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PREVALENCE AND ANTIMICROBIAL RESISTANCE PROFILE OF *ESCHERICHIA COLI* O157:H7 IN GOAT SLAUGHTERED IN DIRE DAWA MUNICIPAL ABATTOIR AS WELL AS FOOD SAFETY KNOWLEDGE, ATTITUDE AND HYGIENE PRACTICE ASSESSMENT AMONG SLAUGHTER STAFF, ETHIOPIA

MSc Thesis

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LIST OF ABBREVIATIONS

a.s.l	Above sea level
AE	Attaching and effacing lesions (*eae*A)
Aw	Water activity
CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute
Cpds	Compounds
DDAC	Dire Dawa Administration council
DNA	Deoxyrobinucleic acid
E. coli	*Escherichia coli*
EAggEC	Enteroaggregative *Escherichia coli*
EDEC	Oedema disease *E. coli*
EHEC	Enterohemorrhagic *Escherichia coli*
Ehly	Enterohemolysin
EIEC	Enteroinvasive *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GIT	Gastrointestinal tract
GMPs	Good Manufacturing Practices
HACCP	Hazard Analysis Critical Control Point
HC	Hemorrhagic colitis
HUS	Hemolytic uremic syndrome
IMS	Immunomagnetic seperation
IMViC	Indole Methyl red Voges proskauer Citrate
KDal	Kilo Dalton
Km	Kilo metre
LEE	Locus of enterocyte effacement
LPS	Lipopolysaccharides

LIST OF ABBREVIATIONS (Continued)

LT	Heat liable toxin
MDal	Mega Dalton
ml	Mill litre
mm	Mill metre
Nacl	Sodium chloride
NM	Non Motile
NSF	Non-Sorbitol Fermenting
P	Plasmid
PAs	Peasant Associations
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
SMAC	Sorbitol MacConkey agar
Spp	Species
SPSS	Statistical Package for Social Science
STEC	Shiga-toxigenic *Escherichia coli*
stx (*stx*1, *stx*2)	Shiga toxin gene
TTP	Thrombotic Thrombocytopaenic Purpura
UK	United Kingdom
USA	United States of America
Vero	Monkey Kidney Cells
VT	Verocytotoxin
VT1	Verotoxin 1
VT2	Verotoxin 2
VTEC	Verotoxin-producing *E. coli*
WHO	World Health Organization
µg	Microgram

ABSTRACT

Escherichia coli O157:H7 serotype is worldwide zoonotic pathogens responsible for the majority of severe cases of human enterohemorrhagic *Escherichia coli* (EHEC) disease. The aim of this study was to investigate the prevalence and antimicrobial resistance pattern of *E. coli* O157:H7 in goat slaughtered at Dire Dawa municipal abattoir, Ethiopia. A total of 235 samples were collected from cecal contents, carcass and environment sample (slaughter house worker's hand, knife and carcass wash water) as 93, 93 and 49, respectively through the months of January and April. *E. coli* O157:H7 was identified by the method slightly modified to ISO 16654:2001.The samples were initially enriched in modified trypticase broth containing novobiocin supplement, followed by plating onto sorbitol MacConkey agar. Consequently, the suspected non-sorbitol fermenting (NSF) colonies were confirmed as *E. coli* biochemically using indole test and selected for serotyping. Out of 235 samples collected, the overall prevalence of 2.55% (comprising of 2.15%, 3.22% and 2.04 of cecal contents, carcass swab and environmental samples respectively) had positive results for Dryspot *E. coli* O157 latex test kit (Oxoid, DR120M). Eighteen different antibiogram belonging to 10 pharmacological groups including ampicillin, amoxycillin-clavulanic acid, cefotaxime, ceftriaxone, cefoxitin,cefuroxime Sodium, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, kanamycin, nalidixic acid, nitrofurantoin, norafloxacin, streptomycin, sulfamethoxazole-trimethoprim, sulfonamides cpds, tetracycline were used for antimicrobial susceptibility test. Resistance to erythromycin was noted in all of the isolates (100%, n=6/6). Most of *E. coli* O157:H7 isolates also showed high resistance to ampicillin (83.3%, n=5/6) and moderate resistance to nitrofurantoin (50%, n=3/6). All the isolates were resistant to at least two of the antibiotics tested. No isolated *E. coli* O157:H7 strain resistance noted to cefotaxime, ceftriaxone, cefuroxime sodium, chloramphenicol, ciprofloxacin, gentamicin nalidixic acid and norfloxacin. This study concludes that the occurrence of *E. coli* O157:H7 and *E. coli* O157:H7 multiple antibiotic resistant profiles in goat slaughtered at Dire Dawa municipal abattoir and this may show a risk for public health and food safety. Regulatory control of antibiotics usage in livestock production, meat hygiene and pharmaco-epidemiological surveillance in food animals is hereby recommended to ensure consumer safety.

Keywords**:** *Antimicrobial resistance, Carcass, Cecum, Environment, E. coli O157:H7, Goat*

1. INTRODUCTION

1.1. General introduction

Microbial food-borne illness still remains a global concern despite the extensive scientific progress and technological developments achieved in recent years in developed countries (Mersha *et al*., 2009). Food-borne disease also occur commonly in developing countries particularly in Africa because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory system, lack of financial resources to invest in safer equipment and lack of education for food-handlers (Haileselassie *et al*., 2013).

Food-borne diseases often follow the consumption of contaminated food-stuffs especially from animal products such as meat from infected animals or carcasses contaminated with pathogenic bacteria (Nouichi and Hamdi, 2009; Pal, 2012). One of the most significant food-borne pathogens that have gained increased attention in recent years is *E. coli* O157:H7. It is an enterohemorrhagic strain of the bacterium *Escherichia coli* and a cause of food borne illness (Pal, 2007). Typical illness as a result of an *E. coli* O157:H7 infection can be life threatening, and susceptible individuals show a range of symptoms including haemolytic colitis, hemolytic-uremic syndrome, and thrombotic thrombocytopenia purpura (Sima *et al*., 2009; Chileshe and Ateba, 2013).

Domestic and wild animals are the sources of *E. coli* O157, but the major animal carriers are healthy domesticated ruminants, primarily cattle and, to lesser extent, sheep, and possibly goat (Sima *et al*., 2009; Kiranmayi *et al*., 2010; Rahimi *et al*., 2012a). Transmission of *E. coli* O157:H7 to humans is principally via contamination of food by animal faeces, with cattle considered to be the primary reservoir (Hancock *et al*., 1997). Sporadic cases and outbreaks of human diseases caused by *E. coli* O157 have been linked to ground beef, raw milk, meat and dairy products, vegetables, unpasteurized fruit juices and water (Sima *et al*., 2009). There are also traceable links between human infection and ruminant faeces via water or direct contact (Licence *et al*., 2001; Strachan *et al*., 2001), and evidence that contact with animal faeces is a strong risk factor for sporadic *E. coli* O157:H7 infection (Locking *et al*., 2001).

Red meat animals can be infected or carry a wide range of microorganisms, which are potentially pathogenic for man (Pal, 2012). The most important of these are zoonotic bacteria, principally pathogenic serotypes of *E. coli*, such as O157:H7, *Salmonella* and *Camplylobacter* spp (Humphrey and Jorgensen, 2006; Pal, 2007). The major source of carcass contamination is contact with the skin during hide removal or contamination by spillage of stomach contents during evisceration (Humphrey and Jorgensen, 2006; Mersha *et al*., 2009). Moreover, during hide stripping, some bacteria originating from the animal hide become suspended in the abattoir atmosphere. This contaminated air may come into contact with food products, i.e. carcasses, containers, equipment and other food contact surfaces during processing, where they may adhere strongly (Sutton, 2004).

The enteric habitat of *E. coli* in animals provides easy access to animal-derived meats at slaughter and at points downstream in the food production process (Olatoye e*t al*., 2012). Possible contamination of edible carcass tissue is the most significant challenge to food safety, and the extent and nature of such contamination are related to the *E. coli* O157:H7 status of the pre slaughter animal, and any processes which distribute the organism within or between carcasses during dressing operations (McEvoy *et al*., 2003).

Antimicrobial resistance has emerged in the past few years as a major problem and many programs have been set up for its surveillance in human and veterinary medicine. These programs are aimed mainly at human pathogens, agents of zoonoses, and indicator bacteria of the normal intestinal flora from animals (Lanz *et al*., 2003). However, little attention has been paid to the resistance in specific animal pathogens (Lanz *et al*., 2003). Limited studies on the ecology of *E. coli* O157:H7/NM have been reported, particularly from developing countries (Rahimi and Nayebpour, 2012).

The magnitude of the public health burden due to resistant food borne pathogens is complex and is influenced by a number of variables such as antimicrobial use practices in farming, process control at slaughter, storage and distribution systems, the availability of clean water, and proper cooking and home hygiene, among others (WHO, 2000). The major concern on the public health threat of food borne illness is infection by antimicrobial resistant strains that lead to more intractable and severe disease (Helms *et al*., 2002; Martin *et al*., 2004).

This situation is further complicated by the potential of resistant bacteria to transfer their resistance determinants to resident constituents of the human microflora and other pathogenic bacteria (Olatoye *et al*., 2012). Several studies have suggested that foods might be a source of human acquired antimicrobial-resistant *E. coli*. The food supply is an established vehicle for certain other antimicrobial resistant and/or pathogenic bacteria including *E. coli* O157:H7 (Mohle-Boetani *et al*., 2001; Lanz *et al*., 2003; Oliver *et al*., 2011; Rahimi and Nayebpour, 2012).

In developing countries of the world, where there is still an alarming rate of insanitary conditions, malnutrition and poor health facilities, there is an urgent need to study this organism and its characteristics with an aim to reduce the human hazard caused by this emerging pathogen (Isibor *et al., 2013*).

It might seem paradoxical to discuss on the subject of food safety when millions are suffering from lack of food and of the most inferior quality. In Ethiopia at a national level however, both food shortage and lack of appropriate food safety assurance systems are problems that have become obstacles to the country's economic development and public health safety (FAO/WHO, 2007; Ayalew *et al*., 2013).

Food borne diseases commonly occur without being reported and Ethiopia is no exception. The lack of vigorous surveillance of food pathogens in Ethiopia meat and meat products presents a challenge for risk-based approaches to improve food safety, as it becomes difficult to demonstrate the magnitude of contamination with this pathogen. There is a need to generate more data from abattoirs, supermarkets, street vendors and butcheries to ascertain the prevalence of *E. coli* O157:H7 in the meat industry and such information must be made available to the public.

1.2. Objectives

The objectives of this research were:-

- To isolate and identify *E. coli* O157:H7 from goat carcass swab, cecal content and environmental sample at Dire Dawa municipal abattoir.
- To find out the prevalence of goat carcass contamination with *E. coli* O157:H7 in healthy goat slaughtered at Dire Dawa municipal abattoir.
- To determine the antimicrobial susceptibility pattern of isolates by disc diffusion method.
- To assess slaughter house worker's knowledge, attitudes and practices towards slaughtering hygiene.

2. LITERATURE REVIEW

2.1. Historical background

Escherichia coli were first isolated by a German paediatrician, Theodore Esherich, in 1884 from faeces of human neonates (Khan and Steiner, 2002). For the genus *E. coli*, there are hundreds of serotypes of *E. coli* which are classified on the bases of various surface antigens referred to as Somatic (O), Capsular (K), Flagellar (H) and Fimbrial (F).

The first confirmed isolation of *E. coli* O157:H7 in the United States of America was in 1975 from a Californian woman with bloody diarrhoea, while the first reported isolation of the pathogen from cattle was in Argentina in 1977, while the bacterium was first identified as a human pathogen in 1982 (Fernandez, 2008). The spread of *E. coli* O157:H7 in North America coincided with the importation of infected cattle from Argentina, where the rates of human infection were previously about three times higher than those found in North America (McMichael, 2001).

The first outbreaks caused by *E. coli* O157 occurred in Oregon and Michigan, USA, in 1982, when it was isolated from individuals who developed bloody diarrhoea and severe abdominal cramps after eating hamburgers in a restaurant chain (Besser *et al*., 1999; Pennington, 2010). Outbreaks caused by EHEC serotype O157:H7 have mostly involved undercooked ground meat products and occasionally raw milk (Adams and Moss, 2008). The first published study on the prevalence in meats of EHEC strains was that of Doyle and Schoeni in 1987, who tested for *E. coli* O157:H7 and found this strain in 3.7% of 164 beef, 1.5% of 264 pork, 1.5% of 263 poultry, and 2.0% of 205 lamb samples (Jay, 2000).

2.2. Nomenclature

E. coli strains that produce the Stx toxins have been referred to as VT-producing *E coli* (VTEC), shiga-toxigenic *E. coli* (STEC) and enterohaemorrhagic *E. coli* (EHEC) (Karmali, 1989). These three toxin nomenclatures have been used interchangeably in the literature which was further complicated by the existence of two major types of Stx (Stx1 and Stx2), with substantial sequence variation between them (Paton and Paton, 1998).

E. coli O157:H7 produce toxins which are toxic to vero (African green monkey kidney) tissue culture cells and are similar to shiga toxin of *Shigella dysenteriae*. They have been known as verotoxin 1 and 2, and as shiga-like toxin I and II. The strains of *E. coli* that produce these toxins have been known as verotoxin- producing *E. coli* (VTEC) or as shiga-like toxin producing *E. coli* (STEC). "Stx-producing *E. coli* O157" is synonymous with *E. coli* O157: H7 (Constantiniu, 2002; Effler *et al*., 2002). The term VTEC is still widely used in United Kingdom and many European scientific publications. The term STEC is used especially in American scientific papers. The term enterohaemorrhagic *E. coli* (EHEC) was originally coined to denote strains that cause HC and HUS (Constantiniu, 2002). The classification of shiga toxin producing *E. coli* is summarized in Table 1.

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

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

Source: Adopted from Gyles, 2007.

a) not as yet associated with disease in animals or humans; few data are available on the characterisation of the virulence factors associated with these strains. (http :// www. microbionet. com.au/vtectable.htm). b) strains of some serotypes also cause haemorrhagic enteritis in cattle. c) probably an accidental host.

2.3. The organism and its characteristics

2.3.1. Shiga-toxin producing E. coli

Escherichia coli are considered as the normal bowel flora of different species of mammals and birds (Zinnah *et al*., 2007). For the most part, *E. coli* is a group of harmless bacteria that are most often used as indicator organisms for faecal contamination and breaches in hygiene. However, several *E. coli* clones have acquired virulence factors that have allowed them to adapt to new niches and in some cases to cause serious disease (Farrokh *et al*., 2012).

The pathogenic group of *E. coli* are divided into six groups on the basis of their virulence properties such as enterotoxigenic (ETEC, causative agent of diarrhea in humans, pigs, sheeps, goats, cattle, dogs and horses), enteropathogenic (EPEC, causative agent of diarrhoea in humans, rabbits, dogs, cats and horses), enteroinvasive (EIEC, found only in humans), verotoxigenic (VTEC, found in pigs, cattle, dogs and cats), enterohaemorrhagic (EHEC, found in human, cattle, and goats) and enteroaggregative *E. coli* (EAggEC) which found only in human (Biswas *et al*., 2006; Xia *et al.*, 2010). In terms of the zoonoses, the most important category is the enterohemorrhagic, which is also the most severe (Acha and Szyfres, 2001).

All STEC including serotype O157:H7 have the same morphology. They are Gram-negative, facultative anaerobic bacteria that belong to the *Enterobacteriaceae* family and the *Escherichia* genus (Xia, 2010; Farrokh *et al*., 2012). *Escherchia coli* O157:H7 produce shiga toxin which is an important cause of food borne illness in human and ruminants where they appear to be more frequently colonized by *E. coli* STEC than other animals, but the reason for this is unknown (Cornick *et al*., 2000).

2.3.2. Growth and inactivation

E. coli is a typical mesophile growing from 7-10 ^{0}C up to 50^{0}C with an optimum around 37 ^{0}C (Adams and Moss, 2008; Xia, 2010), although there have been reports of some ETEC strains growing at temperatures as low as 4 ^{0}C. It shows no marked heat resistance, with a D value at 60 ^{0}C of the order of 0.1 min, and can survive refrigerated or frozen storage for extended periods. A near neutral pH is optimal for growth but growth is possible down to pH 4.4 under otherwise optimal conditions. The minimum aw for growth is 0.95 (Adams and Moss, 2008).

Serotype O157:H7 has been shown to grow well in broth media within the usual laboratory temperature range of 30-42°C and it survives freezing in ground beef quite well. At temperatures above 44-45°C serotype O157:H7 grows poorly and as these temperatures are often used for the detection of *E. coli* in food samples, such conditions probably will negatively impact on the recovery of this serotype from food (Hui *et al*., 2001). A recent publication has also shown that *E. coli* O157 strains possess inherent genetic mechanisms which enable growth at low temperatures (<15 °C), compared to non-pathogenic *E. coli* (Vidovic *et al*., 2011).

2.3.3. Biochemical properties

E. coli can be differentiated from other members of the *Enterobacteriaceae* on the basis of a number of sugar-fermentation and other biochemical tests. Classically an important group of tests used for this purpose are known by the acronym IMViC (Table 2). These tested for the ability to produce: indole from tryptophan (I); sufficient acid to reduce the medium pH below 4.4, the break point of the indicator methyl red (M); acetoin (acetylmethyl carbinol) (V); and the ability to utilise citrate (C) (Adams and Moss, 2008). Despite *E. coli* can be identified with a variety of biochemical reactions, the indole test remains the most useful method to differentiate *E. coli* from other members of the *Enterobacteriaceae* (Xia, 2010).

Table 2: The IMViC tests

	Indole	Methyl Red	Voges proskauer	Citrate
Escherichia coli	+	+	-	-
Shigella	V	+	-	-
Salmonella Typhimurium	-	+	-	+
Citrobacter freundii	-	+	-	+
Klebsiella pneumonia	-	-	+	+
Enterobacter aerogenes	-	-	+	+

Source: Adams and Moss, 2008.

The majority of *E. coli* O157:H7 strains can be distinguished from most *E. coli* by their inability to ferment sorbitol rapidly and by their lack of production of b-glucuronidase. Although rapid sorbitol-fermenting strains of *E. coli* O157:H7 have been associated with colitis and HUS in Germany, these strains are rarely isolated in the United States (Besser *et al*., 1999).

2.3.4. Acid and salt tolerance

Escherichia coli O157:H7 is a highly acid-resistant food-borne pathogen that survives in the acidic environment of stomach and to colonise the gastrointestinal tract (Price *et al*., 2004). Furthermore, it also increases the survival of STEC O157:H7 in acidic foods, enabling survival for extended periods, particularly at refrigeration temperature (Meng *et al*., 2007). Hence, contaminated cultured and fermented foods such as yoghurt and cheese have been implicated in sporadic cases and outbreaks (Baylis, 2009; Farrokh *et al*., 2012).

The doubling time of *E. coli* O157: H7 increases by three fold in 4.5% NaCl in broth where as at 6.5% a 36 hours lag was noted with a generation time of 31.7 hours and no growth occurred at ≥ 8.5% NaCl (Jay, 2000).

2.3.5. Antibiotic resistance

Most strains tested during the early and mid-1980s were susceptible to ampicillin, trimethoprim-sulfamethoxazole, tetracycline, and quinolones, and resistant to erythromycin, metronidazole, and vancomycin. More recently, investigators have reported increasing rates of resistance to streptomycin, sulfimethoxazole, and tetracycline, possibly as a result of the prevalence of this organism in food animals that receive these antibiotics (Besser *et al*., 1999).

In recent study conducted in the central parts of Ethiopia, Hiko *et al.* (2008) determined the antibiotic resistance of *E. coli* O157:H7 strains from meat samples obtained from legally registered butcher shops, municipal abattoirs, and selected export abattoirs at Debre Zeit and Modjo towns. Their results demonstrated multidrug resistance (MDR) to three or more drugs was detected in 7/31 (22.6%) strains. Of the 7 MDR strains, 3 were resistant to three drugs (streptomycin, tetracycline and ampicillin), 2 were resistant to four drugs (streptomycin, cepahlothin, tetracycline and ampicillin), and 2 were resistant to five drugs (streptomycin, cephlaothin, tetracycline, ampicillin and trimethoprim). The other most recent study done by Taye *et al*. (2013) reported 100% resistance to ampicillin (AMP10 μg) and amoxicillin (AML10 μg) and 33.33% resistance to tetracycline (Te30 μg).

2.3.6. Carriage of a 60-MDa plasmid

E. coli O157:H7 isolates associated with human illness harbour a plasmid (pO157) of approximately 60 MDa that contains DNA sequences common to plasmids present in other serotypes of VTEC isolated from patients with haemorrhagic colitis. The plasmid is believed to play a role in the pathogenicity of disease (Fernandez, 2008; Tshabalala, 2011).

2.4. Epidemiology of enteric EHEC O157:H7

2.4.1. Distribution

The first STEC O157 infections were reported in 1982, when *E. coli* O157:H7 was involved in outbreaks associated with two fast food chain restaurants in the United States. These isolates were obtained from fecal samples taken from sporadic cases of hemorrhagic diarrhea submitted to public health or hospital laboratories for examination (Acha and Szyfres, 2001). Since then, ever-increasing numbers of cases and outbreaks due to STEC O157 have been reported worldwide. *E. coli O157:H7* was the causative agent of many out-breaks worldwide (Xia *et al.*, 2010). For instance, serotype O157:H7 has been isolated in outbreaks in Canada, Great Britain, and the United States. It has also been isolated in Argentina, Australia, Belgium, the former Czechoslovakia, China, Germany, Holland, Ireland, Italy, Japan, and South Africa. Reports from Africa (Effler *et al*., 2001) have shown that rates of O157:H7 infections but in countries lacking diagnostic capabilities might be underestimated (Tarr *et al.*, 2005). Annual incidence rates of 8 per 100,000 inhabitants or greater have been reported in the region of Scotland, Canada and USA (Constantiniu, 2002).

2.4.2. Susceptibility

Cattle are generally regarded as the main natural reservoir of EHEC. All ages of cattle are susceptible to colonization with EHEC, although peak shedding is observed in subadult cattle from weaning to 24 months of age (Hussein and Sakuma, 2005; Joris *et al*., 2012).

People of all ages are susceptible to infection with STEC. However, the young and the elderly are more susceptible and are more likely to develop more serious symptoms (FDA, 2012).

2.4.3. Mode of transmission

E. coli O157 is transmitted by food and water, directly from one person to another, and occasionally through occupational exposure. Most food borne outbreaks have been traced to foods derived from cattle, especially ground beef and raw milk (Constantiniu, 2002; Fairbrother and Nadeau, 2006; Gyles, 2007).

Among many foods and dairy products acted as vectors (Fig. 1)-ground beef hamburgers; steak tenderised by injection; steak tartare; kebabs; ready-to-eat cold meats including poultry, pork, and beef products; salami and other fermented meat products; venison jerky; cheese; milk; butter; yoghurt; ice cream; apple juice; grapes; coleslaw; lettuce; spinach; radishes; alfalfa sprouts; and melons are mentioned (Pennington, 2010).

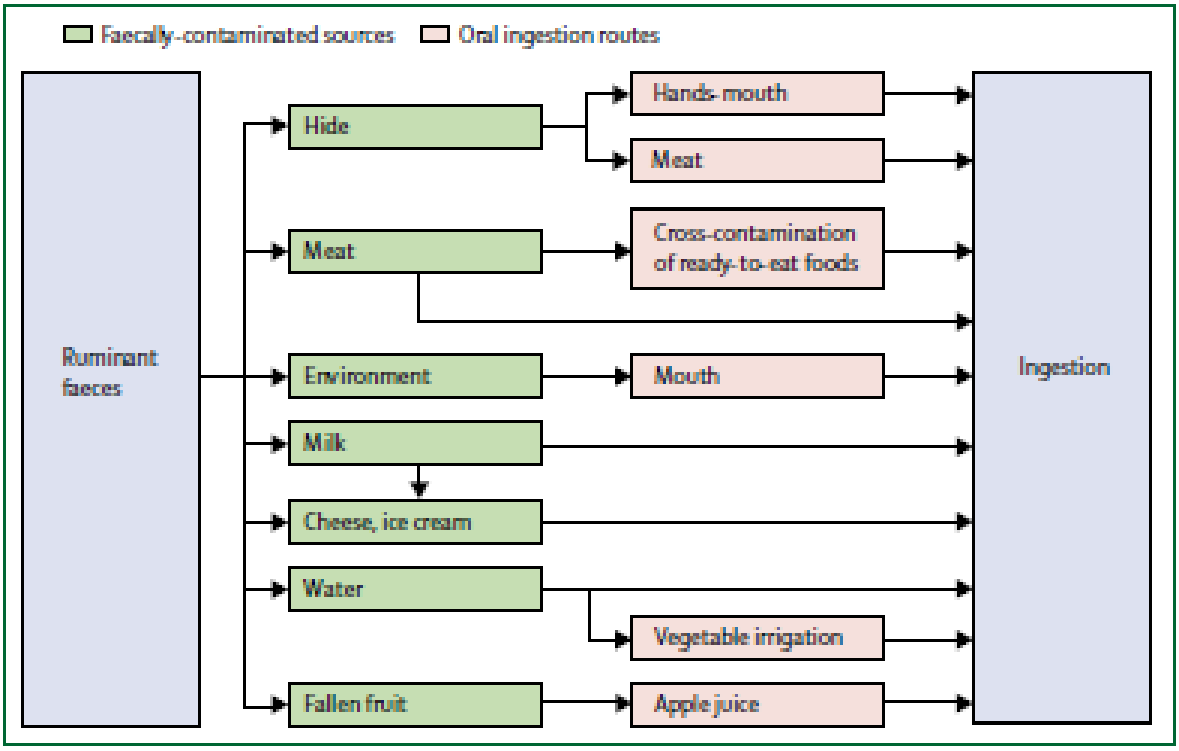

Figure 1: Among many foods and dairy products acted as vectors for *E. coli* O157
Source: Pennington, 2010.

Outbreaks of O157 STEC most commonly occurred in restaurants, often due to cross-contamination during food preparation. Person-to-person transmission via the faecal-oral

route has been an important mode of transmission, particularly since the early 1990s, and occurs mostly in child day care centres, individual homes, communities, and schools.

Waterborne outbreaks of O157 STEC associated with recreational waters, such as lakes, swimming pools, and contaminated drinking water, have been increasingly reported since the early 1990s. Outbreaks associated with contaminated water tend to be larger in size and have been attributed to local well, municipal, and spring water systems. Since 1996, outbreaks resulting from a new transmission mode have been recognised, i.e. direct contact between humans and cows or calves at farms, fairs, or petting zoos. For the most part, the modes of transmission in other industrialised countries appear to be similar to those observed in the USA (Effler *et al*., 2001; Fairbrother and Nadeau, 2006).

As more data become available from developing countries, other modes of transmission specific for the environmental, demographic, and farming conditions in these countries will certainly be elucidated. For instance, a large outbreak of bloody diarrhoea due to O157 STEC in South Africa in 1992 was the result of a combination of carriage of O157 STEC by pastured cattle, cattle deaths due to drought, and ensuing heavy rains resulting in contamination of surface waters (Effler *et al*., 2001; Fairbrother and Nadeau, 2006).

2.4.4. Carrier and sources of infection

Domestic and wild animals are sources of EHEC O157:H7 but the major animal carriers are healthy domesticated ruminants, primarily cattle and to a lesser extent, sheep, and possibly goats (Kiranmayi *et al*., 2010; Rahimi *et al*., 2012a). Faeces and hides of cattle are considered to be the main sources of *E. coli* O157 contamination of carcasses during slaughter (Elder *et al*., 2000; Aslam *et al*., 2003).

The main sources of STEC infection in cattle are drinking water, feed, and the environment of the animal. The environment may be contaminated by cattle carrying the bacteria as well as by production animals of other species (e.g. sheep, goats, or pigs), by companion animals (e.g. dogs, cats, or horses), by wild animal species (e.g. deer), or by insects (e.g. flies). Infection may also occur through direct contact with other cattle or animals of other species (Fairbrother and Nadeau, 2006).

A plethora of fecal-contaminated food items including ground meat, unpasteurized dairy products, unpasteurized refreshments, fruits and vegetables (such as sprouts, lettuce, coleslaw) have been well-known vehicles for EHEC infections (Karmali, 2004; Schlundt *et al*., 2004, Caprioli *et al*., 2005). In addition, waterborne infections (Garcia-Aljaro *et al*., 2005), and infections associated with rural settings have been of growing importance (Karmali, 2004). In particular, environment-related exposures have been associated with EHEC infections during summer and fall (Karmali, 2004; Caprioli *et al*., 2005).

2.4.5. Pathogenesis and clinical features

Pathogenicity of *Escherichia coli* O157:H7 is encoded by a variety of plasmid, bacteriophage and chromosomal genes (Kiranmayi *et al*., 2010). The key virulence factor for subset of EHEC is Stx which consists of five identical B subunits that are responsible for binding the holotoxin to the glycolipid globotriaosylceramide (Gb3) on the target cell surface, and a single A subunit that cleaves ribosomal RNA, causing protein synthesis to cease (Kaper *et al*., 2004).

The ability to produce shiga toxin was acquired from a bacteriophage presumably directly or indirectly from *Shigella* (Kiranmayi *et al*., 2010). The Stx family contains two subgroups -Stx1 and Stx2-that share approximately 55% amino acid homology (Kaper *et al*., 2004). The production of Shiga toxin is central to the pathogenesis of bloody diarrhoea and haemolytic uremic syndrome (Fig. 2) (Pennington, 2010). Stx is produced in the colon and travels by the bloodstream to the kidney, where it damages renal endothelial cells and occludes the microvasculature through a combination of direct toxicity and induction of local cytokine and chemokine production, resulting in renal inflammation. Stx also mediates local damage in the colon, which results in bloody diarrhoea, haemorrhagic colitis, necrosis and intestinal perforation (Kaper *et al*., 2004).

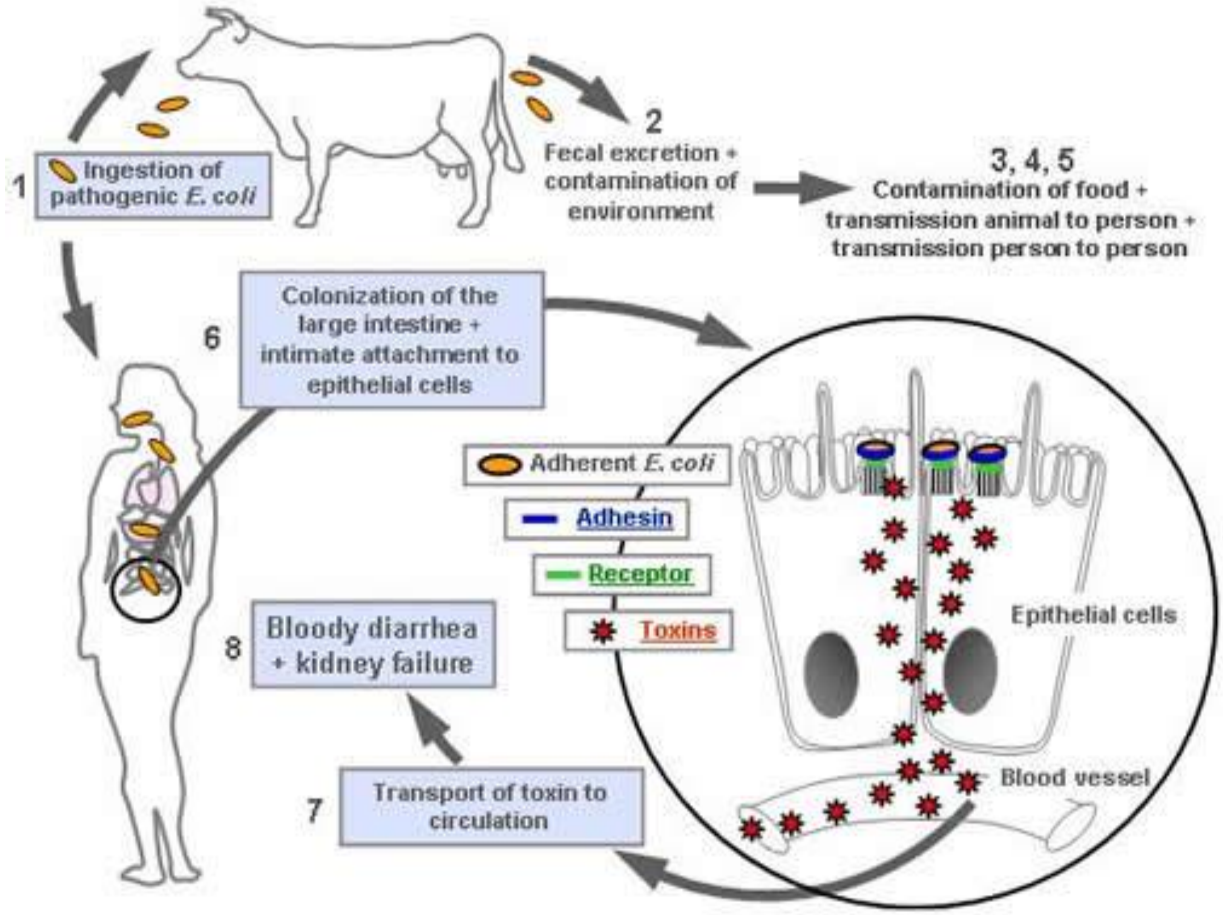

Figure 2: How zoonotic shiga toxin-producing *Escherichia coli* (STEC) cause bloody diarrhoea and haemolytic uraemic syndrome in humans (www.ecl-lab.ca).

Potentially pathogenic bacteria are ingested by cattle and other ruminants (1) and colonize the intestinal tract, but do not cause any disease in these animals. The bacteria are excreted in the feces and contaminate the environment, including the drinking and swimming water of the human population (2). There may also be contamination of foods such as fruits, vegetables, sprouts, lettuce, and raw milk and juice (3). There may be contamination of the carcass at slaughter, and bacteria will be mixed into ground beef. Persons in direct contact with animals, who are working on farms or in slaughter-houses, may also be contaminated by the bacteria (4). There may also be spread of bacteria from person to person (5). In humans, these bacteria colonize mostly the large intestine and cause similar attaching and effacing lesions (6) (Fig. 2).

Bacteria produce their own specific receptor which is injected into the host epithelial cell via a syringe-like bacterial apparatus. A bacterial adhesin then mediates a very intimate attachment of the bacteria to the cell receptors and bacterial signals stimulate effacement of the microvilli, or brush border, and reorganization of the cell cytoskeleton. The adherent bacteria produce a toxin which is transported across the epithelial cells to the circulation (7). This toxin acts on the endothelial cells of blood vessels, resulting in non-bloody to bloody diarrhea and abdominal cramps (8). There may be a complication of hemolytic uremic syndrome which may lead to acute kidney failure, especially in children (www.ecl-lab.ca).

The infective dose of *E. coli* O157:H7 is estimated to be very low, in the range of 10-100 cells. The infective dose of other STEC serotypes is suspected to be slightly higher (FDA ,2012).The pathogenicity of *Escherichia coli* O157:H7 is associated with a number of virulence factors, including shiga toxins (Stx1 and Stx2; encoded by the stx1 and stx2 genes), intimin (encoded by the eae gene) and the enterohaemolysin (encoded by the hlyA gene) (Manna *et al.*, 2006; Kiranmayi *et al*., 2010; Xia *et al.*, 2010).

The toxin is a 70.000 dalton protein composed of a single A subunit (32 kDal) and five B subunits (7.7 kDal). The A subunit has an N-glycosidase that inactivates the 28S ribosome, thus blocking protein synthesis. The B subunits provide tissue specificity by binding to globotriaosylceramide (Gb3) receptors on the surface of eukaryotic cells. Endothelial cells high in Gb3 receptors are the primary target, accounting for the toxin's affinity for colon and renal glomeruli, associated with HC and HUS. The toxin can also indirectly damage cells by releasing cytokines, such as tumour necrosis factor (Constantiniu, 2002).

Within the Stx2, there are additional antigenic variants. The Stx2v (variant)-producing *E. coli* is associated with diseases in domestic animals, such as edema disease of swine. Enterohemorrhagic *E. coli* that commonly cause human illnesses produce Stx1, Stx2, or both. The presence of the Stx2 in these EHEC has a profound influence on the progression of the disease from hemorrhagic colitis to HUS. As is common for many bacterial toxins, Stx consists of 2 subunits. The Stx-A subunit contains the enzymatic activity responsible for inhibiting protein synthesis, and the B-subunit acts as a lectin, binding the toxins to intestinal epithelial and kidney endothelial cells. The Stx is believed to be the major factor

contributing to the lesions in HUS, although the O157 lipopolysaccharide may also contribute to this disease syndrome (Sanchez *et al*., 2002).

The clinical manifestations of *E. coli* O157 and other VTEC serotypes infections range from symptom-free carriage to non-bloody diarrhoea, haemorrhagic colitis (a triade of severe abdominal pain, diarrhoea and frank red blood), HUS and death. The course of events in VTEC infection starts with the ingestion of the pathogen (Constantiniu, 2002). Haemolytic uremic syndrome is characterized by three features, acute renal failure, haemolytic anaemia (reduction in the number of red blood cells) and thrombocytopaenia (a drop in the number of blood platelets), sometimes preceded by a bloody diarrhoea. Thrombotic thrombocytopaenic purpura is a less common complication which is largely confined to adults. It is related to HUS but causes less kidney damage and includes fever and neurological symptoms resulting from blood clots in the brain (Adams and Moss, 2008).

2.6. Host responses to EHEC O157:H7 infection

Infection of the gastrointestinal tract of adult cattle, weaned calves and 5-day-old gnotobiotic calves by EHEC serotype O157:H7 is asymptomatic (Wray *et al*., 2000). Histological analysis of intestinal epithelia from calves and cattle infected with *E. coli* O157:H7 reveals intimate bacterial adherence in some but not all cases and a mild inflammatory response characterized by diffuse infiltration of neutrophils into the lamina propria (Stevens *et al*., 2002).

Serum antibody responses against the O157 lipopolysaccharide and Shiga toxin 1 have been detected in some but not all experimentally infected calves (Wray *et al*., 2000) and sheep (Cornick *et al*., 2000; Stevens *et al*., 2002).

It is likely that immunity plays a role in the susceptibility to infection with *E. coli* O157:H7, as evidenced by the increased rates of infection and HUS in young children and the elderly. Although antibodies to O157 LPS and shiga toxin 1 rise after acute infection, protective immunity has not been demonstrated in humans, and *E. coli* O157:H7 infection has caused recurrent hemorrhagic colitis and HUS in children without apparent immunodeficiencies (Besser *et al*., 1999).

2.7. Diagnosis

Detection of *E. coli O157:H7* is based on phenotypic differences from most other serotypes: its inability to ferment sorbitol on MacConkey sorbitol agar and absence of b-glucuronidase activity in most strains. Presumptive *E. coli* O157:H7 from these tests must then be confirmed serologically for which a latex agglutination kit is commercially available (Adams and Moss, 2008).

Identification of diarrhoeagenic *E. coli* can be based on detection of their associated virulence factors. For example, procedures are available to detect the ST and LT of ETEC serologically, and the LTI and Stx genes in ETEC and EHEC using gene probes and the polymerase chain reaction (PCR) (Adams and Moss, 2008).

2.8. Treatment

The use of antibiotics in the treatment of STEC infection is controversial (Panos *et al*., 2006; Ochoa *et al*., 2007). Some authors reported that antibiotics may have beneficial effects in STEC infection and reduce the risk of STEC-associated complications (Yoshimura *et al*., 1999; Kurioka *et al*., 1999) while others reported an increase in the level of shiga toxin production and a greater risk of fatal complications following administration of antibiotics in STEC infection (Zhang *et al*., 2000; Wong *et al*., 2000). In vitro studies showing most strains are susceptible to various antibiotics, although certain antibiotics, at sublethal concentrations may increase the release of Shiga-like toxin which has been associated with the development of HUS. No clinical studies have indicated that antibiotics are effective in reducing the duration of *E. coli* infection or duration of bloody diarrhea **(**Collins and Green, 2010). In vitro data have demonstrated that ciprofloxacin or subinhibitory concentrations of trimethoprim-sulfamethoxazole induce shiga toxin production by *E. coli* O157:H7 (Besser *et al*., 1999).

Treatment of HUS is supportive, with particular attention to the management of fluids and electrolytes. With meticulous care, the mortality rate for HUS is approximately 4%. Numerous other treatment modalities have been tried but are of unproven efficacy. These include plasma infusion, plasma exchange, intravenous immunoglobulin, Shiga toxin

inhibitors, prostacyclin, antithrombotic therapy, vitamin E, recombinant tissue plasminogen activator, and transfusion with P1-positive erythrocytes (Besser *et al*., 1999).

2.9. Control and prevention of EHEC O157:H7 infection

An effective control program to substantially reduce *E. coli* O157:H7 infections will require the implementation of intervention strategies throughout the food continuum, from farm to table. Promising intervention measures at the farm include competitive exclusion bacteria, bacteriophage, and targeted animal management practices addressing common points of contamination. Consumers also have a role in implementing intervention controls in food handling and preparation. Unfortunately, many consumers eat high-risk foods, improperly handle and store foods, and ignore warnings regarding foods known to be unsafe (Sanchez *et al*., 2002).

Ground beef should be cooked until it is no longer pink. Meat from cattle, like that of other mammalian and avian species, can be contaminated by feces during slaughter and processing. Thus, all precautions should be taken to minimize this risk, and foods of animal origin should be well cooked before they are eaten. Personal hygiene, particularly hand washing after relieving oneself, is also important (Acha and Szyfres, 2001; Pal, 2007).

To control the risk of human infection through direct contact with farm animals, strict hygiene practices should be established, including controlling the movement of visitors to farms, restricting access to farm animals., making washing facilities readily available, providing a means of disinfection in case visitors come into contact with the animals, and segregating eating areas from areas where the animals are kept (Fairbrother and Nadeau, 2006).

The commonly accepted rules of herd management should be followed in animals. For calves, colostrum is important for the prevention of white scours, and for pigs, all unnecessary stress should be avoided during weaning in order to prevent edema (Acha and Szyfres, 2001).

2.10 Public health and economic significance

A small fraction of *E. coli* is human pathogens and has been implicated in food borne illnesses with increasing frequency over the last 2 decades. Among this, *Escherichia coli* O157 is the most common member of a group of pathogenic *E. coli* strains known variously as entero- haemorrhagic, verocytotoxin producing or Shiga-toxin producing organisms (Chapman *et al*., 1997; Abongo and Momba, 2009; Rahimi *et al*., 2012b).

The severity and long-term sequelae of infection with *E. coli* O157 and other verocytotoxin-producing *E. coli* result in high costs. The medical, productivity loss, and outbreak control costs of the 1994 West Lothian outbreak in Scotland (milk pasteurisation failure, 71 cases, 11 with haemolytic uremic syndrome, one death) were estimated to be £3.2 million for the first year. Over 30 years, the costs were projected to be £11.9 million. The medical and productivity loss costs of the 1995 outbreak of *E coli* O111 in South Australia (contaminated mettwurst, about 200 cases, 23 with haemolytic uraemic syndrome, one death) were estimated at AUS$5.6 million. In both outbreaks haemolytic uraemic syndrome and premature death accounted for much of the costs. The directly measurable costs of the Walkerton outbreak (excluding costs attributable to premature deaths) was CAD$64.5 million (Kiranmayi *et al*., 2010).

Escherichia coli O157:H7 strains carrying stx2 gene along with enterohaemolysin gene are potentially dangerous to human health (Manna *et al.,* 2006; Kiranmayi *et al*., 2010). Stx2 producing strains appear to be more commonly responsible for serious complications such as HUS than those only Stx1 producing (Kiranmayi *et al*., 2010).

There have been a number of very large outbreaks around the world and their public impact has often been dramatic. Six hundred people became ill and four children died in a major US outbreak in 1993 caused by undercooked beef hamburgers. In August 1997, a cluster of cases in Colorado prompted the largest food recall in US history when more than 12 000 tons of ground beef were recalled. A large outbreak in Scotland in 1996 had a similar impact in the UK. Nearly 500 were affected and 20 elderly patients died. The cause was thought to be cross-contamination of cooked meats from raw meat in a butcher’s shop (Adams and Moss, 2008).

Estimates by the Centers for Disease Control and Prevention (CDC) indicate that entero-hemorrhagic *E coli* (EHEC) serotype O157:H7 is responsible for approximately 62,500 cases of food borne infection annually in the United States. These estimates include hospitalizations and 52 deaths, which are largely associated with cases of pediatric hemolytic uraemic syndrome (HUS), a leading cause of renal failure in children (Sanchez *et al*., 2002).

2.11. Antimicrobial resistance

In animal production antimicrobial drugs are used for therapy, prophylaxis and growth promotion. The use of such drugs causes a selective pressure to be imposed on bacterial populations and antimicrobial resistances are selected. The pool of resistance genes is thus spread in the environment (WHO, 2004).

Drug resistance in food borne bacterial enteric pathogens is an almost inevitable consequence of the use of antimicrobial drugs in food-producing animals, and specifically in the developing countries by use of medicines in humans (Bogaard and Stobberingh, 2000; Threlfall *et al.,* 2000). A major concern is that the high levels of antibiotic resistance are a result of the use of antibiotics in food animals. A recent estimate in the United States suggests that 24.6 million pounds of antibiotics are given to animals each year as growth promoters at sub-therapeutic amounts in their feed compared to 3 million pounds consumed by humans (White *et al.,* 2001).

Over the last two decades, development of antimicrobial resistance resulting from agricultural use of antibiotics that could impact on the treatment of diseases affecting the human population that require antibiotic intervention has become a significant global public health concern (Oliver *et al*., 2011; Rahimi and Nayebpour, 2012). Different antibiotic resistance profiles have been detected in *E. coli* O157:H7 isolates from different sources, including humans, animals and foods (Magwira *et al.,* 2005; Ju-Yeon *et al.,* 2006).

3. MATERIALS AND METHODS

3.1 Informed consent

The research project was approved by the Academic Commission of the College of Veterinary Medicine and Agriculture, Addis Ababa University, Addis Ababa, Ethiopia.

Moreover, ethical clearance to use human subjects for this study was got from the Ministry of Science and Technology after the study proposal was considered and approved by the Research and Ethics Committee (Appendix 9). Subjects enrolled for this study were those who gave their consent after the purpose of study was explained to them.

3.2. Study area

The Dire Dawa Administration council is geographically located in the Eastern part of the country specifically lying in range of 09^0 28.1'' to of 09^0 49'' N latitude of 41^0 38.1'' to of 42^0 19.1'' E longitude and the town is 515 Km from Addis Ababa the capital city of Ethiopia and 333 Km from the international port of Djibouti. The DDAC has nine urban *kebeles* and 33 rural PAs (Tefera, 2013).

Dire Dawa Administrative council enjoys bi-modal type of rainfall with April as a peak for the scanty rainfall and July for the heavy rains. The rain pattern is characterized by scanty rains in spring and heavy rain in summer. With June as a dry spell month, the rainy season is from October to January. From the seven rainy months only in the months of July and August the rainfall exceeds half the potential evapo- transpiration. The mean annual rainfall in the study area various from 550 mm in the lowland northern part to above 650 mm in the southern mountain ranges (Tefera, 2013).

The temperature in the study area is generally high. The monthly mean maximum temperature ranges from 28.1 ºc which is recorded in the month of December and January, to 34.6 ºc recorded in the month of June. Likewise, the monthly mean minimum temperature varies from 14.5 ºc in December to 21.6 ºc in June (Tefera, 2013).

There are two major climatic zones in the DDAC. *Kola*, areas with altitude ranging from 500- 1500m a.s.l. covering 1173km^2; and *Weyna dega*, areas with altitude ranging between 1500- 2300m a.s.l. covering 160km2. *Kola* has an average annual temperature of 20-27.5^{0}c and *Weyna dega* with 17.5-20^{0}c. The average annual rainfall is 640.3mm with highest 1257.7mm and a minimum of 357.3mm (Tefera, 2013).

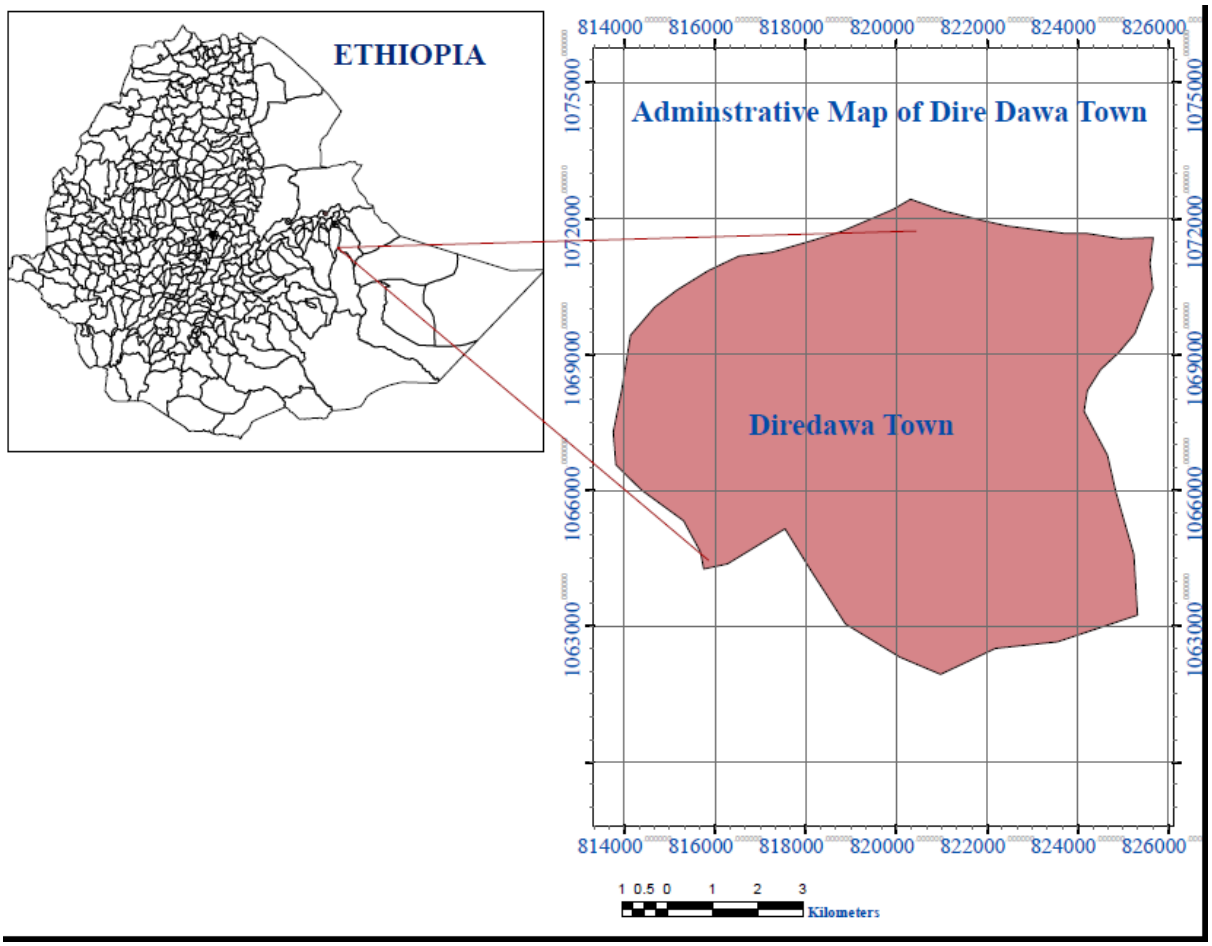

Figure 3: Map of Dire Dawa Administrative Council

3.3. Study design

A cross-sectional study was conducted to determine the prevalence of *Escherichia coli* O157: H7 and antibiotic susceptibility test from January to April 2014 in goat slaughtered at Dire Dawa municipal abattoir.

3.4. Questionnaire Survey

A cross sectional design was used to answer questions concerning the current status of slaughtering hygiene practiced in the abattoir studied. Hygiene and sanitation were determined by the use of structured interview and through direct observations of the hygienic status and practices by abattoir workers. The target population constituted all the abattoir workers.

3.5. Sample size determination

Sample size was determined using the formula by Thrusfield (2005).

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

Where, **n**=required sample size.

Pexp=expected prevalence of *E. coli O157:H7* in goat faeces, which was estimated at 3.3% following Mersha *et al*. (2009).

Z= z statistic for level of confidence

d=desired absolute precision of 0.05.

$$\mathbf{NZ^2P (1-P) / M^2 (N-1)+Z^2P(1-P)}$$

N was adjusted according to Lavrakas, (2008)

Where N= total population

P= expected prevalence

M= Precision value

Z= z statistic for level of confidence

The Dire Dawa municipal slaughter house had a minimum capacity of slaughtering approximately 350 goats per week and also slaughtered sheep, cattle and camel. Goats were always slaughtered first on each collection day at the facility that slaughters for Christian's people. Sampling was carried out over a period of 2 months. Total population N was calculated 21,000 (50 goats x 7 days x 2 months) which gave required sample size of 49. The Dire Dawa municipal abattoir slaughtered 2100 goats (N) through the sampling month of January and April.

Calculated sample size was 49 but 93 samples were taken deliberately in order to maximize the precision of the study. The origin of goats presented for slaughter was from Shinille which is geographically close to Dire Dawa administrative council and where goat as well as sheep ownership is high.

3.6. Study samples

The study was conducted on a total of 235 samples collected from goat carcass, cecal contents and environmental samples (slaughter house worker's hand, knife and carcass wash water) as 93, 93 and 49 respectively.

3.7. Sampling strategy

Carcass swab and fecal samples were collected using systematic random sampling method from the goat population slaughtered on each visit to Dire Dawa municipal abattoir. In addition to this, environmental sample were taken during each visit. Matched samples were collected from each animal. For labelling purposes, fecal (cecal content), and carcass samples (Goat meat swab) from each animal were given the same number (differentiated by CC and GMS), and animals were labelled consecutively as the de-skinning process completed. For environmental sample i.e. slaughter staff's hand swab, knife swab and water sample differentiated by HS, KS and W respectively.

Table 3: Number and types of sample collection

Sample types	Unit/sample	N
Carcass surface	400cm^2	93
Cecal content	10g	93
Workers' hand	2 hands	20
Knife	2 sides	15
Carcass wash water	10 ml	14
Total		235

3.8. Sample collection procedure and transportation

3.8.1. Carcass sampling

During each visit, four different sites of the carcass (thorax, brisket, flank and crutch) were swabbed using the method described in ISO17604 (2003), one site covering 100 cm2 by placing sterile template (10 x 10 cm) on a carcass. For each sampling area, a sterile cotton tipped swab (2 X 3 cm) fitted with shaft was moistened in an approximately 10 ml of buffered peptone water (Oxoid Ltd., Hampshire, England), was rubbed first horizontally and then vertically several times across the carcass surface. 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.

The four swabs were put into one screw cupped test tube containing 10 ml of sterile bacteriological peptone samples were transported to the laboratory in a cool box with frozen gel packs within twenty four hours of sampling for microbiological analysis at Ethiopian Nutrition and Health Research Institute (EHNRI).

3.8.2. Faecal sampling

The fecal sample was collected immediately after evisceration from cecal contents of slaughtered goats; an aseptic incision was made with surgical blade in the cecum to obtain a representative sample of the cecal content. The faecal material was aseptically compressed and the resultant liquor decanted in sterile universal bottle, labelled, transported on ice to the laboratory and held in a cold storage over night and processed the following day.

3.8.3. Environmental sampling

At each slaughter visit, three types of environmental samples were collected by swabbing the slaughter house worker's hand and carcass environments (carcass wash water and knives). For carcass wash water, 10 ml were collected before and during operation from the bucket. For knives, composite samples were collected from the blade and handle of the knives.

3.9. Culture and isolation of E. coli O157:H7

3.9.1. Faecal samples

Approximately 1ml/1g of fecal pellet (homogenized when possible) was suspended into 9 ml of modified tryptone soya broth supplemented with novobiocin (Oxoid) (10 mg/l). Samples were vortexed and incubated for overnight at 37°C. After selective enrichment, 50µl of product was streaked onto sorbitol MacConkey agar (Oxoid) and the plates incubated at 37°C for twenty-four hours. Up to six colourless colonies (non- Sorbitol fermenters) were picked and separately sub-cultured on MacConkey agar (Oxoid) for twenty-four hours at 37°C for purification.

After overnight incubation, the purified and intensely red colonies with a pale periphery were tested for indole production (Oxoid) and indole forming isolates were seeded onto nutrient agar for serological confirmation. The indole test was carried out as follows. One colony was inoculated into 4ml of tryptone soya broth (Oxoid) (appendix1), using a straight inoculation wire. Incubation was done for overnight at 37°C. After this one drops of indole reagent were added to the tryptone soya broth culture to test for indole production (red ring-positive) (Appendix1).

3.9.2. Carcass bacterial swabs

The carcass bacterial swabs were incubated overnight at 37 ^{0}C after being suspended into modified tryptone soya broth supplemented with novobiocin (Oxoid) (1:9) and subjected to similar tests for bacteriological analysis as faecal samples.

3.9.3. Environmental samples

Environmental samples were incubated overnight at 37 ^{0}C after being suspended into modified tryptone soya broth supplemented with novobiocin (Oxoid) (1:9) and subjected to similar tests for bacteriological analysis as faecal samples.

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

Non-sorbitol fermenting (NSF) isolates inoculated onto nutrient agar for testing. Then, NSF and indole positive colonies were then serotyped using Oxoid Dryspot *E. coli* O157 latex test kit. The Dryspot *E. coli* O157 latex test demonstrated by agglutination of *Escherichia* strains possessing the O157 serogroup antigen. One drop of saline was dispensed to the small ring (at the bottom of each oval) in both the test and control reaction areas ensuring that the liquid did not mixed with the dried latex reagents (Appendix 2).

Using a sterile single use plastic loop, a portion of the colony to be tested was picked and carefully emulsified in the saline drop until the suspension was smooth. Then, using paddle the suspension was mixed into the dry latex spots until completely suspended and spread to cover the reaction area. The test card picked up and rocked for up to 60 seconds and looked for agglutination under normal lighting conditions.

A result is positive if agglutination of the latex particles occurs within 1 minute (Appendix 3). This indicates the presence of *E. coli* serogroup O157. A negative result is obtained if no agglutination occurs and a smooth blue suspension remains after 60 seconds in the test area.

3.10. Antimicrobial susceptibility testing

Antimicrobial resistance tests were performed by standard disc diffusion technique (CLSI, 2012). The selection criteria of antibiotics testing discs depended on the regularly use of antimicrobials in the ruminants, potential public health importance and recommended from the guideline of antimicrobial susceptibility testing from CLSI (2012). Resistance testing discs contained ampicillin (10µg), amoxycillin-clavulanic acid (20/10µg), cefotaxime (30µg), ceftriaxone (30µg), cefoxitin (30µg), cefuroxime sodium (30µg), chloramphenicol (30µg), ciprofloxacin (5µg), gentamicin (10µg), kanamycin (30µg), nalidixic acid (30µg), nitrofurantoin (50µg), norafloxacin (10µg), streptomycin (10µg) , sulfamethoxazole-trimethoprim (25µg), sulfonamides cpds (300µg), tetracycline (10µg) (Oxoid). The isolates were considered resistant if the diameter of inhibition zone was less than or equal to the resistance breakpoint provided by CLSI guidelines (Table 4).

Table 4: Antibiotic discs used to test *E. coli* O157:H7 and their respective concentrations.

NO.	Antibiotic discs	Code	Concentration	Diameter of Zone of inhibition in mili meter (mm)		
				Resistant ≤	Intermediate	Susceptible ≥
1	Ampicillin	AMP	10 µg	13	14-16	17
2	Amoxycillin-Clavulanic acid	AMC	20/10µg	13	14-17	18
3	Cefotaxime	CTX	30µg	22	23-35	26
4	Ceftriaxone	CRO	30µg	19	20-22	23
5	Cefoxitin	FOX	30µg	14	15-17	18
6	Cefuroxime Sodium	CXM	30µg	14	15-17	18
7	Chloramphenicol	C	30µg	12	13-17	18
8	Ciprofloxacin	CIP	5µg	15	16-20	21
9	Erythromycin	E	15µg	13	14-22	23
10	Gentamicin	CN	10µg	12	13-14	15
11	Kanamycin	K	30µg	13	14-17	18
12	Nalidixic Acid	NA	30µg	13	14-18	19
13	Nitrofurantoin	F	50µg	14	15-16	17
14	Norfloxacin	NOR	10µg	12	13-16	17
15	Streptomycin	S	10µg	11	12-14	15
16	Sulfamethoxazole-Trimethoprim	SXT	25µg	10	11-15	16
17	Sulfonamides Cpds	S3	300µg	12	13-16	17
18	Tetracycline	TE	30µg	11	12-14	15

Each isolated bacterial colony from pure fresh culture was transferred in to a test tube of 5 ml tryptone soya broth (TSB) (Oxid, England) 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 was prepared according 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. The antibiotic disks were gently pressed onto the agar to ensure firm contact with the agar surface, and incubated at 37^oC 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 (CLIS, 2012). For the results and discussion, we used the terminology of Knezevic and Petrovic (2008): very high rate of resistance (>75% resistant isolates); high rate (50-75%); moderate rate (30-50%); low rate (10-30%); and very low resistance rate (0-10%).

3.11. Data management and statistical analysis

Data were transferred to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA). The overall prevalence of *E. coli* O157: H7 in cecal contents, carcass swab and environmental sample was determined using standard formula. The number of positive samples were divided by the total number of samples examined multiplied by 100. Descriptive statistics such as frequencies were used to present the findings of the questionnaires. Using SPSS 20 statistical software (SPSS Inc., Chicago, IL, USA), a Pearson chi-square test and Fisher's exact two-tailed test analyses were performed and differences were considered significant at $P < 0.05$.

4. RESULTS

4.1. Knowledge, attitude and practice study

4.1.1. Employment status of the abattoir worker

The respondents comprised of two groups, including permanent and temporary staff. ‘‘Permanent staff’’ refers to staff who are permanently employed at the abattoir, while ‘‘temporary staff’’ refers to those working on a contract basis. Among interviewed, half of the total respondents (50%) were employed on a permanent basis whilst the other half (50%) were temporary staff members. Out of the total 14 abattoir workers interviewed, 7.1% of had no formal education and 21.4% of them had not received any job related training.

4.1.2. Slaughterhouse worker’s Knowledge about food borne Disease and reason for carcass contamination

Majority of workers had not been trained on any job related issues in past time. Those who have received training had a significantly higher knowledge about food safety issue and slaughtering hygiene. Most of the supervision was not hygiene based and not all the workers had done medical tests (about 35.7% had not done the medical tests) which is a requirement for one to work in the slaughterhouse. More details on worker’s knowledge about food-borne disease and reason for carcass contamination summarized in Table 5.

Table 5: Knowledge of slaughter house worker's about food borne disease and reason for carcass contamination at slaughtering, Dire Dawa municipal abattoir.

Knowledge	**Frequency**	**percent**
Heard about food borne disease	10	71
Did not hear about foodborne disease	4	29
Causes for food borne disease		
Germs	11	69
Chemicals	2	12
Do not know	3	19
Mode of food borne disease transmission		
Contaminated food	11	34
Contaminated water	9	28
Vectors like flies and cockroaches	9	28
Do not know	3	9
Reason for goat carcass contamination		
Dirty hands	13	23
Infected slaughter house worker	10	18
Accidental puncture of GIT	12	21
Dirty utensils	11	19
Dirty working area	11	19
Contamination pose any health risk to meat consumers	12	86
Contamination did not pose any health risk to meat consumers	2	14
Report illness to the management	14	100

The proportion of abattoir staff who believed that food borne diseases are caused by germs was 11 (69%) and 3 (19 %) of the respondent didn't know about the causes. Of those who were asked about the mode of transmission of food borne disease; 11 (34%) answered that contaminated food is the vehicle and 9 (28%) responded that vectors and contaminated water are the channels for the transmission similarly. Twelve out of fourteen respondents indicated that accidental puncture of gastrointestinal tract as a cause for goat carcass contamination. Eighty six percent (12) of the slaughter house worker also knew that contamination pose health risk to meat consumers.

4.1.3. Slaughter staff's attitude towards food safety and slaughtering hygiene

Half of the respondent (50%) felt that working quickly is more important than keeping hygiene and health is more important than wealth (100%). This shows that their attitude towards hygiene is poor and 81% of them felt that if meat were well cooked then it would not always cause any harm. Disagreement to the statement that personnel (17%) with abrasions or cuts on fingers or hands should not handle carcass or edible organ was observed during abattoir visit. More results on the workers attitude on food safety and slaughtering hygiene are summarised in Table 6.

Table 6: Summary of results for slaughter house worker's attitude towards hygiene in Dire Dawa municipal abattoir, Ethiopia.

Characterstics	**Strongly Agree (%)**	**Agree (%)**	**Disagree (%)**	**Strongly Disagree (%)**	**Do not Know (%)**
In this job, it is more important to work quickly than keep the carcases clean.	36	14	14	36	0
People doing this job are more likely to get sick	36	7	7	50	0
In this type of working environment, keeping clean is easy	60	7	0	33	0
A small amount of dirt on clothing or utensils will not cause any harm	38	8	0	54	0
Health is more important than wealth	100	0	0	0	0
Ensuring hygiene is mainly the role of management	71	0	0	29	0
If meat is well-cooked then it is always safe to eat	81	0	6	13	0

4.1.4. Hygienic practices at slaughter houses

There was no hot water, adequate supply of tap water, sterilizer, retention room (cooling facilities) change rooms and bathroom facilities in the abattoir. Most surprisingly, slaughter house staff's take shower at slaughtering floor at the end of daily slaughter operation. All staffs were found wearing outer working garments, of which majority of staff's (83%) outer garment were not clean.

Veterinary meat inspectors were always present in the slaughterhouse for inspection. However, all of the workers placed their equipment on dirty surfaces during their work and they washed them in bucket water instead of flowing water. Other attributes on their hygiene practices are summarized in Appendix 8.

4.2. Isolation and identification of *E. coli* O157:H7 by conventional bacteriological method

Out of the total of 235 different samples examined, 6 (2.55%) were found to be contaminated with *E. coli* O157:H7. *Escherichia coli* O157:H7 was isolated in goats from cecal contents 2 (2.15%), carcass swabs 3 (3.22%) and environmental 1(2.04%) samples (Table 7).

No significant difference (P > 0.05) was found in the proportion of *E. coli* O157:H7 in different samples obtained from goats slaughtered and surrounding environment.

Table 7: Distribution of serologically confirmed *E. Coli* O157:H7 and their sources.

Sources	**Number of samples**	**Serologically confirmed**
Cecal content	93	2 (2.15%)
Carcass Swab	93	3 (3.22 %)
Environmental sample (Worker's hand, knife, Water)	49	1 (2.04%)
Total	235	6 (2.55)

4.3 Susceptibility to antimicrobial agents

Antimicrobial susceptibility testing results showed (Fig. 4) that of the 6 isolates, 100% resistance was noted for erythromycin, 83.3% were resistant to ampicillin, 50% were resistant to nitrofurantoin, 33.3% to cefoxitin, streptomycin, sulfamethoxazole-trimethoprim, sulfonamides cpds and tetracycline, 16.5% showed a resistance to amoxycillin-clavulanic acid. None of them were resistant to cefotaxime, ceftriaxone, cefuroxime sodium, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid and norfloxacin. Multidrug resistant to more than two antimicrobial agent was detected in 66.7% of the isolate. Interestingly, one isolate was resistant up to nine antimicrobial tested. Antimicrobial resistance pattern for *E. coli* was shown in Table 8.

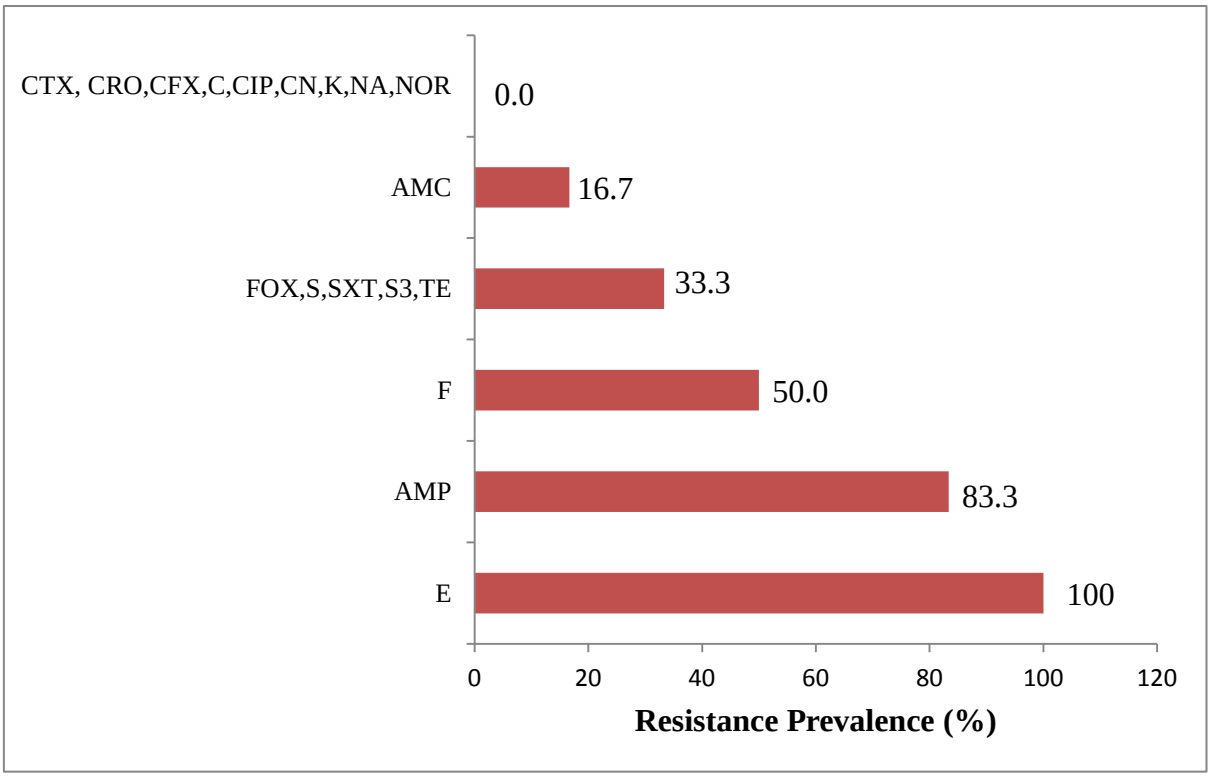

Figure 4: The percentage of *E. coli* O157:H7 resistant to 18 antimicrobial agents.

Key for Figure 4; AMP: ampicillin, AMC: amoxycillin-Clavulanic acid, CTX: cefotaxime, CRO: ceftriaxone, FOX: cefoxitin, CFX: cefuroxime Sodium, C: chloramphenicol, CIP: ciprofloxacin, E: erythromycin CN: gentamicin, K: kanamycin, NA: nalidixic acid, F: nitrofurantoin , NOR: norafloxacin, S:streptomycin, SXT: Sulfamethoxazole-Trimethoprim, S3: sulfonamides cpds, TE: tetracycline.

Table 8: Antimicrobial resistance patterns of *E. coli* O157:H7 isolates.

Resistances	**Patterns**	**No. of Isolates**	**%**
One (antimicrobial drug)	AMC	1	16.5
	E	6	100
Two (antimicrobial drugs)	AMP,E	2	33.3
Three(antimicrobial drugs)	AMP,E,F	1	16.5
More than four (antimicrobial drugs)	AMP,E,F,FOX,S,SXT,S3,TE	2	33.3

Key for Table 8: AMP: Ampicillin, AMC: Amoxycillin-Clavulanic acid, FOX: Cefoxitin, E: Erythromycin, S: Streptomycin, SXT: Sulfamethoxazole-Trimethoprim, S3: Sulfonamides cpds, TE: Tetracycline.

5. DISCUSSION

5.1 Knowledge, attitude and practice Study

A hygienic practice is the major concern of the slaughterhouse but still has poor practice. Basically, hygienic status of dressed carcasses is largely dependent upon the general slaughterhouse hygiene and the skills of the workers (Mothershaw *et al.,* 2006). Slaughterhouse workers play a role in carcass contamination during the slaughter process. Of more importance to avoid carcass contamination are their level of knowledge, attitude and practices towards hygiene.

Food handlers should be trained in the basic concepts and requirements of food and personal hygiene as well as those aspects particular to the specific food-processing operation (Adams and Moss, 2008). The interviews conducted revealed that some of workers at the abattoir where study conducted had no training in safe meat handling, slaughtering and personal hygiene. Although food safety training appeared to be a strong predictor for attitude and food hygiene practices, slaughter staff who have received training and had sufficient "good knowledge" but their attitude and practice was not up to the level. This aspect is very important for programme implementation and policy implication.

Slaughter staff had reasonably good knowledge towards the cause, mode of transmission of food borne disease and the risk factors for carcass contamination. On the other hand their high level of knowledge is incompatible with the personal and slaughtering hygiene practiced. The possible explanation might be multitude; they might have been reluctant to practice what they know due to work overload, lack of attitudinal change, ignorance or lack of encouragement. They are not equipped and/or supplied with the necessary material that enables them to maintain the general hygiene. For instance, some of the slaughter staff indicated that inadequate supply of potable as a challenge towards maintaining hygiene.

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The skin of the animal can contaminate to carcass because of slaughtering processes. From observation, most often butcher's punch his fist forcefully between the skin and the carcass surface to detach the skin ("fisting"). Although fisting is hygienically critical, butchers not

take care to wash frequently their hands and arms and touch the dirty outside of the animal's skin while removing the skin this way as observed visually.

At slaughter area, the slaughter processes are done in the same area without separate dirty and clean zone, thus, the incomplete separation still can make cross contamination. Workers have less concern on hygienic practice from observation and interview. Chewing "Khat", smoking habit and not changing clothes are major points that observed. Slaughterhouses should have worker health check regulation. Even those who have been trained on food safety and proper slaughtering processes; they still less attend to follow.

Apart from the knowledge, attitude is also a crucial factor that may influence food safety behaviour and practice, thus decrease the occurrence of food borne diseases (Sani and Siow, 2014). From the survey conducted, half of the respondent focus on working quickly is more important rather than keeping hygiene; about 46% of the total respondents stated that they concurred to the statement a small amount of dirt on clothing or utensils will not cause any harm. This clearly indicates that slaughter staff's negative attitude towards hygiene though half of the total respondent agrees to statement contamination of carcass always pose risk to the meat consumer. Although all slaughter house worker have a basic responsibility to ensure hygienic practices, about 71% indicated that it as the role of management.

Personal and general hygienic practice is extremely vital to ensure production of safe food to consumers (Sani and Siow, 2014). However, slaughter behaviour in most all of the workers placed their equipment/knife on dirty floor and no frequent washing of hand and equipment was observed.

5.2 Prevalence of *E. coli* O157:H7

To the best of our knowledge this is the first study of the prevalence of *Escherichia coli* O157:H7 in goat slaughtered at Dire Dawa municipal abattoir and the Eastern part of the country. The Dire Dawa town was chosen because, here, consumption of sheep and goat meat was much more frequent, with fresh meat reportedly purchased by the predominantly Muslim population from retail shops 1-5 times a week.

Human infections of *E. coli* O157:H7 have mostly been recognized to be from food products with animal origin (Jo *et al*., 2004). Domestic ruminants, mainly cattle, sheep, and goats, have been established as major natural reservoirs for STEC and play a significant role in the epidemiology of human infections (Griffin *et al*., 1991). Several recent reports have clearly identified (Espie *et al*., 2006; La Ragione *et al*., 2008) or implicated (Chapman *et al*., 2000; Pritchard *et al*., 2000; Rey *et al*., 2006) goats as sources of *E. coli* O157:H7 infection. Not only can goats be colonized with *E. coli* O157:H7, but their innately inquisitive behaviour means that they are much more likely to be in regular direct contact with humans, consequently increasing the risk of the direct faecal–oral transmission of zoonotic infection (La Ragione *et al*., 2008).

The most pressing food safety issues in the food industry are caused by the presence of *E. coli* O157:H7 and *Salmonella* spp in raw meat and poultry products (Sperber, 2005). In present study, presence of *E. coli* O157:H7 on goat carcasses suggests transfer of faecal material onto the sterile carcass during the slaughter process, which may suggest that currently available dressing procedures at abattoir cannot be relied upon to prevent faecal contamination during slaughter.

In the present study, 2 (2.15%), 3 (3.22%) and 1 (2.04%) of cecal contents, carcass swab and environmental sample respectively were *E. coli* O157:H7 positive. There was no statistically significant prevalence variation of the pathogen noted among different sample analyzed in the present study , though, much more data need to be collected to determine whether it is real or simply an artefact of limited sampling.

Our findings do not differ greatly from those reported the isolation of this bacteria from goat meat in other areas of Ethiopia. This has already been reported in two studies, 3% by Mersha *et al.* (2009) in Modjo and 2 % by Hiko *et al.* (2008) in Debre Zeit and Modjo towns of Ethiopia. Moreover, the prevalence rate reported for carcass and faeces in our study was consistent with reports from other parts of the world such as 2.7% from United States (Jacob *et al*., 2013), 1.7% from Iran (Rahimi *et al*., 2012a) 1.2% from Greece (Dontorou *et al*., 2004), 2.5% from Nigeria (Akanbi *et al.*, 2011). On the contrary, 50% prevalence was documented for goat meat in India (Gomashe *et al.* 2011), while 9.1% prevalence was noted in goats in Bangladesh (Islam *et al*., 2008).

The observed differences in the results of the present study from those of other authors could be due to differences in husbandry practices and prevailing climatic conditions which may account for the varied prevalence of STEC from one geographical region to another. The methods and techniques used in the laboratory identification of STEC in this study could also be responsible. Immunomagnetic separation (IMS) technique with enrichment in broth culture has been reported to enhance the isolation of STEC from samples with a low concentration of the organisms (Chapman *et al*., 1994; Ojo *et al*., 2010).

We used modified trypticase soy broth as enrichment stage. It has been proposed that the enrichment before plating on selective agar may increase the sensitivity of *E. Coli* O157:H7 isolation compared to direct plating of test samples on selective agar (Varela-Hernández *et al*., 2007; Hashemi *et al*., 2010). In this study, enrichment without IMS was employed for the isolation of *E. coli* O157:H7. With IMS, the rate of *E. coli* O157:H7 detection could have been enhanced.

During the processing of the carcasses, fecal contamination or transfer of bacteria from the animal's hide to the carcass can facilitate transmission of pathogenic *E.coli* to the meat (Elder *et al*., 2000). Similarly, contamination of carcasses with *E. coli* O157:H7 serotype can occur when gut contents, fecal matter or contaminated hides come in contact with meat surfaces. In this study slightly higher isolation rate (3.22%) was observed for *E. coli* O157:H7 on carcass swab samples in comparison with sample from cecal contents and environment. This seems to be quite logical as the main source of contamination is the skin of the animal which found its way to the surface of the carcass due to poor hygienic

conditions during slaughtering process of the animals or it might be related to cross contamination during the slaughter process which in overall reflect the general unhygienic conditions in employees, utensils and environmental sanitation of the slaughter house under study. From observation, for instance, fisting together with not taking care to wash frequently their hands and arms as well as touching the dirty outside of the animal's skin while removing the skin may still facilitate transfer of the pathogen onto sterile carcass surface.

In slaughterhouse studied, water stored in a plastic bucket was used to wash the floor, carcasses, hands and equipment. The water used to wash the carcasses can be sources of both mesophilic and psychrotrophic microorganisms on carcasses (Tshabalala, 2011). In present study, presence of *E. coli* O157: H7 in environmental sample was noted and it is an indication for fecal contamination originating either from humans or animals. More interestingly, the presence of this pathogen was noted in carcass wash water taken from bucket possibly suggesting carcass-to-carcass spread of this pathogenic bacterium across the slaughter line. Twenty slaughter house worker's hand and knife swab sample were found negative for *E. coli* O157 among the environmental samples analyzed.

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

Emergence and dissemination of antimicrobial resistance is on the increase among enteric bacteria (Sawant *et al*., 2007). Antimicrobial resistance may arise either spontaneously by selective pressure or due to antimicrobial misuse by humans or overuse in feeding or treatment of animals by farmers (Schroeder *et al*., 2002). Resistance development also might be related to exchange of resistance factors between related bacteria (Tenover, 2006).

All the *E. coli* O157:H7 isolated in present study exhibited resistance to two or more antibiotics used in the study. In Ethiopian situation, two studies were reported on the antimicrobial susceptibility of *E. coli* O157:H7 isolated from cattle, sheep and goat. The first study showed that the isolated pathogen is highly sensitive to amikacin, chloramphenicol, gentamicin, kanamycin, nalidixic acid, norfloxacin, polymyxin B and trimethoprim-sulfamethoxazole and highly resistant to streptomycin, cephlaothin,

tetracycline, ampicillin and trimethoprim (Hiko *et al.*, 2008). The second study revealed that all beef isolates were found susceptible to kanamycin, chloramphenicol and spectinomycin and 100% resistance to ampicillin and amoxicillin and 33.33% resistance to tetracycline (Taye *et al*., 2013).

In this study, all *E. coli* O157:H7 isolates were resistant to at least two of the eighteen antimicrobial agents tested. No resistance to newer generation of antimicrobials such as ciprofloxacin and norfloxacin which are important in the treatment of human cases of gastroenteritis was recorded.

Resistance to erythromycin and ampicillin were two of the most common resistance profiles identified among our study isolates. The resistance of all *E. coli* O157:H7 to erythromycin comes in agreement with the results of Harakeh *et al.* (2005) and Osaili *et al*. (2013). The highest resistance prevalence to ampicillin was also noted which is used in human medicine for the treatment of coliform infections, and moderate rate of resistance to tetracycline obtained in this study also in close agreement with the local report of Taye *et al*., 2013 in beef isolates. Furthermore, our results showed that high proportion of *E. coli* O157:H7 isolates were resistant to the nitrofurantoin and other antimicrobial agents. This observation contradicts Hiko *et al*. (2008) who reported 100 % susceptibility for trimethoprim-sulfamethoxazole in *E. coli* O157:H7 isolates from bovine, sheep and goat meat.

Although tetracycline has moderate resistance in this study, it is one of the most commonly available for use as routine chemoprophylaxis among livestock in Ethiopia. They are readily available in different dosage forms and in combination with other antibiotics and vitamins. Interestingly, Galland *et al.* (2001) found that among 57 putative *E. coli* O157:H7 isolates recovered from cattle, 27 (47%) were resistant to amoxicillin-clavulanic acid. This difference may be because Galland *et al*. (2001) used a methodology different from ours as well as a resistance breakpoint (>4/2 μg/ml) that has since been increased (≥18/30μg/ml). Alternatively, it may be the result of temporal and geographical differences between the two studies, Galland *et al*. (2001) having collected samples over an 11- month period from a specific region of south western Kansas.

The increasing developing multi-drug resistant bacteria is signalling a serious alarm from treatment point of view or the possible transforming of resistance genes to other related pathogens (Osaili *et al*., 2013). In this study multiple antimicrobial resistances is also noted among *E. coli* O157:H7 isolates drawn from different sample types which was in agreement with Schroeder *et al*.'s (2002) and Zhao *et al*.'s (2001) report in the USA. They found that out of the twenty nine tested *E. coli* O157:H7, four isolates showed multiple resistances to five antimicrobials: tetracycline, ampicillin, streptomycin, kanamycin, and sulfamethoxazole. Two isolates originated from cattle, and two isolates were from human and ground beef.

The public health significance of these findings is that antimicrobial resistant bacteria from food animals may colonize the human population via the food chain, contact through occupational exposure, or waste runoff from meat production facilities to the neighbourhood

It is essential to keep up with isolate characteristics for any global changes in isolate distribution and similarities and prevalence of common virulence factors. Also, it is essential to track the resistance pattern recorded globally to follow changes in antimicrobial sensitivity patterns that may require a reassessment of zoonotic control strategy. Monitoring of antimicrobial resistance in *E. coli* O157:H7 isolates is valuable for epidemiological uses and for monitoring the increase of antimicrobial resistance among different microbial species (Osaili *et al*., 2013).

6. CONCLUSION AND RECOMMENDATIONS

This study showed that slightly higher isolation rate of *E. coli* O157:H7 in goat meat destined for human consumption in the studied area with some antimicrobial resistance pattern. In addition, the results showed the risk of this pathogen to consumers due to unhygienic meat processing most commonly practiced in Dire Dawa municipal abattoir and the contributions to global epidemiology of bacterial resistance.

The presence of *E. coli* O157:H7 is being reported for all sample types (cecal contents, carcass swab and environmental samples) with slightly higher occurrence in carcass swab, and possibly suggesting skin is the key source of microbial contamination of the goat meat, the study confirmed a need for preventative approach to control *E. coli* O157:H7 in goat meat production chain.

This study has also attempted to cast light on features about the knowledge, attitudes and practices of slaughter staff's pertaining food safety and general hygiene. The results indicated that there were poor personal and general hygiene measures in place and that the workers not focus on hygienic practice.

Generally, this study provides an initial baseline data on the occurrence of *E. coli* O157:H7 in abattoirs studied.

Some recommendations may be made on the basis of the findings:-

- Training of slaughter personnel should be given to ensure that all workers including management take ownership of hygiene practices during animal slaughter and during further processing.

- Management should strive to establish employee commitment regarding personal and general hygiene to ensure a safe meat from the abattoir

- Abattoir facilities such as adequate supply of potable water, knives pouches, hot water, and soap should be fulfilled.

- Clinical data must be collected in order to estimate the real impact of *E. coli* O157:H7 food contamination on human health in Ethiopia.

- Control measures to reduce the public health risk arising from *E. coli* O157:H7 in goat meat chain needs to be addressed at abattoir level by reducing carcass contamination at various stages of the slaughter process.

- *In vitro* antimicrobial susceptibility testing of *E. coli* O157:H7 be performed and appropriate treatment be instituted especially for those cases of food borne *E. coli* O157:H7 with sever or prolonged symptoms or in immunocompromised patients.

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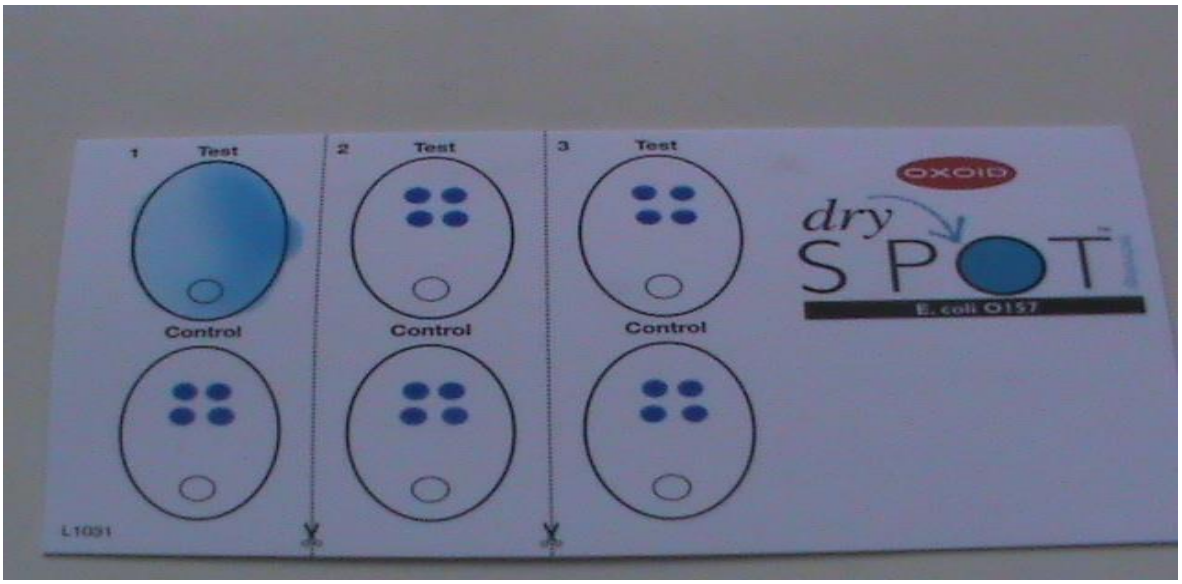
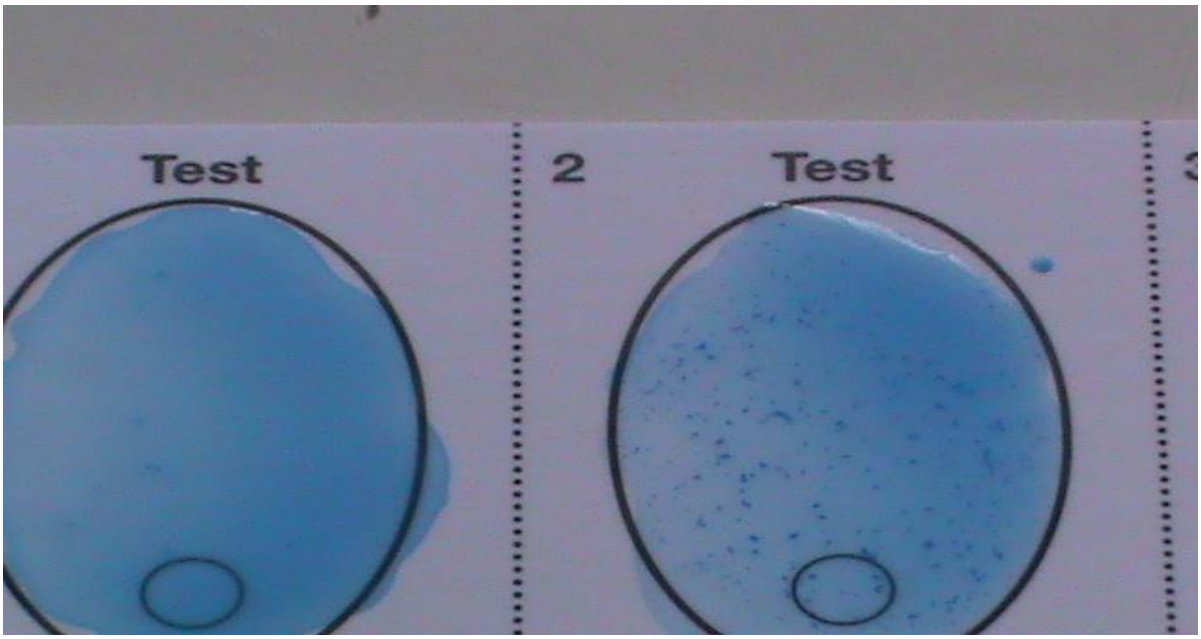
8. APPENDICES

Appendix 1: Indole test; Uninoculated medium (left side) and positive reaction (Red ring).

Appendix 2: Smooth blue suspension (1 test) and blue latex particles coated antigen (2 test) which is ready for identification of *E. coli* serogroup O157.



Appendix 3: Smooth blue suspension (negative result) and agglutination of isolated NSF colonies on SMAC with antibody specifically reactive with the *Escherichia* O157 serogroup (positive result).



Appendix 4: Antimicrobial susceptibility for fresh goat meat swab (GMS).

Appendix 5: Preparation of media and reagents

***MacConkey agar (*CMO115, Oxoid)**.

Formula	grams per litre
Peptone	20.0
Lactose	10.0
Bile salts No.3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0

PH 7.1 (Approximately)

51.5 grams of the powder were suspended in a litre of distilled water brought to boil to dissolve completely and dissolved completely and sterilised at (121^0C for 15 minutes).The molten agar was cooled to 50^0c and approximately 20ml poured into a Petri dish (90mm in diameter) and allowed to cool and solidify at room temperature.

Tryptone soya broth (CMO129, Oxoid)

Formula	grams per litre
Pancreatic digest of casein	17.0
Enzymatic*digest of soy mean	3.0
Sodium chloride	5.0
Di-potassium hydrogen phosphate	2.5
Dextrose	2.5

PH 7.3 (approximately)

*(contains papain)

Dissolve 30 g in 1litre of water (purified, as required) and distributed into final containers. Sterilize by autoclaving at 121^0c for 15 minutes.

Glycerine 25%* tryptone soya *broth

Thirty grams of tryptone soya broth powder and 25ml of glycerine were added to 75ml of distilled water and brought to boil to dissolve completely .The medium was dispensed in to

cry vials in 1.8ml amounts. Sterilize by autoclaving at 121^0c for 15 minutes. These were used for storing culture at-20˚C.

Indole reagent (Ehrlichs reagent)

1-gram para-dimethylaminobenzaldehyde
95 ml absolute ethanol
20ml concentrated hydrochloric acid
1 gram of P-dimethylaminobenzaldehyde was dissolved in 95ml absolute ethanol before adding 20ml of concentrated hydrochloric acid. The solution was stored in an amber bottle. For testing presence of indole few drops were added to the culture medium; and a red colour indicates a positive result and no change in colour a negative result.

Modified tryptone soya broth supplemented with Novobiocin. (mTSB+N)

Pancreatic digest of casein	17.0 g
Papaic digest of soybean meal	3.0 g
Sodium chloride	5.0 g
Di-potassium hydrogen phosphate	4.0 g
Glucose	2.5 g
Bile salts	1.5 g
Novobiocin solution	2.0 ml
Water	1000 ml

Dissolve the dehydrated medium in the water by heating if necessary. Transfer into a bottle and autoclave at 121^{o}C for 15 min. Allow the media to cool to 50°C before adding the novobiocin supplement as appropriate.

Novobiocin solution.

Novobiocin	10mg
Water	2ml

Dissolve the novobiocin in the water and sterilise by membrane filtration. Do not store the solution for more than one day.

Sorbitol MacConkey agar (CMO813, Oxoid)

Formula	grams per litre
Peptone	20.0
Lactose	10.0
Bile salts No.3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0
PH 7.1 (Approximately)	

Suspend 51.5 in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121 ^{0}c for 15 minutes.

Nutrient Agar (DM 1001, microgen)

Formula	grams per litre
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Beef extract	1.50
Yeast extract	1.50
Agar	15.00
PH 7.4 (Approximately)	

Suspend 28.00 gms in 1000ml distilled water. Heat to boiling to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121^{0}C) for 15 minutes. Mix well before pouring.

Peptone water (CM0009)

Formula	grams per litre
Peptone	10.00
Sodium chloride	5.00

PH 7.4 (Approximately)

Add 15g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121 ^{0}C for 15 minutes.

Appendix 6: Questionnaire

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Microbiology, Immunology and Veterinary Public Health

Questionnaire for data collection from Dire Dawa Municipal abattoir worker's to assess their knowledge, attitude and practice concerning slaughter hygiene, Dire Dawa, 2006.

Verbal consent form before conducting interview

Greeting:

Hello, my name is _____. I am working in the research team of Addis Ababa University. I would like to interview you a few questions about the sanitary condition of your slaughter houses and some of the questions require physical observation and taking swab samples from your hands. The objective of this study is to assess practice concerning slaughter hygiene, which is important to improve the sanitary status so as to safeguard the safety of goat meat reaching consumer from slaughter houses. Your cooperation and willingness for the interview and observation is helpful in identifying problems related to the subject matter. Your name will not be written in this form. All information that you give will be kept strictly confidential. Your participation is voluntary and you are not obliged to answer any question you do not wish to answer. If you are not still comfort with the interview please feel free to drop it any time you want. Do I have your permission to continue?

1. If yes, continue to the next page
2. If no, skip to the next participant

General instruction

Almost all questions have pre-coded response. So it is important to follow the following instructions while you are interviewing respondents and recording their answer.

- Ask each question exactly as it is written on the questionnaire.
- Do not rely on the response of respondents only; inspect/observe the areas that need physical observation
- Do not read the pre-coded response to respondents. listen only the response of respondent
- Circle the response in the response column that best matches the answer of the respondent.

Respondent Name: _____Address: _____

Educational status: 1) Illiterate 3) Grade 1-6 2) Grade 7-12 4) Grade >12

Employment status: Temporary ☐ Permanent ☐

Questionnaire code: _____

Section I. General knowledge of slaughter house worker and training information

Sr.No	Question	Response	Code
1	Did you receive any job related training?	1. Yes [ ] 2. No [ ]	/___/
2	If yes to 1; Has the training been helpful?	1. Yes [ ] 2. No [ ]	/___/
3	If yes to 2; in what ways?	1=I have become more efficient in my work 2=I have become more aware of hygienic practices e.g. Cleaning hands, wearing, protective wear, cleaning of equipment 3= I have become more hygienic/	/___/

		cleaner 4= If other, specify	
4	Have you undergone any job related medical tests to work in the slaughter house?	1. Yes [ ] 2. No [ ]	/____/
5	Have you ever heard about foodborne disease?	1. Yes [ ] 2. No [ ] **(If no skip to 1.9)**	/____/
6	What is the cause of foodborne disease? **(Circle all responses)**	1. Germs 2. Chemicals 3. Evil eye 4. Super natural force 5. Do not know 6. If other, specify	/___/ /___/ /___/ /___/ /___/
7	Foodborne disease is transmitted by **(Circle all responses)**	1. Contaminated food 2. Contaminated water 3. Vectors like flies and cockroaches 4. Do not know 5. If other, specify	/___/ /___/ /___/ /___/ /___/
8	At what stage does a carcass get contaminated are more likely in slaughter houses?	1.Stunning 2.Bleeding 3.Flaying 4. Evisceration 5. Splitting 6. Inspection 7. Washing 8. If other, specify	/___/ /___/ /___/ /___/ /___/ /___/ /___/ /___/
9	What is the reason for goat carcass contamination?	1. Dirty hands 2. Infected slaughter house worker 3. Accidental puncture of GIT 4. Dirty utensils 5. Dirty working area	/___/ /___/ /___/ /___/ /___/

		6. Do not know 7.If other, specify	/___/ /___/
10	In your opinion, does contamination pose any health risk to meat consumers?	1. Yes [ ] 2. No [ ]	/___/
11	If No, why?	_____	/____/
12	When you are ill do you still work/ report to the management?	_____	/____/

Section II. Attitude

I will read you some statements about hygiene in the slaughter process. Please indicate whether you agree or disagree. KEY: SA= Strongly Agree, A= Agree, D= Disagree, SD= Strongly Disagree and Dk= Don't know

Question	**SA**	**A**	**D**	**SD**	**DK**
1. In this job, it is more important to work quickly than keep the carcasses clean.					
2. People doing this job are more likely to get sick					
3. In this type of working environment, keeping clean is easy					
4. A small amount of dirt on clothing or utensils will not cause any harm					
5. Health is more important than wealth					
6. Ensuring hygiene is mainly the role of management					
7. If meat is well-cooked then it is always safe to eat					

Section III. Practices (slaughter house observation checklist).

Does the food handler wear outer garments/gown during inspection?	1. Yes [ ] 2. No [ ]
Does the garments/gown is clean?	1. Yes [ ] 2. No [ ]
Cuts/wounds covered with an appropriate	1. Yes [ ] 2.No [ ] 3.N/A [ ]

waterproof dressing	
Smoking or eating or chewing while working	1. Smoking [ ] 2. Eating [ ]
Clothes clean and completely free from any dirt or blood	1. Yes [ ] 2. No [ ]
Hand washing :before, after and during cutting meat	1. Before [ ] 2. After [ ] 3. During [ ]
How washed? Running water or bucket? Hot or cold? Brush or cloth? Soap?	1. Running water [ ] 2.Bucket [ ] 3.Hot [ ] 4.Cold [ ] 5. Brush [ ] 6. Cloth 7.Soap [ ]
Fingernails short and completely clean	1. Yes [ ] 2. No [ ]
All knives are completely clean and free from dirt, cracks and damages	1. Yes [ ] 2. No [ ]
Knives are cleaned before, after and during use	1. Before [ ] 2. After 3. during use[ ]
How cleaned tick as you think it should be used.	1. Running water [ ] 2. Bucket 3. Hot [ ] 4. Cold [ ] 5.Brush [ ] 6. Cloth [ ] 7.Soap [ ]
Is any disinfectant used? Write name of disinfectant	1. Yes [ ] 2. No [ ]
Latrine available nearby	1. Yes [ ] 2. No [ ]
Latrine has water, soap, paper, towels for hand washing (tick all that apply)	1. Water [ ] 2. Soap 3. Paper [ ] 4. Towels [ ] 5. Tissue Paper[ ]
Equipments rested in dirty surfaces during working	1. Yes [ ] 2. No [ ]
Strict separation between clean and dirty areas	1. Yes [ ] 2. No [ ]
Veterinary inspectors present to examine and pass carcass for consumption.	1. Yes [ ] 2. No [ ]

Section IV. Perceptions

1. What constraints do you experience in your work?

2. Do they affect your ability to achieve high levels of hygiene? 1=YES [ ] 2= NO [ ]

3. If yes, in what way(s)?. ....

4. In your opinion, what role do you think the management should play in:

   (a) Setting standards for hygiene in the slaughterhouse?

   (b) Maintaining those standards?

5. In your opinion, what role do you think the workers should play in?

   (a) Maintaining standards for hygiene in the slaughterhouse?

Remark_____

_____

_____

_____

Thank you!

Appendix 8: Summary of observations result on slaughterhouse worker's practices in the Dire Dawa municipal abattoir, Ethiopia.

Practices and observation types	**Value**
Does the slaughter house worker wear outer garments/gown during abattoir visit?	Yes (100%)
Does the garments/gown is clean?	No (83%)
Cuts/wounds uncovered with an appropriate waterproof dressing	17%
Smoking or eating or chewing while working	Not Available
Clothes clean and completely free from any dirt or blood	No
Hand washing :before, after and during cutting meat	0
How washed? Running water or bucket? hot or cold? brush or cloth? Soap?	Bucket
Fingernails short and completely clean	No
All knives are completely clean and free from dirt, cracks and damages	No
Knives are cleaned before, after and during use	No
How cleaned tick as you think it should be used.	Bucket
Is any disinfectant used?	No
Latrine available nearby	Yes
Latrine has water, soap, paper, towels for hand washing	Not Available
Equipments rested in dirty surfaces during working	Yes
Strict separation between clean and dirty areas	No
Veterinary inspectors present to examine and pass carcass for consumption.	Yes

Appendix 8: Record sheet

Sample: Carcass swab #1, cecal content #1, environmental sample #1

	Colour	Result	Comment
Morphology of colonies on SMAC			
Morphology of colonies on MacConkey			
Indole test			
Antigenic reaction			

Sample: Carcass swab #2, cecal content #2, environmental sample #1

	Colour	Result	Comment
Morphology of colonies on SMAC			
Morphology of colonies on MacConkey			
Indole test			
Antigenic reaction			

Antimicrobial susceptibility testing (in diameter)

Isolate Number	Ampicillin	AMC	Cefotaxime	Ceftriaxone	Cefoxitin	CXM	Chloramphenicol	Ciprofloxacin	Erythromycin	Gentamicin	Kanamycin	Nalidixic Acid	Nitrofurantoin	Norfloxacin	Streptomycin	SXT	Sulfonamides	Tetracycline