ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE

SCHOOL OF MEDICINE

DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY

Isolation and Antimicrobial Resistance Determination of Escherichia coli O157:H7 from Raw Meat in Selected Abattoirs and Butcher Shops, Addis Ababa, Ethiopia.

A THESIS SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY, COLLEGE OF HEALTH SIENCES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICAL MICROBIOLOGY

BY: NAHOM MISKER

AUGUST, 2021

ADDIS ABABA, ETHIOPIA

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE

SCHOOL OF MEDICINE

DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY

Isolation and Antimicrobial Resistance Determination of Escherichia coli O157:H7 from Raw Meat in Selected Abattoirs and Butcher Shops, Addis Ababa, Ethiopia.

Advisors:

1 - DR. WOLDAREGAY ERKU ABEGAZ

Associate professor of Medical Microbiology

Department of Medical Microbiology, Immunology and Parasitology

School of Medicine Addis Ababa University, Ethiopia

2- DR. MATIOS LAKEW

Researcher of Veterinary Microbiology
National Animal Health Diagnostic and Investigation Center (NAHDIC)

AUGUST, 2021

Contents

ACKNOWLEDGMENT

Above all, we have grown in faith and know that the strength, the perseverance and the blessing of all wonderful things in our life are a gift from God the almighty and his mother St. marry. First of all, I would like to provide my great gratitude for the almighty GOD who supports me in all activities throughout my life.

I would like to express my deepest gratitude, appreciation and honor to my principal supervisor Dr. Woldaregay Erku Abegaz, Associated professor, for his academic guidance, supervision, encouragement, economical support and inspiration, like true fathers did. I am also highly thankful to, my co-advisor, Dr. Matios Lakew for his academic guidance, supervision, financial and material support, motivation and guidance in finalizing this thesis.

My great appreciation also goes to Mr. Tesfaye Legesse, researcher at EPHI, who had supported me academically, technically, financially and spiritually. My gratitude goes to Food Safety Research Laboratory staffs, Ethiopian Public Health Institution (EPHI); special thanks provided for Kaleab Sebsibe, Tatek Kasim, and from the National Animal Health Diagnostic and Investigative Center (NAHDIC) laboratory workers; special thanks go to Abebe Olani, Leteberhan Yimesgen and Tafese Koran department of Veterinary microbiology. My thank also extends to the Assosa University Collage of Natural and Computational Science for creating helpful environment in order to accomplish this thesis.

Finally, I would like to express my invaluable and worthy gratitude to my lovely families especially to my sister W/o Hiwot Fikru and her family, my wife W/o Berhane Amare and my son Noub Nahom, for their strength in helping me morally, spiritually and financially.

LIST OF FIGURES

LIST OF ABBREVIATIONS

AEEC	Attaching and effacing E. coli
AAAE	Addis Ababa Abattoir Enterprise
CFU	Colony forming unit
CLSI	Clinical and Laboratory Standards Institution
EAEC	Enteroaggregative *E. coli*
Eae	Intimin gene
EHEC	Enterohaemorrhagic *E. coli*
EPEC	Enteropathogenic *E. coli*
EPHI	Ethiopian public health institute
ETEC	Enterotoxigenic *E. coli*
Gb3	Globotriosylceramide
GIT	Gastro intestinal tract
HC	Hemorrhagic colitis
Hly-A	Hemolysin
HUS	Hemolytic uremic syndrome
PCR	Polymerase chain reaction
SLT	Shiga-like toxin
Sta	Heat-stable toxin
STEC	Shiga toxin producing *E. coli*
TSB	Tryptone soya broth
TTP	Thrombotic thrombocytopenic purpura
VBNC	Viable but not culturable
VTEC	Verocytotoxin producing *E. coli*
WHO	World health organization

LIST OF TABLES……………………………………………………………page No.

LIST OF ANNEXES

ABSTRACT

Background: Foodborne illness and death due to foodborne diseases caused by highly dangerous pathogens is common in the world; especially it is widespread in developing nations. In Ethiopia, the consumption of meat as a raw is well practiced among the people, which is a potential cause of foodborne illness. Cattle are considered as reservoir of pathogens like the highly virulent *Escherichia coli* O157:H7 (*E. coli* O157:H7). Low standard of meat handling and safety practices, lack of protecting personal hygiene among workers of meat supply chain and lack of awareness about *E. coli* O157:H7-caused infection are major problems in Ethiopia. Furthermore, there is paucity of information regarding the epidemiology of the pathogen along the beef carcass supply chain at Addis Ababa Abattoir Enterprise (AAAE). Therefore, the purpose of this study was to isolate *E. coli* O157: H7 and determine the site of contamination of raw beef meat at AAAE and its carcass supply chain of butcher shops in Yeka sub-city, Addis Ababa, Ethiopia

Methodology: A total of 210 samples from carcass swab, raw minced beef meat and pooled environmental sources were collected along the supply chain. Collected samples were cultured to isolate *E. coli* O157: H7 and identified using biochemical tests and Biolog bacterial identification system. Antimicrobial susceptibility tests were performed on *E. coli* O157: H7 isolates using the Kirby-Bauer disk diffusion method on Muller-Hinton agar. Observation was used to assess the overall hygiene and sanitation of the beef-selling environment, and a questionnaire was used to assess the workers' knowledge, attitude, and practice. Following data entry into an Excel spreadsheet, the data was exported to and analyzed using statistical software (SPSS version 26.0). Descriptive statistics and the Chi-square (X^2) test were used, and a p-value ($p< 0.05$) was considered statistically significant.

Result: The overall prevalence of *E. coli* O157:H7 was 2/210 (0.95%) and the two isolates were detected from knife swab sample at slaughter house and sample from minced raw beef meat at butcher shop. In the case of the antimicrobial susceptibility test, both isolates were susceptible to ciprofloxacin, gentamicin, meropenem, chloramphenicol, amoxicillin clavulanate and cefotaxime, but resistant to sulfamethoxazole + trimethoprim, amoxicillin, streptomycin and ampicillin.

Conclusion: Overall, although the prevalence of *E. coli* O157:H7 was low, the results indicated that the contamination detected from raw beef meat from butcher shop and the utensil in the abattoir is a matter of concern that calls for an effort to improve the hygienic status. Hence, to ensure the hygienic quality of meat, everyone involved in carcass slaughter, distribution and serving to the customers in the chain should be trained for sanitary and hygienic practices of these meat supply services including providing basic trainings to the personnel involved in the industry.

Key words: *AAAE, Antimicrobial susceptibility, Butcher shop, E. coli* O157:H7.

1. INTRODUCTION

Foodborne illness is a gastrointestinal disease mostly caused by highly infectious pathogenic microorganisms (Agüeria *et al*., 2018). Foodborne disease can be classified into three major groups, namely, food infection, food intoxication and toxin-mediated infection (Teplitski *et al.,* 2009). Food infection is caused by ingestion of food which contains viable pathogens while food intoxication refers to ingestion of secreted toxic chemicals produced by microorganisms. On the other hand, toxin-mediated infection happens due to production of enterotoxins by pathogenic microbes throughout colonization and cell production in gastrointestinal tract (Redmond and Griffith, 2010).

The risk of microbial foodborne disease is increasing throughout the world, even though the scientific and technological development in livestock sector shows progressive improvement (Mersha *et al.,*2009). Developing nation such as Ethiopia, are commonly susceptible to foodborne diseases because of poor food handling and sanitation practice, insufficient food safety management, changing food consumption pattern, weak monitoring system, complex and lengthy of food processing techniques, weakened infrastructure due to limited financial resource to invest on safer equipment and illegal slaughtering practice (Disassa *et al.,* 2017).

Even though almost half of foodborne illness is caused by viruses, bacterial foodborne disease is responsible for causing approximately 15-20% of hospitalizations and deaths occur in the world (Teplitski *et al.,* 2009 and Painter *et al.,* 2013). Currently, foodborne diseases affect almost 1 in 10 people (around 600 million individuals) and 420,000 deaths every year in the world (WHO, 2017). Foodborne pathogens such as non-typhi serotypes of *Salmonella enterica*, *Campylobacter* species, *Listeria monocytogenes*, *Yersinia enterocolitica*, staphylococcal food poisoning and Shiga toxin-producing strains of *Escherichia coli* (*E. coli*) and Enterobacter are the major illness caused bacteria (Adams *et al*., 2010).

E. coli is one of the best studied bacterial species since it was discovered by Theodor Escherich in 1885 (Escherich, 1988). The genus Escherichia is a member of the family *Enterobacteriaceae,* and grouped under gram-negative rod bacteria, non-sporulating, facultative anaerobic which can ferment glucose and a wide range of other sugars (Hazen *et al*., 2013). There are several types of *E. coli* strains; however, only few of them are pathogenic due to their production of a variety of highly effective virulence factors including toxins. These pathogenic types are categorized in to six sub-types based on the antigens they possess (somatic, O, capsular, K, and flagella, H), pathogenicity mechanism, virulence factor, or clinical symptoms of the bacteria (Canizales *et al.*, 2013). The sub-types include; Enterotoxigenic *E. coli*, attaching and effacing *E. coli*, enteropathogenic *E. coli*, enteroinvasive *E. coli*, enteroaggregative *E. coli* and enterohemorrhagic *E. coli* (also known as Vero toxin producing *E. coli* (VTEC), or Shiga-toxigenic *E. coli* (STEC)) (Canizales *et al.*, 2013).

Enterohemorrhagic *E. coli* (EHEC) contains different strains, among which *E. coli* O157:H7 is the most virulent and responsible for serious diseases like hemolytic-uremic syndrome (HUS), hemorrhagic colitis (HC) and thrombotic thrombocytopenic purpura (TTP) (Wang *et al.*, 2014). The strain produces shiga-like toxin (SLT), which is a verotoxin that causes diarrhea both in human and animals. The ability of producing toxic substances by *E. coli* O157:H7 was gained as the result of transduction process with bacteriophage carrying SLT1 and SLT2 genes. The SLT̕s of *E. coli* O157:H7 are cytotoxic to human colon and ileum cells. In addition to SLT toxin production, there is also secretion of intimin (*eae*), which provide the microorganism to attach itself to epithelial part of intestinal tract (O'Loughlin and Robins-Browne, 2001). Due to secretion of stx inside the colon, it may lead to death through the mechanism in which the toxin is transported via blood stream to aggravate massive attack on target cells leading to failure of certain tissues found on the targeted organ (Detzner *et al*., 2020).

The global epidemiology of foodborne disease due to *E. coli* O157:H7 is estimated to be about 2,801,000 million illnesses, with incidence rate of 43.1 cases per 100,000 per- person each year (Fatima and Aziz, 2020). Among those, developing nations of Africa suffer a huge burden leading to a total of 10,200 cases of STEC with incidence rate of 1.4 cases per 100,000 person – years, 10% of this burden developed to HUS (Majowicz *et al.,* 2014 and Lupindu, 2018). According to World health organization (WHO) report, in 2004, in the USA, *E. coli* O157:H7 causes 73,000 illnesses, 2000 hospitalization, and 50-60 deaths occurred each year (WHO, 2015). In Ethiopia, due to poor outdoor livestock management and their products, the country is exposed to be a host for the second highest foodborne disease in the world next to Nigeria (Havelaar *et al*., 2015).

In most cases, cattle are incriminated as primary reservoirs and usually asymptomatic carriers of *E. coli* O157:H7. Therefore, it is transient in gastrointestinal tract of animals, so cattle feces can be the potential source of *E. coli* O157:H7 for humans. However, researches indicated that the main sources of this pathogen can be consumption of raw meat, raw milk and under cooked ground beef (Beyi *et al.,* 2017).

Meat is a nutrient rich food which is composed of water, protein, fat and some essential minerals and vitamins with higher bioavailability than other food contents (Mboto *et al.,* 2012). However, the presence of those essential nutrition values in meat makes it the main vehicle for the transmission of foodborne microbes to humans by creating a good environment favoring growth and reproduction of the pathogens which contaminate the food (Bryan *et al*., 2015). The condition of hygienic practice during slaughtering, meat transportation and distribution, meat handling, storage and processing may create possible *E. coli* O157:H7 contamination of carcass (Abraham *et al.,* 2012). Carcass from cattle, sheep and goats may be exposed to *E. coli* and other bacteria from environment, because of low quality of potable water, improper wastage disposal or poorly constructed wastage outlet, poorly ventilated slaughter house hall, poor handling and use of contaminated equipment for cutting and evisceration process, all of which could compromise the quality of meat through contamination (Ntanga *et al.*, 2013).

In this connection, meat transportation van can be responsible for contamination of their package due to lack of regular cleaning of the track exposed to dust, insects and flies that leads to meat contamination (Ntanga *et al.,* 2013). Workers in abattoirs and butcher shops may also act as a source of contamination of carcass at ante or post mortem due to improper hand washing, unclothing of working dress, lack of awareness on personal hygiene, and collectively taking part in cross contamination of illness caused by EHEC O157:H7 (Sofos *et al.,* 2008). In the past two decades, almost all continents around the world have reported isolation of the pathogens, including *E. coli* O157:H7 from food, animal, clinical sample and environmental samples (Dulo *et al.,* 2014).

In Ethiopia, several antibiotics have been used either for therapeutic or prophylactic purposes to treat unhealthy food-animals for the purpose of reducing potential risk of STEC transmission. In addition, those same drugs are also used for growth promotion in livestock. Such indiscriminate uses of antibiotics like the aforementioned types of treatment are likely to lead to the development of considerable resistant bacterial strains (sebsibe and Asfaw, 2020).

1.1. Statement of the problem

In Ethiopia, the consumption habit of meat as raw or under cooked (*Kurt siga, dulet leb-leb and kitfo leb-leb*) is very traditional, and contaminated carcass is the most common rout of *E. coli* O157:H7 from livestock animals to community (Sebsibe and Asfaw, 2020). In Ethiopia there has been dramatically an increased demand for meat products possibly as a result of shifting of life style, which is a driving force to change feeding habit for most people toward animal products due to rising income, urbanization, and technical development of animal farming sector (Zerabruk *et al*., 2019). However, food safety and hygiene with respect to EHEC-related disease is a great concern for Ethiopia (Disassa *et al.*, 2017) because raw meat can harbor pathogenic bacteria; cattle are a major natural reservoir of *E. coli* O157:H7; and the contamination caused by this pathogen is very common in abattoirs and in butcher shops (Chepkemoi, 2016).

Tissue from healthy cattle is sterile. However, the environmental condition of beef meat supply chain can act as an important source of contamination with *E. coli* O157:H7 during processing and manipulation such as skinning, evisceration, processing, storage and distribution of the carcass (Annan-Prah *et al*., 2012). Improper management of fecal matter can be a major source of contamination through direct contact with carcass or indirect contact with water, floor, knives or different equipment found in abattoir (Ntanga *et al.*, 2013).

Transportation of carcass during its distribution from slaughter house to whole sell is facilitated through specially prepared track known as meat van. The hygienic condition and sanitary practice of this van and the people working therein must be in focus by stakeholders. However, developing countries, like Ethiopia, face challenges due to insufficient availability of meat vans. Even though few trucks are available, still they are old and are not modern that makes hygienic way of transporting meat difficult, especially where standardize legal framework is lacking (Getachew *et al.,* 2008). In addition, lack of regular cleaning to remove dust, insects and flies from old trucks can promote meat contamination (Ntanga *et al.*, 2013). Furthermore, insufficient personal hygiene among staff members in meat supply chain may act as a source of cross contamination of the pathogen (Sofos *et al.*, 2008).

Meat handling practices among most butcheries of developing nations do not meet the minimum sanitation and hygiene standard. For instance, the study which conducted in two states of Kenya reported that 60% and 69% of meat operators in Isiolo and Nairobi butcheries, respectively, didn't wash their hands before handling meat; 60% and 34%, respectively, of them used regular and reused sponges or cloths to clean utensils; and 90% and 87% of operators, respectively, handled meat concurrently with handling money (Chepkemoi, 2016).

There are some practices associated with meat handling that can be risk factors for causing foodborne illness including meat hanging in open space in butchery, using chopping board or table that is not cleaned after work, poor supply of safe water found in abattoir and butcher shops that is used for general purpose, lack of having medical certificate, absence of training on meat handling, and lack of protecting personal hygiene among staff members working in the meat supply chain (Ntanga *et al*., 2013).

According to World Bank report in 2018, there were 600 million cases reported on foodborne disease globally, and the world spent 110 billion dollars for combating the disease. It is a common health problem in developing nations, including Ethiopia, where it is considered as one of the highest foodborne disease-burden country and where *E. coli* O157:H7 has the potential to be one of the major causes of diarrheal disease (Kibret and Abera, 2011). Due to lack of awareness about the infection caused by *E. coli* O157:H7, people in Ethiopia usually consume animal products as a raw or undercooked (Adugna *et al.,* 2015).

Considerable number of studies in Ethiopia have reported the occurrence of *E. coli* O157:H7 isolates from food of animal origin, animal surfaces and feces samples taken from slaughter houses, retail shops, street vendors, restaurants, farms and milk vendors (Abdissa *et al.,* 2017; Abebe *et al*., 2014; Atinafe *et al.*, 2017; Bedasa *et al*., 2018; Bekele *et al*., 2014; Beyi *et al.*, 2017; Dulo *et al*., 2014; Haile *et al.*, 2017; Haile Selassie *et al*., 2013; Hiko *et al.*, 2008; Mengist *et al*., 2018; Messele *et al.*, 2017; Mohammed *et al.*, 2014; Taye *et al.*, 2013). Although most of these studies focused on prevalence and antimicrobial susceptibility test of *E. coli* O157:H7, none of them focused on identifying the potential microbial contamination source of *E. coli* O157:H7 and their links along the long chain of meat production process starting from slaughtering up to the point of readying for consumption including transportation. Thus, this proposed research work is intended to investigate the prevalence of *E. coli* O157:H7 isolates, antimicrobial susceptibility and the potential contamination points in raw beef meat processing during its distribution in the route from abattoirs to retailers at Addis Ababa. In addition, the study will evaluate meat handling and hygiene knowledge, attitude and practice among working staff.

1.2. Significance of the study

Data generated from this research will be important for improving stakeholders' ability to design accurate intervention strategy tailored to reduce contamination of raw meat starting from abattoir through transportation in the meat van to butcher shops, where the meat is ready for consumption. In addition, it also generates information that may be used as reference material for future studies.

The study will provide information regarding level of people's awareness on the matter so that concerned bodies will be able to create awareness various parties on how consuming meat as raw brings health problem, how potential contamination of meat by bacterial pathogens reduces its quality, and the importance of improving workers' attitudes, understanding, and hygienic practices for safe services of meat to the consumer. Such better understanding of food safety and hygienic practice among people involved in the meat supply chain may in turn contribute in promoting healthy public life and in reducing millions of dollars in combating the disease. The result of antimicrobial susceptibility pattern of *E. coli* O157:H7 will also provide information about the drug resistance status of the isolates, and so help clinicians in their choice of drugs.

2. LITERATURE REVIEW

2.1. Composition and importance of meat in human health

Meat is the flesh of animals that plays a key role in human nutrition. People have hunted and slaughtered animals for food since the pre-Neolithic revolution around 9000 BC. With the advent of civilization, the sheep became the first domesticated livestock, followed by goats, pigs, camels, and cattle. This gradually contributed to their use in industrial-scale meat processing with the help of slaughterhouses (McNeill *et al.*, 2017). Meat is primarily composed of water, protein, and fat from a nutritional standpoint. Zink, vitamin B12, selenium, phosphorus, niacin, vitamin B6, choline, riboflavin, and iron are among the most important micronutrients contained in it (Ahmad, 2018).

People in many parts of the world consume meat as their primary protein source, and it plays an important role in the human body, such as wound healing, muscle strengthening, and immune function. Red meat, in particular, is a good source of iron, which is needed for the formation of red blood cells. Red meat and meat products are contributing 30% of the readily absorbable zinc in a human's diet. Zinc is essential for immune system fitness, development, cell maintenance, and fertility (Kebede *et al.,* 2014).

Although vitamin B can be found in a few plants, animal products are the most common source. Vitamin B is essential for the growth, development, and release of energy in healthy blood cells. Meat also plays an important part in vitamin D absorption, which allows calcium and phosphorus to be enabled in the development of healthy bones and teeth (Ahmad *et al*., 2018). Over the past two decades, the fat content of red meat has decreased dramatically, and the level of fat in red meat is now much smaller than most people believe. Breeding methods on the field and modern butchering techniques that cut off the fat have helped to accomplish these reductions. Eating too much fat and cholesterol can cause hypercholesterolemia, or an accumulation of cholesterol in the bloodstream, increasing the risk of heart disease in consumers (Hassan *et al.*, 2019).

2.2. Hygienic quality of meat and microbial contamination

Foodborne diseases are currently a global public health concern; developing countries, in particular, are adversely impacted due to the prevalence of poor food handling and sanitation practices, inadequate food safety laws, shortage of resources to invest in safer equipment, and lack of food handling education, which makes meat and its products extremely prone to contamination (Abreham *et al.,* 2019).

2.2.1. Contamination from abattoirs and butcher shops

Slaughter house is a suitable location licensed by an accredited agency and registered for routine hygienic inspection and slaughtering of healthy animals, as well as preparation and delivery of the carcass using effective operative procedures and guidance (Shamsul *et al.*, 2016). Contamination of carcasses is heavily influenced by the environment at the slaughterhouse and at the meat counter. The availability of high-quality water for washing carcasses, as well as careful site selection for abattoirs or retail meat outlets, are essential considerations that can influence meat quality. Meat poisoning occurs in abattoirs as a result of inadequate preslaughter livestock care, unsanitary slaughtering equipment procedures, and a squalid slaughtering environment (Ntanga, 2013).

The length of time that cattle spend at the abattoir before being slaughtered could be the first time they are exposed to pathogens, raising the risk of disease and infection. The type of water source used to clean the pen surface, railings, and feed may lead to an increase in pathogen load in the slaughterhouse area (Bersisa *et al*., 2019). In the later stages of slaughtering process, dirt, mud, body discharges, and excreta from animals in holding pens or lair may often be considered as the principal causes of pollution of carcasses. In addition, cutting knives, intestinal contents, chopping boards, skins, meat handlers, bins, vehicles for shipping carcasses, and the meat sale atmosphere are also potential causes of pollution (Ntanga, 2013). Knives, meat grinders, axes, hooks/meat hanging branches, sponge/towels, wooden boards, and measuring scales from retail shops can also be a cause of bacterial infection. Generally, poorly managed abattoir and retail facilities are a source of contamination and pollution; they attract domestic and wild carnivores, mice, and flies, all of which being vectors of microbes (Bersisa *et al.,* 2019).

2.2.2. Meat van as a source for contamination

Meat vans are vehicles that carry carcasses from abattoirs to wholesalers. Due to inadequate or irregular track washing, which prevents microbial infection from dust and insects, the meat van may be a source of contamination. Furthermore, in developing countries, especially in their municipal abattoirs, the tracks used for carcass delivery are still limited in number and old, with no additional accessories to sustain a standard level of hygienic quality in the van, resulting in carcass contamination with high microbial loads (Ntanga, 2013).

2.2.3. Abattoir and retail meat workers as sources of contamination

Cross contamination of beef before and during processing could be caused by un hygienic condition of abattoir and retail meat outlet workers. Microbial infection was attributed to improper hygienic practices, such as unclean slaughter men's hands, butcher arms, garments, and tools used in carcass dressing. Most of the abattoir and butcher shops employees are contract workers in which the work experience due to the daily practices for long period of time is unfortunate, as a result of the above conditions, this sector has little or no people with adequate expertise on food protection and sanitary procedures during the slaughtering, packaging, and delivery of cattle carcasses (Zelalem *et al.*, 2005).

2.3. The common pathogenic microbes present in meat and meat products

Helminths, moulds, bacteria, and viruses are important microorganisms in terms of meat hygiene. Despite the fact that viruses cause half of all foodborne diseases worldwide, bacteria remain the most common cause of hospitalization and death (Timothy *et al.,* 2015). *Salmonella* spp., *Campylobacter* spp., *Clostridium perifringes*, *Yersinia enterocolitica*, *Shigella* spp., *Listeria* spp., *Staphylococcus aureus*, and *E. coli* are the most common bacteria found in beef products (Bakhtiary *et al.,* 2016). In addition, member of the fecal coliform group, such as *E. coli*, is often used as markers of fecal contamination of food and water (Adamu *et al.,* 2014).

2.3.1. *Escherichia coli*

E. coli was first named by a German pediatrician, Theodore Escherich, in 1885, after being isolated from human neonates (Shulman *et al.*, 2007). *E. coli* is grouped under Enterobacteriaceae family, class gamma enterobacteria. It is a gram-negative, rod-shaped bacterium, oxidase negative, non-sporulating facultative anaerobe, flagellated, and has the ability to ferment glucose and lactose sugars (Croxen *et al.,* 2013). *E. coli* is a common microbe found in the gastrointestinal tract (GIT) of warm-blooded animals and humans. Any pathogenic *E. coli* variants, on the other hand, can cause debilitating and often lethal disease in humans (Hajian *et al.,* 2011). *E. coli* spp. are also useful markers of the hygienic condition of food and water that are associated with fecal matter (Songe *et al.,* 2017). The pathogenic form of *E. coli* can be divided into two categories: gastro intestinal diarrheagenic *E. coli* and extraintestinal *E. coli* (Jafari *et al.*, 2012).

Depending on the different surface antigens they exhibit (somatic, O, capsular, K, and flagellar, H), the mechanism of pathogenicity, virulence factors, or clinical manifestations of the disease, there are six main pathotypes of diarrheagenic *E. coli* (Canzales *et al.,* 2013). These sub types include enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), adherent invasive *E. coli* (AIEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and enterohaemorrhagic *E. coli* (EHEC) or STEC/VTEC (Croxen *et al*., 2013). The most significant pathotype in public health is Shiga toxin producing *E. coli* (STEC) or Vero toxin producing *E. coli* (VTEC) (Shahrani *et al.,* 2014).

ETEC, a toxin-producing bacterium that causes diarrhea in humans and animals, is often commonly linked to traveler's diarrhea. Abdominal cramping, nausea, low fever, and watery diarrhea of mucus or pus are signs and symptoms of infection (Shahrani *et al.,* 2014). Enterotoxin and colonization factors (fimbriae) are two virulence factors produced by this pathogen. In developed countries, infection by this pathogen is very common (Christina and Bertil ,2005).

EPEC was the first *E. coli* strain found and is the most prevalent pathotype of infantile diarrhea. EPEC infections have decreased significantly in developed countries as a result of technological advancements in protection and hygienic practice. However, this is not the case in developing countries, where the strain is also a major cause of potentially lethal infantile diarrhea (Shahrani, 2014).

EAEC is a category of *E. coli* strains that are heterogeneous in nature. EAEC is the most common cause of bacterial diarrhea in children and diarrheal associated with HIV patients. This pathotype causes watery diarrhea (usually without blood), nausea, and moderate fever, as well as tenesmus borborygmi (Jensen *et al.*, 2014).

EIEC is, the pathogen that infects M-cells, macrophages, and epithelial cells in the colonic mucosa, causing watery diarrhea. However, in acute cases (inflammatory colitis), the inflammation causes a scanty dysenteric stool with blood and mucus (Jafari *et al.*, 2012).

AIEC is best known for its unusual adherence sequence (HEp-2 in culture), in which it binds to epithelial cells of GIT and macrophages. As a result of AIEC colonization, infection by this pathotype causes strong inflammatory responses in the human gut, especially in the 'ileal lesions' of Crohn's disease (CD) patients (Canizales *et al*., 2013)

EHEC (also known as shiga toxigenic *E. coli* (STEC) or verotoxin forming *E. coli* (VTEC) (STEC)) releases verotoxins or shiga-like toxin (stx-1) and shiga-like toxin (stx-2), which induce diarrhea in both humans and animals. A transduction mechanism of bacteriophages containing the SLT1 and SLT2 genes resulted in the capacity to generate toxic substances (O'Loughlin and Robins-Browne, 2001). The two types of toxins produced by VTEC suppress protein synthesis and cause apoptosis in target cells, with stx-1 having a 10-fold greater cytotoxic effect than stx-2 (Grey *et al.*, 2014). EHEC is the most common zoonotic agent, with many serotypes (e.g., O111, O26, O26:H11, O113:H21, O157:H7, and others) that play an important role in meat pollution and causing serious diseases in humans (Sebsibe and Asfaw, 2020).

2.4. General over view on *E. coli* O157:H7

E. coli O157:H7 is the most common and dangerous bacterial pathogen linked to life-threatening infections including bloody and non-bloody diarrhea, abdominal pain, kidney failure, hemorrhagic colitis (HC), and hemolytic uremic syndrome (HUS) (Pal *et al*., 2016). *E. coli* O157:H7 is a more significant pathogen than other well-known foodborne pathogens since it can cause serious infections in people of all ages with a low infection dosage and can survive in an acidic environment (Haile *et al*., 2014).

E. coli O157:H7 has several virulence genes, including *stx-1, stx-2, eae, ehxA, and saa*, which allow the pathogen to attach to the epithelial part of the intestine and produce cytotoxic within the colon, potentially leading to death through a mechanism in which the toxin is transported via bloodstream to aggravate massive attack on target cells, or resulting in the failure of certain tissues found on the targeted organ (Peng *et al.,* 2019).

E. coli O157:H7 strains are unable to ferment D-sorbitol, and the lack of β-glucuronidase activity can be used to selectively isolate the pathogen from 90% of other GIT bacteria (Ghimire, 2018). As a result of the pathogen's properties, as well as its resistance to tellurite, sorbitol MacConkey (SMAC) agar was developed as a selective medium. SMAC agar is an effective tool for conventional detection of *E. coli* O157:H7 from various sample sources (Priyanka *et al.*, 2016). A commercially available latex agglutination test can be used to validate *E. coli* O157:H7 (Lim *et al.*, 2010). However, there are also several other developed methods of isolation and identification of *E. coli* O157:H7 that can be used for experimental or routine purposes, the methods including; immunological method, molecular method such as PCR and Loop-mediated isothermal amplification (LAMP) (Reza *et al*., 2016), and conventional selective culture methods that depend primarily on biochemical features observed and serotype identification of O157 and H7 antigens (Ranjbar *et al*., 2016).

Due to heavy growth of background flora, mostly other *E. coli* strains and proteus species, the differential medium for *E. coli* O157: H7 cultivation may have shortcomings (Muller *et al*., 2005). One of the limitations is that as a result of the confusion that arose during the isolation of STEC O157: H7 from a mixed culture of other non-sorbitol fermenter bacteria, the media's precision could be questioned (Islam *et al.,* 2018).

The second limitation in *E. coli* O157: H7 isolation may occur due to the fact that this pathogen remains viable but not culturable (VBNCs). The VBNC state is a situation in which non-sporulating bacteria survive in harsh environments without forming colonies on regular media (Liu *et al.*, 2016).

Somatic (O) antigen 157 and flagella (H) antigen 7 are expressed by *E. coli* O157:H7. The most important feature that contributes to serotype recognition is the antigenic structure of the outer cell membrane. *E. coli* O157:H7 has a Gram-negative cell envelope structure, so it has an outer membrane with a lipopolysaccharide component apart from the cytoplasmic membrane. The carbohydrate structure and composition of the lipopolysaccharide characterize the O157 antigen. The particular polypeptide structure of flagella determines the H7 antigen (Beneduce *et al*., 2003).

2.4.1. Geographical distribution

Foodborne contamination caused by *E. coli* O157: H7 is found all over the world. Since the first epidemic in the United States, a multitude of cases have been recorded from countries around the globe, with the exception of Antarctica (Ma, 2019). The pathogenic *E. coli* O157:H7 serotype was a common cause of large outbreaks in countries around the world, including the United Kingdom, Canada, the United States, Japan, China, the Czech Republic, Austria, Argentina, Germany, Holland, Ireland, Italy, and Belgium (Eklund *et al*., 2005). Due to a lack of modern laboratory facilities, high costs, and a scarcity of well-trained human resources, *E. coli* O157:H7 related infections in Africa could be underestimated. However, diarrheal cases have been recorded in some African countries, including the Central African Republic, Ethiopia, Gabon, Ivory Coast, Kenya, Nigeria, South Africa, and Swaziland (Raji *et al*., 2006).

Even though the distribution of *E. coli* O157:H7 in Ethiopia is not well understood, isolation of the pathogen, detection, and characterization from various sources in different parts of the country have been studied. For example, Kibret and Abera (2011) reported that *E. coli* O157:H7 was found in 14.2% of 446 clinical samples including urine samples, ear discharges, eye discharges, and wound swabs. Bekele et al. (2014) have published the findings on the occurrence of *E. coli* O157:H7 at A. A municipal abattoir, which showed a pooled prevalence of 10.2% (13.3% from beef followed by 9.4% mutton and 7.8% goat meat).

2.4.2. Reservoir of *E. coli* O157:H7

Mammals, especially ruminants, are thought to be a natural reservoir for *E. coli* O157:H7. Domesticated ruminants are known as reservoirs, and they are usually asymptomatic carriers of this bacteria, especially cattle being the most common cause of infection. In fact, more *E. coli* O157:H7 strains are excreted by peak-shedding cattle than by other animals (Botkin *et al.*, 2012). Other ruminants, such as horses, goats, pigs, and deer, may also carry the strain to a lesser degree (Schlundt *et al.*, 2004). Possibly, other animals including dogs, cats, gees, chicken, turkey, rodents, birds, and fish may also act as a carrier for the pathogenic strains (Ferens and Hovde, 2011). House flies and blow flies, on the other hand, are believed to be potential transport hosts for virulent *E. coli* especially during the summer months (Kiranmayi and Krishnaiah, 2010).

2.4.3. Susceptibility to and Mode of transmission of *E. coli* O157:H7

Cattle, as previously mentioned, serve as a reservoir host for *E. coli* O157:H7, posing a major public health threat. Thus, cattle of all ages are vulnerable to *E. coli* O157:H7 invasion and colonization, including indications of super shedding in the excreta of sub-adult cattle (Jufare, 2018). On the other hand, *E. coli* O157: H7 infections can affect people of any age group. As a result, infants, the elderly, and people with weakened immune systems are more susceptible to a variety of illnesses, ranging from mild diarrhea to life-threatening infections (Engdaw and Temesgen, 2016).

E. coli O157:H7 is transmitted via fecal-oral route, through contamination of food and water, direct contact with infected animals, contaminated environment, and occasionally through occupational exposure (Caprioli *et al*., 2005). Several outbreaks have been linked to foods derived from raw undercooked ground beef meat and raw milk (Hessain *et al.*, 2015). In addition, foodborne disease outbreaks have been traced to contaminated vegetables including lettuce, spinach, various sprouts and etc. Contaminated irrigation water plays a great role in infecting both animals and human beings through the pathogen transmission to vegetables (Tesfaye *et al*., 2019). The conditions of food preparation in restaurants were the main area in which most *E. coli* O157:H7-associated outbreaks commonly occurred (Lim *et al.*, 2010). Since the early 1990s, many outbreaks especially occurred, in day care facilities, schools, communities and individual homes through person-person or via fecal-oral route which have been an important mode of transmission for *E. coli* O157:H7 (Lim *et al.*, 2010). For instance, in a study reported on the outbreaks of diarrheal infections that occurred in 2014 due to *E. coli* O157:H7 in Canada, Alberta, it was estimated that 4(3%) of the patients acquired the disease through person-person contact with an outbreak associated with household patients (Honish *et al.*, 2017).

Contamination of water with virulent bacteria is the main public health concern in most nations of the world. Polluted water surface which is contaminated with *E. coli* O157:H7 and used for irrigation in vegetable plants could harbor the pathogens responsible for foodborne outbreaks (Uddin *et al*., 2019). Many outbreaks have been largely attributed to local well, municipal, and spring water system or have been associated with direct contact between humans and dairy cows or cattle at farms, fairs, or petting zoos. For example, during 1992, in South Africa, combination of carriage by pastured cattle, cattle deaths due to famine, and contaminated surface water due to heavy rain were identified as the likely vehicle which resulted in a large outbreak of bloody diarrhea in the country (Fairbrother and Nadeau, 2006). Figure 1 summarizes the various ways that *E. coli* O157:H7 use to contaminate food/water to get to susceptible hosts.

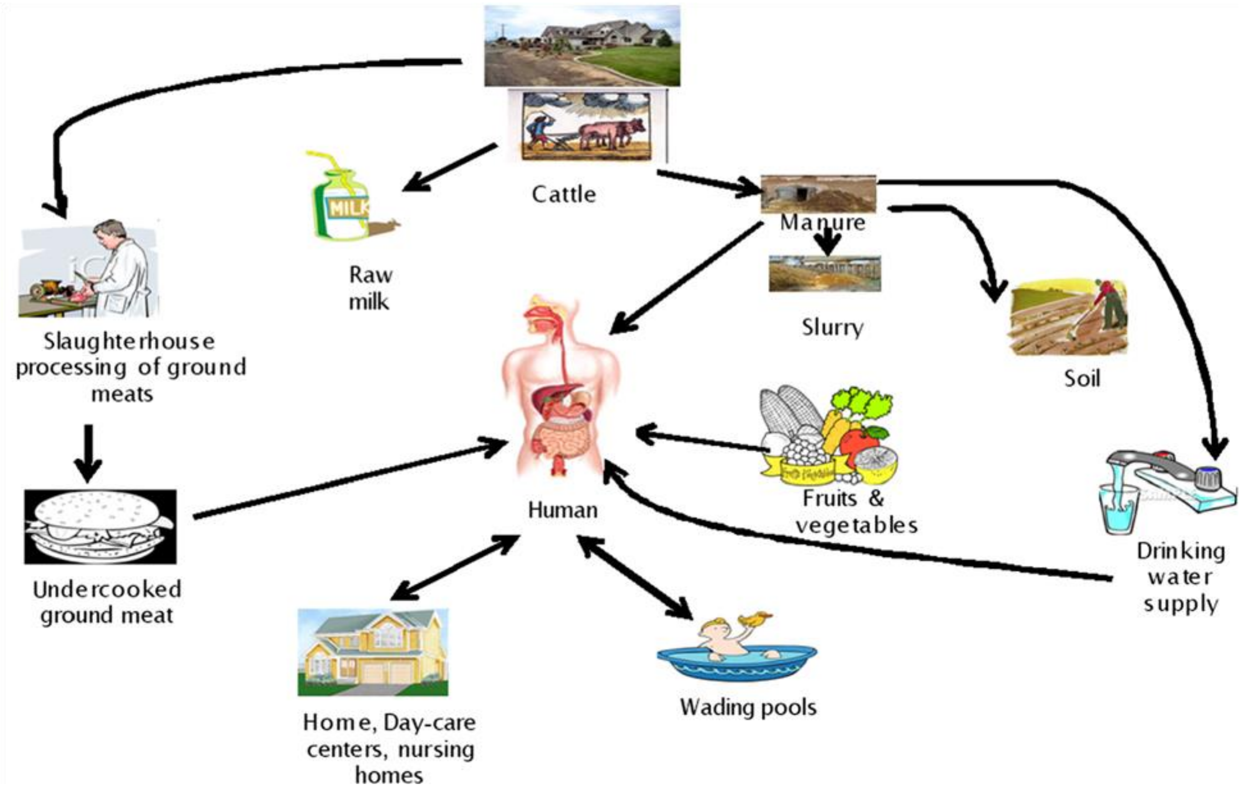


Figure 1: Sources of *E. coli* O157:H7 infection. From Petridis et al. (2002).

2.4.5. Occurrence of *E. coli* O157:H7

The type of herd and its care, seasonal variation, geographical range, and detection system can affect the prevalence of *E. coli* O157:H7 in cattle and their excreta. As a result, the pathogens prevalence ranges from 0% to 67 percent (Lennox *et al*., 2020). *E. coli* O157:H7 seems to be more common in fed-lot animals than in dairy cows (Islam *et al.,* 2018). The status of *E. coli* O157:H7 in Ethiopia have been reported by some researchers when they undertook studies to determine the occurrence of the serotype associated with beef in different area of the country (Sebsibe and Asfaw, 2020). Gutema et al. (2020), have reported a total prevalence of 6.3% of *E. coli* O157:H7 from beef at Bishoftu municipal abattoir and butcher shops. Furthermore, a prevalence of 9.33% was documented from beef meat samples in western Ethiopia by Haile et al. (2017). Another research looked at the prevalence of *E. coli* O157:H7 in beef and meat products, and found that it was 2.5% in raw chicken and 5 % in cooked chicken (Hessain *et al*., 2015).

2.4.6. Pathogenesis of *E. coli* O157:H7

Despite the fact that *E. coli* O157:H7 is potentially pathogenic to humans, ingestion of the pathogen and invasion of the GIT in cattle and other animals do not cause disease (Ferens *and* Hovde, 2011). *E. coli* O157:H7's pathogenicity is linked to a combination of plasmid, bacteriophage, and chromosomal genes. Shiga toxin (*stx1* and *stx2*), intimin (encoded by the *eae* gene), and enterohemolysis (encoded by the *hly A* gene) are the main virulence factors. Shiga toxin is made up of a single A subunit and five equivalent B subunits that bind the holotoxin to the Glico lipid Globo triaosylceramide (Gb3) and stop protein synthesis (Tassew, 2015).

The pathogenesis of a wide range of illnesses caused by *E. coli* O157: H7, from mild diarrhea to HC, HUS, and TTP, depends on the development of shiga toxin at the infection site (Pennington, 2010). The pathogen adheres to and colonizes the human large intestine after being consumed along with contaminated food or water. Infection with *E. coli* O157:H7 takes around 3-4 days to establish. Following colonization of the bowel mucosa, shiga toxin causes target cells to express Gb3, allowing the toxin to be absorbed into the bloodstream and spread to the target organ. As the result, the toxin can inhabit the protein synthesis through inactivation of ribosomal RNA found in the cell (Kiranmayi *and* Krishnaiah, 2010).

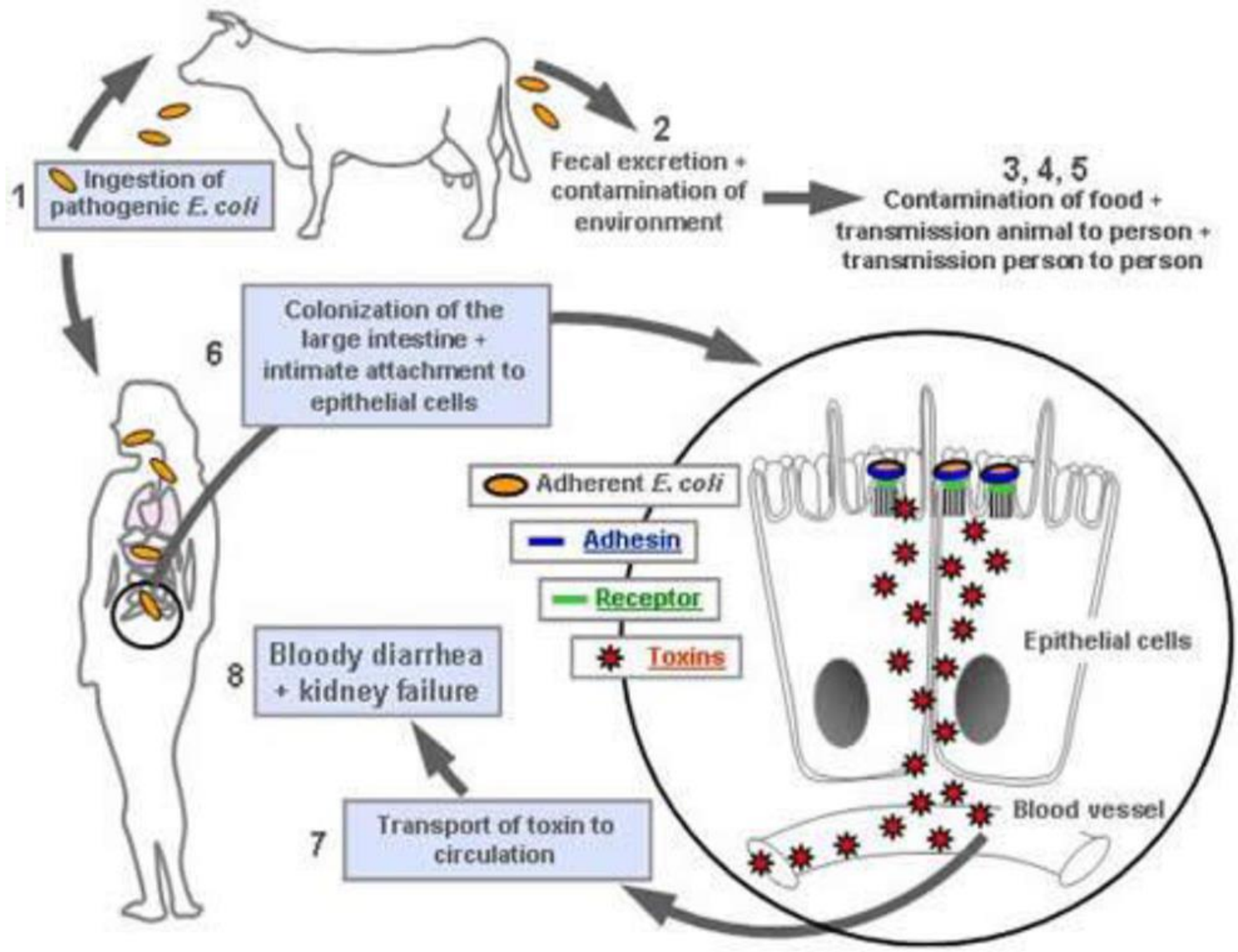


Figure 2: The mechanism by which shiga toxin-producing *E. coli* causes bloody diarrhea and hemolytic uremic syndrome in humans. Source: http://www.ecl-lab.ca/en/ecoli/pathogenesis.asp

2.4.7. Clinical signs and symptoms

Since *E. coli* O157: H7 has no clinical symptoms in the digestive tract of cattle other than diarrhea in cattle calves, these animals should be called carriers of the pathogen. Most cattle infections cure without more complications (in 1-4 weeks), and in some circumstances, *E. coli* O157: H7-carrier cattle may excrete varying concentrations of the bacteria and shed it their manure in less than one day (Messele *et al*., 2017). In humans, Infected people may have no symptoms or experience watery diarrhea, hemorrhagic colitis, and/or Hemolytic Uremic Syndrome (HUS). The most common symptom is diarrhea. Some cases clear up without medication after about a week, while others quickly progress to hemorrhagic colitis.

Hemorrhagic colitis is distinguished by bloody diarrhea, stomach tenderness, and, in some cases, extreme abdominal cramps. Fever is present in some patients, although it is absent in others. It is likely to experience nausea and vomiting, as well as dehydration. Hemorrhagic colitis is mostly self–limiting and goes away after about a week. Intestinal necrosis, perforation, and colonic strictures are also possible outcomes of severe colitis (Tassew, 2015).

Up to 16 percent of patients with hemorrhagic colitis have hemolytic uremic syndrome. This condition is most common in children, the elderly, and people who have compromised immune systems. It normally appears a week after the diarrhea has started, when the patient is feeling better. HUS will strike children without prodromic diarrhea on rare occasions. Kidney disease, hemolytic anemia, and thrombocytopenia are all symptoms of HUS (Bekele *et al*., 2014) (Figure 2). These symptoms have varying degrees of importance. Some HUS patients have hemolytic anemia and/or thrombocytopenia but little or no renal disease, while others have severe kidney disease but no thrombocytopenia and/or mild hemolysis CNS (Central nerves system) association with lethargy, irritability, and seizures are all typical extra renal symptoms (Fan *et al.*, 2019). TTP is a term used to describe a type of HUS that affects adults, particularly the elderly. Thrombocytopenia, hemolytic anemia, azotemia, fever, thrombosis in the terminal arterioles and capillaries, and neurological symptoms rule the clinical presentation of thrombocytopenic purpura. Brain clots are inevitable, and they often result in death (Fan *et al.*, 2019).

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

The source of *E. coli* O157:H7 infection can be detected through diagnosis of fecal samples, food and water samples, environmental samples, etc. Several techniques have been developed to isolate all *E. coli* serotypes (CDC, 2016). Most other serotypes can be distinguished from the *E. coli* O157:H7 strain by phenotypic difference. The strain's non-sorbitol fermenting nature on MacConkey sorbitol agar and lack of glucuronidase activity are the pathogen's main future prospects for its selective isolation (Rahal *et al.*, 2012). The isolated colonies of *E. coli* O157:H7 from the traditional procedure must then be confirmed with a serological test, with a positive outcome detected when the isolated forms agglutinate with the test reagent (Al-Dragy and Baqer, 2014). However, newer and automated methods, that offer advantages in terms of rapid and reliable identification of bacterial species, such as automated biochemical methodologies, immunoassays, and polymerase chain reaction methods, are commonly used to diagnose *E. coli* O157:H7 in animal diarrhea, stool, predictable food products and water samples (Parsons *et al*., 2016).

Biolog's Omni Log Identification System (Biolog, Hayward, CA) uses automated biochemical techniques to identify bacteria at the species and strain level. The Biolog Omni Log Identification evaluates a microorganism's capacity to utilize or oxidize previously prefilled and dried carbon source nutrients and biochemical in 96 wells of a microplate (OMNILOG, 2010). In addition, each of microtiter plate wells containing substrates is infused with tetrazolium violet. During the test a bacterium respires when it begins to utilize the carbon sources in particular wells of the microplate, where the tetrazolium redox dye is reduced, and the wells turn purple. The ultimate result is a microplate with a pattern of colored wells that is unique to that bacterial species. When the data are analyzed with a computer, they establish unique biological pattern or "fingerprint". The fingerprint information is examined, compared to a database, and a result is generated.

The technology also includes PCR and other DNA-based methods, immunomagnetic separation, and enzyme-linked immunosorbent assays (ELISAs). The evolution of molecular detection of *E. coli* O157:H7 has provided many benefits to molecular-based techniques, including sensitivity (detecting the pathogen even though it is present in small quantities), selectivity (detecting only the target pathogen), and speed (detecting the pathogen quickly) (Dhama *et al*., 2014).

2.4.9. Treatment of *E. coli* O157:H7 infections

Antimicrobial therapy for *E. coli* O157:H7 infections can exacerbate the illness. As a consequence, the use of antibiotics for treatment can result in the lysis of bacteria that cause *E. coli* O157:H7 to produce shiga toxin (Dulo *et al.,* 2014). As *invitro* data demonstrated that Ciprofloxacin or sub-inhibitory concentrations of trimethoprim-sulfamethoxazole have been shown to increase stx secretion in *E. coli* O157:H7, but in order to minimize the time it takes for symptoms to appear and to avoid systemic complications, antimicrobials, probiotics, antibodies, and toxin neutralizers must be used selectively against the key pathogenic pathway (i.e., called supportive therapy) (Rahal *et al.,* 2012). To balance electrolytes in the body fluid of a person with diarrhea, clear liquids of oral rehydration salts are recommended as a treatment (Dulo *et al.*, 2014).

2.4.10. Prevention and control of *E. coli* O157:H7 infection

The appropriate hygienic steps to mitigate *E. coli* O157:H7 infections should be implemented and managed effectively at all levels of the food supply chain, from farm to fork. Food handlers must take adequate hygienic measures, such as properly frying meat (until no longer pink in color), avoiding unpasteurized milk, washing fresh edible fruits and vegetables, consuming chemically processed water, and washing hands with soap and running water. (Dulo *et al*., 2014).

E. coli O157:H7 has been observed in dairy farms on a regular basis, and the animals show no evidence of disease. As a result, it is difficult to distinguish infected cattle from healthy cattle. (Desta *et al*., 2012). To reduce the risk of pathogen infection from animals to humans or the ecosystem, strict hygiene procedures should be taken. Thus, the most effective methods of prevention and monitoring of *E. coli* O157:H7 infection in the farm are proper waste disposal, using clean source of water and drainage for washing the pens and bedding, enhancement of regular veterinary inspection practices, certainty of inhibitory feeds, use of probiotics, and immunization (Desta *et al*., 2012).

2.4.11. Public health and economic significance

Foodborne diseases caused by *E. coli* have been more common in the world over the last three decades. *E. coli* O157:H7, in particular, is the most common and pathogenic serotype of EHEC. (Lim *et al*., 2010). Foodborne diseases caused by this microorganism resulted in a significant public health risk and financial damage for the countries (Liu *et al*., 2016). The cost of outbreak prevention in West Lothian, Scotland, was estimated to be about £3.5 million in 1994, the year the first incident occurred. Then, after 30 years, the annual expenses were raised to £11.9 million (Tassew, 2015). An epidemic in Australia resulted in a $5.6 million expenditure to contain the extreme cases (which affected over 200 people, 23 of whom were diagnosed with HUS and one patient died). Despite the fact that HUS only affected a small percentage of patients, both outbreaks suffered significant medical and productivity losses due to the complications (Kirnmayi *et al*., 2010).

The incidence of *E. coli* O157:H7 reported in several countries affected by large outbreaks ranged from 0.08 to 4.1 per 100,000 populations. The countries included in this report were Japan (12,000 cases), USA (in 1993, 600 illness and 4 children were died), Germany, Australia, Korea republic and Scotland (where the highest incidence rate was reported) (Tassew, 2015).

According to the WHO, in the united states *E. coli* O157:H7 causes approximately 73,000 cases, 200 hospitalizations, and 50-60 deaths per year. The report also mentioned that the cases were mostly linked to HUS, the leading cause of renal failure (Tassew, 2015).

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

Researchers in Ethiopia have performed a considerable amount of research to determine the prevalence of *E. coli* O157: H7 in cattle, animal products (meat and dairy), environmental pooled samples, and samples from humans in various parts of the country. In a recent study conducted in Sebeta town with the aim of evaluating the occurrence and antimicrobial tolerance of *E. coli* O157:H7 from beef, an overall prevalence of 9 (3.4 percent) 9/267 was observed (Berkutawit, 2020).

Few studies have been performed to assess the prevalence of *E. coli* from cattle faces, including Jufare, 2018; Atinafe *et al.*, 2017; Abdisa *et al.*, 2017; and Haile *et al*., 2017. According to the findings, 7%, 4.7%, 1.89%, and 7.3% were considered positive for *E. coli* O157: H7. Beef samples were collected to assess the status of *E. coli* O157: H7 in various research areas across the country, (Bedasa *et al*., 2018; Beyi *et al*., 2017; Bekele *et al.*, 2014; and Mengistu *et al.*, 2017). In addition, diarrhea patients were studied in various parts of the country to assess the prevalence of pathogenic *E. coli* O157:H7 (Kahsay *et al.*, 2008; Temesgen and Engdaw, 2016;

Adugna *et al*., 2015; Abdisa *et al.,* 2017 and Jufare, 2018). Details of findings from the above studies on the occurrence of *E. coli* O157:H7 are summarized as follows (Table 1).

Table 1: summary on the prevalence of *E. coli* O157:H7 in different parts of Ethiopia.

Authors	Study area	Sample type	Prevalence %
Hiko et al., 2008	Debre-Zeit and Modjo towns	Beef Lamb and mutton meat Goat meat	8 2.5 2
Mersha et al., 2010	Mojo	Feces Skin swabs Carcasses before washing Carcasses after washing Water samples	4.7 8.7 8.1 8.7 4.2
Taye et al., 2013	Haramaya University Slaughter House	Carcass swab	2.65
Bekele et al., 2014	Addis Ababa	Beef Sheep meat Goat meat	13.3 9.4 7.8
Dulo et al., 2014	DireDawa	Cecal contents Carcass swab Environmental samples	2.15 3.22 2.04
Balcha and Gebretinsae, 2014	Mekelle Quiha Wukro	Meat sample	0.04 0.06 0.01
Tassew, 2015	Afar Arsi-bale Harar Ogaden Yabello Wollo Wolayta	Meat from abattoirs	1.32 9.17 4.62 9.80 0.76 9.57 5.20
Disassa et al., 2017	Asosa	Traditionally marketed raw cow milk	2.9
Abdissa et al., 2017	Addis Ababa/ Debre Birhan	Fecal swab Intestinal mucosal swab Skin swab Carcass internal swab Cutting board swab Carcass swab	1.89 0.81 0.54 0.54 0.8 0.8
Atnafie et al., 2017	Hawassa	Fecal sample Carcass swabs Meat sample from butcher shop Cutting board swabs	4.7 2.7 2 3.3
Shecho et al., 2017	Haramaya	Cloacae samples	13.4
Beyi et al., 2017	Central Ethiopian	Carcass swabs	4.5

		Cutting board swabs	3.6
Bedasa et al., 2018	Bishoftu	Raw milk Cheese Meat	12 5.71 3.07
Abreham et al., 2019	Mojo	Carcass	4.9
Sebsibe and Asfaw, 2020	Jima	Meat and environmental sample	5.4
Geresu and Regassa, 2021	Arsi	Animal origin foods	2.1
Desalegn et al., 2021	Ambo	Carcass	9.1
Getaneh et al., 2021	Eastern Ethiopia	stool	13.5

2.5. Antimicrobial resistance

Antibiotics are not recommended for treating *E. coli* O157:H7 infections in humans, though widespread use of antibiotics in humans and farm animals can disperse antibiotic resistance genes into the environment (Munita and Cesar, 2016).

The genes responsible for the development of antibiotic resistance can be gained through the continuous genetic alterations that occur in bacterial generations as a result of various horizontal gene transfer mechanisms such as transduction, mutation, conjugation, or transformation (Munita and Cesar, 2016). According to White et al. (2002), in the United States, 24.6 million pounds of antibiotics were given to food animals for growth promotion, while 3 million pounds were used by humans each year, posing a serious public health problem in the world. To further recognize the pathogen's antimicrobial susceptibility, researchers studied *E. coli* O157 isolates dating back to1985 from humans, cattle, swine, and food from the United States. Tetracycline resistance was observed in 27% of swine isolates, followed by sulfamethoxazole resistance in 26%, cephalothin resistance in 17%, and ampicillin resistance in 13% (Tadesse *et al*., 2012). Antimicrobial susceptibility of *E. coli* O157: H7 obtained from 380 raw milk was studied by Disassa et al. (2017), and the antimicrobial susceptibility profile revealed that 81.8 percent of *E. coli* O157:H7 was resistant to kanamycin, streptomycin, and tetracycline.

3. The Research Project

3.1 The Objectives

3.1.1 General objective

To isolate and determine the antimicrobial susceptibility of *E. coli* O157:H7 isolated from raw beef meat through the route of abattoirs to retailers at Addis Ababa.

3.1.2 Specific objectives

- To isolate *E. coli* O157:H7 from raw beef meat.
- To assess the status of antimicrobial susceptibility of *E. coli* O157:H7 isolates
- To assess the hygienic situation and condition of beef meat processing, handling facilities and selling environment associated with the isolates of *E. coli* O157:H7.
- To assess the *knowledge, attitude and practices* among workers from both abattoirs and butcher shops regarding processing and handling of meat.

3.2 MATERIALS AND METHODS

3.2.1 Study Location

One abattoir, Addis Ababa Abattoirs Enterprise (AAAE), was selected by lottery system from three municipal slaughter houses that provide slaughtering service for the country’s capital, Addis Ababa. In the cases of meat retail outlets, the study was conducted at Yeka sub city administration, Addis Ababa.

Addis Ababa Abattoirs enterprise located around ‘Kera’ was founded in 1950’s in Kirkos sub city of Addis Ababa. Thirty percent of the meat demand in the country is found in the capital and AAAE is the largest slaughter houses which supply for the majority of the Addis Ababa’s demand. The enterprise has a total daily capacity of slaughtering 1200 cattle, 1500 sheep and goats (Zerabruk *et al*., 2019). Also, the enterprise owns vehicles for transporting meat (carcass) to the customers and vehicles for transporting live animals from different nearby markets to the abattoir (Gudeta *et al*., 2012). The main source of cattle to be slaughtered are brought to the city from different regions of the country including Southern Nation Nationalities People Region (SNNPR), Oromia and Amhara regions.

Yeka is one of the eleven sub-cities in A.A administration. It is situated in northern part of Addis Ababa with a total area of 85.98 Km square. This sub-city is located within latitude and longitude of 9^{0}01' 30.73" North and 38^{0} 46' 27.55" East, respectively. Currently, the sub city is divided into 13 woredas and 124 sub woredas (kebele). According to Ethiopian CSA (central statistics agency) population projection of the year 2016, the total population of the sub city is estimated to be 424,217; and it is the largest densely populated area with 4284.9 people living in one Km square (Eshetu and Assefa, 2019). There are 103 legally registered butcher shops in the sub-city and each of them has at least an average of meat retailer employed employees (source from Yeka sub-city admin office). The figure below shows the study area of Yeka sub city (Figure 3)

Legend

1. Yeka
2. Bole
3. Akaki Kality
4. Nifas silk Lafto
5. Kolfe Keranio
6. Gulele
7. Arada
8. Addis Ketema
9. Lideta
10. Kirkos
11. Lemi Kura

Fig.3: Addis Ababa city map, Yeka sub-city administration.

Source: The land development and management office of Lemi Kura sub-city, Addis Ababa.

3.2.2 Study design

A cross-sectional study was conducted from October 2020 to May 2021, to isolate and determine the antimicrobial susceptibility of *E. coli* O157: H7, and to assess the potential sources of *E. coli* O157: H7 contamination in raw beef meat at abattoirs and butcher shops. The samples were processed at Food Safety Research Laboratory of Ethiopian Public Health Institute (EPHI). The general hygienic and sanitation of the beef selling environments were evaluated through observation and the knowledge, attitude and practice of the workers along the chain were administrated by questionnaire.

3.2.3 Sample size determination and sampling technique

Sample size was determined by using the formula given by Thrusfield (2005).
Therefore, it was calculated based on 5% precision, 95% level of confidence interval and expected prevalence of 13.3% (Bekele *et al.*, 2014) which was found from beef carcass swabs at Addis Ababa. The minimum sample size was 177, but deliberately we sampled 210 to increase the precision.

$N=Z^2 x\ P\ ex\ (1- P\ ex)\ /d^2$

Where, n= required sample size

Pex = expected prevalence

d =desired absolute precision = 0.05

Z=statistic for level of confidence =1.96

Then, the calculated sample size n=210 was distributed purposely among pre planed sample types. Accordingly, the calculated sample sizes required from each points of beef supply chain were 40, 160 and 10 for beef carcass swab, environmentally pooled samples at abattoir and butcher shops and minced raw beef meat at selected butcher shops, respectively.

3.2.4. Study population

All healthy cattle ready for slaughter and workers who participated along carcass supply chain (at AAAE and butchers at Yeka sub –city) were the study population and each participant was selected randomly. The study was conducted by collecting a total of 210 samples from different sources like carcass swab, raw minced beef meat, and environmental samples (worker's hand, knife swab, carcass wash water sample, hook swabs from meat transporting vans, table swabs, meat balance swabs, floor swabs, worker's protective cloth swabs and sponge swabs).

3.2.5 Participant recruitment

The source of carcass swab sample was selected based on simple random sampling technique. There are four different slaughter lots found in AAAE. In each lot, average of 30 cattle were slaughtered at a time, then during sample collection only one slaughtered cattle was selected at a time by lottery method. But before slaughtering operation begun, the anti-mortem inspection of the selected animal was checked by gathering the data from the slaughter house laboratory information desk in order to confirm and collect the samples without doubt. The butcher man hand, knife, water, towel/sponge, floor and catting table swab samples were collected based on the role they had in manipulation and processing of the selected cattle carcass. The meat vans were purposely selected for sampling, based on which track distribute the targeted carcass to the retailers and the samples from butcher shops were also collected from the retailer which received this carcass.

3.3. Data collection and transportation of the samples

3.3.1. Sampling methods

Simple random sampling was undertaken to select the slaughtered beef carcass from the Abattoir and followed them at the respective butcher shops. This was done after lists of cattle that passed through ante-mortem inspection were found from the laboratory supervisors of the enterprise and the list of meat retailers found at Yeka sub-city which were included in the meat supply chain of the enterprise. The collected samples were transported and processed at EPHI, after sampling the samples were cross-checked for the sample ID, sample source, sample type and sampling date in the sample tag.

Carcass swab sampling from slaughter hall and meat van

Depending on the calculated sample size, from a total of 210 swab samples, 40 beef carcass swab samples were collected throughout beef meat supply chain of Addis Ababa Abattoirs Enterprise. The carcass swab which was collected from abattoir and meat transportation van was selected by using simple random sampling technique. Lottery method was used to select one killed beef cattle to follow its carcass up to the time for it to be ready to eat. There were ten visiting times for sample collection at abattoir (twice a week, mainly Wednesday and Friday afternoons) and simultaneously ten sample collection days were used at butcher shops (mainly, Thursday and Saturday mornings). During each of the ten-visiting time at AAAE, a total of 30 carcass swab samples (3x10) were collected and 10 (1x 10) beef carcass swab samples were taken at list 12hr later after arrival of the carcass at ten purposely selected butcher shops in Yeka sub-city. Samples were collected based on the technique described in ISO16654 (2001).

At AAAE, carcass swab samples were collected at three points following carcass processing in each sampling day. The first carcass swab was collected just after skinning or before evisceration, where the carcass may be cross contaminated from the skin. The second was taken after splitting or evisceration of the carcass, assuming that the carcass might get contaminated with GIT matters during splitting. The third one was collected after evisceration or just after carcass wash and loaded on the transporting track. According to ISO 6888-3, there are four specific carcass sites with the highest rate of contamination to be identified and samples taken from. These include the thorax, flank, crutch, and breast sites, four separated double concentrated tryptone soya broth (TSB) containing test tubes and sterile cotton swabs were used. For each sampling sites of the carcass, a sterile cotton tipped swab fitted with shaft was moistened in 10 ml of double concentrated TSB and then each specific site was rubbed first 10 times in the horizontal and then 10 times in the vertical direction by using separated cotton swabs. On completion of the rubbing process, each of the cotton tipped swab was return and soaked in the former test tube with 10 ml of double concentrated TSB and it was labeled after the four specific carcass site samples containing tubes were mixed in a single test tube as one carcass sample (Sheng *et al.*, 2006). Similar technique was followed to collect the rest beef carcass swabs at both abattoir and the butcher shops. Finally, the carcass swabbed samples at both study sites were transported using ice box in cold chain to EPHI food safety and research laboratory within three hours.

Environmental sampling from abattoir and butcher shops

To determine the cross contamination of carcass with the pathogen derived from contaminated environment, a total of n=160 environmental pooled samples were collected and for each visiting time at both abattoir and butcher shops, different types of environmental pooled samples were collected.

A total of 80 pooled environmental samples were collected at the slaughter house including; workers hand swabs before and mid operation (who were involved in slaughtering of the cattle and evisceration of the carcass), knives swabs (before and at mid operation), floor swab samples at the slaughter hall, water samples, swab samples from the protective cloth of carcass transporters, and hooks swab samples (the carcass hanging hook in meat van).

A total of 80 environmental samples were aseptically collected at butcher shops from chopping table swabs, pooled knife swabs, towel / sponge swabs, balance swabs, butchers hand swabs, floor swabs, protective coat swabs and water sample. Then all collected samples were transported to laboratory for examination within three hours.

Minced raw beef meat sampling at butcher shops

By following the carcass which was derived from the selected cattle, the swab samples were collected from the previous mentioned carcass sites based on the ISO reference number 6888- 3, (ISO, 2003). The minced raw beef meat samples from the specified carcass were also collected at butcher shop. So that, in each ten-visiting time at purposively selected butcher shops at Yeka sub-city, about 25 grams of raw minced beef meat was taken from the beef carcass for microbiological analysis by using sterilized zipped plastic bag, and the samples were transported using ice box in cold chain to EPHI food safety and research laboratory within 24 hrs.

Water sampling

A total of 40 ml of carcass washing water samples was collected from AAAE using sterile screw capped glass container and the same amount of water samples were collected from each selected 10 meat retailers found at Yeka sub-city. This water was mainly used for washing the workers hand and utensils of the butcher shops. The water sample collected at both study site was immediately transported using ice box to EPHI laboratory within 24 hrs.

3.3.2. Inclusion and Exclusion criteria in the sampling techniques

Inclusion criteria: - Carcass handler or workers who were directly in contact with or involved at any point of meat supply chain (i.e., the staff worker in slaughtering, transporting and distribution of carcass department). But only the workers who were presumptively selected were considered as a study participant. And also, Cattle slaughtered on the day of sampling were considered for sampling.

Exclusion criteria: - In the carcass supply chain, worker who were unable to communicate well due to disability were excluded from the study.

3.4. Laboratory works and analyses

3.4.1. Sample processing

The carcass and other environmental pooled swab samples collected at both abattoir and meat retailers underwent sample preparation and enrichment according Lejeune et al. (2001), Have been concluded that use of high temperature 44.5°C incubation for 24 hrs. in double concentrated TSB improved the sensitivity of *E. coli* O157:H7 culture from food and environmental samples.

Therefore, immediately after arrival at EPHI laboratory, the cotton tipped swabs in the test tube were shaken by using hand and taken out and discarded. the suspension in test tube with double concentrated TSB were incubated overnight at 44.5^0C for 24hr.

The 25-gm minced raw beef meat sample collected were taken out from the sterile zipped plastic bag, and then weighed aseptically for accuracy. The weighed sample was then placed in sterile flask with 225 ml of double concentrated TSB. Finally, the mixture was placed on shaker machine for 5 minutes and the suspension was incubated for 24 hr. at 44.5^0C (USDA, 2013; Lejeune *et al.*, 2001).

The 40 ml water sample collected at both abattoir and meat retailers were mixed with equal amount of 40 ml double concentrated TSB, then it was incubated at 44.5 ^{0}c for 24hr. for further isolation of non-sorbitol fermented bacteria (USDA, 2013; Lejeune *et al.*, 2001).

3.4.2. Isolation of *E. coli* O157:H7 from all samples

Each enriched sample was cultured on sterilized CT- SMAC (Sorbitol MacConkey with cefixime and tellurite) and incubated at 37 ^{0}c for 24 hours. The agar plates were used for plating out and examined for the presence of non-sorbitol fermenting colorless colonies (potentially *E. coli* O157:H7). However, other *E. coli* strains that formed pink colonies due to their sorbitol fermenting ability were also found mixed in the medium. The suspected colorless colonies were again streaked on CT-SMAC medium to get pure presumptive colonies of *E. coli* O157:H7. Those presumptively identified as colonies as *E. coli* O157:H7 were transferred to tryptic soya agar (TSA) to be further identified using biochemical tests such as indole, methyl red, vouges-proskacuer and citrate (IMViC and other tests) (Quinn *et al.*, 2011 and Beneduce *et al.*, 2003).

3.4.3. Biochemical test

Each presumptively identified colony was examined using various biochemical tests for identification and confirmation of *E. coli* O157:H7. The biochemical identification testes used were Kliger iron agar (KIA), sorbitol, indole, motility, citrate utilization, methyl red, vouges-Proskauer, lysine iron agar (LIA) and hydrogen sulphide.

For this purpose, the sub-cultured and purified colonies were inoculated in each biochemical test medium and incubated at 37 ^{0}c, and examined after overnight incubation for identification of *E. coli* O157:H7 by observing the color changed developed in the test tubes, online biochemical test identification protocols for *Enterobacteriaceae, Pseudomonas* and Non-fermenters identification database (https://www.tgw1916.net/bacteria_logare_desktop.html) was applied.

3.5. Confirmatory identification of *E. coli* O157:H7 using Biolog bacterial identification system

The confirmation of *E. coli* O157:H7 was performed using Biolog bacterial identification test at the National Animal Health Diagnostic and Investigation Center (NAHDIC), Bacteriology laboratory. The *E. coli* O157:H7 suspected isolates from the biochemical tests were streaked onto nutrient agar (NA) and transported in ice box to microbiology laboratory of NAHDIC. Up on arrival, the presumptive isolates were preserved in refrigerator at 4^{0}C until the Biolog universal medium agar (BUG) were prepared, then the suspected isolates from the NA were plated on the BUG agar and were incubated at 37^{0}C for 24 hrs. Then, BIOLOG/OMNILOG system (fully automated coated microplate based bacterial identification system) using GEN III micro plate (Lot number 3303141, BIOLOG, USA) with protocol A method was used to further confirm *E. coli* O157:H7 colonies. A single colony grown on Biolog Universal Growth (BUG) agar medium was selected and emulsified into 'Inoculating Fluid A' (IF A). According to the manufacturer's instructions, cell density of the bacterial inoculum was measured and adjusted for a specified transmittance (90 to 98%) using a turbidimeter. For each isolate, 100 µl of the bacterial cell suspension was inoculated in to each of the 96 well coated micro plates, using automatic multichannel pipette and incubated aerobically at 33^{0}C for 22 hrs. BIOLOG MicroStation reader was used to read the incubated microplate and provides species/serotype Identification (ID), and then the results were printed out (OMNILOG, 2010).

3.6. Antimicrobial susceptibility test against *E. coli* O157:H7

The antimicrobial susceptibility test against the *E. coli* O157:H7 isolates was examined using the Kirby-Bauer disk diffusion method on Muller-Hinton agar, based on the test protocol of National Committee for Clinical Laboratory Standards (CLSI). The selection criteria for the antibiotic discs were their accessibility, potential public health importance and the reference of CLSI antimicrobial susceptibility test (AST) guideline (CLSI,2020).

The preparation of Muller-Hinton agar medium was conducted aseptically. The prepared medium was poured into Petridish (20 ml) and left at 33^0C for 24 hr. to check for sterility. In this test, four to five well isolated colonies of already identified *E. coli* O157:H7 were taken using a sterile inoculating loop, transferred in a test tube containing 2 ml of sterile normal saline and were gently vortexed to form suspension with smooth texture. The turbidity of the broth was adjusted to 0.5% McFarland (as measured using Grant-bio DEN-1 McFarland Densitometer). The sterile and solidified Muller-Hinton agar plates were seeded using dipped sterile cotton swab into broth culture and the swab was streaked over the entire agar surface to ensure an even distribution of the inoculum. After the inoculation, the plates were left for 5 minutes to dry. Then, the predetermined antimicrobial disks were placed on to the surface of the inoculated agar plate by using sterile forceps. The forceps were also used to press the disks slightly and ensured the appropriate contact with agar surface. Each plate was incubated at 37^0C for 18 hours, after which it was taken out from the incubator and the inhibition zone diameter formed around each disk was measured using a caliper to the nearest whole millimeter. The interpretation of the result was done according to CLSI manual (CLSI,2020) as shown in Table 2.

Table 2: Shows of antibiotic disks used along with their respective concentration and zone diameter break points in millimeter (CLSI, 2020).

NO.	Antibiotic disc	code	concentration	Diameter Zone of inhibition in mm		
				Resistant ≤	Intermediate	Susceptible ≥
1	Sulfamethoxazole-trimethoprim	SXT	25 µg	10	11-15	16
2	Chloramphenicol	C	30 µg	12	13-17	18
3	Streptomycin	S	10 µg	11	11-14	15
4	Ciprofloxacin	CIP	5 µg	15	16-20	21
5	Amoxicillin clavulanic acid	AMC	30 µg	13	14-17	18
6	Amoxicillin	AML	2 µg	14	15-17	18
7	Gentamicin	CN	10 µg	11	12-14	15
8	Cefotaxime	CTX	30 µg	10	11-15	16
9	Ampicillin	AMP	10 µg	13	14-16	17
10	Meropenem	MEM	10 µg	19	20-22	23

3.7. Assessment of knowledge, attitude and practice among the workers at the chain

A structured questionnaire was used to collect additional data on the knowledge, attitudes and practices of meat handling along beef carcass supply chain. Questionnaires were administered to 10 retail meat outlet workers and 20 abattoir workers. All the observational and questionnaire data collections were done by the principal researcher of the study.

Moreover, the on-site observation of AAAE was conducted using observational checklist prepared for the purpose of direct assessment of the abattoir. The checklist was related with observational findings associated with lay out of the abattoir, the number of workers-to-toilet ratio, source of water supply, carcass handling practices of the workers and their food safety knowledge, status of meat transportation, availability of meat cold chain, frequency of health checkup for staff and others were checked during the ten visiting times at AAAE. (Annex IV).

The general quality of butcher shops was also assessed using a structured questionnaire regarding the workers knowledge on hygienic practice of handling and processing of the meat. One worker from each meat retailer was administrated with the questionnaire to respond accordingly. The key elements of the data collection included in the assessment were: gender, age, educational background, exposure and frequency of training, having medical certificate, personal hygiene, washing and sanitization of butchery utensils, apron wearing, hair cover, money handling and jewelry wearing. The interview was prepared in English and Amharic languages, (Annex II and III).

3.8. Data management and interpretation

The generated data was arranged, coded and entered to Excel spread sheet (Microsoft® office excel 2010). Following data entry, the data was then exported to and analyzed using statistical software program (SPSS version 26.0) with appropriate statistical analysis. Chi-square (X^2) test was performed to calculate the association between sample sources and the prevalence of *E. coli* O157:H7. Descriptive statistics were used to present the responses to the questionnaire. The overall occurrence of *E. coli* O157:H7 in beef meat carcasses, butcher shops and environmental pooled samples were determined using the standard formula of dividing the number of positive isolates by the total number of samples and multiplied by 100. A p-value ($p < 0.05$) was considered as statistically significant.

The food hygiene and safety principles and ratings used in this study were selected from the recommended international code of practice: general principles of food hygiene developed by the FAO/WHO (2009). hence, the rating of workers' knowledge, attitude, and practice was done based on the total number of parameters. The total possible score on the food hygiene knowledge questionnaire was 28. The correct answer received a score of "1," while the wrong answer received a score of "0", then the total number of scores was counted and converted to a percentage. For the sake of interpretation, If the rate was less than 50%, the knowledge, attitude, and practice were rated as poor; decent if the rate was 50-69 percent; and good if the rate was 70% or higher. A five-point Likert scale was used to assess respondents' attitudes toward food hygiene. The maximum score that could be achieved was 13. The scales for the Likert response items were 4,3,2, 1, and 0. The following was the range of scores: A score of 50% or less indicates an unfavorable attitude, A score of 11 to 51-75% indicates a somewhat favorable attitude and A score of >75% indicates a positive mindset.

3.9. Ethical consideration

The study was conducted after the project was approved and the ethical clearance obtained by Department Research Ethics Review Committee (DRERC) of Department of Microbiology, Immunology and Parasitology; (Annex VI) (Certificate Ref. No: DRERC /006/2020, Date: 14 Oct, 2020. Moreover, A permission letter was obtained from the Addis Ababa Abattoir Enterprise Office and the study participant consent were obtained after the purpose of study was explained to them. (Annex II).

4. Results

4.1. Knowledge, attitude and practice study

4.1.1. Socio-demographic characteristics of respondents at abattoir and butcher shop

The general profile of socio-demographic characteristics of respondents are summarized in table 3. The result indicates that all butchers participated on interviewee at slaughter house were male, of 20 respondents at abattoir enterprise the majority were young employee; 29-39 (55%) followed by 20% of age between 21-28 and >39 years (20%) old, and only 5% of the respondent was < 21years old. Regarding to the educational status of the respondents at the slaughter house, majority of the workers (70%) had completed primary school followed by considerable number of (25%) of them were attend secondary school and only one (5%) respondent was at the level of tertiary and none of them were illiterate.

Hundred percent of (n=10) respondents at butcher shops was represented only male employee, and those respondents within the age of 21-28 and 29-39 of them were accounted 40%. The educational status of many respondents (60%) had obtained primary education followed by 30% of secondary school and 10% of the worker didn't attended to any of formal education.

Table 3: Socio-demographics characteristics of respondents at both AAAE and butcher shops

Variables	Categories	Abattoir workers(n=20)		Butcher shop workers (n=10)	
		Frequency (%)	Percent	Frequency	Percent (%)
Age in years	<21	1	5	1	10
	21-28	4	20	4	40
	29-39	11	55	4	40
	>39	4	20	1	10
Educational status	Illiterate	0	0	1	10
	Primary	14	70	6	60
	Secondary	5	25	3	30
	Tertiary	1	5	0	0
Employment status	Temporary	15	75	9	90
	Permanent	5	25	1	10
Health certificate	Yes	8	40	5	50
	No	12	60	5	50

4.1.2. Observational survey of Addis Ababa Abattoir Enterprise

Addis Ababa abattoir enterprise has two separate and independent Christian and Muslim slaughter operation lines located on a common site. Both slaughtering lines are serviced by the same central facilities, which include the plant office, technical services, potable water supply and reserves, hot water supply, electric power supply, waste water treatment and maintenance. This abattoir has four distinct ritual slaughter halls (three Christian and one Muslim), each hall divided into cattle and sheep/goat lines totally separated, and rendering site located beneath the halls. Even though the abattoir's slaughtering capability varies greatly due to religious observances (fasting periods and feasting days), an average of more than 5,000 heads of cattle are slaughtered each week, and more than 500 slaughter workers are assigned to beef carcass preparations. As a result, the AAAE has the greatest capacity of all local abattoir in the country to meet domestic Addis Ababa markets' fresh meat demands.

The abattoir's stunning technique was not assisted by the stunning package; therefore, cattle were stunned on the floor with a sharp knife (summarized in Annex IV). During the sample collection, it was observed that the majority of the slaughtering operations were done by bare hand, with only a few butchers using latex gloves. The slaughter house carcasses are washed by potable water before dispatching. Almost all the abattoir staff wore protective clothing, but only a few of them wore hair covers. The floor was made of a concrete and impervious material that allowed a simple drainage system and quick cleaning. The slaughterhouse, on the other hand, were not cleaned of their dust's regular basis. In abattoir, there was a retention area, a dressing room for employees, and bathroom facilities. Despite the fact that AAAE had 40 meat delivery tracks, all of the distribution vans lacked chilling equipment, and the carcasses were transported with other offal, such as kidney, lung, heart, liver, and other intestinal contents, in an unclean metal container found on the track board. Table 4 summarizes data collected from the observational checklist.

Table 4: Observational survey of butchers, carcass transporters and environmental condition at AAAE. (N=10 Visiting time at slaughter house).

Observation		Remark	frequency	%
Hand washing before, after and during touching the carcass		No	10	100
knives are clean and completely sterile from contamination		No	10	100
The carcass dressing techniques		mixed	10	100
Method of stunning used		sticking knife	10	100
Availability of hot water supply		Yes	10	100
Shortening of the finger nails and its cleanness		No	8	80
Regular usage of worker appropriate clothing	Garment dressing	Yes	10	100
	Head cover	No	7	30
	Glove	No	8	80
	Boots	Yes	10	100
Do they have enough amount of toilet?		No	10	100
Do workers wash their hand after visiting the toilet?		Yes	10	100
Latrine has water, soap, paper for hand washing	Water	Yes	10	100
	Tissue Paper	No	7	70
	Soap	No	6	60
Strict separation between dirty and clean area of slaughter house		No	10	100
General personal hygiene of the butchers		Poor	8	80
Eating or chewing while working		Yes	8	80
Is there any domestic animal or rodents seen?		Yes	1	10
Does the meat van have refrigerator or chiller?		No	10	100
Is there a specified gap between the line of hanged carcass in the track		No	10	100
Do they deliver both beef carcass and other offal contents using the same transportation truck?		Yes	10	100
Do the workers who load the carcass in to the van wear clean garments and safety materials		No	9	90

4.1.3. Physical observation of butcher shops

According to the survey results, the majority of retail shop staff (80 %) did not receive standardized food safety training. When it came to medical examinations, 50 % of meat handlers had no idea about their health status or whether they were fit to work in the meat processing industry, while the remaining 50% had health certificate. Around 70 percent of butchers wore a clean working uniform, while 30 percent did not wear a gown and only wore casual clothing. During the observation, none of the retail shop staff wore a hairnet and their nails were untidy. In addition, 40 % of the butchers worked with money when preparing the beef meat. The sanitary condition of lawfully licensed butcher shops was poor. Meat processing in butcher shops was not separated from offal (internal organs), and raw meat was left out in the open for at least 6 hours a day, attracting thousands of houseflies.

Most surprisingly, none of the butcher houses had refrigerators. Some of this and other observational survey regarding the attribute on the overall condition of butcher shops are summarized in Table 5 below.

Table 5: Check list for observation of butcher shops (N=10 visiting time).

Observations	remarks	frequency	%
Do all the food handler wear appropriate apron?	No	3	30
Do the meat retailers use an appropriate hair cover?	No	10	100
Are finger nails of the workers clean and short?	No	10	100
Any visible cuts and wounds covered with an appropriate procedure seen at time of visit	not observed	10	100
Do they have refrigerator in the retailer shop	No	10	100
The sanitary condition of butcher shops	Poor	10	100
Are latrine available nearby the meat retailer?	No	6	60

4.1.4. Knowledge, attitude and skill of abattoir workers on the hygienic practices.

To evaluate the experience, mindset, and hygienic procedures at AAAE, about 20 slaughterhouse employees were interviewed. Thirteen out of twenty employees were unaware of any form of microorganism that could cause food poisoning, although seven had at least basic knowledge of food poisoning. Sixty-five percent of respondents didn't know the transmitting source of foodborne disease to the population, while 1 (5%), 4 (20%) and 2 (10%) thought polluted food, contaminated food and water, and imported/canned foods could be the way of transmission of foodborne disease, respectively. Most of the participants (13 (65%)) wash their hands always with cold water and soap before and after the end of the process, and the carcass and other equipment (knife, axe, cutting table etc.) are washed after evisceration. All of the respondents answered that they wash their protective clothes and boots regularly. Out of 20 abattoir workers, 0% of them were illiterate, but all of them took work related and personal hygiene training. In this study, none of the slaughter staff received regular medical checkups because, as they complained, medical tests for workers were administered only prior to employment at AAAE. In general, the following were the over-all observations based on the standardized WHO tool, 77.4 percent of food handlers had poor food hygiene knowledge (less than 50 percent), while 19.6 percent had a moderate level of knowledge (50-69%). Only 3% (70% and more) of abattoir workers had a satisfactory degree of attitude toward food hygiene practices in terms of the overall score.

(Table 6).

Table 6: Questionnaire for slaughter house workers to assess their general knowledge and hygienic trainings at the Abattoir. (N=20 respondents from AAAE).

Knowledge		**frequency**	(%)
Had you been given any job-related training	Yes	20	100
	No		
Have you ever undergone regular medical checkup	Yes		
	No	20	100
Do you have any idea about foodborne disease	Yes	7	35
	No	13	65
What do you think could be the possible cause of the foodborne disease?			
	Germs	4	20
	Toxic chemicals	3	15
	Evil eye		
	Don't know	13	65
What do you think about the ways of transmission of the foodborne disease?	Via contaminated food	1	5
	Via contaminated water	4	20
	Via contaminated food and water	2	10
	Via tinned food		
	Through vectors, like house flies	13	65
	Don't know		
Do you have an awareness about carcass contamination	Yes	16	80
	No	4	20
Which stage of slaughtering process do you think is more likely the possible source of contamination?	Stunning	1	5
	Bleeding	2	10
	Flaying	7	35
	Evisceration	6	30
	Splitting		
	Inspection		
	Carcass washing	4	20
	Don't know		
Do you think that, carcass contamination could bring any health risk to consumers?			
	Yes	4	20
	No	16	80

4.1.5. Knowledge, attitude and general handling practice at retail shops

A total of ten meat retail shop meat cutters were interviewed parallel to sample collection in order to determine their experience, attitude, and hygienic practices. Thirty percent of respondents said they didn't wear protective clothing on a daily basis, and 20% said they didn't wash their hands with soap and water at each work interval. When the butcher shops received the carcasses from AAAE, 90% of them placed the meat directly on the wall cabinet, while the remaining 10% used temporary storage for the carcass. This may contribute to contamination of meat. 100% of respondents believed that the hygienic status of the working environment and meat retailer personnel could contribute to foodborne infection. However, none of the butcher shop employees had a regular medical checkup and only 50% of them had health certificate, ensuring that the shops provided appropriate meat hygiene service. In general, the food handlers' knowledge regarding food hygiene in the butcher shops can be categorized as moderate with rate of 58.9% and respondents also showed positive attitudes in food safety and hygiene (76%). Results concerning butcher shops workers Knowledge, attitude and personal hygiene are summarized in (Table 7).

Table 7: Knowledge, attitude and the general hygienic status of butcher at butcher shop, N=10

Activity	Responses	frequency	(%)
Do you display the meat on the wall cabinet immediately after being received from the abattoir?	Yes No	9 1	90 10
Is the carcass covered or uncovered?	Covered open	2 8	20 80
Do you wear an apron regularly?	Yes No	7 3	70 30
Frequency of washing	Daily Once a week	0 9	0 90

	Twice a week	1	10
Do you clean knives, axes, and cutting boards on a regular basis?	Yes No	5 5	50 50
Cleaning frequency	Once a day Twice per day Three times a day	1 7 2	10 70 20
Means of cleaning	Only with water Water and detergent	3 7	30 70
Before handling beef, do you still wash your hands with soap?	Yes No	10 0	100 0
Are the cabinets and hooks in the butcher shop cleaned on a daily basis?	Yes No	10 0	100 0
Cleaning methods for display or cabinet	Water only Water and detergent	6 4	60 40
collecting money during serving	Yes No	4 6	40 60
Where does water come from?	Tap water Tanker well	8 1 1	80 10 10
Did you take the medical test before being employed at a meat retailer?	Yes No	5 5	50 50
Take a regular medical checkup?	Yes No	0 10	0 100

4.2. Laboratory test finding

4.2.1. Occurrence of *E. coli* O157:H7

In this study, a total of 210 samples were collected and processed from various sample sources such as: 40 carcass swabs and 10 minced raw beef meat from the meat supply chain of AAAE, 80 pooled environmental samples at the slaughterhouse and 80 pooled environmental samples from meat retail shops at Yeka sub-city. After conducting a series of bacteriological examination and biochemical tests, 9 isolates were suspected as *E. coli* O157:H7. Farther confirmation by BIOLOG indicated that two of them were found to be positive for *E. coli* O157: H7 and the rest seven isolates were found to be other non-sorbitol fermenting *E. coli* species. Therefore, the current study prevalence of *E. coli* O157: H7 was 2/210 (0.95%) of the total collected samples

None of the pathogens were isolated from the 40 carcass swab samples collected in the supply chain, at both abattoirs and butcher shops. One positive sample was found from a total of 10 minced raw beef meat samples collected at butcher shops, while the other *E. coli* O157: H7 was isolated from one of the 80 abattoir pooled environment samples (1/10 swabs of knife before slaughter operation started). The complete results presenting the occurrence of *E. coli* O157:H7 from carcass swabs and minced raw beef meat samples along the carcass supply chain of AAAE are indicated in (Table 8).

Table 8: Isolation rate of *E. coli* O157: H7 form different types of samples that indicate bacterial status along carcass supply chain in Addis Ababa Abattoir Enterprise.

Sample sources	Types of samples	No. of samples Tested	No. of positive samples (%)	95% CI	χ2	p-value
Abattoir house	Carcass swab before evisceration	10	0			
	Carcass swab after evisceration	10	0			
	Carcass swab at meat van	10	0			
	Hook swab from meat van	10	0			
	Hand swab before operation	10	0			
	Knife swab before operation	10	1(10%)			
	Hand swab at mid operation	10	0			
	Knife swab at mid operation	10	0			
	Water sample	10	0			
	Floor swab	10	0			
	Protective cloth swabs	10	0			
	Sub - total No.	110	10%			
Butcher shops	Carcass swabs	10	0			
	Knife swab	10	0			
	Hand swab	10	0			
	Chopping table swab	10	0			
	Meat balance swab	10	0			
	Water sample	10	0			
	Floor swab	10	0			
	Sponge swab	10	0			
	Protective cloth swab	10	0			
	Minced raw beef meat sample	10	1(10%)			
	Sub - total No	100	10%			
Total N (%)		210	2 (0.95%)	0-2.4	0.008	0.928

4.3. Antimicrobial susceptibility pattern

Antimicrobial susceptibility tests were done on both isolates of *E. coli* O157: H7. After the two isolates were confirmed, the isolates were tested with 10 antimicrobial discs according to CLSI (2020) manual. The test result showed that Ciprofloxacin (CIP), Gentamicin (CN), Meropenem (MEM), Chloramphenicol(C), Amoxicillin Clavulanates (AMC) and Cefotaxime (CTX) were all effective against both isolates. On the other hand, both isolates showed complete resistance to Sulfamethoxazole + Trimethoprim (STX), Amoxicillin (AML), Streptomycin (S) and Ampicillin (AMP).

5. DISCUSSION

The present study focused on the isolation of *E. coli* O157: H7, assessment of critical point contamination in raw beef meat at AAAE and selected butcher shops found at Yeka sub-city, Addis Ababa. Also, the knowledge, attitude and practice of workers regarding safety and meat handling along the carcass supply chain were assessed. In addition, the antimicrobial susceptibility of isolated pathogens was tested using different antibiotic drugs.

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

Meat is a perishable food that is high in micronutrients, making it ideal for microbial growth. Although the human and animal intestinal tracts are thought to be a reservoir for *E. coli*, the possible discovery of *E. coli* in any food could be a major public health concern it may be linked to the production of toxins that can devastate the targeted cells of the organ and lead to severe GIT illness with subsequent life-threatening diseases (Engdaw and Temesgen, 2016). Using SMAC as differential and selective culture medium and various biochemical studies, 9 samples were suspected of being *E. coli* O157 out of a total of 210 samples collected in this study. Following biochemical analysis, Biolog bacterial identification system was used where only two of the nine presumptive bacterial isolates were identified phenotypically as *E. coli* O157: H7 strains, one from a butcher shop and the other in a slaughterhouse from knife swab sample collected before the slaughter process started. The rest seven were known to be other sorbitol negative E. coli species, of which two were isolated from carcass swab samples after evisceration, one from water samples collected at the abattoir, one from knife swab samples collected before operation at the slaughter house, two from floor swab samples taken at the slaughter hall and the last one was isolated from chopping table swabs at butcher shops. The presence of these species in the aforementioned sample types indicates that there might be fecal contamination, which is potentially unhygienic situation.

Sample types including water samples, hand swabs, protective wear swabs, knives swab in the middle of the process, sponge swabs, cutting tables swab, knife swab and balance swabs at both butcher shops and abattoir were considered as environmental sources, and they were all negative for the target infectious agent.

No statistically significant differences were seen in the prevalence of the pathogen among the abattoir and butcher shops ($\chi 2 = 0.008$ and p value became p=0.928). This could be due to the fact that only a few samples were found positive for the target pathogen.

In this study, the 0.95% overall prevalence of *E. coli* O157:H7 is in agreement with the low prevalence rate of 1.3% reported in Addis Ababa by Abdisa et al. (2017), 1% in Ireland, reported by EFSI (2011), and 1.2% in USA (Barkocy-Gallagher *et al.*, 2013). However, other studies have reported slightly higher values than the present finding: 2.7% from Haramaya University (Taye *et al.*, 2013), 2% reported from Jima (Shumi *et al.*, 2021), 2.4% from Hawassa (Atnafie *et al.*, 2017), 3.4% reported from Sebeta (Beruktayet, 2020), 5.4% from Jima (Sebsibe and Asfaw, 2020), 6.7% in Mekele city (Mekonnen *et al.*, 2013), and 4.9% from Mojdo (Abreham *et al.*, 2019). On the other hand, the result is considerably lower than the 9.1% reported by Tadese et al. (2021) from Ambo, 10.4% by Abebe et al. (2014) from Tigray, 10.2% reported by Bekele et al. (2014) from Addis Ababa, and 8% reported by Hiko et al. (2008) from Debrzeyit. From outside the country, more reports came with higher isolation rate: 8.8% reported by Abongo and Momba (2009) from South Africa, 8.9% by Hajian et al. (2011) and 9.6% by Tahamtan et al. (2006) from Iran, 13.3% from China (Zhang *et al.*, 2015), and 53% from Nigeria by Dahiru et al. (2008). On the contrary, other scholars reported slightly lower prevalence rate than the finding in this study: 0.22 % by Haile et al. (2014) at butcher shop from Addis Ababa; 0.2% reported by Mailafia et al. (2017) from Nigeria, 0.3% by Dontorou et al., (2003) from the Netherlands, 0.3% in European Union (EFSA, 2013).

The occurrence of *E. coli* O157: H7 from the knife swab sample before operation in the current study is 10% (1/10), which is consistent with reports of 9.57% and 9.3% reported at abattoir environment samples at AAAE by Asmelash (2015) and Haile et al. (2017) from Jima municipal abattoir, respectively; much higher than 5 % reported by Abreham et al. (2019) and Sebsibe and Asfaw, (2020) from Ethiopia, and by Inat and Sirken (2010) from Turkey. Contrarily, the occurrence observed in the present study it was slightly lower than 12% reported from a study by Hamid et al. (2018) in central Ethiopia. However, interpretation of the 10% finding in the current study needs caution because the number of knife swab samples from which the pathogen was isolated were very few (N=10).

The current study revealed a prevalence of 10% (1/10) *E. coli* O157:H7 isolation from raw minced beef meat from the selected butcher shops. The result is in line with a report of 9.8% by Asmelash (2015) but higher than the rate found in some other studies: for instances, 6.3% by Geresu and Regassa (2021), 3.07% by Bedasa et al. (2018), and 5.6 % by Sebsibe and Asfaw, (2020) from Ethiopia, and much higher than 0.82% isolated from minced beef meats in USA (Kiermeier *et al.*, 2011). Both lower (1.7%) in central Ethiopia by Beyi et al. (2017); 1.38% in Jima by Shumi et al. (2021), and higher than the current *E. coli* O157:H7 isolation rate from beef meat (13.3% reported by Bekele et al. (2014)) were reported previously from elsewhere in Ethiopia. It is important here also to take the small sample number of meat sources examined (N=10) in the current study into account when interpreting the finding.

Likewise, the zero percent *E. coli* O157:H7 isolation rate from various retail meat utensils such as knife swab, chopping table swab, and sponge swab at the butcher shop in this study was similarly reported from other recent studies in Ethiopia (Shumi *et al.*, 2021). Closer isolation rate (0.2%) from the same sources was reported from Nigeria (Mailafia *et al.*, 2017). This is in contrast to remarkably very high isolation rate (91.2%) reported by Al-Rudha et al. (2016) from Iraq. These disagreements in prevalence among different studies could be attributed to differences in geographical areas, or differences in the number of samples considered (as the case in the current study) or may be due to divergence in husbandry practices leading to distinguishable rate of exposure of the animals to the pathogen.

The fact that isolation rate of *E. coli* O157:H7 from knives at abattoir (10%) was equal to that from minced meat at retail shops (10 %) may show the risk of passing meat contamination from the source (abattoir) all the way to consumers at retail outlets, signifying the importance of avoiding raw meat consumption. However, no increase in contamination took place when the carcass was transported from abattoir to retail shops, as it is evident from absence of increased isolation rate from butcher shops. This observation is in contrast to what was reported by Beruktayet, (2020) who found that isolation rate at abattoir was lower (2.9%) than the one at retail shops (7.4%), which is not surprising because contamination of the carcass increases during transport due to uncleaned vehicle or unhygienic handling and storage at butcher shops.

5.2. Antimicrobial resistance profiles of the isolates

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

In this study, both isolates from minced raw beef meat and knife swab (N=2) were tested with ten distinct antimicrobial agents. The isolates were susceptible to ciprofloxacin, chloramphenicol, meropenem, gentamicin, amoxicillin-clavulanate, and cefotaxime. This result is in agreement with the work of Shumi et al. (2021) and partially agree with findings from the study of Hiko et al. (2008) in which all the above mentioned and other additional five antimicrobials (including the drugs in which resistance was observed in this case) were 100% effective. However, *E. coli* O157:H7 strains isolated in Saudi Arabia were found resistant to both ciprofloxacin and chloramphenicol (Nasir *et al.,* 2007). This variation might be attributed to the differences between countries in regards to extensive use of antibiotics in their husbandry practices, which in turn may lead to a difference in rate of emergence and horizontal transfer of resistance genes among isolates (Reuben and Owuna, 2013).

On the other hand, both *E. coli* O157: H7 isolates in this study showed 100% resistance to sulfamethoxazole + trimethoprim, amoxicillin, Streptomycin and ampicillin. This result is in agreement with findings from different other studies in Ethiopia: Disassa et al. (2017) and Mohammed et al. (2014) for sulfamethoxazole + trimethoprim resistance report; Atnafie et al. (2017); and Tadesse et al. (2012) for Streptomycin resistance report; Atnafie et al. (2017) and Bekele et al. (2014) for amoxicillin resistance report; and Fuh et al. (2018) and Messele (2017) for ampicillin resistance report.

5.3. Assessment of Knowledge, attitude and practice

The result from questionnaire revealed that all of the workers at the abattoir had a training in the basic safe meat handling technique, hygienic practice at the abattoir and personal hygiene. But according to their response, the slaughter staff received training which was mainly focused on general slaughtering techniques. This agrees with the finding of Zerabruk et al. (2019), who found that trainings related about food handling and personal hygiene were given in AAAE but in contrast good practices was poor, rather they were focusing on the managerial aspects. Most slaughter house workers were unaware of the cause, ways of transmission, risk factors and public health importance of foodborne disease due to carcass contamination. Therefore, the knowledge or awareness of workers about foodborne diseases is compatible with the situation observed around their working environment and personal hygiene. Similarly, Haileselassie et al. (2013) reported that only 9 out of 26 abattoir workers in Mekele had an idea about cause and transmission of foodborne disease.

Most of the abattoir workers responded that they were good at carcass handling practice. But the observational survey did not support their words due to the fact that none of the workers were engaged in washing their hands frequently during direct contact with the carcass. Similar finding was reported by Beruktayet (2020), who reported that even if the workers from Sebeta municipal abattoir responded that they were good in keeping personal hygiene, the observations made revealed that none of them were involved in the practice of hand washing while trying to touch carcass and had bad experience in keeping working equipment clean. The stunning and bleeding process was conducted on the floor, which could be a principal source of carcass contamination. This outcome is in line with the report of Haileselassie et al. (2013), who reported the carcass dressing on the floor could clearly worsen the contamination rate since it increases carcass-floor contact.

But the carcass was skinned and eviscerated while hanging over the floor to reduce carcass contact with the floor. Since stunning and other slaughtering processes are carried out without a designated work zone, fecal matter excreted during the process may contribute to fecal-carcass contamination, and worker boots may disperse excreta from the dirty floor to other free slaughter areas. In the same way, Dulo et al. (2014) have showed that the slaughter processes are done without clear division in to stunning, bleeding, skinning, evisceration and hanging for delivery.

Based on observation and interviews done at the slaughter house, workers were less concerned about keeping their personal hygiene. Chewing gum while slaughtering, muddy finger nails and dirty working clothes and boots were major points that were observed. This was in line with the report of Beruktayet, (2020).

The slaughterhouse's waste management was insufficient, and during the slaughter operation there was no regular washing of the slaughtering floor or sterilizing of the equipment at all stages of the slaughtering. Surprisingly, during the first day of visiting the abattoir, a cat was observed entering the slaughter hall corridor, which is unsafe. Tadese et al. (2021) from Ambo and Beruktayet, (2020) from Sebeta both also reported that stray dogs were observed at the entrance of the slaughter house, showing the lack of awareness among the abattoir workers regarding the significance of animals in contaminating animals.

In the case of carcass distribution from AAAE to meat retailers, most of the tracks used in the abattoir are unfit for safe and hygienic meat distribution. Despite the fact that the carcass was dispatched from the slaughterhouse in less than an hour, the meat van lacks a chilling facility to prevent bacterial growth and maintain the hygienic quality of the meat. Furthermore, since both the carcass and offal contents were placed in the same vehicle, the hygiene of the delivery tracks was compromised. Personal hygiene and the unclean appearance of the protective cloth worn by carcass-loading employees may also be factors in carcass contamination. These findings are largely matched with the report of Shumi et al. (2021) from Jima, who reported that the poor sanitary environment of carcass transporting tracks and lack of personal hygiene of the transporters could contribute a lot for higher contamination.

In the perspective of countries with high risk of foodborne disease, the butcher shops and meat handlers or processors should be in a good hygiene status for serving the safest meat for consumers. Seven out of ten meat handlers said that they had practical experience in maintaining the safety and hygiene of retail shops, including personal hygiene. But the observation clearly showed that overall sanitation and hygienic practice were poor. Activities such as using the same knife to cut both meat products and offal, frequent use of sponges, poor cleaning of the table and floor, none of the food handlers wearing hair covers and lack of frequent hand washing practice were common phenomena. Other investigators also had the same observations.

For example, Zerabruk et al. (2019) reported that, the general hygiene of meat retailer was very poor, in which both the meat products and offal were not placed separately in proper manner and that only one of the retailer shops had a refrigerator.

Generally, most butchers lacked professional qualifications and had no experience in hygienic meat handling, potentially resulting in the sale of contaminated beef to customers. Lack of implementation of the "one man one job" principle were seen, where butchers were collecting money while handling the meat in parallel, which is the recipe for contamination of ready to eat raw meat, since paper currencies and coins are loaded with bacteria responsible with foodborne diseases (Girma *et al.*,2014), which is in agreement with the findings by Beruktayet (2020) who reported that the dual activity of meat cutters', frequent handling of money at the same time as serving food would make butcher shops unsafe place for avoiding foodborne infections.

5.4. Strength and limitation of the study

The strength of the study

Determining the antimicrobial resistance of *E. coli* O157:H7 isolated from raw beef meat and assessment of critical point of contamination along the supply chain of AAAE to the butcher shops found at Yeka sub city was attempted for the first time. Therefore, it will provide baseline information for further study to determine the prevalence by using different molecular techniques.

Limitations of the study

The study has some limitations. First, the serological identification test was not done due to the outdate or expired of the antisera kit. However, out of the nine isolated presumptive only two were found positive for the target strain of shiga toxin producing *E. coli*, the rest seven biochemically isolated and identified using biolog were only at species level due to the missing of the software database line of the Biolog reader. As a result, additional confirmation tests were required. Unfortunately, the second attempt to use the MALDI-TOF (Matrix-assisted laser desorption ionization-time of flight) machine for fast screening failed due to a shortage of a machine accessories for installation at the time. Second, all samples were taken from a sole slaughter house (AAAE) and a small number of legally registered butcher shops in one sub-city of Addis Ababa, Ethiopia, which may not reflect the situation throughout the city. Lastly, the fact that low number of target meat retailers and carcasses could be responsible for dealing with low number of isolates examined, which in turn might cast a shadow on the generalizability of the finding. Financial constraint was the principal reason for having lower number of target samples.

.

6. CONCLUSION AND RECOMMENDATIONS

Pathogens from animals and/or agricultural products, such as beef and meat products, are responsible for the majority of human illnesses. Infected animals, on the other hand, serve as a source of emerging potentially pathogenic microorganisms such as *E. coli* O157:H7, necessitating preventative measures at any stage in the food production chain. The present study indicates that the majority of beef carcass delivered to the sub-city were handled in unhygienic conditions at ambient temperatures, with inadequate sanitation in carcass transporting truck. Most of the stakeholders were handling the raw beef meat with little knowledge of carcass contamination and the public health implications of pathogens found in animal products. The presence of *E. coli* O157: H7 has been reported in knife swabs prior to slaughter, possibly indicating that poor sanitary conditions at slaughter houses are the primary source of microbial contamination of beef meat. The sanitation and hygiene services were insufficient, perhaps resulting in pollution, which indicates the need for a preventative approach to control *E. coli* O157: H7 in the beef meat production chain.

The fact that an isolate was detected from raw minced meat indicated how raw meat could be a significant factor for the occurrence of *E. coli* O157:H7 in the community where consumption of raw meat is highly practiced. Generally, this study could give an insight on the low prevalence of *E. coli* O157:H7 and its public health significance associated with the consumption of unsafe food at the study area.

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

- Slaughterhouse staff and meat handlers in retail establishments should be trained on foodborne pathogens and their public health implications, and personal hygiene and hygienic procedures during slaughtering.

- Retail meat handlers should be qualified in basic hygienic and proper meat handling techniques.

- Slaughterhouse administrators and retail outlet owners should collaborate together with meat inspection groups to deliver and provide safe beef meat to consumers.

- Veterinarians and healthcare professionals should collaborate to create public awareness about the serious health risks involved with eating raw meat.

- Antimicrobial use in the animal community must be closely monitored in order to discourage bacteria from developing antimicrobial resistance and posing a public health risk.

- Additional large-scale researches covering all abattoirs and a good number of representative butcher shops of Addis Ababa need to conducted to come up with more reliable data on the prevalence of *E. coli* O157:H7 in the city.

7. REFERENCES

Abdissa, R., Haile, W., Fite, A.T., *et al.* (2017). Prevalence of *Escherichia coli* O157: H7 in beef cattle at slaughter and beef carcasses at retail shops in Ethiopia. *Biomedical Central research notes for Infectious Diseases*. 17(1), pp.1-6.

Abebe, M., Hailelule, A., Abrha, B., *et al.* (2014). Antibiogram of *Escherichia coli* strains isolated from food of bovine origin in selected Woredas of Tigray, Ethiopia. *African Journal of Bacteriology Research*. 6(3), pp.17-22.

Abongo, B. O. and Momba, M. N. B. (2009). Prevalence and characterization of *Escherichia coli* O157:H7 isolates from meat and meat products sold in Amathole District, Eastern Cape Province of South Africa. *International Journal of Food Microbiology*. 26: 173-176

Abraham, A.G., Wellington, T.T. and Victoria, A. (2012). Microbiological quality of chicken sold in Accra and determination of D 10-value of *E. coli*. *Food and Nutrition Sciences*. 20(12).

Abreham, S., Teklu, A., Cox, E. and Tessema, T.S. (2019). *Escherichia coli* O157: H7: distribution, molecular characterization, antimicrobial resistance patterns and source of contamination of sheep and goat carcasses at an export abattoir, Mojdo, Ethiopia. *Biomedical Central Research notes for Microbiology*. 19(1), pp.1-14

Adams, M.C., Baines, S.K. and Ranadheera, R.D. (2010). Importance of food in probiotic efficacy. *Food Research International*. 43(1), pp.1-7.

Adamu, M.T., Shamsul, B.M.T., Desa, M.N. and Khairani-Bejo, S. (2014). A Review on *Escherichia coli* O157:H7-The Super Pathogen. *Journal of Health Environment.*5(2):118-134

Adugna, M., Seifu, E., Kebede, A. and Doluschitz, R. (2015). Quality and safety of camel milk along the value chain in eastern Ethiopia. *International Journal of Food Studies*. 2:2.

Adzitey, F. (2020). Incidence and antimicrobial susceptibility of *Escherichia coli* isolated from beef (meat muscle, liver and kidney) samples in Wa Abattoir, Ghana. *Cogent Food & Agriculture*. 6(1), p.17-18.

Agüeria, D.A., Terni, C., Baldovino, V.M. and Civit, D. (2018). Food safety knowledge, practices and attitudes of fishery workers in Mar del Plata, Argentina. *Food Control*.91, pp.5-11.

Ahmad, R.S., Imran, A. and Hussain, M.B. (2018). Nutritional composition of meat. *Meat Science and Nutrition*. 61.

Al-Dragy, W. and Baqer A. (2014). Detection of *Escherichia coli* O157:H7 in human patients stool and food by using multiplex PCR assays targeting the *rfbE* and the *eae A* genes compared with detection by biochemical test and serological assay. *Journal of Al-Nahrain University*. 17**(**3), 124-131.

Al-Rodham, A.M.H., Al-Rubaie, E.M. and Khalil, N.K. (2016). Distribution of *E. coli* O157:H7 in fecal and urine samples of cattle. *Iraqi Journal of Veterinary Medicine*. 40(1): 79-82.

Al-Rudha, A.M.H., Al-Rubaie, E.M. and Khalil, N.K. (2016). Distribution of *E. coli* O157:H7 in fecal and urine samples of cattle. *Iraqi Journal of Veterinary Medicine*. 40(1): 79-82.

Annan-Prah, A., Mensah, A.A., Akorli, S.Y., Asare, P.T. and Kumi-Die, I.D. (2012). Slaughterhouses, animal slaughter and slaughter hygiene in Ghana. *Journal of Veterinary Advances*. 2(4), pp.189-198.

Asmelash, T. (2015). Isolation, identification, antimicrobial profile and molecular characterization of Enterohaemorrhagic *E. coli* O157:H7 isolated from ruminants slaughtered at Debre Zeit Elfora export abattoir and Addis Ababa abattoirs enterprise. A thesis submitted to the college of veterinary medicine and agriculture of Addis Ababa.

Atnafie, B., Paulos D., Abera M., *et al.* (2017). Occurrence of *E. coli* O157:H7 in cattle faces and contamination of carcass and various contact surfaces in abattoir and butcher shops of Hawassa, Ethiopia. *Biomedical Center of Microbiology.* 17 (1), pp.20-37.

Bakhtiary, F., Sayev, H.R., Remely, M., Hippe, B., Hosseini, H. and Haslberger, A.G. (2016). Evaluation of bacterial contamination sources in meat production line. *Journal of food quality*. 39(6), pp.750-756.

Balcha, E. and Gebretinsae, H. (2014). Assessment of bacteriological quality of meat contact surfaces in selected butcher shops of Mekelle city, Ethiopia. *Journal of Environmental Occurrence Science*. 2:61-66.

Barkocy-Gallagher, G.A., Arthur, T.M., Rivera-Betancourt, M., *et al*. (2003). Seasonal prevalence of Shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and Salmonella in commercial beef processing plants. *Journal of Food Protection*. 66: 1978-1986.

Bedasa, S., Shiferaw, D., Abraha, A. and Moges, T. (2018). Occurrence and antimicrobial susceptibility profile of *Escherichia coli* O157: H7 from food of animal origin in Bishoftu town, Central Ethiopia. *International Journal of Food Contamination*. 5(1), p.2.

Bekele, T., Zewde, G., Tefera, G., Feleke, A. and Zerom, K. (2014). *Escherichia coli* O157: H7 in raw meat in Addis Ababa, Ethiopia: prevalence at an abattoir and retailers and antimicrobial susceptibility. *International Journal of Food Contamination*.1(1), p.4.

Beneduce, L., Spano, G. and Massa, S. (2003). *Escherichia coli* O157: H7 general characteristics, isolation and identification techniques. *Annuals of microbiology*. 53(4), pp.511-528.

Beruktayet, W. (2020). Occurrence and antimicrobial resistance of *E. coli* O157:H7 in cattle, beef and diarrheic patients in Sebeta town, Ethiopia. A thesis submitted to the college of veterinary medicine and agriculture of Addis Ababa.

Bersisa, A., Tulu, D. and Negera, C. (2019). Investigation of bacteriological quality of meat from abattoir and butcher shops in Bishoftu, Central Ethiopia. *International journal of microbiology*. 2(19).

Beyi, A.F., Fite, A.T., Tora, E., *et al.* (2017). Prevalence and antimicrobial susceptibility of *Escherichia coli* O157 in beef at butcher shops and restaurants in central Ethiopia. *Biomedical Central research notes for microbiology*. 17(1), pp.49.

Botkin, D.J., Galli, L., Sankarapani, V., Soler, M., Rivas, M. and Torres, A.G. (2012). Development of a multiplex PCR assay for detection of Shiga toxin-producing *Escherichia coli*, enterohemorrhagic *E. coli*, and enteropathogenic *E. coli* strains. *Frontiers in Cellular and Infection Microbiology*. 2, pp.8.

Brektawit, A. (2020). Occurrence and antimicrobial resistance of *E. coli* O157:H7 in cattle, beef and diarrheic patients in Sebata town, Ethiopia. MSc Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre- Zeit, Ethiopia

Bryan, A., Youngster, I. and McAdam, A.J. (2015). Shiga toxin producing *Escherichia coli*. *Clinical science in laboratory medicine.* 35(2), pp.247-272.

Canizales, R.A., Gonzalez N.E., Vidal J.E., Flores V.H. and Leon-S.N. (2013). Prevalence and antibiotic resistance profile of diarrheagenic *Escherichia Coli* strains isolated from food items in Northwestern Mexico. *International Journal of Food Microbial*. 164, pp.36-45.

Caprioli, A., Morabito, S., Brugère, H. and Oswald, E. (2005). Enterohaemorrhagic *Escherichia coli:* Emerging issues on virulence and modes of transmission. *Veterinary research*. 36(3), pp.289-311.

CDC (Centers for Disease Control and Prevention) (2016): Enterohemorrhagic *Escherichia coli* and other *E. coli* causing Hemolytic Uremic Syndrome. Iowa state university, Institute for international Cooperation in Animal Biolocs.

Chapman, P.A., Siddons, C.A., Cerdan-malo, A.T. and Harkin, M.A. (2000). An evaluation of rapid methods for detecting *Escherichia coli* O157 on beef carcasses United Kingdom. *Epidemiology of Infectious disease.* 124: 207-213.

Chepkemoi, S. (2016). Handling practices, microbial quality and weight loss of beef in small and medium enterprise butcheries in Nairobi and Isiolo counties, Kenya (Doctoral dissertation, University of Nairobi).

Christina, W.O. and Bertil, K. (2005). Review Enterohemorrhagic *Escherichia coli* (EHEC). *Journal of infectious disease.* 37:405-416

CLSI (Clinical and Laboratory Standards Institute) (2020): Performance standards for antimicrobial susceptibility testing. 30 th ed. CLSI supplement M 100 Wayne, PA. *Clinical Laboratory standard Institution*.

Croxen, M.A., Law, R.J., Scholz, R., Keeney, K.M., Wlodarska, M. and Finlay, B.B. (2013). Recent advances in understanding enteric pathogenic *Escherichia coli*. *Clinical microbiology reviews*. 26(4), pp.822-880.

Dahiru, M., Uraih, N., Enabulele, S.A. and Shamsudeen, U. (2008). Prevalence of *Escherichia coli* O157:H7 in fresh and roasted beef in Kano city, Nigeria. *Bacteriological journal of pastoralist.* 1(1): 39 – 42.

Desta, H, Cattaneo, D., Martino, P.A., *et al*. (2012). Occurrence of pathogenic species of Enterobacteriaceae, Listeria monocytogenes, Staphylococcus aurous and Brucella in bovine raw bulk milk in the selected milks heds, Asella Dairy Union and Ada Dairy Cooperatives, Ethiopia. *International Food Safety Conference.*

Detzner, J., Pohlentz, G. and Müthing, J. (2020). Valid Presumption of Shiga Toxin-Mediated Damage of Developing Erythrocytes in EHEC-Associated Hemolytic Uremic Syndrome. *Toxins*. 12(6), pp.373.

Dhama, K., Karthik, K., Chakaraborty, S. *et al.* (2014). Loop mediated isothermal amplification of DNA: Anew diagnostic tool light the world of diagnosis of animal and human pathogen: a review. *Pakistan Journal of Biological Science*. 17(2): 151- 166.

Disassa, N., Sibhat, B., Mengistu, S., Muktar, Y. and Belina, D. (2017). Prevalence and antimicrobial susceptibility pattern of *E. coli* O157: H7 isolated from traditionally marketed raw cow milk in and around Asosa town, western Ethiopia. *Veterinary medicine international*. 20:17.

Dontorou, C., Papadopoulou, C., Filioussis, G., *et al.* (2003). Isolation of *Escherichia coli* O157: H7 from foods in Greece. *International journal of food microbiology*. 82(3), pp.273-279.

Dulo, F., Feleke, A., Szonyi, B., Fries, R., Baumann, M.P. and Grace, D. (2014). Isolation of multidrug-resistant *Escherichia coli* O157 from goats in the Somali region of Ethiopia: a cross-sectional, abattoir-based study. *Veterinary medicine international.* 10(11)

EFSA (European Food Safety Authority) (2011): The European union summary report on trends and sources of zoonoses, trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in 2011. *European Food Safety Authority Journal*. 11, (4), pp. 3129, 2013.

Eklund, M. (2005). Enterohemorrhagic *Escherichia coli* (EHEC) findings from humans in Finland. *Emerging infectious diseases*. 8(5), p.334.

Engdaw, T.A. and Temesgen, W. (2016). O157:H7 Serotype of *Escherichia coli* as an important emerging zoonosis. *International Journal of Microbiolgy Research.*7 (1): 9-17.

Escherich, T. (1988). The intestinal bacteria of the neonate and breast-fed infant. *Clinical Infectious Diseases*. 10(6), pp.1220-1225.

Eshetu, W. and Assefa, B. (2019). Location A case of primary school in Yeka sub-city, Addis Ababa, Ethiopia. *Allocation Analysis for Public Services Using GIS Technique*. 26-38.

Fairbrother, J.M. and Nadeau, E. (2006). *Escherichia coli*: on-farm contamination of animals. *Review of Science and Technology*. 25: 555-569

Fan, R., Shao, K., Yang, X., *et al*. (2019). High prevalence of non-O157 shiga toxin producing *Escherichia coli* in beef cattle detected by combining four selective agars. *Biomedical Center for Microbiology*. 19:13-235.

FAO/WHO (2009). "Basic texts on Food Hygiene" Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Rome, Italy.

Farasat, T., Bilal, Z. and Yunus, F.U.N. (2012). Isolation and biochemical identification of *Escherichia* coli from wastewater effluents of food and beverage industry. *Journal of Cell and Molecular Biology*. 10(1).

Fatima, R. and Aziz, M. (2020). Enterohemorrhagic Escherichia Coli (EHEC). *Foodborne pathogens and disease.* 9: 443-487

Ferens, W.A. and Hovde C.J. (2011). *Escherichia coli* O157:H7: animal reservoir and sources of human infection. *Foodborne pathogens and disease*. 8: 465-487.

Fuh, N.J., Christiana, O.M., Attah, O.G., *et al*. (2018). Risk Analysis and Antibiogram Spectrum of *Escherichia coli* O157: H7 Serotype from Children Stool and Raw

Bovine Meat in Households Across Cross River State, Nigeria. *Eastern Journal of Center for Biological Science.* 4(3): 39-45.

Geresu, M.A. and Regassa S. (2021). *Escherichia coli* O15: H7 from food of animal origin in Arsi: occurrence at catering establishments and antimicrobial susceptibility profile. *The Journal of Scientific World*. 10.

Getachew, L., Hailemariam, T., Dawit, A. and Asfaw, N. (2008). Live animal and meat export value chains for selected areas in Ethiopia: Constraints and opportunities for enhancing meat exports. *Veterinary Epidemiology*. 2, pp,104-106.

Getaneh, D.K., Hordofa L.O., Ayana D.A., Tessema T.S. and Regassa L.D. (2021). Prevalence of *Escherichia coli* O157:H7 and associated factors in under-five children in Eastern Ethiopia. PLoS ONE. 16(1).

Ghimire, S. (2018). Antibiogram profiling of sorbitol non-fermenting *E. coli* isolated from raw meat (Doctoral dissertation).

Girma, G., Ketema, T. and Bacha, K. (2014). Microbial load and safety of paper currencies from some food vendors in Jimma Town, Southwest Ethiopia. *Bio Medical Center of Research*. Note.

Gökhan, I. and Belgin S. (2010). Detection of *Escherichia coli* O157 and *Escherichia coli* O157:H7 by the immunomagnetic separation technique and *stx1* and *stx2* genes by multiplex PCR in slaughtered cattle in Samsun province, Turkey. *Journal of Veterinary Science*. 11(4): 321-326.

Gray, M.D., Lampel, K.A., Strockbine, N.A., Fernandez, R.E., Melton-Celsa, A.R. and Maurelli, A.T. (2014). Clinical isolates of Shiga toxin 1a–producing Shigella flexneri with an epidemiological link to recent travel to Hispaniola. *Emerging infectious diseases*. 20(10), pp.1669.

Gudeta, B. (2012). Optimizing logistic chain of animal transport and meat distribution: studies on livestock markets and abattoirs in Addis Ababa City. *International Conference of Food Engineering and Biotechnology*. 6, pp. 178-5.

Gutema, F.D., Rasschaert, G., Agga, G.E. *et al*. (2020). Occurrence, Molecular characteristics, and Antimicrobial Resistance of *Escherichia coli* O157 in Cattle, Beef, and Humans in Bishoftu Town, Central Ethiopia. *Foodborne Pathogen Diseases.* 17.

Haile, W. (2014). Prevalence and source of contamination of cattle meat at municipal abattoirs and butcheries as well as its public health importance in Addis Ababa, Ethiopia. [Thesis]

Haile, A.F., Kebede, D. and Wubshet, A.K. (2017). Prevalence and antibiogram of *Escherichia coli* O157 isolated from bovine in Jimma, Ethiopia: Abattoir-based survey. *Ethiopian Veterinary Journal*. 21(2): 109-120.

Haileselassie, M., Taddele, H., Adhana, K. and Kalayou, S. (2013). Food safety knowledge and practices of abattoir and butchery shops and the microbial profile of meat in Mekelle City, Ethiopia. *Asian Pacific journal of tropical biomedicine*. 3(5), pp.407-412.

Hajian, S., Rahimi, E. and Mumtaz, H. (2011). A 3-year study of *Escherichia coli* O157:H7 in cattle, camel, sheep, goat, chicken and beef minced meat. *International Conference of Food Engineering and Biotechnology*. 9, pp. 163-5.

Hamid, M., Tefera, Y., Eguale, T. and Worku, Y. (2018). *Escherichia coli* O157:H7: Prevalence, identification and antimicrobial resistance in cattle slaughter at Addis Ababa municipal abattoir, Ethiopia. *International Journal of Advanced Research in Biological Sciences.* 5.

Hassan, A., Din, A.U., Zhu, Y. *et al.* (2019). Updates in understanding the hypocholesterolemia effect of probiotics on atherosclerosis. *Applied Microbiology and Biotechnology*. 103(15), pp.5993-6006.

Havelaar, A.H., Kirk, M.D., Torgerson, P.R., *et al.* (2015). World Health Organization: Global estimates and regional comparisons of the burden of foodborne disease in 2010. *Pennsylvania laboratory science and medicine*. 12(12).

Hazen, T.H., Sahl, J.W., Fraser, C.M., Donnenberg, M.S., Scheutz, F. and Rasko, D.A. (2013). Refining the pathovar paradigm via phylogenomic of the attaching and effacing *Escherichia coli*. *Proceedings of the National Academy of Sciences*. 110(31), pp.12810-12815.

Hessain, A.M., Al-Arfaj, A.A., Zakri, A.M., El-Jakee, J.K., Hemeg, H.A. and Ibrahim, I.M. (2015). Molecular characterization of *Escherichia coli* O157: H7 recovered from meat and meat products relevant to human health in Riyadh, Saudi Arabia. *Saudi Journal of Biological Sciences*. 22(6), pp.725-729.

Hiko, A., Asrat, D. and Zewde, G. (2008). Occurrence of Escherichia coli O157: H7 in retail raw meat products in Ethiopia. *The Journal of Infection in Developing Countries*. 2(05), pp.389-393.

Honish, L., Punja, N. and Nunn, S., *et al.* (2017). Enteric disease outbreaks: *Escherichia coli* O157:H7 infections associated with contaminated pork products—Alberta, Canada, July–October 2014. *Canada Communicable Disease Report*. 43(1), p.21.

Inat, G. and Siriken, B. (2010). Detection of *Escherichia coli* O157 and *Escherichia coli* O157: H7 by the immunomagnetic separation technique and *stx1* and *stx2* genes by multiplex PCR in slaughtered cattle in Samsun Province, Turkey. *Journal of veterinary science*. 11(4), p.321.

Islam, M.M., Sokolova, E. and Hofstra, N. (2018). Modelling of river fecal indicator bacteria dynamics as a basis for fecal contamination reduction. *Journal of hydrology*. 563, pp.1000-1008.

ISO (International Organization for Standardization) (2001): Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Escherichia coli* O157. ISO/FDIS 16654.

ISO (International Organization for Standardization) (2003): Microbiology of food and animal feeding stuffs -Horizontal method for isolation and identification of *E. coli* O157:H7-Part 3: Detection and MPN technique for low numbers. ISO reference number 6888- 3: 2003 (E). Geneva, Switzerland: ISO.

Jafari, A., Aslani, M.M. and Bouzari, S. (2012). *Escherichia coli*: a brief review of diarrheagenic pathotypes and their role in diarrheal diseases in Iran. *Iranian Journal of Microbiology*. 4 (3): 102-117.

Jensen, B.H., Olsen, K.E.P., Struve, C., Krogfelt, K.A. and Petersen, A.M. (2014). Epidemiology and clinical manifestations of Enteroaggregative *Escherichia coli*. *Clinical Microbiology Review.* 27(3): 614 -630.

Jufare, A. (2018). Occurrence and Antimicrobial susceptibility profile among *E. coli* O157:H7 isolated from the Abattoir, retail shop and diarrheic patient in Bishoftu town, East Shoa Ethiopia. MSc Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre-Zeit, Ethiopia

Kahsay, H., Gashaw, A., Afework, K., Feleke, M. and Moges, T. (2008). Prevalence of bacteria and intestinal parasites among food-handlers in Gondar Town, Northwest Ethiopia. *Journal of Health for Population and Nutrition.* 26:451-455.

Kaspar, C., Doyle, M.E. and Archer, J., 2010. White paper on non-O157: H7 Shiga toxin-producing E. coli from meat and non-meat sources. *Food Research Institution Food Safety Review*. pp.1-26.

Kebede, T., Afera, B., Taddele, H. and Bisrat, A. (2014). Assessment of bacteriological quality of sold meat in the butcher shops of Adigrat, Tigray, Ethiopia. *Applied Journal of Hygiene*. 3(3), pp.38-44.

Kibret, M. and Abera, B. (2011). Antimicrobial susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia. *African health sciences*. 11, pp.40-45.

Kiermeier, A., Mellor, G., Barlow, R. and Jenson, I. (2011). Assumptions of acceptance sampling and the implications for lot contamination: *Escherichia coli* O157 in lots of Australian manufacturing beef. *Journal of Food Protection*. 74(4):539–44.

Kiranmayi, Ch.B. and Krishnaiah, N. (2010). Detection of *Escherichia coli* O157:H7 prevalence in foods of animal origin by cultural methods and PCR technique. *Veterinary World*. 3(1): 13-16.

Lejeune, J.T., Besser, T.E., Rice, D.H. and Hancock, D.D. (2001). *Letters in Applied Microbiology*. 32:316-320

Lennox, J. A., John G. E. and Echa C. (2020). Isolation of *Escherichia coli* O157:H7 from selected food samples sold in local markets in Nigeria. *African Journal of Food Science*. 14(2), pp.32-30.

Lim, J.Y., Yoon, J.W. and Hovde, C.J. (2010). A brief overview of *Escherichia coli* O157:H7 and its Plasmid O157. *Journal of Microbiology and Biotechnology*. 20(1): 5–14

Liu, J., Zhou, R. and Li, L., *et al.* (2016). Viable but non-culturable state and toxin gene expression of enterohemorrhagic *E. Coli* O157 under cryopreservation. *Research in microbiology*. 168(3), pp.188-193

Lupindu, A.M. (2018). Epidemiology of Shiga toxin-producing *Escherichia coli* O157:H7 in Africa in review. *Southern African Journal of Infectious Diseases*. 33(1), pp.24-30.

Ma, Y.Y.H. (2019). Determination of the antibiotic resistance and bacteriophage infectivity of verocytotoxigenic *Escherichia coli* (Doctoral dissertation, University of British Columbia).

Magwira, C., Gashe B. and Collison E. (2005). Prevalence and antibiotic resistance profiles of *Escherichia coli* O157: H7 in beef products from retail outlets in Gaborone, Botswana. *Journal of Food Protection.* 68:403-406.

Mailafia, S., Madubuike, S. A.L. and Raji, M. A. (2017). Phenotypic identification of *Escherichia coli* O157:H7 isolates from cattle at Suleja Abattoir, Nigeria. *African Journal of Microbiology Research.* 11(21): 845-850.

Majowicz, S.E., Scallan, E., Jones-Bitton, A., *et al.* (2014). Global incidence of human Shiga toxin–producing *Escherichia coli* infections and deaths: a systematic review and knowledge synthesis. *Foodborne pathogens and disease*. 11(6), pp.447-455.

Mboto, C.I., Agbo, B.E., Ikpoh, I.S., *et al.* (2012). Bacteriological study of raw meat of Calabar Abattoir with public health and veterinary importance. *Journal of Microbiology and Biotechnology Research*. 2(4), pp.529-532.

McNeill, S.H., Belk, K.E., Campbell, W.W. and Gifford, C.L. (2017). Coming to terms: meat's role in a healthful diet. *Animal Frontiers*.7(4), pp.34-42.

Mekonnen, H., Habtamu, T., Kelali, A. and Shewit, K. (2013). Food safety knowledge and practices of abattoir and butchery shops and the microbial profile of meat in Mekelle City, Ethiopia. *Journal of Tropical Biomedicine*. 3:5, pp. 407–412.

Mengist, A., Aschale, Y. and Reta, A. (2018). Bacterial and parasitic assessment from finger nails in Debre Markos, Northwest Ethiopia. *Canadian Journal of Infectious Diseases and Medical Microbiology*. 21(8).

Mengistu, S., Abayneh, E. and Shiferaw, D. (2017). *E. coli* O157:H7 and *Salmonella* Species: Public Health Importance and Microbial Safety in Beef at Selected Slaughter Houses and Retail Shops in Eastern Ethiopia. *Journal of Veterinary Science and Technology*. 115: 468.

Mersha, G., Asrat, D., Zewde, B.M. and Kyule, M. (2009). Occurrence of *Escherichia coli* O157:H7 in feces, skin and carcasses from sheep and goats in Ethiopia. *Letter of Applied Microbiology*. 50(1): 71-76.

Messele, Y.E., Abdi, R.D., Yalew, S.T., Tegegne, D.T., Emeru, B.A. and Werid, G.M. (2017). Molecular determination of antimicrobial resistance in *Escherichia coli* isolated from raw meat in Addis Ababa and Bishoftu, Ethiopia. *Annuals of Clinical Microbiology and Antimicrobials*. 16(1), p.55.

Mohammed, O., Shimelis, D., Admasu, and P Feyera, T. (2014). Prevalence and antimicrobial susceptibility pattern of *E. coli* isolates from raw meat samples obtained from abattoirs in Dire- Dawa City, Eastern Ethiopia. *International Journal of Microbiological Research*. 5(1), pp.35-39.

Müller, E.E. and Ehlers, M.M. (2005). Biolog identification of non-sorbitol fermenting bacteria isolated on *E. coli* O157 selective CT-SMAC agar. *Water Science and Analysis*. 31(2), pp.247-252.

Munita, J. M. and Cesar, A. A. (2016). Mechanisms of antibiotic resistance. *Microbiological Spectrum*. 4(2).

Nasir, M.N.H. (2007). Aetiology of Diarrhea in Infants in Khartoum State (Doctoral dissertation)

Ntanga, P.D. (2013). Assessment of microbial contamination in beef from abattoir to retail meat outlets in Morogoro municipality, Tanzania (Doctoral dissertation, Sokoine University of Agriculture).

O'Loughlin, E.V. and Robins-Browne, R.M. (2001). Effect of Shiga toxin and Shiga-like toxins on eukaryotic cells. *Microbes and Infection*. 3(6), pp.493-507.

OMNILOG (2010): OMNILOG data collection software, bacterial and fungi identification system, user guide part no. 90311, 2:3.

Painter, J. A., Hoekstra, R. M., Ayers, C. R., *et al*. (2013). Attribution of Foodborne Illnesses, Hospitalizations, and Deaths to Food Commodities by using Outbreak Data, United States, 1998–2008. *Emerging Infectious Diseases.* 19(3): 407–415.

Pal, M., Mulu, S., Tekle, M., Pintoo, S.V. and Prajapati, J. (2016). Bacterial contamination of dairy products. *Beverage and food world*. 43(9), pp.40-3.

Parsons, B., Zelyas N., Berenger B. and Chui L. (2016). Detection, Characterization, and Typing of Shiga Toxin-Producing *Escherichia coli*. *Frontier Microbiology.*7:478.

Paul, N., G. and Karl, A., B. (2012). Goldwater and Bettelheim Treatment of enterohemorrhagic *Escherichia coli* (EHEC) infection and hemolytic uremic syndrome (HUS). *Biomedical Central Research Notes of Medical Science.* 10(12).

Peng, Z., Liang, W., Hu, Z., *et al.* (2019). O-serogroups, virulence genes, antimicrobial susceptibility, and MLST genotypes of Shiga toxin-producing *Escherichia coli* from swine and cattle in Central China. *Biomedical Central Research Notes for veterinary Science*. 15(1), pp.1-13.

Pennington, H. (2010). Review: *Escherichia coli* O157. *Lancet.*376: 1428-35.

Petridis, H., Kidder, G. and Ogram, A. (2002). *Escherichia coli* O157:H7 A Potential Health Concern. Institute of Food and Agricultural Sciences, University of Florida, Florida.

Power, C.A., Johnson, R.P. and McEwen, S.A. (2000). Evaluation of the reversal and Safe Path rapid *Escherichia coli* O157 detection tests for use on bovine feces and carcasses. *Journal of Food Protection*. 63: 860–866.

Priyanka, B., Rajashekhar, K. P. and Sulatha, D. (2016). A review on detection methods used for foodborne pathogens. *Indian Journal of Medical Research*. 144. 327-338.

Quinn, P.J., Markey, B.K., Leonard, F.C., Hartigan, P., Fanning, S. and Fitzpatrick, E. (2011). *Veterinary microbiology and microbial disease*. John Wiley & Sons.

Rahal, E., Kazzi N., Nassar F. and Matar G. (2012). *Escherichia coli* O157:H7-Clinical aspects and novel treatment approaches. *Medical and Health Science.* 2(138).

Raji, M.A., Minga, U. and Machangu, R. (2006). Current epidemiological status of Enterohaemorrhagic *Escherichia coli* O157:H7 in Africa. *Chinese Medical Journal*. 119(3), pp.217-222.

Ranjbar, R., Erfanmanesh, M., Afshar, D., Mohammadi, M., Ghaderi, O. and Haghnazari, A. (2016). Visual detection of Enterohemorrhagic *Escherichia coli* O157:H7 using loop-mediated isothermal amplification. *Electronic physician*. 8(6), pp.2576.

Redmond, E.C. and Griffith, C.J. (2010). Home food safety and consumer responsibility. *Medical and health sciences*. 10, pp.88.

Reza, R., Maryam, E., Davoud, A., Mohsen, M., Omar, G. and Ali, H. (2016). Visual detection of Enterohemorrhagic *Escherichia coli* O157:H7 Using Loop-Mediated Isothermal Amplification. *Electro Physician.* 8: 2576-2585.

Reuben, R. C. and Owuna, G. (2013). Antimicrobial resistance patterns of *Escherichia coli* O157:H7 from Nigerian fermented milk samples in Nasarawa state, Nigeria. *International Journal of Pharmaceutical Science Inventory*. 2(3): 38-44.

Schlundt, J., Toyofuku, H., Jansen, J. and Herbst, S.A. (2004). Emerging foodborne zoonoses. *Revue scient fique et technique-office international des epizooties*. 23(2), pp.513-534.

Schroeder, C.M., Zhao, C. and Deb-Roy, C. (2002). Antimicrobial resistance of *Escherichia coli* O157 isolated from humans, cattle, swine, and food. *Applied and environmental microbiology*. 68(2), pp.576.

Sebsibe, M.A. and Asfaw, T.E. (2020). Occurrence of Multi-Drug Resistant *Escherichia Coli* and *Escherichia Coli* O157: H7 in Meat and Swab Samples of Various Contact Surfaces at Abattoir and Butcher Shops in Jimma Town, Southwest District of Ethiopia. *Infection and Drug Resistance*. 13, pp.3853.

Shahrani, M., Dehkordi, FS. and Momtaz, H. (2014). Characterization of *Escherichia coli* virulence genes, pathotypes and antibiotic resistance properties in diarrheic calves in Iran. *Biological Researches*. 47: 1–13.

Shamsul, B.M.T., Adamu, M.T., Desa, M.N.M. and Khairani-Bejo, S. (2016). Prevalence of *Escherichia Coli* O157: H7 and Enterobacteriaceae on hands of workers in halal cattle abattoirs in Peninsular Malaysia. *The Malaysian Journal of Medical Sciences.* 23(5), pp.65.

Shecho, M., Thomas, N., Kemal, J. and Muktar, Y. (2017). Cloacal Carriage and Multidrug Resistance *Escherichia coli* O157:H7 from Poultry Farms, Eastern Ethiopia. *Journal of Veterinary Medicine.* 17: 1-9.

Sheng, H, Lim, J.Y., Knecht, H.J., Li, J. and Hovde, C.J. (2006). Role of *Escherichia coli* O157:H7 virulence factors in colonization at the bovine terminal rectal mucosa. *Infectious and Immunology*. 74: 4685-4693.

Shulman, S.T., Friedmann, H.C. and Sims, R.H. (2007). Theodor Escherich: the first pediatric infectious diseases physician. *Clinical infectious diseases*. 45(8), pp.1025-1029.

Shumi, E., Tolosa, T., Abdurrahman, M., Olani, A., Lekew, M. and Taddese, D. (2021). Phenotypic characterization, antimicrobial susceptibility patterns profile and risk factors of *Escherichia coli* O157:H7 isolated from cattle meat at Jimma, Ethiopia. *American Journal of Bioscience and Bioengineering.* 9(2): 40-48.

Sofos, J.N., 2008. Challenges to meat safety in the 21st century. *Meat science*. 78(1-2), pp.3-13.

Songe, M.M., Hang'ombe, B.M., Knight-Jones, T.J. and Grace, D. (2017). Antimicrobial resistant enteropathogenic *Escherichia coli* and *Salmonella spp*. in houseflies infesting fish in food markets in Zambia. *International Journal of Environmental Research and Public Health*. 14(1), pp.21.

Tadese, N.D., Gebremedhin, E.Z. and Moges, F. (2021). Occurrence and antibiogram of *Escherichia coli* O157: H7 in raw beef and hygienic practices in abattoir and retailer shops in Ambo town, Ethiopia. *Veterinary Medicine International*. 20(21): 12.

Tadesse, D.A., Zhao, S., Tong, E., *et al.* (2012). Antimicrobial drug resistance in *Escherichia coli* from Human and Food animal, United States, 1950-2002. *Emerging Infectious Deases*. 18:5.

Tahamtan, Y.E., Pourbakhsh S.A. and Shekar-foroush S.S. (2006). PCR detection of *Escherichia coli* O157:H7 directed from slaughtered cattle in Shiraz. *Iranian Journal of Archives Razi Institute*. 1:1.

Tassew (2015). Isolation, identification, antimicrobial profile and molecular characterization of Enterohaemorrhagic *E. coli* O157:H7 isolated from ruminants slaughtered at Debre Zeit ELFORA Export Abattoir and Addis Ababa Abattoirs Enterprise [dissertation].

Taye, M., Berhanu, T., Berhanu, Y., Tamiru, F. and Terefe, D. (2013). Study on carcass contaminating *Escherichia coli* in apparently healthy slaughtered cattle in Haramaya University slaughter house with special emphasis on *Escherichia coli* O157: H7, Ethiopia. *Journal of Veterinary Science and Technology*. 4 (1), pp.132.

Temesgen, W. and Engdaw, T.A. (2016). O157:H7 Serotype of *Escherichia coli* as an Important Emerging Zoonosis. *International Journal of Microbiolgy Research.* 7 (1): 9-17.

Teplitski, M., Wrigh, A.C. and Lorca, G. (2009). Biological approaches for controlling shellfish-associated pathogens. *Current Opinion in Biotechnology.* 20 (2): 185-190.

Tesfaye, H., Alemayehu, H., Desta, A.F. and Eguale, T. (2019). Antimicrobial susceptibility profile of selected Enterobacteriaceae in wastewater samples from health facilities, abattoir, downstream rivers and a WWTP in Addis Ababa, Ethiopia. *Antimicrobial Resistance and Infection Control*. 8(1), pp.134.

Thrusfield, M. (2005). *Veterinary epidemiology*. John Wiley and Sons.

Timothy, L., Switaj, M.D., Kelly, J., Winter, D.O., Scott, R. and Christensen, M.D. (2015). Diagnosis and Management of Foodborne Illness. *American Family Physician*. 92 (5): 358-365.

Uddin, M., Hossain, M.T., Proshad, R. and Rimi, T.A. (2019). Identification of pathogenic *Escherichia coli* strain from river and sewage water in Bangladesh. *Archiological, Agricultural and Environmental Science*. 4(1): 39–44.

UN (2013). Urban inequality report; Addis Ababa: Cities and citizen's series. Retrieved from. https://www.google.ch/search?q=Addis+ababa,+Yeka+subcity+water+suply+ sanitation. Date: 12-04-20.

USDA (United States Department of Agriculture) (2013). Food Safety and Inspection Service. Laboratory Guidebook on detection, isolation and identification of *Escherichia coli* O157:H7 from meat products and carcass and environmental sponges. 1–14.

Vijayan, C., Ajaykumar, V.J., Bhattacharya, A. and Bhanurekka, V. (2017). Detection of enterohaemorrhagic *E. coli* O157:H7 from beef and chevon sold in and around Puducherry. *Journal of Environment and Zoonotic Science.* 5(6): 1395-1403.

Wang, J.J., Yang, G.X., Zhang, W.C., *et al*. (2014). *Escherichia coli* infection induces autoimmune cholangitis and anti- mitochondrial antibodies in non- obese diabetic (NOD). B 6 (I dd10/I dd18) mice. *Clinical and Experimental Immunology*. 175(2), pp.192-201.

White, D.G., Zhao, S., Simjee, S., Wagner, D. and McDermott, P.F. (2002). Antimicrobial resistance of foodborne pathogens. *Microbes of Infectious disease.* 4(4): 405-412.

WHO (World Health Organization) (2015). *WHO estimates of the global burden of foodborne diseases: Executive summary* (No. WHO/FOS/15.02). World Health Organization.

WHO (World Health Organization) (2019). *Shiga Toxin-producing Escherichia Coli (STEC) and Food: Attribution Characterization and Monitoring* (Vol. 19). World Health Organization.

Zhang, S., Zhu, X., Wu, Q., Zhang, J., Xu, X. and Li, H. (2015). Prevalence and characterization of *Escherichia coli* O157 and O157:H7 in retail fresh raw meat in South China. *Annuals of Microbiology*. 65:4, pp. 1993–1999.

Zelalem, Y., Gerard, L. and Bernard, F. (2005). Synopsis of Entero virulent *Escherichia Coli* O157:H7. *Ethiopian Veterinary Journal.* 9(2): 1-26

Zerabruk, K., Retta, N., Muleta, D. and Tefera, A.T. (2019). Assessment of microbiological safety and quality of minced meat and meat contact surfaces in selected butcher shops of Addis Ababa, Ethiopia. *Journal of Food Quality*. 20:19.

8. APPENDICES

Annex I: Composition and preparation of bacteriological medias used for isolation, identification and antimicrobial susceptibility test of *E. coli* O157:H7

1. **Triptone Soya Broth (TSB) (Oxide, England)**

Composition: Casein pancreatic digest (17.0g), soyabean meal peptic digest (3.0g), sodium chloride (5.0g), Di-Base potassium phosphate (2.5g), glucose (2.5g).

Preparation: To prepare double concentrated TSB, 60g of powder was suspended in 1 litter of filtered water. Thoroughly mixed and boiled for 10 minutes after heating with frequent agitation. Autoclaved for 15 minutes at 121°C.

2. **CT- Sorbitol MacConkey agar (Oxide, England**)

Composition (g/liter): Peptone 15.5g, Proteose peptone 3g, D-sorbitol 1.0g, Bile salts 1.5g, Sodium chloride 5.0g, Neutral red 0.03g

Preparation: In 1 liter of purified water, 55.75g of powder was suspended. boil for 10 minutes with frequent agitation to thoroughly dissolve the powder. Autoclave for 15 minutes at 121°C. Then, on the prepared base media tempered at 50-55°C, potassium tellurite (2.5 mg/l) and cefixime (0.05 mg/l) were added. shacked softly and dumped into Petri dishes.

3. **Muller-Hinton agar preparation (Oxoid, England)**

Preparation: In one liter of deionized water, 38g of the powder was suspended. Then, boiled for ten minutes with frequent agitation to fully dissolve the medium. Then, autoclaved for 15 minutes at 121°C.

4. **BUG**

In a 2–3 L flask, 57 grams of BUG Agar was mixed with 1,000 mL deionized water. To dissolve the agar and other ingredients, the solution was gently boiled without shaking and the homogenized mixture was waited for cooling, and the solution undergone a PH reading. pH was adjusted with NaOH or HCl to 7.3 + 0.14. Finally, Sterilization took place by autoclaving for 15 minutes at 15 pounds of pressure and 121 °C for 5 minutes. Then, it allowed to cool to 45-50°C till it was dispensed on Petridish.

5. **Nutrient agar (OXOID)**

Composition: Nutrient agar (OXOID ®Ltd., Basingstoke, U.K.) containing 1g/l of powder, 2g/l of yeast extract, 5g/l of peptone, 5g/l of sodium chloride and 15g/l of agar.

Preparation: 28g of powder was suspended in 1 liter of distilled water, thoroughly mixed, and boiled until fully dissolved before being sterilized in a 121^{o}C autoclave for 15 minutes.

6. **Normal saline solution**

The solution was made by dissolving 0.85 g of sodium chloride (Sigma-Aldrich, Co., USA, and Cat. S5886, Lot SLBC3215V) in 100 ml of deionized water, mixing thoroughly, and sterilizing by autoclaving at 121°C for 15 minutes and cooling to below 45°C.

7. **Methyl red test (OXOID, UK)**

The aim of this test was to see whether positive isolates of the pathogen produce strong acids (lactic, acetic, and formic) from glucose. The procedure was as follows: A bacterial colony was taken and placed in MR-VP broth medium. It was incubated at 37°C for 48 hours before adding 6 drops of Methyl red. Finally, the medium's surf has developed a consistent red color. In the case of VP test: - 12 drops of alpha naphthalol were added, mixed well and 4 drops of KOH negative result was detected.no color change.

8. **Indole test**

Peptone water was prepared and 3ml of it was pipetted into test tubes using an aseptic pipette. After that, fresh sterile loops were used to inoculate a well-isolated colony of bacteria into test tubes, which were then incubated for 24 hours at 37^{o}C. After a 24-hour incubation period, 3 drops (1 ml) of Kovacs reagent were applied, and the tube was gently shaken for a minute to check for any red color formation at the surface of the media.

9. **Simmone citrate test**

The media contains C as the only source and *E. coli* didn't utilize it, so the green color of the media indicated the negative result.

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE

SCHOOL OF MEDICINE

DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY

Annex: II English version of information sheet and questionnaire

Questionnaire for data collection from Addis Ababa Abattoir Enterprise (AAAE) and butcher shop workers to assess their knowledge, attitude and practice concerning slaughter hygiene, Addis Ababa, 2021.

Verbal consent form for Abattoir and butcher shopworkers

Greetings,

Title: Prevalence of *E. coli* O157:H7, antimicrobial susceptibility and assessment of critical point of its contamination in beef raw meat along carcass supply chain among selected abattoir and butcher shops in Addis Ababa.

My name is Nahom Misker. I'm postgraduate student at Addis Ababa University, Collage of Health Science, School of Medicine, Department of Medical Microbiology, Immunology and Parasitology. Currently, I'm working a research for my thesis project on the title mentioned above. The aim of this study is to investigate the prevalence and potential contamination site of *E. coli* O157:H7 isolates from raw meat through the rout of abattoirs to retailers. I will also look into the status of Antimicrobials Susceptibility of *E. coli*O157:H7 isolates obtained from A.A abattoir enterprise and some selected butcher shops found at Yeka sub-city. In addition, the knowledge, attitude and practices of workers at both study sites regarding processing and handling of meat will be assessed. We may need to visit you and your establishments for five times in the time span of five months to collect samples.

I would like to invite you to assist me in conducting this research. Before you decide, however, you need to understand why the research is done and what it would involve for the participant and for the community. Please take time to read the following information carefully, and you can ask clarifications if anything you read is not clear

1. If yes, continue to the next page

2. If no, skip to the next participant.

Consent form

I………………….. Voluntarily agree to help facilitate this research study.

I understand that even if I agree to help now, I can withdraw at any time without any consequence of any kind.

I have had the purpose and nature of the study explained to me in writing and I have had the opportunity to ask question about the study.

I understand that all data collected in this study is confidential and anonymous.

I understand that I am free to contact any of the people involved in the research to seek further clarification and information.

Name of researcher: Nahom Misker e- mail: nahommisker21@gmail.com

Advisor: Dr. W/Aregay Erku e-mail: woldearegay.erku@aau.edu.et

Signature of the gatekeeper date

________________________ __________________

I believe the participant is giving informed consent to participate in this study.

Signature of the researcher date

______________________ __________________

Section I: Questionnaire for slaughter house workers to assess their general knowledge and hygienic trainings at the Abattoir

General information

Questionnaire code date ___________

Respondent Name: ___________________________ Address_________

Sex □M □F Age___________

Educational Status □None □Primary □Secondary □Tertiary

Employment standing: □Permanent □Temporary

Health certificate: □ Yes □No

No.	Questions	Response	Skip
1	Had you been given any job-related training?	1.Yes □…………2.No□	
2	If Yes to 1, who gave you the training?	1.Approved organization 2.Governmental health office 3.Others, specify----------------------	
3	How it has been useful in your job?	1. I have developed my work efficiency 2. I have increased my awareness on hygienic practices 3.I have become more hygienic 4.other, specify-------------------------	
4	Have you ever undergone work related medical test?	1.Yes □…………2. No□	
5	Do you have any concepts about foodborne disease?	1.Yes □…………2. No□	
6	If yes to 5, what do you think could be the possible cause of the foodborne disease?	1.Germs □ 2.Toxic chemicals □ 3. Evil eye □ 4. other, specify ------------------	
7	What do you think about the ways of transmission of the foodborne disease?	1.Via contaminated food 2. Via contaminated water 3. Via tinned food 4. Through vectors, like house flies 5. Through other, specify------------	

8	Do you have an awareness about carcass contamination?	1.Yes □…………2. No□	
9	If yes to 5, which stage of slaughtering process do you think is more likely the possible source of contamination?	1.Stunning□ 2.Bleeding□ 3.Flaying□ 4. Evisceration□ 5. Splitting□ 6. Inspection□ 7. Washing□ 8. If other, specify-----------------	
10	What is the reason for beef carcass contamination? (Circle all responses)	1.Dirty butcher hands and clothing 2.Feces due to infected workers 3.Dirty utensils of slaughter house 4.Dirty floor of the slaughter house 5.Dirty carcass wash water 6.If other, specify------------------	
11	Do you think that, carcass contamination could bring any health risk to consumers?	1.Yes □…………2. No□	
12	Do you wash your hands and slaughtering equipment?	1.Yes 2. No□	
13	If yes, when do you wash? (Circle all responses)	1.Only before slaughtering 2.Before and after slaughtering 3. Before and at mid operation 4.Only after slaughtering 5.If other, specify----------------	
14	Do you wash protective cloths and boots regularly?	1.Yes □ 2. No□	
15	If yes, when?	1. Every day 2. In every two days 3. Once per week 3. Twice a week	
16	What is the source of water for sanitation?	1.City supplied □ 2. pipe□ 3. Tanker□ 4. other, specify-----------------	

Section II. Questionnaire for abattoir workers to assess their general Attitude

I will read some ideas concerning safety and hygiene in the slaughtering process for you and please show whether you agree or disagree. KEY: Dk= Don't know, SD=Strongly Disagree, D= Disagree, A= Agree and SA= Strongly Agree

Question	DK	SD	D	A	SA
1.In this profession, keeping the safety and hygiene of carcass is less important than working fast					
2. Employments working this job are more likely to suffer for foodborne disease.					
3. In this type of working area, controlling the condition of environment is easy					
4.The amount of dirt on slaughter equipment or personal protective is nothing to do with causing any risk.					
5.Having good health is more important than treasure					
6.management staffs are the only stakeholders to ensure the hygiene					
7.well-cooked meat is always safe to eat					

Section III. English version of questionnaire for meat retailers to assess their knowledge, attitude and hygienic practices at the butcher shops.

General information

Questionnaire code date ___________

Respondent Name: ___________________________ Address_________

Sex □M □F Age____________

Educational Status □None □Primary □Secondary □Tertiary

Employment standing: □ Permanent □ Temporary

Health certificate: □ Yes □No

No	Questions	Response
1	Where is the source of your meat?	1. AAAE □ 2. Cattle slaughtered at backyard □ 3.Both □ 4. Other, specify---------------
2	Do you display the meat on the wall cabinet immediately after being received it from the abattoir/ carcass slaughtered at backyard?	1.Yes □ 2. No□
3	Do the carcass covered or uncovered?	1.covered □ 2. Uncovered □
4	If covered, which covering material do you used	1.plastic □ 2.newspaper □ 3.leaf □ 4.other, specify………………..
5	Do you keep the hygiene of butcher house, display cabinet and hooks?	1.Yes □ 2. No□
6	If yes, what material do you use for cleaning?	1.water and detergent □ 2. only water □
7	Do you wear apron?	1.Yes □ 2. No□
8	If yes, how often do you wear?	1.Everyday□ 2 Sometimes □
9	Do you wear head cover?	1.Yes □ 2. No □
10	If yes, how often do you wear?	1. Everyday □ 2 Sometimes□
11	How often do you wash your apron and head cover?	1.Every day□ 2.Twice a week □ 3.Once a week □ 4. Others, specify ………………..
12	Do you wear jewelry during work?	1.Yes □ 2. No□
13	Do you handle money/birr during work	1.Yes □ 2. No □
14	Do you have the habit of hand washing while on job?	1.Yes □ 2. No □
15	If Yes how frequently?	1.once per day 2.twice per day

		3. Three times a day 4. Other,Specify……………………
16	What do you use for washing your hands?	1.water only 2.water and soap
17	Do you wash the meat retailer's equipment regularly, such as knives, axe, cutting board and sponges?	1.Yes □ 2. No □
18	If yes, how often do you wash?	1.Once per day 2.Twice per day 3. Three times a day 4. Other,Specify……………..
19	How do you wash the equipment at butcher shops?	1.Only with water 2.with water and soap 3.Other, specify………………
20	What is the source of water?	1.Tap water 2.Pipe 3.Tanker 4.others, specify…………………..
21	Do you have refrigerator in your shop?	1.Yes □ 2. No □
22	Have you trained on Sanitation and hygienic handling of the meat?	1.Yes □ 2. No □
23	Did you take the medical test before being employed at meat retailer shop?	1.Yes □ 2. No □
24	Do you undergo regular medical checkup?	1.Yes □ 2. No □
25	Do you check the carcasses examination test result when you receive it?	1.Yes □ 2. No □

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE

SCHOOL OF MEDICINE

DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY

Annex III. Amharic version of information sheet and questionnaire

ርዕስ፡- Prevalence of E. coli O157:H7, antimicrobial susceptibility and assessment of critical point of its contamination in beef raw meat along carcass supply chain among selected abattoir and butcher shops in Addis Ababa.

እኔ ተማሪ ናሆም ምስክር የተባልኩ በ አ. አ ዩኒቨርስቲ በጤና ሳይንስ ኮሌጅ በሜዲካል ማይክሮባዮሎጂ ኢሚዩኖሎጂ እና ፓራሳይቶሎጂ ት/ት ክፍል የሁለተኛ ዲግሪ ተመራቂ ተማሪ ስሆን በአሁኑ ሰዓት የመመረቂያ ጽሁፌን ከላይ በርዕሱ በተገለጸው ሀሳብ ላይ አተኩሬ እየሰራው እገኛለው። የዚህ ጥናታዊ ጽሁፍ ግብ እንደሚከተለው ነው፡-

ለ ምግብነት በሚቀርበዉ የጥሬ ስጋ ላይ '*E. Coli* O157:H7' የተባለው ባክቴሪያ ያለውን የብክለት መጠን እና የስርጭቱን ዋና መነሻ ሰንሰለቱን ጠብቆ ከቄራ እስከ ለተጠቃሚዎች የሚደርስበት ስጋ ቤቶች ድረስ ይመረምራል፤ የተለየውንም ተዋህሲ የጸረ-ባክቴሪያ ተጋላጭነት እና መቋቋም ብቃቱን ያጠናል፤ በተጨማሪም በስጋ ውጤቶች አቅርቦት ሰንሰለቱ ውስጥ የሚሰሩ ሰራተኞች በስራው ላይ ያላቸውን እውቀት፣ አመለካከት እና ልምድ ይገመግማል፤ ስለሆነም እኛ ለዚሁ ምርምርና ሙና ለመሰብሰብ ድርጅታችሁን በአምስት ወራት ውስጥ ለ 10 ጊዜያት ብቻ እንጎበኛለን።

ለዚህ ሳይንሳዊ ምርምር መሳካት ሲባል እኔ የእናንተን እርዳታ ጠይቃለው፤ ነገር ግን የመጨረሻውን ውሳኔ ከማሳለፋችሁ በፊት እባክዎ የምርምሩን አጠቃላይ አስፈላጊነት እና ለህብረተሰቡ የሚያስገኘውን ትሩፋት ይረዱለኝ። እባክዎ ከዚህ በመቀጠል ያለውን መረጃ ጊዜ ወስደው በጥንቃቄ ያንብቡት፤ ካነበቡት ውስጥ ግልጽ ላልሆነ ነገር ማብራሪያ መጠየቅ ይችላሉ።

ጥቅም፡- በጥናቱ ውስጥ የግለሰብ ተሳትፎ ግለሰባዊ ጥቅምን ባያስገኝም ለማህበረሰቡ ዘርፈ ብዙ ውጤቶችን ለማምጣት ትልቅ አሰተዋጽዎ ያበረክታል፤ ከነዚህም መካከል መንግስት ለሚያወጣቸው ማህበረሰብ ተኮር ለሆኑ ፖሊሲዎች እና ህግጋቶች በተለይም በምግብ አቅርቦት ሰንሰለት ውስጥ የሚታየውን የምግብ ብክለትን ከመቆጣጠር አንጻር የጥናቱ ውጤት የራሱ የሆነ ሚናን ይጫወታል።

በሌላ በኩል ደግሞ የዚህ ጥናት ግኝት በተለያዩ የህብረተሰ ብጤና ምርምር መስኮች ላይ አተኩረው ለሚሰሩ ባለሙያዎች በዘርፉ

ያለውን ክፍተት ለማሳየት ይረዳል። በተጨማሪም ለጤና ባለሙያዎች በ '*E. Coli* O157:H7' ተዋህሲ ምክኒያት ለሚከሰቱ በሽታዎች ትክክለኛውን ያልተላመደውን መድሀኒት ለበሽተኛው ለመስጠት ይረዳቸዋል። የተሳትፎ መመዘኛዎች፡- በጥናቱ ላይ የሚደረገው ተሳትፎ ሙሉ በሙሉ በፍቃደኝነት ላይ የተመሰረተ ነው።

በጥናቱ ላይ ሙሉ በሙሉ አለመሳተፍም ሆነ በከፊል መሳት ፍይቻላል፤ በዚህም ምክኒያት ምንም አይነት አሉታዊ ተጽዕኖ በተሳታፊው ላይ አያሳድርም፤ ስምም ሆነ የምትሰሩበት መስሪያ ቤት ስም ምስጥራዊ በሆነ መልኩ የተመራማሪው ንብረት ብቻ በመሆን በየትኛውም መረጃ አያያዝ እና አተገባበር ውስጥ በ አስተማማኝ መልኩ ድብቅ ይሆናል። አመሰግናለሁ

የፍቃደኝነት ፎርም

እኔ_________________________________በዚህ ምርምር በሙሉ ፍቃደኝነት ልተባበር ተስማምቻለሁ።

ነገር ግን ምንም እንኳ አሁን ልተባበር ፍቃደኛ ብሆንም በሌላ ጊዜ ሀሳቤን ቀይሬ ትችዋለው ብል ምንም አሉታዊ ተጽዕኖ እንደማይፈጠርብኝ ተረድቼነው፤ ስለ ጥናታዊ ጽሁፉ አጠቃላይ አሰራር እና ግብ በጽሁፍ ተገልጾልኝ ጥያቄም ካለኝ እንድጠይቅ እድል ተሰጥቶኛል፤ በዚህ ሳይንሳዊ ምርምር የሚሰበሰብ እያንዳንዱ መረጃ በአስተማማኝ ሁኔታ በተመራማሪው ቁጥጥር ውስጥ ብቻ እንደ ሆነ ተረድቻለሁ። ስለ ጥናቱ ሰፊ ማብራሪያ ከፈለኩ በዚህ ምርምር ውስጥያሉትን አካላት የመጠየቅ ነጻነት እንዳለኝ ተረድቻለሁ።

የተመራማሪው ስም____________________________e-mail ›

የአማካሪ ስም____e-mail

የተሳታፊው ፊርማ____________________________________ቀን______________________

ተሳታፊው በፈቃደኝነት ለመሳተፍ ስለ ጥናቱ በቂ የሆነ መረጃ ማግኘቱን አምናለሁ

የተመራማሪው ፊርማ__________________________ቀን______________________

Sction I: Amharic version of questionnaire

ለቁራ ሰራተኞች በጠቅላላ እውቀት እና ንጽህና አጠባበቅ ዙሪያ የሚቀር ብቃ ለመጠየቅ

አጠቃላይ መረጃ

የቃለ መጠየቁ ቁጥር ________ ቀን.....................

ስም ____________________ አድራሻ-______________________

ፆታ □ ወንድ □ ሴት

እድሜ...___________________

የጋብቻሁኔታ_______________

ትምርት ደረጃ.................□ የለም □ አንደኛ ደረጃ □ ሁለተኛ ደረጃ □ ሶስተኛ ደረጃ .

የስራውመደብ □ ቋሚ................□.ጊዜያዊ

የጤና ሰርተፍኬትህ አለህ?...□.አዎ □ አይ..................

ተ.ቁ	ጥያቄ	መልስ	ይለፍ
1	ስለ ምግብ ኣያያዝ የተሰጠህ ስልጠና ካለ?	1.አዎ□ 2. አይ□	
2	አዎ ካሉ፤ስልጠና የሰጠህ ኣካልማነው?	1.እውቅና ካለው ድርጅት 2. ጤና ቢሮ 3.ሌሎች፤ግለጽ----------------	
3	ስልጠናው ምን ያህል ጠቀመህ ?	1.የስራ አፈጻጸሜ ጨምሩዋል 2.የስራ እውቀቴ ዳብሮዋል 3.በጽዳት ጉዳይ ጠንቃቃ ሆኛለሁ 4.ሌሎች፤ግለጽ	
4	ለስራዎ ሲባል የጤና ምርመራ አድርገው ያውቃሉ?	1.አዎ□ 2. አይ□	
5	በምግብ ምክኒያት ስለሚመጡ በሽታዎች እውቀቱ አለህ?	1.አዎ□ 2. አይ□	
6.	አዎ ካሉ፤መንስኤዎቹ ምን ይመስልዎታል?	1.ጀርም 2.መርዛማ ኬሚካል 3. የሰው አይን 4.ሌሎች፤ግለጽ	
7	በሽታዎቹ በምን የሚተላለፉ ይመስልሀል?	1.በተበከለ ምግብ 2.በተበከለ ውሃ 3.በተበከለ ምግብና ውሃ 4.በታሸገ ምግብ 5.በበሽታ አስተላላፊ ነብሳት 6. ሌሎች፤ግለጽ	
8	ስለ ስጋ ብክለት እውቀቱ አለህ?	1.አዎ • 2. አይ •	
9	አዎ ካሉ፤በ የትኛው የእርድ ደረጃ ይመስሎታል ስጋ ሊበከል የሚችለው?	1.እርድ ላይ • 2.ደም ሢፈስ • 3.ቆዳ ሲገፈፍ • 4.ሆድ እቃው ሲወጣ • 5.ብልት ሲበለት • 6.ምርመራ ወቅት • 7.ስጋው ሲታጠብ • 8.ሌሎች፤ግለጽ•	
10	የስጋው ብክለት ምክኒያት ምን ይመስሎታል ?	1.የቆሸሸ የአራጅ እጅ እና ልብስ • 2.በታመመ ሰው የሰግራ ንክኪ • 3.በቆሸሹ የእርድ እቃዎች • 4.በቆሸሸ የእርድ ስፍራ •	

		5.ንጹህ ባልሆነ የስጋ ማጠቢያ ውሃ • 6.ሌሎች፤ግለጽ•	
11	የስጋ መበከል በተመጋቢው ላይ በሽታ የሚያመጣ ይመስልሀል?	1.አዎ • 2. አይ •	
12	እጅዎን እና ለዕርድ ሥራ የዋሉትንዕቃዎችን ያጸዳሉ?	1.አዎ • 2. አይ •	
13	አዎ ካሉ፤መቼ ያጸዳሉ?	1.ከማረድዎበፊትብቻ• 2. ከማረድዎ በፊት እና በኃላ• 3.ከማረድዎ በፊት እና በመካከል ላይ• 4. ከእርድ በዋላ ብቻ • 5. ሌሎች፤ግለጽ	
14	የተጠቀሙበትን የመከላከያ አልባሳት ያጸዳሉ?	1.አዎ • 2. አይ •	
15	አዎ ካሉ፤ መቼ ?	1.በየቀኑ• 2.በ 2 ቀን አንዴ • 3.በሳምንት አንድ ጊዜ• 4.በ 2 ሳምንት አንድ ጊዜ• 5.ሌሎች፤ግለጽ	
16	ለማጽጃ የሚጠቀሙበት የዉሀ ምንጭ ምንድ ነው?	1.የቧንቧ • 2. የጉድጓድ • 3.የማጠራቀሚያ ውሃ • 4.ሌሎች፤ግለጽ	

Section II- ለቄራ ሰራተኞች በ ንጽህና እና በ ጤና አጠባበቅ ዙሪያ ያላቸውን አመለካከት ለመረዳት የሚቀርብ ቃለ መጠየቅ

ከዚህ በመቀጠል በጤና እና በንጽህና ዙሪያ የሚጠየቁ ጥያቄዎችን አነብላችዋለው እናም እባክዎትን ጥያቄውን በመረዳት እስማማለው ወይም አልስማማም በማለት አመልክቱ ፡፡

የመልስ ቁልፎች (አላውቅም=አላ፤ በጣም አላስማማም=በአ፤ አልስማማም=አል፤ እስማማለው=እስ፤ በጣም እስማማለው=በእስ)

ተ.ቁ	ጥያቄ	አላ	በአ	አል	እስ	በእስ
1	በዚህ የስራ መስክ ውስጥ ከእርዱ ደንነትና ጤንነት ይበልጥ የስራ ፍጥነት አስፈላጊነት አለው፡፡					
2	በዚህ የስራ መስክ ውስጥ ያሉ ሰዎች ለምግብ ብክልት በሽታ በይበልጥ የመጋለጥ እድሉ ከፍተኛ ነው፡፡					
3	በዚህ የስራ ምህዳር ውስጥ የስራ አካባቢውን የጽዳት ሁኔታ መቆጣጠር ቀላል ነው፡፡					
4	በእርድ እቃዎች እና በራስ ጤንነት አጠባበቅ ላይ የሚኖር የብክለት መጠን ብዙም አስጊነት የለውም፡፡					
5	የጥሩ ጤንነት ባለቤት መሆን ክብዙ ሀብት ይበልጣል					
6	የስራ ቦታ ጤንነት አጠባበቅን ማረጋገጥ የሚመለከተው በአስተዳደር ስራ መስክ ላይ ያለ ሀላፊ ብቻ ነው					
7	ሁሌም በደምብ የበሰለ ስጋን መመገብ አስተማማኝ ነው፡፡፡					

Section III: ለልኳንዳ ቤት ሰራተኞች በ ጠቅላላ እውቀት በአመለካከት እና ንጽህና አጠባበቅ ዙሪያ የሚቀርብ ቃለ መጠየቅ

አጠቃላይ መረጃ

የቃለ መጠየቁ ቁጥር______ ቀን....................

ስም ________________ አድራሻ-__________________

ፆታ • ወንድ • ሴት

እድሜ..........................

የጋብቻ ሁኔታ____________

ትምርት ደረጃ................• የለም • አንደኛ ደረጃ • ሁለተኛ ደረጃ • ሶስተኛ ደረጃ .

የስራው መደብ • ቋሚ................•.ጊዜያዊ......................

የጤና ሰርተፍኬትህ አለህ?…☐.አዎ ☐ አይ

ተ.ቁ	ጥያቄ	መልስ
1	.የስጋ ውጤቶችን ከየት ትረከባላችሁ?	1.ከቄራ(አአቄድ)• 2.ዕርዱን በጓራችን በመፈጸም • 3.ከሁለቱም ቦታዎች• 4.ሌላ ፤ግለጽ____
2	የእርድ ስጋውን ከተረከባችው በሁዋላ ወዲያውኑ የስጋ ማሳያ መደርደሪያ ላይ ትሰቅሉታላችሁ?	1.አዎ • 2.አይ•
3	በልኳንዳ ቤት ውስጥ ያለው ስጋ ይሸፈናል?	1. አዎ • 2.አይ •
4	መልስህ አዎ ከሆነ፤በምን ይሸፈናል?	1.ላስቲክ 2.ወረቀት 3.ቅጠል 4. ሌላ ፤ግለጽ
5	ጠቅላላ የልኳንዳ ቤቱ ፤የስጋ ማሳያው እና ሜንጦ ጤንነቱ ይጠብቃለው	1. አዎ • 2.አይ •
6	መልስህ አዎ ከሆነ፤ ለማጽጃ ምን ይጠቀማሉ ?	1.ውሀ 2.ውሀ እና ማጽጃ ሳሙና
7	በመስተንግዶ ወቅት ጋዎን ይለብሳሉ?	1. አዎ • 2.አይ •
8	አዎ ካሉ መቼ?	1.ሁሌም• 2.አልፎ አልፎ•
9	ጋዎኑን በምን ያህል ጊዜ ያጥባሉ?	1.በየቀኑ• 2.በሳምንት ሁለቴ• 3.በሳምንት አንዴ• 4.. ሌላ ፤ግለጽ

10	በመስተንግዶ ወቅት የጸጉር መሸፈኛ ይለብሳሉ?	1. አዎ • 2.አይ •
11	አዎ ካሉ መቼ?	1.ሁሌም• 2.አልፎ አልፎ •
12	በስራ ላይ እያሉ የተለያዩ ጌጣጌጦችን ይጠቀማሉ?	1. አዎ • 2.አይ •
13	በስራ ላይ አያሉ ገንዘብ ይይዛሉ?	1. አዎ • 2.አይ •
14	እጆትን የመታጠብ ልምድ አለዎት?	1.አዎ • 2.አይ •
15	መልሱ አዎ ከሆነ መቼ?	1.በቀን አንድ ጊዜ • 2. በቀን ሁለት ጊዜ • 3. በቀን ሶስት ጊዜ • 4. ሌላ ፤ግለጽ
16	መልስህ አዎ ከሆነ፤ ለማጽጃ ምን ይጠቀማሉ ?	1. ውሀ 2. ውሀ እና ማጽጃ ሳሙና
17	የተጠቀሙበትን ቢለዋ፤ መጥረቢያ መክተፊያ እና ፎጣ/ስፖንጅ በቋሚነት ያጥባሉ.	1.አዎ • 2.አይ •
18	መልሱ አዎ ከሆነ፤ድግግሞሹ ምን ያህል ነው	1.በቀን አንድ ጊዜ • 2. በቀን ሁለት ጊዜ • 3. በቀን ሶስት ጊዜ • 4.ሌላ ፤ግለጽ
19	የልኳንዳ ቤቱን ንጽህናን እንዴት ነው የሚጠብቁት?	1. ውሀ 2. ውሀ እና ማጽጃ ሳሙና 3ሌላ ፤ግለጽ .
20	ለማጽዳት የሚጠቀሙበት የዉሀ ምንጭ ምንድ ነው?	1.የቧንቧ • 2. የጉድጓድ • 3.የማጠራቀሚያ ውሃ• 4. ሌሎች፤ግለጽ.
21	በልኳንዳ ቤቱ የማቀዝቀዣ መሳሪያ አላችሁ?	1.አዎ • 2.አይ •
22	በስጋ አቅርቦት ላይ የሙያ ደህንነት እና ጤንነት ስልጠና ወስደው የውቃሉ ?	1.አዎ • 2.አይ •
23	ለስራ ቅጥር ሲባል የጤና ምርመራ አድርገሀል?	1.አዎ • 2.አይ •
24	የጤና ሰርተፍኬትህን በየግዜው በመመርመር ታሳድሳለህ?	1.አዎ • 2.አይ •
25	ስጋዉን ስትረከቡ ስለጤንነቱ ማረጋገጫ ውጤት ትቀበላላችሁ?	1.አዎ • 2.አይ •

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE

SCHOOL OF MEDICINE

DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY

Annex IV- Check list for observations

Section: I- slaughter house observation checklist

	Observation		Remark
1	Hand washing before, after and during touching the carcass		A. Yes B. No
2	knives are clean and completely sterile from contamination		A. Yes B. No
3	The carcass dressing techniques		A. hanging above the floor B. on the floor C. mixed
4	Method of stunning used		A.Bolt B. sticking knife C. other
5	Availability of hot water supply		A.Yes B. No
6	Tidy and short finger nails of the workers		A. Yes B. No
7	Using all appropriate protective materials	gown	A.Yes B. No
		Head cover	A.Yes B. No
		Glove	A.Yes B. No
		Boots	A.Yes B. No
		Over all	A.Yes B. No
8	Do they have enough amount of toilet?		A.Yes B. No
9	Does workers wash their hand after visiting the toilet?		A.Yes B. No
10	Latrine has water, soap, paper, towels for hand washing (Circle all that apply)		A. Water B. Soap C. Paper D.Tissue Paper
11	Strict separation between dirty and clean area of slaughter house		A.Yes B. No

12	The presence of veterinary experts to inspect and pass the safe carcass for human consumption	A. Yes B. No
13	General personal hygiene of the butchers	A. poor B. good C. better
15	Eating or chewing while working	A.Yes B. No
16	Does any domestic or rodents seen?	A.Yes B. No Specify the name----- ---------------

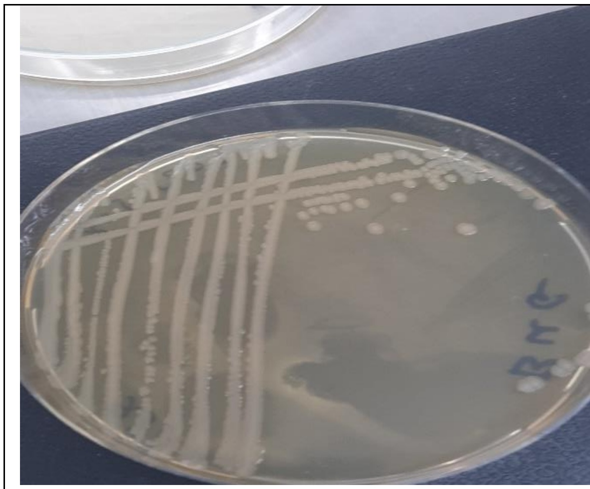
Section: II- Check list for observation of carcass transportation

1	Does the meat van consist refrigerator or chiller?	A. Yes B. No
2	Is there a specified gap between the line of hanged carcass in the track	A. Yes B. No
3	Do they deliver both beef carcass and other offal contents using the same transportation truck?	A.Yes B. No
4	Do the vehicles washed before shipment?	A. Yes B. No
5	The sanitary condition of the carcass loading environment	A. Poor B. Good C. better
6	The system of carcass loading or unloading from the meat van follows--------	A. Manually B. through conveyer C. other
7	Does the workers who transport the carcass to the vehicle wear clean garments and safety materials.	A.Yes B. No

Section: III- Check list for observation of butcher shops

1	Does the food handler wear appropriate apron?	A.Yes B. No
2	Do the meat retailers used an appropriate hair cover?	A.Yes B. No
3	Does finger nails of the workers are clean and short?	A.Yes B. No
4	Do the workers wear a mask during serving the customers?	A. not observed B. Observed
5	Any visible cuts and wounds covered with an appropriate procedure seen at time of visit	A. not observed B. Observed
6	Do they have refrigerator in the retailer shop	A.Yes B. No
7	The sanitary condition of butcher shops	A. Poor B.Good C. better
8	Do latrine available nearby the meat retailer?	A.Yes B. No

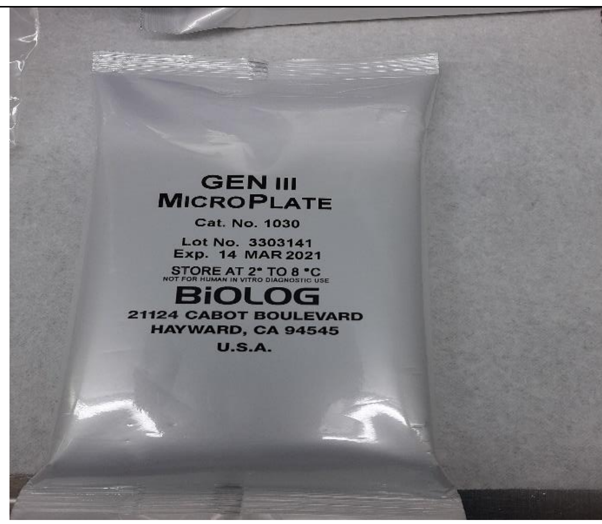
Annex V: Pictures of Laboratory equipment, laboratory work and results



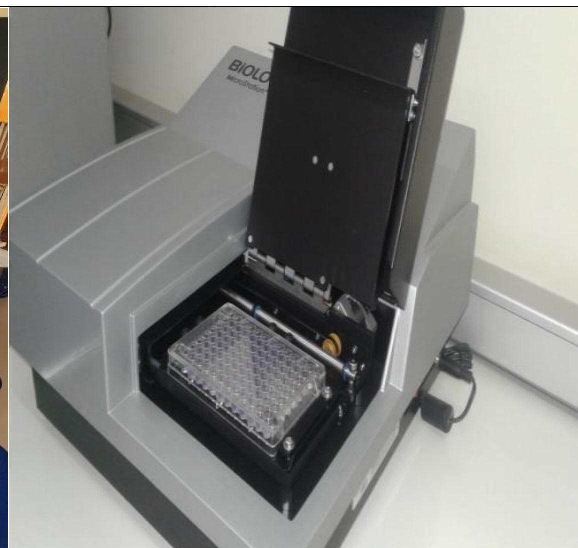
Picture 1: Presumptive E. coli O157:H7 growth on BUG agar

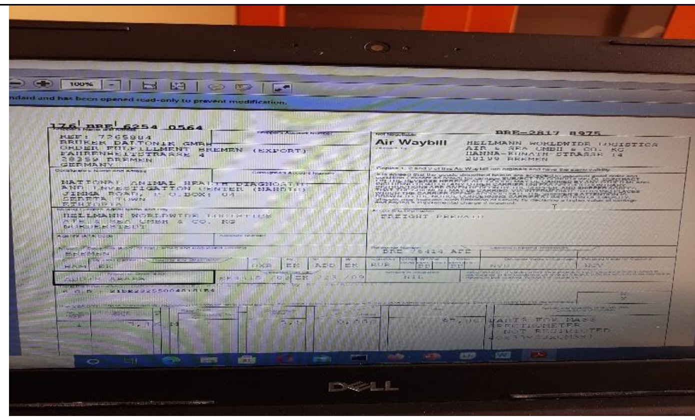
Picture 2: Selecting and transferring single colony from BUG agar to IFA.

Picture 3: IF A: inoculating fluid A.

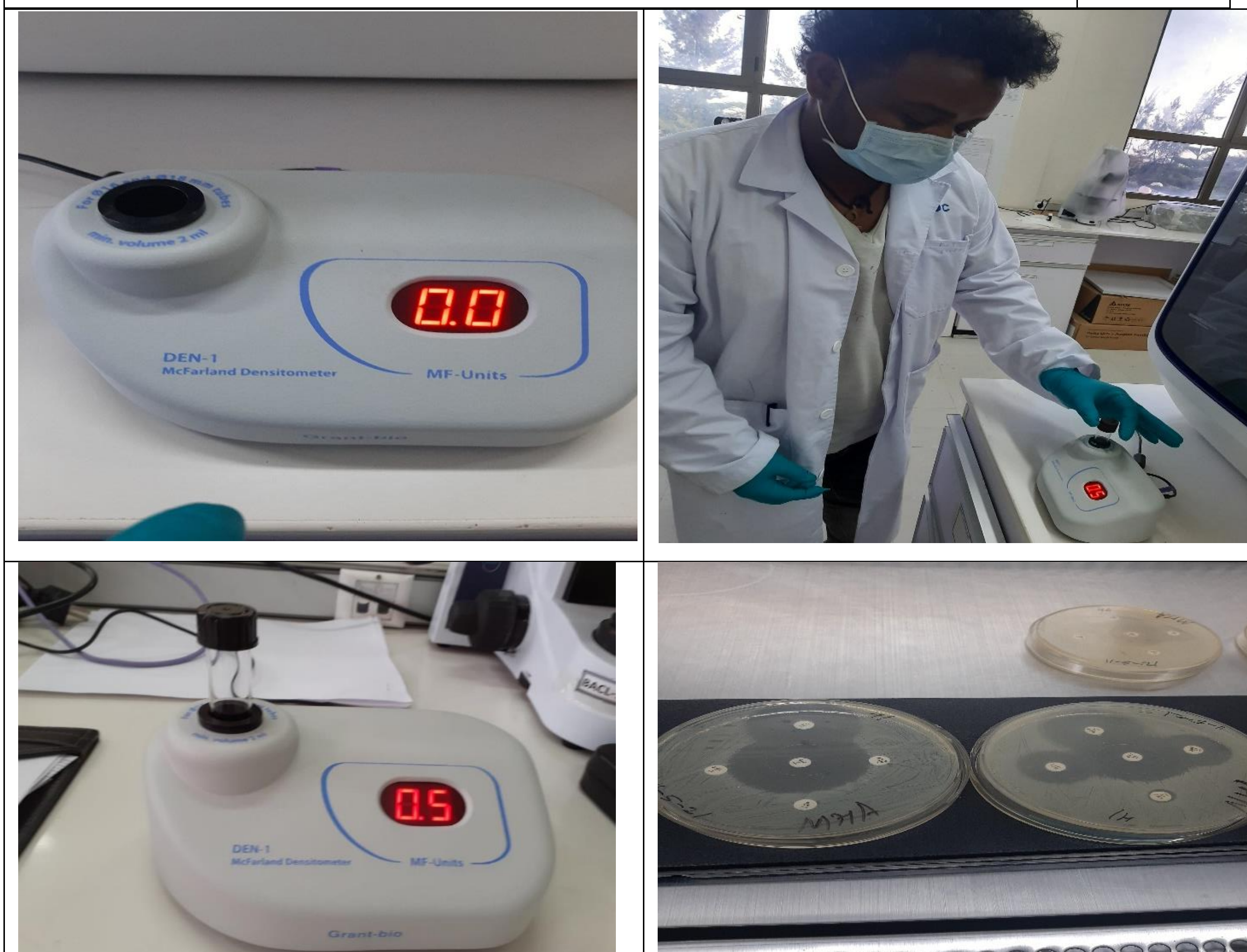


Picture 4: GEN III, fully automated coated micro plate.

	
Picture 5: Inoculating the emulsified solution from IFA to the GEN III microplate	**Picture 6:** Omni Log incubator
	
Picture 7: Placing the incubated microplate in microstation reader	**Picture 8:** *E. coli* O157:H7 confirmed by Biolog reader after 22 hours incubation

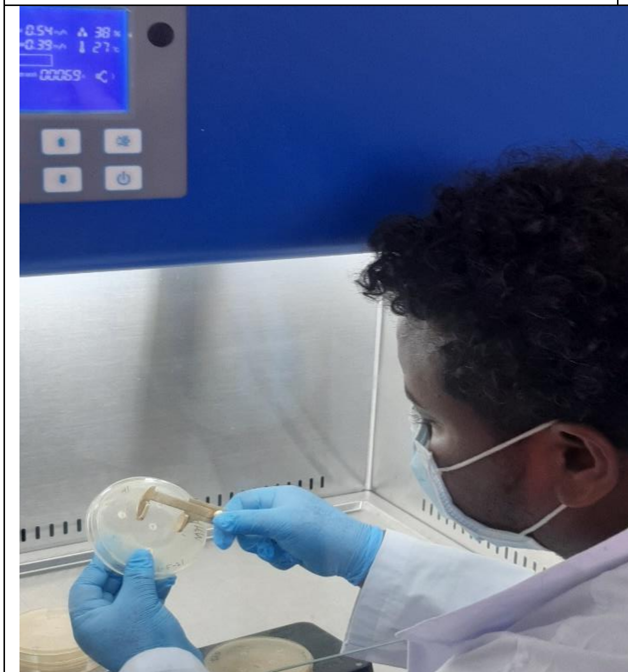


Picture 9: The secreen displayed the species identification result(ID.)



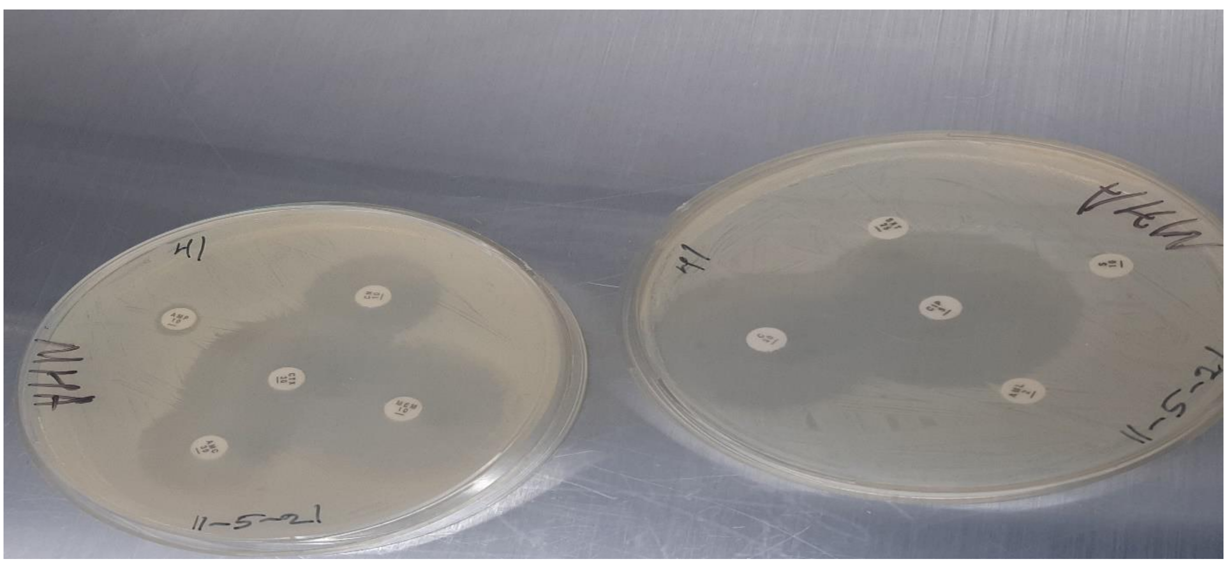
Picture 10: McFarland Densitometer used for checking turbidity during antimicrobial testing

Picture 11: Measuring the diameter of inhibition zone by using caliper



Picture 12: The antimicrobial sesceptiblity test result for the two isolates

Picture 13: Antimicrobial susceptibility result of the ten drugs for raw minced beef meat sample.



Annex VI: Ethical clearance

	Department of Microbiology, Immunology andParasitology (DMIP) Department Research Ethics Review Committee (DRERC)

Meeting No: DRERC/006/2020 Date: 14 Oct 2020

Protocol title: Prevalence of E. coli O157:H7, antimicrobial susceptibility and assessment of critical point of contamination in raw beef meat along carcass supply chain at selected abattoir and butcher shops in Addis Ababa			
Principal Investigator	Nahom Miskir		
Institute/Department	CHS-AAU/DMIP		
Type of review	[x] Initial Review:	[ ] Amendment	[ ] Other (specify):__________
Elements Reviewed	[ ] Attached	[ ] Not attached	
Decision of the meeting	[ ] Approved [ ] Revision requested	[ ] Approved with Recommendation [ ] Disapproved	
Action Required	[ ] Send to IRB	[x] Authorize Implementaion	

Obligations of the PI:

i. Should comply with the standard international and national scientific and ethical guidelines
ii. All amendments and changes made in protocol and consent form needs DREC approval
iii. The PI should report Serious Adverse Events (SAE) within 10 days of the event
iv. End of the study, including thesis work and manuscript should be reported to the DREC

Follow up report expected in:

3 Months______ 6 Months __X__ 9 Months ______ one year ______

Asrat Hailu (Prof)

Chair, DRERC

Signature

Date: 14/10/2020

Department Stamp

DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY & PARASITOLOGY (DMIP)

ADDIS ABABA UNIVERSITY-FACULTY OF MEDICINE

Declaration

I, Nahom Misker Mekonnen, declare that this thesis, title "**Isolation and Antimicrobial Resistance Determination of *Escherichia coli* O157:H7 from Raw Meat in Selected Abattoirs and Butcher Shops, Addis Ababa, Ethiopia**", is my own original work, that it has not been presented for a degree in any other university, and that all sources of materials used for the thesis have been duly acknowledged.

Name: Nahom Misker Mekonnen

Date: August 30, 2021

Signature: ______________________________

Supervisor: Dr. Woldaregay Erku Abegaz

Date: August 30, 2021

Signature ______________________________

Co-Supervisor: Dr. Matios Lakew

Date: August 30, 2021

Signature ______________________________