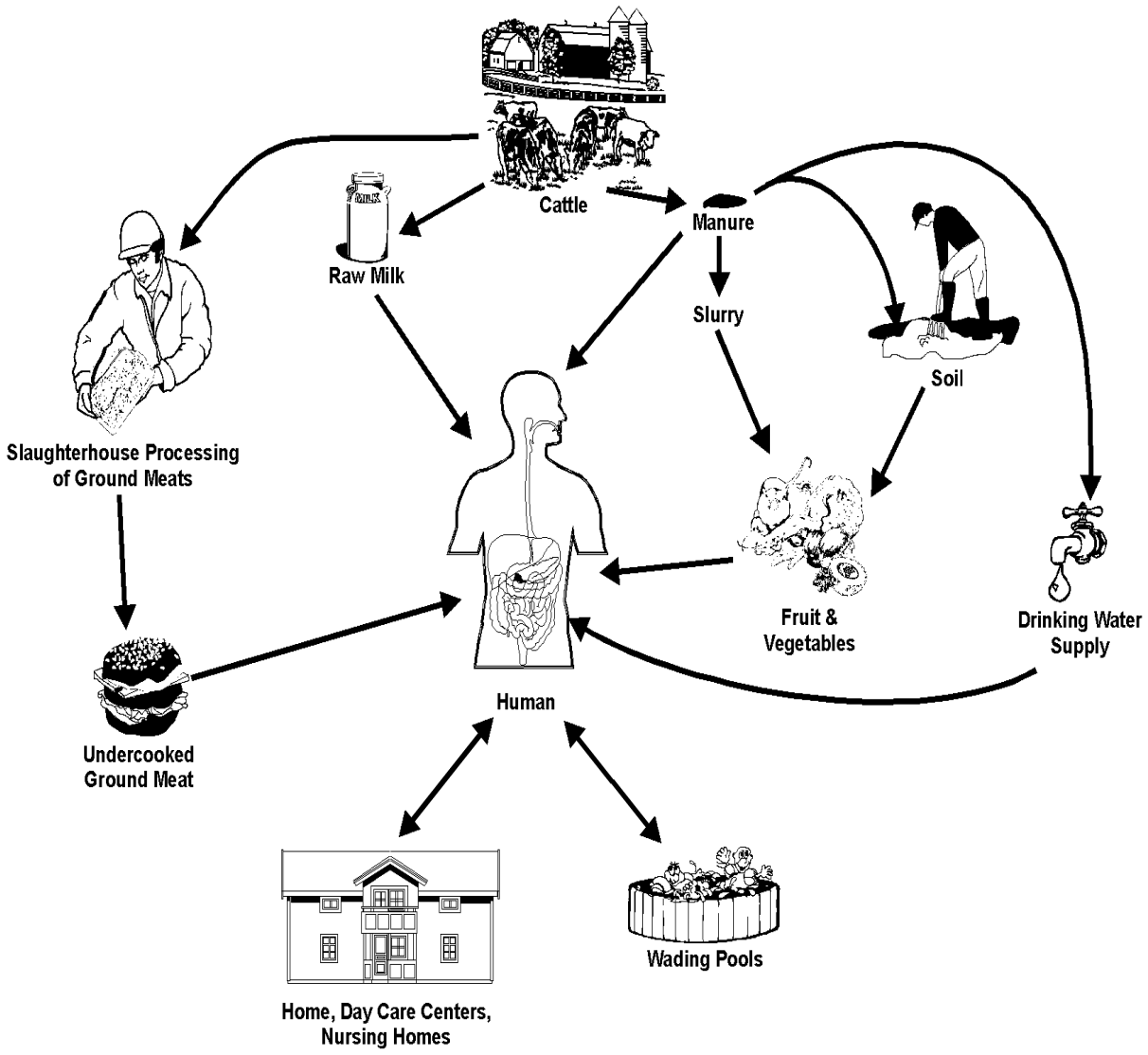

Figure 1: Model for transmission of *E. coli* O157:H7 from cattle to humans

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

Transmission has been primarily been linked to undercooked meat. Beef may become contaminated during slaughter, and the process of grinding beef may transfer pathogens from the surface of the meat to the interior. If ground beef is then incompletely cooked, the bacteria can survive and be ingested (Richard, 2006).

The bacteria can also spread from one food item to another on hands, cooking tools, cutting boards, and food preparation surfaces that have not been thoroughly cleaned and disinfected after contact tools with raw meat (Annoynmous, 2006).

Any food that can be contaminated by beef, cow manure, contaminated water, or an infected food handler can be a source of infection. Fruits and vegetables have recently accounted for an increasing number of outbreaks. Radish sprouts were implicated in several outbreaks in Japan, including the massive Sakai City outbreak in 1996, which affected more than 6000 school children. In the United States, recent outbreaks of *E. coli* O157:H7 infections have been caused by lettuce and alfalfa sprouts (Richard, 2006).

Human or animal feces harbouring *E. coli* bacteria can contaminate water. People can be infected with contaminated city or town water supply that has not been properly treated with chlorine or when people accidentally swallow contaminated water while swimming in a lake, pool, or irrigation canal (Annoynmous, 2006).

Bacteria in stools of infected persons can be passed from one person to another if hygiene or hand washing habits are inadequate. This is particularly likely among toddlers who are not toilet trained (IFT, 2003; CDC, 2005). The bacteria can also be passed from person to person in day care centers and nursing homes, at communal bathroom, through touching things, especially food (IFT, 2003; CDC, 2005). Thus, fecal-oral person-to-person transmission is often reported in family members who contracted the disease from food or water (MOH, 2001; Gansheroff and O' Brien, 2005).

Occupationally, outbreaks due to direct contact with animal's visits (Durso *et al*., 2005; OIE, 2008) among nurses, microbiologists and laboratory workers (Mead and Griffin, 1998) have been reported. While some produce-associated outbreaks may be due to cross-contamination from meat products, others are more likely to reflect direct contamination in the field with feces of wild or domestic animals (Johnson *et al*., 2001).

2.5.4. Prevalence

Prevalence of *E. coli* O175:H7 in the world

The intestinal carriage of *E. coli* O157:H7 in bovine was 4.7% in Great Britain (Milnes *et al*., 2008), and the prevalence of *E. coli* O157:H7 at slaughterhouse level was 27.2% at lairage and 28.8% on cow hides (Small *et al.,* 2002). In Northern Spain, the herd prevalence of *E .coli* O157:H7 amounted to 7.0% and 1.6% in dairy and beef, respectively. While the prevalence of non *E .coli* O157:H7 in dairy and beef was 20.7% and 46.0% (Oporto *et al*., 2008). In beef products of Australia, *E. coli* was detected in 17.8% of chill ground beef samples at retail outlet, of which 0.3% were *E. coli* O157:H7 (Phillips *et al.*, 2008).

Prevalence in Ethiopia

Very few attempts have been made to identify *E. coli* O175:H7 under Ethiopia conditions. Isolation of the agents from stool samples at Bishoftu Hospital and from minced beef from Addis Ababa supermarket by Demissie (2005), from cattle, sheep and goat meat at export and municipal abattoirs in Debre-Zeit and Modjo by Hiko *et al.,* (2008) and from feces, skin and carcasses as well as total and fecal coliforms on carcasses of sheep and goat's at export abattoir in Modjo by Mersha *et al.,* (2010), from beef, sheep and goats meat at Dira Dawa by Lula (2011) have been reported. These reports indicated the presence of *E. coli* O175:H7 in the country. On other hand, *E coli* O157:H7 has been isolated and studied from "Borde" and "Shamita", a traditional fermented beverages (Tadesse *et al.,* 2005), its survival in the processes of yoghurt and cheese makings (Tsegaye and Ashenafi, 2005), concerning its growth potential in fresh tropical fruit juices (Mutaku *et al*., 2005) and isolation of the agent at Dil-chora hospital from patients with complication of urinary tract infection at Dire Dawa (Faris, 2011) in the country.

2.6. Pathogenesis

Following the ingestion of contaminated foods, *E. coli* O157:H7 withstands the acidic environment of the human stomach and begins the arduous and complex process of infection (Figure 2). From the point of ingestion, the incubation period of *E. coli* O157:H7 ranges from 8 hours to 16 days, but the typical incubation period is three to four days (Robinson and McKillip, 2010).

First, *E. coli* O157:H7 must initially adhere to the microvilli of the host epithelial cells (Mainil and Daube, 2005). The association between the bacterial and host cells consequently induces the expression of the TTSS genes located on the LEE (Robinson and McKillip, 2010).

The alterations in signal transduction are accomplished through the activities of the bacterial effector proteins, and, by selective phosphorylation, the effector proteins force actin to polymerize and the cytoskeleton to reorganize (Frankel and Phillips, 2008). Reorganization results in the effacement of host cell microvilli. At this point, the bacteria may superficially adhere to the host cell while simultaneously preparing for intimate adherence (Robinson and McKillip, 2010).

Intimate adherence of the bacterial cell to the host cell requires the orchestrated activities of LEE-encoded intimin and the specialized Tir. Once inserted, intimin, located on the surface of the bacterial cell, associates with Tir and causes a more exaggerated rearrangement of the host cytoskeleton. This rearrangement forms the characteristic pedestal of the A/E lesion and enables the bacterial cell to intimately adhere to the host cell. As more *E. coli* O157:H7 intimately adhere to host epithelial cells and form the A/E lesions, the bacteria begin to overwhelm the normal microflora of the intestinal tract and successfully colonize the host (LeBlanc, 2003). However, the exact means by which *E. coli* O157:H7 establishes and sustains colonization in the host remains elusive (Robinson and McKillip, 2010).

Once it has successfully colonized and established itself within the host, *E. coli* O157:H7 produces and releases its Stxs in the intestinal lumen (Welinder-Olsson and Kaijser, 2005; Mainil and Daube, 2005). Furthermore, the Stxs can translocate from intestinal epithelial cells into the bloodstream. Here, the Stxs bind to the Gb3 receptors on glomerular endothelial cells. The Stxs

injure the glomerular cells and cause platelets and fibrin to deposit within the glomeruli. Eventually, the deposits decrease renal filtration and lead to the acute kidney damage characteristic of HUS (Robinson and McKillip, 2010).

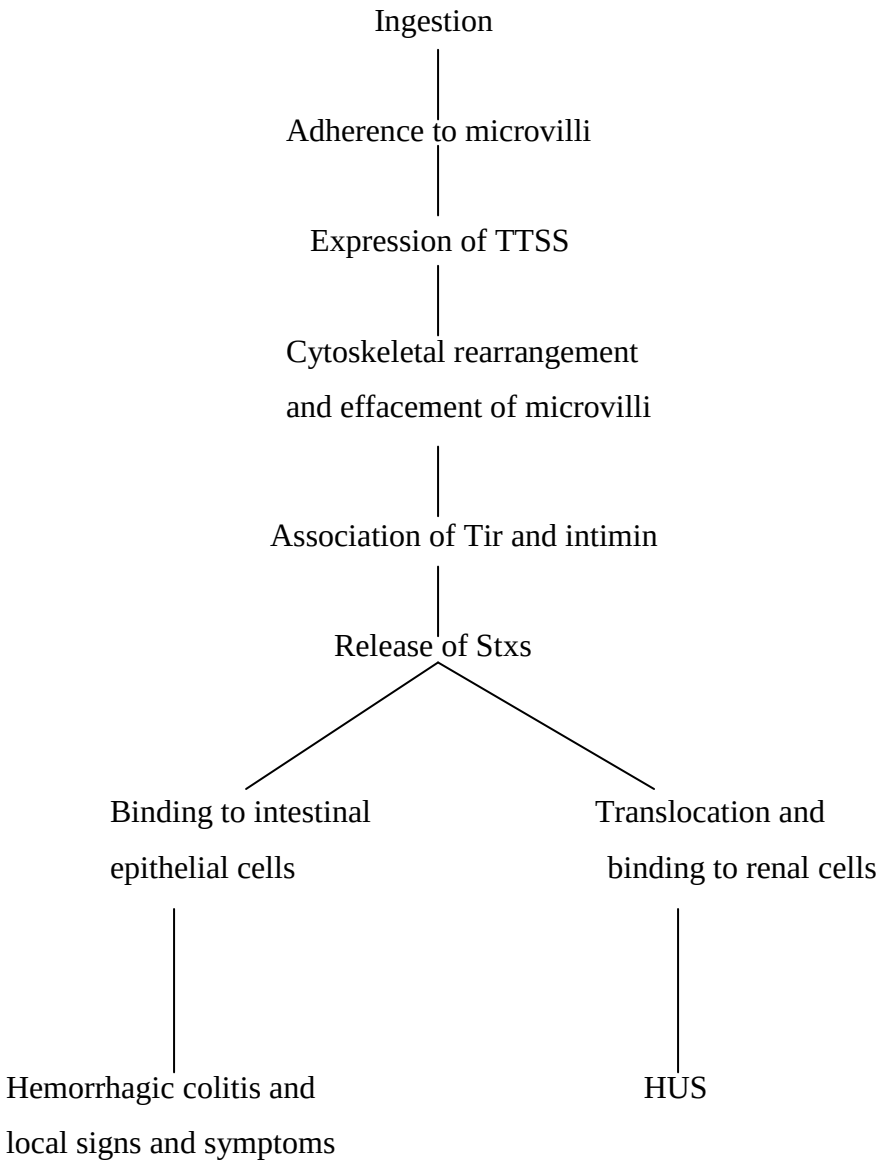

Figure 2: A schematic outline of the progression of *Escherichia coli* O157:H7 infection beginning with ingestion of the bacteria to the development of signs and symptoms (Robinson and McKillip, 2010).

2.7. Disease pattern

Escherichia coli O157:H7 infection in humans shows clinical manifestations that range from carrier state to more complicated forms of the disease that includes hemorrhagic colitis (HC), hemolytic uraemic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) and finally death. The disease affects any age groups however, most frequently in infants (less than 4 years), elders (greater than 65 years) and immunocompromized individuals (Boyce *et al*., 1995; Mead and Griffin, 1998; CDC, 2005; Moses *et al.*, 2006).

2.7.1. Hemorrhagic Colitis

Hemorrhagic colitis caused by *E. coli* O157:H7 is a clinical syndrome that consists of abdominal cramps; diarrhea that progresses to become bloody; radiologic or endoscopic evidence of clonic mucosal edema, erosion, or hemorrhage; and the absence of conventional enteric organisms in the stool. Infection with *E. coli* O157:H7 usually begins with the sudden onset of severe abdominal cramps, which are followed within hours with watery diarrhea that progresses to grossly bloody stools. Upper gastrointestinal symptoms such as nausea and vomiting occur early and may be prominent (Su and Brandt, 1995). Furthermore, the absence of a fever or the presence of only a very low-grade fever is another hallmark of hemorrhagic colitis. Although the illness is agonizing, it is typically self-limiting and resolves itself in approximately one week (Robinson and McKillip, 2010).

2.7.2. Hemolytic Uremic Syndrome

The hemolytic uremic syndrome (HUS) is a renal disorder that primarily affects children and the elderly and is considered to be the leading cause of childhood acute renal failure (Petridis *et al.*, 2002). HUS targets renal endothelial cells and is distinguished by the development of microangiopathic hemolytic anemia accompanied by fragmentation of red blood cells, thrombocytopenia, uremia, and acute renal failure (Robinson and McKillip, 2010). Most patients with hemolytic uremic syndrome have gastrointestinal prodromes, and non-bloody and bloody

diarrhea is the most common presenting symptoms. Other presenting symptoms include abdominal cramps, vomiting, fever, lethargy, seizure, pallor and respiratory distress; some patients have no distinct prodromes. Rectal prolapse, toxic megacolon, and ascitis have also been reported. Common neurologic manifestations include irritability and lethargy, but serious complications, such as seizure and coma, can occur (Petridis *et al.*, 2002).

2.7.3. Thrombotic Thrombocytic Purpura

Thrombotic thrombocytopenic purpura (TTP) is similar to hemolytic uremic syndrome, except that it more commonly afflicts adults instead of children. In addition to the renal attack characteristic of hemolytic uremic syndrome thrombotic thrombocytopenic purpura has an associated fever with the addition of neurological symptoms including sever headaches, convulsions, lethargy, and encephalopathy result from aggregation of platelets in various organs (Petridis *et al.*, 2002).

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

Because humans do not normally carry EHEC, clinical cases can be diagnosed by finding these organisms in fecal samples. Food and environmental samples may also be tested to determine the source of the infection. EHEC are sometimes difficult to identify. They are a minor population in the fecal flora or food. They also closely resemble commensal *E. coli* except in verocytotoxin production. However, the verocytotoxin alone does not necessarily identify an organism as EHEC; additional virulence factors must also be present. Many diagnostic laboratories can detect verocytotoxin-producing *E. coli* (VTEC) and identify EHEC O157:H7. There is no single technique that can be used to isolate all EHEC serotypes (OIE, 2008).

2.8.1. History and clinical finding

The diagnosis of EHEC can be based on history of patients and clinical findings both in humans and animals. *E .coli* O157: H7 is an important enteric pathogen causing symptoms ranging from mild watery diarrhea, bloody cramps, fever, and nausea or vomiting to hemorgic colitis and

hemolytic uremic syndrome (Acha and szyfres, 2001; IFT, 2003). Thus, definitive diagnosis is necessary for differentiation from these and other enteric diseases (Radostits *et al.,* 2000; IFT, 2003).

2.8.2. Identification

Isolation

Escherichia coli O157:H7 is usually isolated by detecting the bacterium from appropriate respective sample. Common sample are diarrheic feces in animals, expected food item in both animal and human food, stool of infected individual in human with hemolytic-uremic syndrome and from foodborne outbreaks (IFT, 2003; Davis *et al.*, 2005). Most hospital laboratories perform test for this particular strain, especially infected individual has bloody diarrhea (Leclereq *et al*., 2002; Quinn *et al.,* 2002; IFT, 2003). However, antibiotic treatment decreases the chance of recovery of *E. coli* O157: H7; therefore, when follow-up specimens are being obtained, the patient should have history of not receiving antibiotic for a minimum of 48 hours before culture (CDC, 2005).

MacConkey agar is the most frequently used primary selective and differential agar. Gram-negative bacteria that grow on MacConcay agar can be differentiated by the ability to ferment lactose. They are bright pink on MacConcay plates which contain pH indicator neutral red owing to the production of acids (Quinn, *et al.,* 2002; Todar, 2005), and they may be surrounded by a zone of precipitated bile. While non-lactose utilizers, remain uncolored (Todar, 2005). MacConkey agar containing 1% D-sorbitol instead of lactose is a useful and inexpensive medium for the isolation of *E. coli* O157:H7. On this media non-sorbitol fermenting *E. coli* grow as small, round, shinny, grayish-white to colorless colonies (Chapman *et al.,* 2001; Feng *et al*., 2002). Selectivity is improved by the addition of 0.05mg/litre cefixime and 2.5 mg/liter potassium tellurite on CT-SMAC, which has a greater inhibitory effect against non-O157 *E. coli* and other non-sorbitol fermenters, such as *Aeromonas, Plesiomonas, Morganella* and *Providencia* (Zadik *et al.,* 1993; Chapman, 2001; Sargeant and Smith, 2003; OIE, 2008).Besides, Rainbow agar O157 is used in

screening pathogenic and non-toxigenic *E. coli* strain including other particular species resembling to *E. coli* sterains (Table 1) (BTS, 2003).

Table 1: Strain identification using Rainbow Agar O157.

Organism	Colony coloration
E. coli O157: H7	Black or gray
E. coli O157: H7	Purple-blue glucuronidase positive
E. coli O26: H11	Purple-magenta
E. coli O48: H21	Purple
E. coli O111: H- or O111: H8	Violet or gray
Non-toxigenic *E. coli*	Pink or magenta

Source: (BTS, 2003)

Specimens from which sorbitol-negative colonies have been isolated that agglutinates in O157 antiserum or O157 latex reagent, and is biochemically *E. coli*, may be reported as presumptively positive for *E. coli* O157:H7 (Feng *et al*., 2002).

2.8.3. Serology

Immunoassays to identify O and H antigens and VT may be used to confirm the identity of the organisms once isolated from clinical, food or environmental samples, while others, including dipstick and membrane technologies, microplate assays, colony immunoblotting, immunofluorescence and enzyme linked immuno sorbent assay ELISA, are used as rapid methods for detecting the presence of potential pathogens in samples prior to isolation, thus shortening the time for a presumptive diagnosis. Most assays for somatic and flagellar antigens are designed to detect the O157 LPS and H7 flagellar antigen. Toxin assays have the advantage of detecting all VTEC. Enzyme immunoassays for O157 and VT, visual immunoassays for O157 and agglutination tests for O157, H7 and VT are available commercially as kits (Clifton-Hadley, 2000; Boer and Heuvelink, 2001). Not all have been validated for use with feces. Specialized reagents in which anti-O157 LPS antibodies are conjugated to fluorescein, peroxidase or phosphatase are also

available. Of the enzyme immunoassays, the most commonly used format is a sandwich assay. Antibody is bound to a carrier surface to capture a specific VTEC antigen; following the addition of an appropriate substrate, a second antibody with an enzyme label binds to this antigen and produces a colour reaction. The kits have been validated with specific pre-enrichment protocols and reagents to ensure reproducible results. Some use heat-treated samples thus improving the safety of the test, and some incorporate an automated processing system to screen large numbers of samples. Others are blot ELISAs developed to screen colonies for O157 antigen. The commercial kits have the advantage of being easy to perform in routine laboratories, and tests should be carried out according to the manufacturers' instructions. Kits validated for food and carcass samples or for human clinical samples may lack sensitivity for animal feces samples. Immunological assays only give a presumptive result, which must be confirmed by isolation and characterization of the organisms producing the O157 antigen or the toxin (OIE, 2008).

2.8.4. Molecular techniques

DNA probes and others PCR assays and microarrays have been developed to detect genes in VTEC shown to be associated with virulence in humans, including *eae* (encoding for intimin), *ehx* (encoding for enterohaemolysin production), *fliC* (encoding the H7 antigen), O157 *rfb* (encoding O157 LPS), *uidA* (the mutant glucuronidase gene in beta-glucuronidase-negative *E. coli* O157:H7) and *katP* (a gene carried on the large plasmid of *E. coli* O157:H7 encoding a novel catalase peroxidase) (Bekal, 2003; OIE, 2008). A variety of multiplex assays has been developed to detect simultaneously several diagnostic genes. These assays are of value in the characterisation of pure cultures. On mixed populations of bacteria in food or feces samples, they may have a use in identifying samples to which isolation procedures should be targeted (OIE, 2008).

2.9. Treatment

Antimicrobial agents have no proven value in the treatment of *E. coli* O157:H7 infections (Riemann and Cliver, 2006). The use of antibiotics in the treatment of infection is controversial, since antimicrobial therapy may increase the risk of development of HUS (Molbak *et al*., 2002). Antimicrobial may induce the expression of the Shiga toxins, and/or bacterial injury caused by the

antibiotic may result in increased release of preformed toxins. Wilkerson *et al*. (2004) reported that some antimicrobial agents, particularly quinolones, trimethoprim, and furazolidone, were shown to induce toxin gene expression and should be avoided in treating patients with confirmed *E. coli* O157 infections Therefore, management of patients with *E. coli* O157:H7 infection is supportive. Patients with documented infection should be monitored for dehydration and other signs of symptoms that might suggest HUS. Patients with complications may require intensive care including dialysis, transfusion and/or platelet infusion. Patients who develop irreversible kidney failure may need a kidney transplant (CFSPH, 2009).

Selection of antibiotic for the treatment of *E. coli* O157:H7 has to be done based on antimicrobial susceptibility test. Empirical treatment end with increase risk of infection and resistance to antimicrobials. Over the past 20 years, there has been an increase in antimicrobial resistance observed in *E. coli* O157:H7 isolates (Schroeder *et al*., 2002; Wilkerson *et al.,* 2004). Resistance to streptomycin was the most common resistance found in bovine and human *E. coli* O157:H7 isolates (Wilkerson *et al.,* 2004).

2.10. Prevention

The prevention of infection requires control measures at all stages of the food chain, from agricultural production on the farm to processing, manufacturing and preparation of foods in both commercial establishments and the domestic environment (WHO, 2010).

The HACCP system continues to be the most effective means for systematically developing food safety protocols that can reduce the risk of *E. coli* O157:H7 infections. These pathogens however, pose some unique problems when developing and implementing HACCP plans. For example, the low incidence of *E. coli* O157:H7 in food makes direct microbiological testing for the pathogen as a means of verifying the effectiveness of a HACCP program of limited benefit. In such instances, verification based on microbiological analysis would have to depend on the use of an appropriate indicator microbe that could provide a measure of how well a process controls factors associated with risk of *E. coli* O157:H7 contamination. An important component of HACCP application in animal production at farms is reducing the carriage of *E. coli* O157:H7 in animals. Two

approaches that have potential are competitive exclusion and vaccination (Sargeant and Smith, 2003).

At slaughterhouse**,** the ultimate source of *E. coli* O157:H7 on carcasses is fecal contamination. Traditional trimming procedures of the visible fecal contamination, pre-evisceration washing, rinsing of carcass surfaces with solutions of organic acids on carcass surfaces and equipment can reduce *E. coli* O157:H7, with different degree of effectiveness (Besser, *et al.,* 2003). Most desirable are a process that is lethal to the pathogen and a critical control points associated with preventing pathogen growth. Such plan shall include skinning, post-skinning rinsing/bactericidal spray, evisceration, final bactericidal spray, chilling and maintenance of refrigeration as likely critical control points (Sargeant and Smith, 2003).

At food processing center, *E. coli* O157:H7 can be controlled readily through traditional thermal processing techniques; however, the organism's low infectious dose requires effective processing that eliminates the pathogens completely (MOH, 2001).

Proper food handling practices at food service and home preparation are the most and last line of defense in the prevention of *E. coli* O157:H7 infection. Generally, adequate cooking temperatures and time, prevention of cross contamination between raw and cooked, and proper storage are key factors for reducing the risks associated with *E. coli* O157:H7 (CFSPH, 2009).

Contamination of public water supplies is prevented by standard water treatment procedures. Livestock-should be kept away from private water supplies. Microbiological testing can also be considered. To the extent possible, people should avoid swallowing water when swimming or playing in lakes, ponds and streams (CFSPH, 2009).

Moreover, good hygiene, careful hand-washing and proper disposal of infectious feces can reduce person-to-person transmission. Thorough hand washing is especially important after changing diapers, after using the toilet, and before eating or preparing food. Bed linens, towels and soiled clothing from patients with hemorrhagic colitis should be washed separately, and toilet seats and flush handles should be cleaned appropriately (CFSPH, 2009).

On the other hand, a number of vaccine protocols for use in cattle and humans are being investigated (Horne *et al*., 2002). A plant cell-based intimin vaccine tested in mice showed the development of an intimin-specific mucosal immune response and a reduced duration of shedding of *E. coli* O157:H7 (Judge *et al*., 2004). This plant-based vaccine system is being explored for oral administration to cattle to decrease shedding of the pathogen. Vaccination of cattle with type-III secreted proteins reduced the duration of shedding and numbers of *E. coli* O157:H7 in feces (Potter *et al*., 2004). Additionally, the prevalence of the organism in cattle was reduced in a clinical trial conducted under conditions of natural exposure in a feedlot setting. A vaccine consisting of liposomes incorporating monophosphoryl lipid A and antigens from an *E. coli* O157:H7 lysate induced IgG and IgA serum-antibody and mucosalantibody responses in immunized mice A number of other vaccine strategies, including toxoid and O-specific polysaccharide-protein conjugate vaccines, for prevention of EHEC disease are under investigation (Tana *et al*., 2003).

3. MATERIALS AND METHODS

3.1. Study area

The study was carried out in Addis Ababa central Ethiopia. Addis Ababa is the capital city of Federal Democratic Republic of Ethiopia and it has an area of 51,000 hectare in the central highlands with an average altitude of 2000-2560 meters above sea level. The area is characterized by bimodal rainfall with an average of 1100 mm, the highest percentage of rain falls during the long rainy season from June to September. The short rainy season is from February to April. Addis Ababa has an estimated human population of 3.15 million (CSA, 2007).

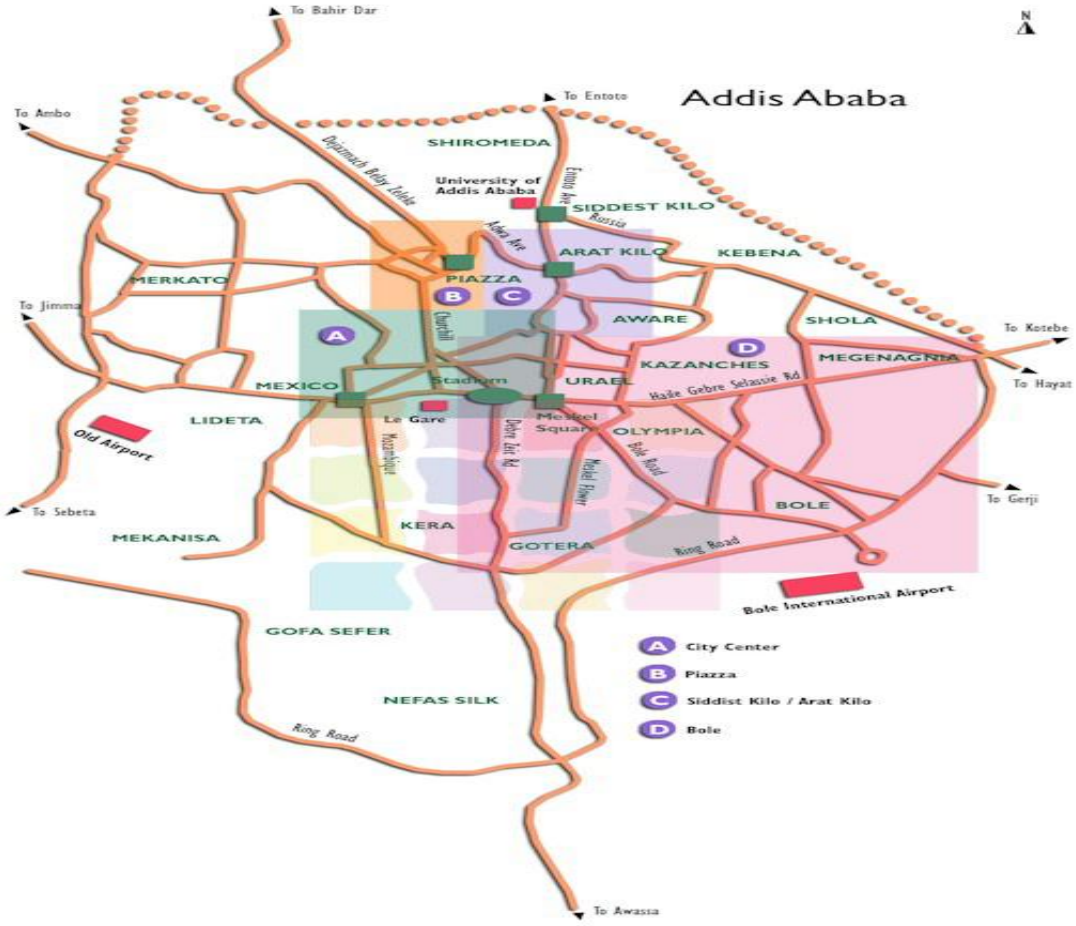

Figure 3: Map of Addis Ababa

3.2. Study design

A cross-sectional study was conducted to determine the prevalence of *Escherichia coli* O157: H7 strains and antibiotic susceptibility test from August 2011 to April 2012 in bovine, sheep and goat's carcasses slaughtered at Addis Ababa Abattoir Enterprise and meat presented for sale in different retail shops in the city.

3.3. Sample size determination

The approximate sample size required was determined, according to Thrusfield (2005), from expected prevalence of 50% with defined precision of 5% and level of confidence of 95%.

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

Where, n=required sample size.

Pexp=expected prevalence.

d=desired absolute precision.

Therefore, the calculated sample size was 384 samples. This is because there is no previous study on the prevalence and antimicrobial test at Addis Ababa abattoir enterprise and retail shops.

3.4. Sampling technique

Swab samples were collected using systematic random sampling technique from the carcasses of daily slaughtered animals at Addis Ababa Abattoir Enterprise. Legally registered meat retailer shops were selected randomly. From the selected meat retail shops raw meat samples were aseptically collected using simple random sampling technique as it was sold to the consumer.

3.5. Study samples

The study was conducted on a total of 384 raw meat samples consisting of 64 beef, 64 mutton and 64 chevon were collected each from Addis Ababa abattoir enterprise (n=196) and retail shops (n=196) that means with the total of 128 beef,128 mutton and 128 chevon .

3.6. Sample collection procedure

Selected carcasses were swabbed using the method described in ISO17604 (2005) by placing sterile template (10 x 10 cm) on specific sites of a carcass. A sterile cotton tipped swab (2X3 cm) fitted with shaft, was first soaked in an approximately 10 ml of buffered peptone water (Oxoid Ltd., Hampshire, England) rubbed first horizontally and then vertically several times on the carcasses. The abdomen (flank), thorax (lateral) and breast (lateral) which are sites with the highest rate of contamination (ISO 17604, 2005) were chosen for sampling. On completion of the rubbing process, the shaft was broken by pressing it against the inner wall of the test tube and disposed leaving the cotton swab in the test tube. A second dry sterile cotton swab of the same type was used as before over the entire sampled area. Twenty five g of raw meat sample was collected from retail shops following aseptic techniques. The samples were put in a sterile universal bottle filled with peptone water. Finally, the samples were transported to the Institute of Biodiversity Conservation Laboratory, Addis Ababa using ice box in cold chain for microbiological analysis. Up on arrival, the samples were stored in refrigerator at 4 ^{O}C.

3.7. Sample preparation

Carcass swabs were homogenized with vortex mixer and 25 grams of raw meat sample collected from each retail shops were chopped and the meat was placed in a plastic bag containing 225 ml of buffered peptone water and homogenized using a homogenizer (Stomacher 400, Seward Medical, England) at high speed for two minutes. The resulting suspension was used for isolation of *Escherichia coli* O157:H7.

3.8. Isolation Procedure

Two hundred µl of prepared sample of a meat rinse solution was streaked onto MacConkey agar (IVD, UK) plates and incubated at 37° C for 24 hrs (Appendix 3a). Following incubation, lactose-positive (pink) colonies were streaked onto Sorbitol-MacConkey Agar (IVD, UK) plates and incubated at 37° C for 24 hrs (Appendix 3b). The sorbitol negative colorless colonies were sub-cultured on Rainbow agar.

Rainbow Agar O157 (Hayward, USA) has both selective and chromogenic properties that make it particularly useful for isolating pathogenic *E. coli* strains. The medium was inoculated by spreading a sample suspected of containing *E. coli* on its surface. The plates were then incubated for 20 to 24 hours, or longer, at 37 °C and observed for the presence of colored colonies. The distinctive black or gray coloration of *E. coli* O157:H7 colonies were easily viewed by laying the Petri dish against a white background (Appendix 4). Upon sub-culturing, the isolated *E. coli* O157:H7 colonies showed their typical black or gray coloration (BiOLOG User Guide, 2008).
This medium was not used as the sole basis for identification of microorganisms. Any colony suspected of being *E. coli* O157:H7 or another toxin producing serotype was tested further to verify its identity using BiOLOG identification system (BiOLOG User Guide, 2008).

3.9. Identification

Pure colonies from Rainbow agar were inoculated on BUG agar (Hayward, USA) with 5% sheep blood and incubated at 37°C for 24 hrs. Sub-culture was made using the same culture media to have pure culture colonies before identification was done by OmniLog (appendix 5). The BUG (Biolog Universal Growth Medium) is a recommended medium for aerobic bacteria and it was employed to isolate *E. coli*. Following this, the identification of *Escherichia coli* O157:H7 was performed using the Omnilog plus Identification System (BiOLOG User Guide, 2008).

After getting pure culture colonies, identification was carried out using BiOLOG Standard Operation Protocols (SOP). For each inocula preparation, the turbidimeter was blank with the

uninoculated inoculating fluid tube (wiped clean of dirt and fingerprints) by adjusting the 100% transmittance adjustment knob so that the meter reads 100% T (i.e. Inoculating Fluid and Cell Density). For aerobic, enteric bacteria identification, having 61% turbidity in 18-20 ml inoculating fluid was adjusted. A sterile cotton swab was used and the top of a colony was gently touched to pick up a 3 mm diameter area of cell growth from the surface of the agar plate and the organisms were emulsified into the solution using a vigorous motion on the bottom of the inoculating fluid tube to release the bacteria into the inoculating fluid. Any bubble that may have formed was allowed to disperse. The turbidity was read using turbidimeter (annex, 5). The 61% turbidity suspension was adjusted by adding more cells (to increase density) or more inoculating fluid (to lower density).

The MicroPlate was labeled to the side of the MicroPlate itself, not the lid (Biolog imprint) side. Using aseptic technique, the cell suspension was poured into the 8-Channel repeating pipetter (Annex 5). As shown (Annex 5) all MicroPlate wells were filled with 150 (µL/well) by avoiding contamination and touching the bottom of the wells, which could transfer carbon sources then the MicroPlate was covered with its lid. The plates were placed in OmniLog machine (Annex 5) at 37 °C for 22 hrs (BiOLOG User Gide, 2008; Appendix 5). After an appropriate incubation time, OmniLog Identification System automatically read each MicroPlate. The patterns formed from the wells were automatically entered to the system and used for identification (BiOLOG User Gide, 2008).

3.10. Antimicrobial susceptibility pattern

The antimicrobial susceptibility test was performed following the standard agar disk diffusion method according to CLSI (2008) using commercial antimicrobial disks (Table 2).

Table 2: Antibiotic disks used to test *E. coli* O157:H7 and their respective concentrations

NO.	Antibiotic disks	Disc code	Concentration	Diameter of Zone of inhibition in mili meter(mm)		
				Resistant ≤	Intermediate	Susceptible ≥
1	Amikacin	AK	30µg	14	15-16	17
2	Amoxycillin-Clavulanic acid	AMC	20/10µg	13	14-17	18
3	Ceftriaxone	CRO	30µg	14	15-17	18
4	Cephalothin	KF	30µg	14	15-17	18
5	Chloramphenicol	C	30µg	12	13-17	18
6	Ciprofioxacin	CIP	5µg	15	16-20	21
7	Nalidixic acid	NA	30µg	13	14-18	19
8	Streptomycin	S	10µg	11	12-14	15
9	Tetracycline	TE	30µg	11	12-14	15
10	Sulfamethoxazole-Trimethoprim	SXT	30µg	10	11-15	16

Each isolated bacterial colony from pure fresh culture was transferred in to a test tube of 5 ml Tryptone Soya Broth (TSB) (Oxid, England) (Annex-3) and incubated at 37^{o}C for 6 hours. The turbidity of the culture broth was adjusted using sterile saline solution or added more isolated colonies to obtain turbidity usually comparable with that of 0.5 McFarland standards (approximately 3x10^{8} CFU per ml). Mueller-Hinton agar (Bacton Dickinson and Company, Cockeysville USA) plates were prepared according to the manufacturer. A sterile cotton swab was immersed into the suspension and rotated against the side of the tube to remove the excess fluid and then swabbed in three directions uniformly on the surface of Mueller-Hinton agar plates. After the plates dried, antibiotic disks were placed on the inoculated plates using sterile forceps (Appendix 4). The antibiotic disks were gently pressed onto the agar to ensure firm contact with the agar surface, and incubated at 37^{o}C for 24 hours. Following this the diameter of inhibition zone formed around each disk was measured using a black surface, reflected light and transparent ruler

by lying it over the plates. The results were classified as sensitive, intermediate, and resistant according to the standardized table supplied by the manufacturer (CLSI, 2008). *E. coli* ATCC 25922 type strains was used as a positive control.

3.12. Data management and analysis

The coded data was entered in MS Excel and then analyzed using SPSS version 15 (2006). The overall prevalence of *E. coli* O157: H7 in raw meat was determined using standard formula. The number of positive samples was divided by the total number of samples examined multiplied by 100. In addition to these, the prevalence in each meat type as well as at retail shops and abattoir was determined in the same way by dividing positive value with corresponding total examined samples. Difference among and between proportions of the groups with certain determinant factor was determined by chi- square (χ^2) test. A p-value <0.05 was considered indicative of a statistical significant difference.

4. RESULTS

4.2. Prevalence

A total of 384 raw meat samples (128 beef, 128 mutton and 128 chevon) collected from Addis Ababa abattoir enterprise and retailer shops were investigated from August 2011 to April 2012 cross sectionally to determine the magnitude of *E. coli* O157:H7.

The overall prevalence of *E. coli* O157:H7 in three types of raw meat samples (beef, mutton, and chevon) was 39 (10.2%). Of which, 13.3% (17/128) were from beef, 9.4% (12/128) were from mutton and 7.8% (10/128) were from chevon. Beef was found to be highly contaminated than mutton and chevon. The test statistics among raw meat samples with Chi-square ($\chi 2$) = 2.226; degree of freedom (*df*) = 2; P = 0.329 (Table 3) indicated that there was no significance difference in prevalence rate ($p > 0.05$) among three types of raw meat types.

Table 3: Prevalence of *Escherichia coli* O157:H7 in raw meat

Type of raw meat	Total samples examined	Number positive	Prevalence (%)	$\chi 2$	*df*	p-value
Beef	128	17	13.3			
Mutton	128	12	9.4	2.226	2	0.329
Chevon	128	10	7.8			
Total	384	39	10.2			

Out of 39 isolates, the prevalence of *E. coli* O157:H7 in raw meat samples was 11 (5.7%) from abattoir and 28 (14.6%) from retailer shops (Table 4). Higher prevalence of *E. coli* O157:H7 was found in retailer shops than Addis Ababa Abattoir Enterprise. The test statistics between raw meat sample source with $\chi 2 = 8.24$; *df= 1,* P= 0.04, showed significant difference in prevalence ($p<0.05$).

Table 4: Prevalence of *Escherichia coli* O157:H7 between meat source

Meat source	No of samples examined	Number positive	Prevalence (%)	$\chi 2$	*df*	p-value
Abattoir	192	11	5.7			
Retailer shops	192	28	14.6	8.24	1	0.04
Total	384	39	10.2			

The percentages of *E. coli* O157:H7 isolated from beef, mutton and chevon in the abattoir were 3 (4.7%), 4 (6.3%) and 4 (6.3%), respectively (Table 5). There was no statistically significant difference among in three meat types in the abattoir with $\chi 2$= 0.193; *df*= 2, P= 0.908 (p>0.05).

Table 5: Prevalence of *Escherichia coli* O157:H7 in abattoir

Type of raw meat	Total samples examined	*E .coli* O157:H7 positive (%)	$\chi 2$	*df*	p-value
Beef	64	3 (4.7)			
Mutton	64	4 (6.3)	0.193	2	0.908
Chevon	64	4 (6.3)			
Total	192	11 (5.7)			

On the other hand the prevalence of *E. coli* O157:H7 isolated from beef, mutton and chevon in the retailer shop were 14 (21.9%), 8 (10.9%) and 6 (9.4%) respectively (Table 6). The high prevalence was found in beef than goat's meat and sheep meat. The $\chi 2$= 4.348; *df*= 2, P= 0.114 (p<0.05) indicated that there was no significance relationships among tree types of raw meat in retailer shops.

Table 6: Prevalence of *E. coli* O157:H7 in retail shops

Type of raw meat	Total samples examined	*E .coli* O157:H7 positive (%)	$\chi 2$	*df*	p-value
Beef	64	14(21.9)			
Mutton	64	8(10.9)	4.348	2	0.114
Chevon	64	6(9.4)			
Total	192	28(14.6)			

4.2. Antimicrobial susceptibility pattern

The result of antimicrobial susceptibility test of 39 *E. coli* O157:H7 isolated from raw meat samples with 10 selected antimicrobial agents is shown in Table 7. The current study on antimicrobial sensitivity test of *E. coli* O157:H7 recovered from different raw meat types revealed a varying degree of susceptibility to antimicrobial agents tested. *E. coli* O157:H7 was highly susceptible to chloramphenicol (100%), ceftriaxone (100%), sulfamethoxazole -trimethoprim (100%), tetracycline (94.9%), amoxacilin-clavulanic acid (92.3%), cephalothin (82.1%) nalidixic acid (79.5%), ciprofloxacin (71.8%), and amikacin (69.3%). Susceptibility as low as 48.7%, was shown to streptomycin. Intermediate susceptibility was also observed in streptomycin (17.9%), nalidixic acid (15.4%), ciprofioxacilin (10.3%) and cephalothin (5.1%). Furthermore, resistance of 33.4% and 30.8% was developed to streptomycin and amikacin, respectively. Similarly, 5.1%, 5.1%, 7.7%, 12.8% and 17.9% resistance were developed against nalidixic acid, tetracycline, Amoxacilin-clavulanic acid, cephalothin and ciprofloxacin, respectively (Table 7).

The antibiotic susceptibility profile showed a significant difference ($p<0.05$) in the susceptibility pattern of various antimicrobial agents among *E. coli* O157:H7 isolates recovered from different raw meat types. Isolates recovered from chevon were found to be 40% to 80% susceptible to five antimicrobial agents tested except amoxicillin-clavulanic acid, ceftriaxone, cephalothin, chloramphenicol, and sulfamethoxazole-trimethoprim antimicrobial agents showed 100%

susceptibility (Table 7). Similarly, *E. coli* O157:H7 isolates from mutton showed 100 % susceptiblity to amikacin, ceftriaxone, chloramphenicol, sulfamethoxazole-trimethoprim and tetracycline. However, the remaining isolates showed a susceptibility ranging from 58.3% to 91.7%. *E. coli* O157:H7 recovered from beef revealed a susceptibility to antibiotic which ranged from 17.6% to 100%.

Table 7: Antimicrobial susceptibility pattern of *E. coli* O157:H7 isolates (n=39)

Antimicrobial used	Type of raw meat and *E. coli* O157:H7 isolates											
	Beef (n=17)			Mutton (n=12)			Chevon (n=10)			Total (n=39)		
	S No. (%)	I No. (%)	R No. (%)	S No. (%)	I No. (%)	R No. (%)	S No. (%)	I No. (%)	R No. (%)	S No. (%)	I No. (%)	R No. (%)
AK	10(58.8)	0(0)	7(41.2)	10(83.6)	0(0)	2(16.6)	7(70)	0(0)	3(30)	27(69.3)	0(0)	12(30.9)
AMC	17(100)	0(0)	0(0)	9(75)	0(0)	3(25)	10(100)	0(0)	0(0)	36(92.3)	0(0)	3(7.7)
CRO	17(100)	0(0)	0(0)	12(100)	0(0)	0(0)	6(60)	4(40)	0(0)	39(100)	0(0)	0(0)
KF	15(88.2)	2(11.8)	0(0)	7(58.3)	0(0)	5(41.7)	10(100)	0(0)	0(0)	32(82.1)	2(5.1)	5(12.8)
C	17(100)	0(0)	0(0)	12(100)	0(0)	0(0)	10(100)	0(0)	0(0)	39(100)	0(0)	0(0)
CIP	13(76.5)	0(0)	4(23.5)	9(75)	0(0)	3(25)	6(60)	4(40)	0(0)	28(71.8)	4(10.3)	7(17.9)
NA	17(100)	0(0)	0(0)	10(83.6)	2(16.4)	0(0)	4(40)	4(40)	2(20)	31(79.5)	6(15.4)	2(5.1)
S	4(23.5)	7(4.1)	6(35.3)	11(91.6)	0(0)	1(8.4)	4(40)	0(0)	6(60)	19(48.7)	7(17.9)	13(33.4)
TE	17(100)	0(0)	0(0)	12(100)	0(0)	0(0)	8(80)	2(20)	2(20)	37(94.9)	0(0)	2(5.1)
SXT	17(100)	0(0)	0(0)	12(100)	0(0)	0(0)	10(100)	0(0)	0(0)	39(100)	0(0)	0(0)

=See Table 2 for key abbreviations; S= Sensitive, I= Intermidiate, R= Resistant

Out of 39 *E. coli* O157:H7 isolates subjected to antimicrobial susceptibility test using ten different antimicrobials, 9 (23.1%) were susceptible to all antimicrobials used. Out of nine *E. coli* O157:H7 isolates, 5 (29.4%), 2 (16.7%) and 2 (20%) originated from beef, mutton and chevon, respectively (Table 8).

Table8: *E. coli* O157:H7 isolates susceptible to all antimicrobials

Type of raw meat	Number of isolates Tested	Number of isolates susceptible to all antimicrobials sensitive No. (%)
Beef	17	5 (29.4)
Mutton	12	2 (16.7)
Chevon	10	2 (20.0)
Total	39	9 (23.1)

Of the 39 *E. coli* O157:H7 isolates, 7 (17.9%) were found to be resistant to three or more drugs tested (Table 9). Out of the isolates developed multiple drug resistant, 4 (40%) were recovered from beef samples.

Table 9: Multidrug (MDR) resistance of *E. coli* O157:H7

Type of drugs registered as MDR	Source of resistant isolates			Total No. (%)
	Beef (No)	Mutton (No)	Chevon (No)	
AK, S, TE	-	-	2	2 (28.6)
AK, CIP, S	2	-	-	2 (28.6)
AMC, CIP, S	1	-	-	1 (14.3)
AK, KF, CIP, S	1	-	-	1 (14.3)
AK,AMC,KF,S	-	1	-	1 (14.3)
Total MDR No. (%)	4 (57.1)	1 (14.3)	2 (28.6)	7 (17.9)

=See Table 2 for key abbreviations; S= Sensitive, I= Intermidiate, R= Resistant

5. DISCUSSION

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

Raw meat and its products are commonly consumed in traditional Ethiopian diets, but *E. coli* O157:H7 is rarely studied compared to other countries. In the present study, *E. coli* O157:H7 was isolated from beef, mutton and chevon at both raw meat sources at different recovery rates. The overall prevalence of *E. coli* O157:H7 in the present study was 10.2%. This present finding was almost comparable to that reported by Lula (2011) in Dire Dawa (11.2%). When compared to the observation of Hiko *et al.* (2008) in Modjo and Debre Zeit (4.2%) and Mersha *et al.* (2010) at Modjo (5.1%), the present finding is higher. One of the probable reasons could be due to the study sites, meat source and method applied.

The difference in prevalence observed among the three types of meat samples in present study is high (13.3%) in beef, but relatively similar between mutton (9.4%) and chevon (7.8%). Similar prevalence of *E. coli* O157:H7 was reported from cooked beef samples collected from various restaurants in Kars-Turky with 11.25% (Ulukanli *et al.,* 2006) and with 14% (Kiranmayi and Krishnaiah, 2010). In contrast, the prevalence of *E. coli* O157:H7 in this study was lower than 20%, 20%, 19%, 20%, 35% and 53% prevalence reported by Abong (2008) in South Africa, Hajian *et al*. (2011) in Iran, Zhao *et al*. (2011) in USA, Zschock *et al*. (2000) in Europe and Elder *et al.* (2000) and Dahiru *et al*. (2008) in fresh beef meat in Nigeria, respectively. This could be due to the fact that bovine has been implicated as the principal reservoir of this pathogen when it is compared with other food animals (Kim *et al*., 2004). Wide variation (1-90%) in prevalence of *E. coli* O157:H7 in beef had been reported from many countries (De Boer and Hauvelink, 2007). The consumption of raw meat with *E. coli* O157:H7 (Jay, 2005) and consumption of contaminated such raw undercooked meat is the principal source of *E. coli* O157:H7 that cause hemorrhagic colitis, hemorrhagic uremic syndrome, and trombotic thrombocytopenic purpura in human worldwide. Studies have shown that the main source of contamination of *E. coli* O157:H7 is from the skin and hides, rumen and feces. The presence of *E. coli* O157:H7 in mutton and chevon might be due to contamination either from gastrointestinal content and/or skin (McEvoy *et al.,* 2003).

In this study, the prevalence of *E. coli* O157:H7 in mutton and chevon were 9.4% and 7.8 %, respectively (Table 3). The results obtained in this study were higher than the previous study made by Hiko *et al*. (2008) in Ethiopia who reported a prevalence of 2.5% and 2% in sheep and goat's meat, respectively.

The higher prevalence of *E. coli* O157:H7 was found at retailer shops (14.6 %) than Addis Ababa Abattoir Enterprise (5.7%). Statistical analysis of this result showed significant variation ($p<0.05$) in prevalence (contamination) rate between abattoir and retailer shops (the two meat sources) .This difference could be due to the difference in hygienic standard used in the abattoir is better than in the retailer shops. For instance, there was no disinfection of knives or other equipments and the hygiene status of butcher seems to be poor. In addition to this, there could be risk of carcass contamination and cross and subsequent contamination, during transportation, environment, handling of meat at retailer shops. Similar views were expressed by Keen *et al.* (2009).

The prevalence of *E. coli* O157:H7 was similar at abattoir in the beef, mutton and chevon. Statistical analysis at abattoir indicated no significant variation ($p>0.05$) among three raw meat samples. The similarity in the prevalence of *E. coli* O157:H7 among three types of raw meat types might be due to similar slaughtering processes and meat handling at both cattle and small ruminant slaughtering hall in the abattoir.

In the present study, the difference in prevalence was observed among the three types of meat samples in retailer shops. The high prevalence was recorded in beef (21.9%) than chevon (9.4%) and mutton (10.9%). This could be due to the fact that bovine has been implicated as the principal reservoir of this pathogen as compared with other food animals (Kim *et al*., 2004). The prevalence in beef in the present study is comparable with previous study done by Mora *et al*. (2007), but it was higher than the reported prevalence data from Ethiopia in slaughterhouse (8.1%) by Hiko *et al*. (2008) and UK (1.1%) by Chapman *et al*. (2001).

Differences in the reported prevalence could be due to difference in the number of sampling techniques and laboratory methodologies used, and it may also be due to differences in geographical locations. It is interesting to mention that MacEvoy (2003) recovered only one

among the nine positive samples by swabbing but by excision the author recovered six among the nine positive samples. This showed a difference in the prevalence of a given study due to difference in sampling methods.

5.2 Antimicrobial susceptibility pattern of *E. coli* O157:H7

The use of antibiotics in the treatment of infection with *E. coli* O157:H7 is controversial, since antimicrobial therapy may increase the risk of development of HUS (Molbak *et al*., 2002). Although some studies do not advice antibiotic treatment for infections caused by such bacteria (Wong *et al.,* 2000), others suggest that disease progression may be prevented by administrating antibiotic at early stage of infection (Shiomi *et al.*, 2007). Thus, for the better response, antimicrobial susceptibility test is necessary (Quinn *et al*., 2002). Hence, on the basis of this necessity, antimicrobial susceptibility test was conducted on the isolates recovered from raw meat.

Antimicrobial resistance of *E. coli* O157:H7 isolates from animal and human sources have been reported in Ethiopia by Hiko *et al.* (2008), Faris (2011) and Lula (2011). In the present study *E. coli* O157:H7 showed resistance to seven antimicrobials which varied from 5.1% to 33.4% except to ceftriaxone, chloramphenicol, and sulfamethoxazole-trimethoprim to which 100% susceptibleity was noticed. From these *E. coli* O157:H7 isolates, high resistance to streptomycin was identified. This finding support the results of Hiko *et al.* (2008) who reported antimicrobial resistance to *E. coli* O157:H7 isolates from raw meat samples to some of above mentioned antimicrobials especially to streptomycin. The significantly high level of resistance to these antimicrobials was probably an indication of their extensive usage in the veterinary sector for therapeutic and prophylactic purpose both for *E. coli* and other infections. Resistance to these and relatively cheep and commonly available antimicrobials is distributing in developing countries like Ethiopia.

Recently, multidrug resistant phenotypes have been spread widely among Gram negative bacteria (Ahemed *et al*., 2005). Concurrent resistance of *E. coli* O157:H7 to some antimicrobials may complicate the therapeutic management of infection. In the present study, multidrug resistance was observed among amikasin, amoxycillin-clavulanic acid, cephalothin, ciprofloxacin and streptomycin and tetracycline antimicrobials. From the above mentioned antimicrobials

streptomycin is found in all MDR *E. coli* O157:H7 isolates. This finding was supported by Hiko *et al*. (2008).

The susceptibility to chloramphenicol, ceftriaxone, sulfamethoxazole-trimethoprim, tetracycline, amoxacilin-clavulanic acid, cephalothin, nalidixic acid, ciprofloxacin and amikacin in the present study might have contributed to the effectiveness of these antimicrobials mostly against Gram negative bacteria like those of the family of Enterobacteriaceae to which *E. coli* O157:H7 belongs. In this study, all isolates were 100% susceptible to chloramphenicol, ceftriaxone and sulfamethoxazole –trimethoprim. This finding is consistent with the results of Rangel and Marin (2009) and Rahimi and Nayebpour (2012). Most of these antimicrobials are not commonly used in Ethiopia in the treatment of animals that served as a source of meat. Thus, there was low chance of development of drug resistance.

Antibiotic resistance varies from one country to another depending on antibiotic use and there is a correlation between antibiotic use and subsequent resistance (Tanagho *et al.,* 2004). The application of antibacterial is increasing in many countries around the world, and it is increasingly recognized as the main reason for the emergence of resistance (Lin *et al.,* 2008). The extensive use of antimicrobial agents has invariably resulted in the development of antibiotic resistance. Since their first use in the early 1940s it has been known that bacteria carry mechanisms that allow them to resist antimicrobials (Zhanel *et al.,* 2006).

6. CONCLUSION AND RECOMMENDATIONS

One of the most significant food-borne pathogens that have gained increased attention in recent years is *E. coli* O157:H7. In this study, isolation of *E. coli* O157:H7 from raw Beef, mutton and chevon was conducted at Addis Ababa Abattoir Enterprise and retail shops. Beef, mutton and chevon were contaminated with *E. coli* O157:H7. The prevalence of *E. coli* O157:H7 was higher in retail shops than in abattoir. Beef was more contaminated with *E. coli* O157:H7 than mutton and chevon. Furthermore, susceptibility isolates against a ten of antibiotics was determined invitro. Accordingly, pattern of the *E.coli* O157:H7 was highly susceptible to ceftriaxone, chloramphenicol, sulfamethoxazole-trimethoprim, tetracycline, amoxacilin-clavulanic acid, cephalothin, nalidixic acid, ciprofloxacin, and amikacin. Streptomycin was low susceptible. Intermediate susceptibility was also observed in streptomycin, nalidixic acid, ciprofioxacilin and cephalothin and *E. coli* O157:H7 was resistant to amikacin, streptomycin, ciprofioxacin, cephalothin, nalidixic acid and tetracycline. The existence of *E. coli* O157:H7 in raw meat and isolation of resistant isolates highlight the threat to public health. This is higher in consumers who have habit of eating raw or undercooked meat and meat products, imunocompromised individual and elders.

Based on the findings of present study, the following recommendations are forwarded.

- Implementation of hazarded analysis critical control point (HACCP) principle under the umbrella of good manufacturing and hygienic practice for the Addis Ababa abattoir enterprise should be adopted for efficient food safety codes.

- Food handlers at Addis Ababa abattoir enterprise and retail shops need to be educated about appropriate slaughter procedures, risk of food borne diseases, and sanitary and hygienic method of production. They should also be monitored and inspected regularly by the health authorities, to reduce public heath hazards associated with food borne pathogens.

- Awareness should be created among the public about the risk associated with consumption of raw or under cooked meat and immediate treatment of cases especially infection with *E. coli* O157:H7 should be practiced.

- The prudent use of antimicrobials should be encouraged to prolong their effectiveness by preventing a serious public health problem

- There should be regular antimicrobial sensitivity testing to select effective antibiotics and also to help to reduce the problems of drug resistance development towards commonly used antibiotics.

- Detailed and coordinated epidemiological studies should be undertaken in different part of Ethiopia in both the veterinary and public health sectors to generate a nation wide data on the status of *E. coli* O157:H7 infection both in animals and humans for effective control and preventive measures.

- Further studies should be conducted to establish the stages at which contamination occur along raw meat production chain so that corrective measures can be taken.

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8. APPENDICES

Appendix 1: Sample collection and laboratory activities work sheet

No	Data collection				Laboratory activities					
	Sample. ID	Date	Species of animals	Sample type	MacConkey Agar	Sorbitol MacConkey	Rainbow O157 Agar	BUG		BiOLOG Identification
								1st	2nd	

Appendix 2: Medias used in the isolation and identification of *E. coli* O157:H7.

1. Buffered peptone water (Oxoid Ltd., Hampshire, England)

Composition (g/l): Peptone 10.0; Sodium chloride 5. Final pH: 7.2 ± 0.2

Preparation: - Added 20g to 1 liter of distilled water. Mixed it well and sterilized by autoclaving at 121^{o}C for 15 minutes.

2. MacConkey agar (IVD, UK)

Preparation:- Dispersed 52 g in 1 liter of distilled water. Soaked for 10 minutes, mixed and sterilized by autoclaving at 121^{o}C for 15 minutes. Cooled to 47 ^{o}C and mix well before pouring into Petri dishes and then dried the agar surface.

3. Sorbitol-MacConkey Agar (IVD, UK)

Preparation: Dispersed 48.5g in 1 liter of distilled water. Soaked for 10 minutes, mixed and sterilized by autoclaving at 121^{o}C for 15 minutes. Cooled to 47 ^{o}C and mix before pouring into Petri dishes and then dried the agar surface.

4. Rainbow Agar O157 (Hayward, USA)

Preparation: Mixed package contents in to 500ml of purified water. Heated gently to dissolve components and autoclaved for 10 minutes at 15lbs pressure and 121^{o}C. The medium was purred in to Petri plates, dispensing about 20 ml per plate. The medium was ready to use as soon as it is cooled, gelled and the surface as dried.

5. Biolog Universal Growth Medium (BUG^{TM}) agar (Hayward, USA)

Preparation: Mixed 57g of BUG^{TM} agar into one liter of purified water. Boiled gentely to desolve components. Measured pH. If necessary, adjusted with NaOH or HCl. Sterilized by autoclaveing at 15lbs pressure and 121 ^{o}C for 15 minutes and dispensed. When used as blood agar base, mix the same components in to 950 ml of purified water and adjusted the pH as indicated above. After autoclaving, cooled to 45-50 ^{o}C and 50 ml of sterile defibrinated sheep blood, mixed gently and dispensed as desired. pH: 7.3 ± 0.1 at 25 ^{o}C.

6. Tryptone Soya Broth (TSB) (Oxide, England)

Composition: Pancreatic digest of casein (17.0 g), peptic digest of soyabean meal (3.0 g), sodium chloride (5.0 g), Di-Base potassium phosphate (2.5 g), Glucose (2.5 g).

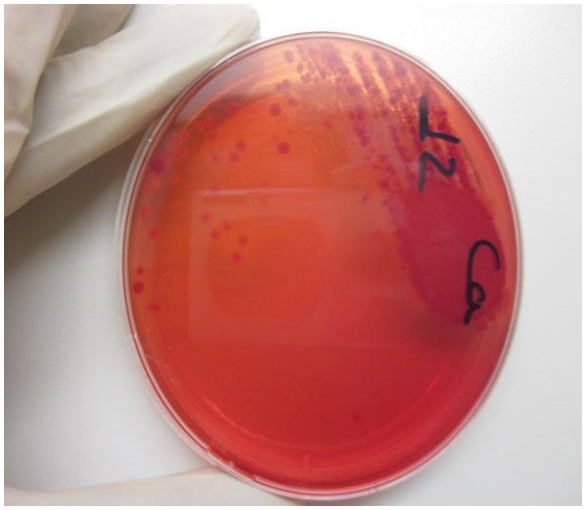
Preparation: - Suspend 30 g of powder in 1 liter of purified water. Mixed thoroughly. Heated with frequent agitation and boiled for 1 minute. Autoclaved at 121 ^{o}C for 15 minutes.

7. Mueller-Hinton agar (Bacton Dickinson and Company, Cockeysville USA)

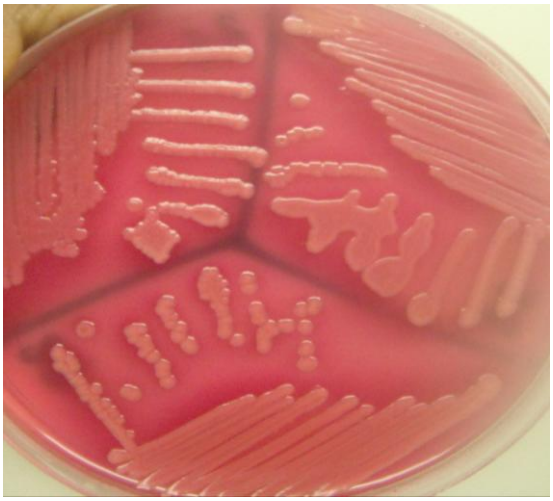
Composition: Beef extract (2.0g), acid hydrolysate of casein (17.5g), starch (1.5g), Agar (17g). Final pH: 7.3± 0.1.

Preparation: Suspended 38 g of powder in 1 liter of purified water mix thoroughly. Heated with frequent agitation and boiled for 1 minute to completely dissolve the powder. Autoclaved at 121 ^{o}C for 15 minutes.

Appendix 3: Colony morphology of *Escherichia coli* O157:H7: on (a)MacConkey agar plate and (b)Sorbitol-MacConkey Agar plate.

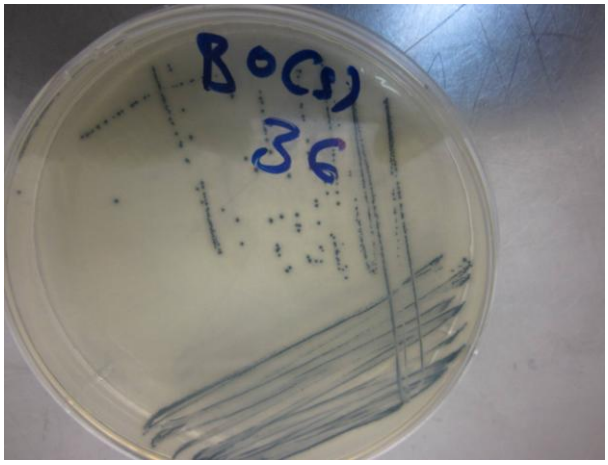


a) **pink colonies** on MacConkey Agar plate

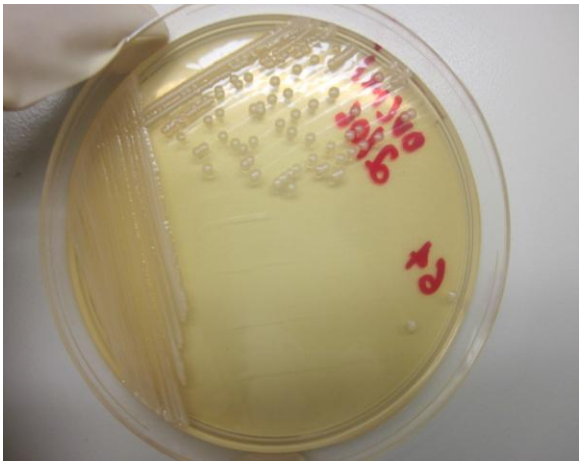


b) **colorless colonies on** Sorbitol-MacConkey Agar plate

Appendix 4: *E. coli* O157:H7 on Rainbow agar plate and pure isolated *E. coli* O157:H7 colonies on BUG agar

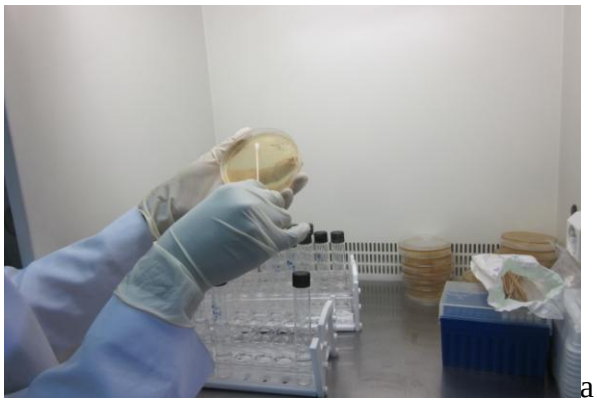


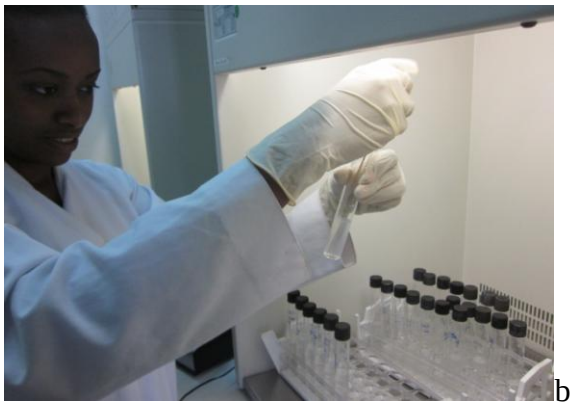
a) **Black colonies** on rainbow Agar plate



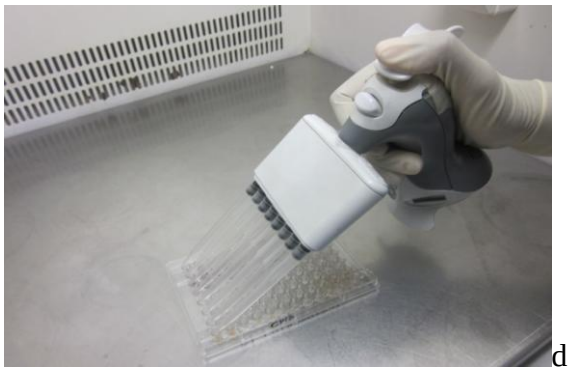
b) *E. coli* O157:H7 colonies on BUG agar

Appendix 5: OmniLog identification procedure

 a

 b

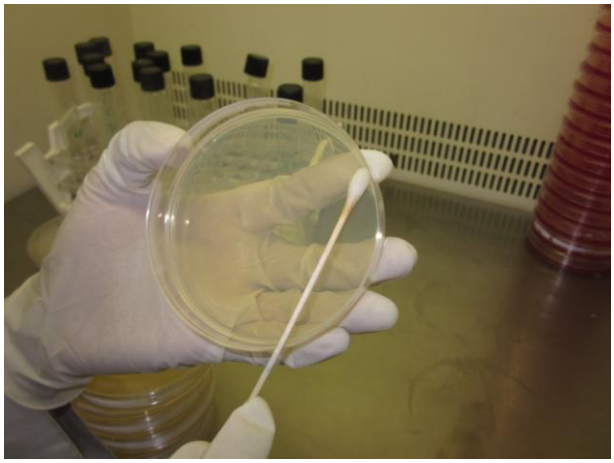
c

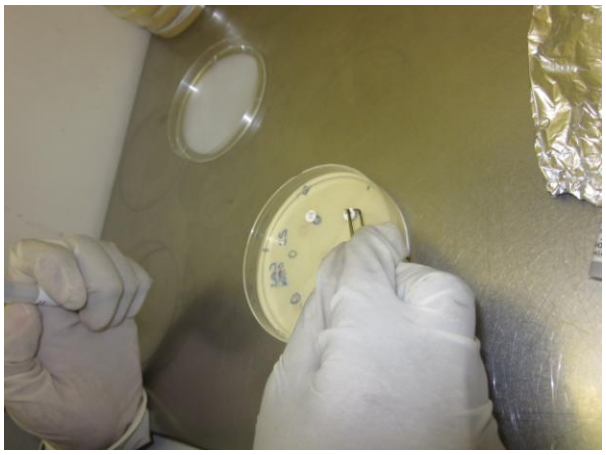
 d

 e

f

Appendix 6: Antimicrobial susceptibility pattern.





10. SIGNED DECLARATION SHEET

This thesis is my original work, has not been presented for a degree in any other university and that all sources of material used for the thesis have been duly acknowledged.

Name _____

Signature _____

Date of submission _____

This thesis has been submitted for examination with my approval as University advisor.

Dr. Girma Zewde (DVM, PhD, Associate Professor) _____

Dr. Genene Tefera (DVM, MSc, PhD) _____