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ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE

INVESTIGATION OF THE OCCURRENCE OF SELECTED FOOD-BORNE PATHOGEN IN THE SLAUGHTER HOUSES AND THEIR DRUG SUSEPTABILITY PROFILES AT BISHOFTU TOWN, CENTRAL ETHIOPIA

BY

SEYUM FIREW WAKO

JUNE, 2019
BISHOFTU, ETHIOPIA

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A thesis submitted to the College of Veterinary Medicine, Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Veterinary Microbiology

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by: Seyum Firew entitled "**investigation of the occurrence of selected food-borne pathogen in the slaughter houses and their drug susceptibility profiles at bishoftu town, central Ethiopia** " and recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters of Science in Veterinary Microbiology**.**

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STATEMENT OF THE AUTHOR

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

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LISTS OF ABBREVIATIONS

^0C	Degree centigrade
A/E	Attaching and effacing
BPW	Buffered peptone water
CDC	Center for Disease Control
CFu	Colony forming Unit
CLIS	Clinical and Laboratory standards Institutes,
DAEC	Diffuse-adherent
DMTSB	Double modified trypton soya broth
DNA	Deoxyribonucleic acid
EHEC	Enterohemorrhagic *Escherichia coli*
ELISA	Enzyme Linked Immunosorbent Assay
EU	European Union
GALT	Gut-associated lymphoid tissue
GB3	Globotriaosylceramide
HUS	Hemolytic-Uremic Syndrome
IMS	Immunomagenetic separation
iNTS	Invasive non-typhoidal Salmonellosis
KIA	Kiligler Iron agar
LPS	Lipopolysaccharide
ml	Milliliter
MSRV	Modified Rappaport Vasilidus
MTSB	Modified Tryptone Soya Bronth
NAD	Nucleic Acid-Based Diagnostics
NCCLS	National Committee for Clinical Laboratory Standard
NSFC	Non-sorbitol fermenting colonies
NTS	Non-Typhoidal *Salmonella*
PCR	Polymerase Chain Reaction
QRDR	Quinolone Resistance Determining Region
RNA	Ribonucleic Acid

SMAC	Sorbitol Mac Conkey Agar
STEC	Shiga toxin producing *Escherichia Coli*
Stx	Shiga toxin
T3SS	Type Three Secretion System
TTP	Thrombotic thrombocytopenic purpura
UK	United Kingdom
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate agar
PNA	Peptide nucleic acid

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ABSTRACT

Escherichia coli O157 and non-typhoidal *Salmonella* are common cause of food-borne diseases worldwide. They cause acute gastroenteritis when ingested with contaminated food such as raw meat and meat products. They are also responsible for the emergence of antimicrobial resistance both in human and veterinary medicines. The objective of this study was to isolate, identify and determine antimicrobial sensitivity profile test of non-typhoidal *Salmonella* and *Escherichia coli* O157 in Bishoftu. Seven hundred (700) samples from abattoirs containing carcass swabs (n=420), pooled hide swab (n=140) and feces (n=140) were collected and processed using standard procedures. The overall occurrence of *Escherichia coli* O157 and non-typhoidal *Salmonella* were 4.29% and 4.57%, respectively. The occurrence in feces, hide and carcass swabs were 10%, 4.29 %, and 2.38% for *Escherichia coli* O157 and 7.14%, 7.14% and 2.86% for non-typhodal *Salmonella,* respectively. There was no statically significant variation among sample types and between abattoirs. The antimicrobial susceptibility test showed that *Escherichia coli* 157 isolates were 100% sensitive to Ciprofloxacin and Gentamicin. All of isolates were resistant to Cloxacillin, Ampicillin, Streptomycin and Polymyxin-B. But, 60%, 73.3%, 93.3%, 73.3%, 53.3%, 6.7% were susceptible to Tetracycline, Trimethoprim, Chloramphenicol, Kanamycin, Rifampicin, and Streptomycin, respectively. All non-typhoidal *Salmonella* species were susceptible to trimethoprim, ciprofloxacin and Gentamicin while resistant to Cloxacillin, Cephalothin, Rifampicin, Ampicillin, Streptomycin and Polymyxin-B. But, 50%, 43.8 % and 56.25% were susceptible to Tetracycline, Chloramphenicol and Kanamycin, respectively. The study showed that the occurrences of *Escherichia coli* O157 and non-typhoidal *Salmonella* in the study area and their resistance to the commonly used drugs. Therefore, adequate hygienic practices at abattoirs, applying food safety procedures and rational use of drug were recommended.

***Key words**: Antimicrobial resistances, Escherichia coli O: 157, Food-borne disease, Isolation, isolation and identification, non- typhoidal Salmonella,*

1. INTRODUCTION

Food-borne diseases are global problems and approximately 600 million cases of illness and 420,000 deaths caused by food-borne diseases (Havelaar *et al*., 2015). In the United States of America (USA), food-borne disease causes 94 million cases per year resulting in 1,351 deaths (Scallan *et al*., 2011). In developing countries, the problem is more serious and up to five million people die per year (Wabeto *et al*., 2017). *E. coli* O157:H7 and non-typhoidal *Salmonella* are zoonotic bacterial diarrheal disease, resulting mainly from the contamination of beef carcass, raw milk, milk products, surface water and consumption of uncooked food (Nzouankeu *et al*., 2010; Chang *et al.*, 2013; Henry *et al.*, 2017).

Non-typhoidal *Salmonella* (NTS) species are a leading cause of food-borne disease in humans worldwide that estimated to cause 93.8 million gastroenteritis and 155,000 deaths annually (Majowicz *et al*., 2010). European Centre for Disease Prevention and Control, reported Salmonellosis as the most commonly reported gastrointestinal infection with 89,883 laboratory-confirmed cases in EU/EEA (ECDC, 2014). In USA, CDC estimated that NTS cause about 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths every year that costs 3.3 billion US dollars per year (Majowicz *et al*., 2010; Delgado-Suárez *et al*., 2018). The disease is endemic in developing countries especially in Southeast Asia, Africa, and South America (Gal-Mor *et al*., 2014). In sub-Saharan Africa, (NTS) are emerging problem in HIV and malaria-infected and malnourished infants and young children (Tennant *et al*., 2010; Ao *et al.*, 2015).

Shiga toxin-producing *E. coli* (STEC), including *E. coli* O157:H7 is also responsible for numerous food-borne outbreaks worldwide (Havelaar *et al*., 2013; Ricke *et al*., 2018). It is mainly responsible for intestinal and extra-intestinal disease syndromes in human both in developed and developing world (Christian Vinueza Burgos, 2017; Croxen *et al*., 2013). In human it causes diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS) (Chekabab *et al*., 2013).

Globally, STEC causes 2.8 million acute illnesses annually, with an incidence rate of 43.1 cases per 100 000 person-years (Athumani, 2018). This burden leads to 3,890 cases of hemolytic uremic syndrome (HUS) and 230 deaths (Athumani, 2018). In USA, Center for Disease Control and Prevention (CDC) estimates that each year's shiga toxin producing *E. coli* (STEC) causes 265,000 illnesses, 3,600 hospitalizations and 30 deaths. STEC O157:H7 causes about 36% of these infections, and non-O157 STEC cause the rest (CDC, 2018). In Africa, a total of 10,200 cases of STEC O157:H7 infections occur with an incidence rate of 1.4 cases per 100,000 person-years contributing 10% to this burden (Athumani, 2018).

Cattle are an important reservoirs and asymptomatic carriers of NTS (Barkocy-Gallagher *et al*., 2003; Cobbold *et al*. 2006; Cummings *et al*., 2009). In Ethiopia, NTS occurrences in apparently health slaughtered cattle were reported by different authors from different areas (Abunna *et al*., 2017; Wabeto *et al*., 2017; Takele *et al*., 2018). Moreover; Cattle are also an important reservoirs and asymptomatic carriers of *E. coli* O157: H7 that contaminated beef carcasses during slaughtering process (Henry *et al*., 2017). The process of evisceration and skinning during meat processing regarded as the most important sources of contamination of beef carcass and organs with *E. coli* O157:H7 and NTS at abattoirs (Alemayehu, 2003; Arthur *et al*., 2010; Jacob and Delgado *et al*., 2018).

Isolation and identification of NTS and *E. coli* O157:H7 are the main important things in prevention of the disease. The detection methods for these bacteria include conventional culturing on selective media, immunological method, and nucleic acid and biosensors (Rodríguez-Lazaro *et al.*, 2007; Brichta-Harhay *et al*., 2007).

Beta-lactam and quinolone antimicrobials are commonly used for treatment of infection caused by NTS and *E. coli* O157 (Momtaz *et al*., 2013). Antimicrobial resistance (AMR) is increasingly recognized as growing global health problem that reduce antimicrobial therapies and the effectiveness of drugs (WHO, 2014). In Africa, multidrug-resistant bacteria are currently circulating in a population with a high proportion of individuals that are susceptible to the disease (Lokken *et al*., 2016). Determination of antibiotic resistance bacteria carried by food-producing animals and in the food chain enables comparison of data and the distribution of antibiotic resistant genes within the population that helps for the controls of antibiotic resistance (Ateba *et al*., 2008).

Few studies were conducted on *E. coli* O157 and NTS in Ethiopia indicated that apparently healthy slaughtered cattle, feces, internal mucosal, beef carcass butcher shops and hide of animals are the main sources of infection to human (Abdissa *et al*., 2017; Atnafie *et al*., 2017; Takele *et al*., 2018). Moreover; raw meat and under cooked meat are widely consumed by many people (Biffa and Skjerve, 2010; Abayneh *et al*., 2014; Thomas *et al.,* 2015). There is limited information regarding the occurrence and antimicrobial resistance profile of *E. coli* O157 and NTS isolated from cattle feces, carcass and hide swabs in the processing plant or abattoirs before skinning and evisceration in Bishoftu Town. Therefore, this study was conducted with the following objectives:-

General objectives: investigation of the magnitude of occurrence of selected food-borne pathogens in the slaughter house and their drug sensitivity profile at Bishoftu Town, central Ethiopia.

Specific objective:

- To isolate and identify *E. coli* O157 and NTS from beef carcass, hide and feces
- To evaluate the antimicrobial sensitivity profile of *E. coli* O157 and NTS isolates using most commonly used antimicrobial drugs.

2. LITERATURE REVIEW

2.1. *Escherichia coli* O157:H7

Food-borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and medical and social costs worldwide (Havelaar *et al*., 2015). Contaminated raw meat is one of the main sources of food-borne illnesses. The risk of the transmission of zoonotic infections is also associated with contaminated meat (Nafisa *et al*., 2010). WHO estimated that in developed countries up to 30% of population suffering from food-borne illness each year, whereas developing countries up to 70% diarrhea diseases are associated with consumption of contaminated food per year (Edget *et al*., 2014).

As estimates of WHO on the global burden of Food-borne disease due to 31 hazards indicated 600 million food-borne illnesses 420,000 deaths and 33 million Disability Adjusted life years (DALYs (WHO, 2015; Havelaar *et al*., 2015). This occur commonly in developing countries particularly in Africa because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory system, lack of financial resources to invest in safer equipment and lack of education for food handlers (Haileselassie *et al*., 2013). It often follows the consumption of contaminated foodstuffs especially from animal products such as meat from infected animals or carcasses contaminated with pathogenic bacteria including *Escherichia coli* (Nouichi & Hamdi, 2009).

E. coli are a normal part of the intestinal micro-flora of many healthy animals and humans. Many *E. coli* strains are harmless or even beneficial to the hosts; however, some strains of *E. coli* can be pathogenic and cause fatal disease in humans (Belanger *et al*., 2011). The pathogenic *E. coli* strains, which cause enteric disease, are grouped into six categories: enterohemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enteroaggregative (EAggEC), and diffuse-adherent

(DAEC). The last two categories are not yet well defined. These categories differ in their pathogenesis and virulence properties, and each comprises a distinct group of O: H serotypes. *E. coli* O157: H7 is the most predominant and most virulent serotype in a pathogenic subset of EHEC (Chapman *et al*., 2001). *E. coli* O157:H7 is gram-negative, facultative anaerobic bacteria which belong to genus *Escherichia* of family Enterobacteriaceae (Farrokh *et al*., 2012; Xia *et al*., 2010). It is an enterohemorrhagic *E. coli* (EHEC), is of the best known pathogenic strain and the most common causes of food-borne infections in humans. It has been linked to foods of cattle origin and fresh produce (CDC, 2017; Al-Dragy & Baqer, 2014; Ferens & Hovde, 2011).The diseases caused by *Escherichia coli* O157: H7 strains in humans are result from the consumption of food and water contaminated with faces of infected animals and/or humans (Ateba & Bezuidenhout, 2008).

Based on the immune status and the general health of the infected individual, and the dose and virulence of the bacteria, infection with *E. coli* O157: H7 can result in mild diarrhea, severe bloody diarrhea, hemorrhagic colitis, or hemolytic uremic syndrome (HUS) leading to kidney failure(Ferens & Hovde, 2011).The virulence of the causative *E. coli* O157:H7 strains determine the illness severity of the infection which ranges from asymptomatic colonization with body, to lethal HUS disease (Pennington, 2010).

Cattle are a major reservoir of *E. coli* O157:H7 (Tourret *et al*., 2016; Martorelli *et al*., 2015) and a diversity of foods has been identified as vehicles of illnesses. The microbiological contamination of carcasses occurs mainly during processing and manipulation, such as skinning, evisceration, storage and distribution at slaughter houses and retail establishments. Fecal matter is a major source of contamination and could reach carcasses through direct deposition, as well as by indirect contact through contaminated and unclean carcasses, equipment, workers, installations and air (Abdulla *et al*., 2009).

The most frequent mode of transmission for *E. coli* O157:H7 infection is through consumption of contaminated food and water, particularly with consumption of uncooked and contaminated beef product. However, it may spread directly from person to person and occasionally through occupational exposure (Sodha *et al*., 2015; Abdalla *et al*., 2009).

Globally, the Food-borne *E. coli* O157:H7 estimated to cause 2.8 million acute illnesses each year (Majowicz *et al*., 2014). In United States only the pathogen is estimated to cause over 60,000 illnesses in the each year, resulting in about 2,000 hospitalizations and 20 deaths (Scallan *et al*., 2011), resulting in an economic burden of $607 million (Scharff, 2012), including $370 million for premature deaths, $30 million for medical care, and $5 million in lost productivity (Frenzen *et al*., 2006).

The case-fatality rate of *E. coli* O157:H7 is quite low at 0.5%, but due to the severe nature of HUS, especially in children under 10 year (CDC, 2016). The hospitalization rate is estimated to be 46.2% (Scallan *et al*., 2011) .The patient who develops TTP carries a fatality rate of 50 %, (Pal & Mahendra, 2016). Infection associated HUS is estimated to be fatal in 1-10% of children and up to 50% of the elderly (CDC, 2016). Most of outbreak of the disease in world is due to consumption of raw or undercooked ground beef (Pal & Mahendra, 2016).

Sanitary operations in abattoirs, good personal hygiene and proper disposal of faces, use of chlorinated water and ingestion of pasteurized milk, thorough washing of fruits and vegetables, and public education about the consumption of cooked meat will positively help to reduce the incidence of this emerging pathogen which poses a global threat to public health (Pal & Mahendra, 2016).

2.1.1. Historical background

E. coli, originally called “Bacterium coli commune,” was first isolated from the feces of a child in 1885 by German microbiologist Theodor Escherich (Escherichia, 1885). In 1982, *Escherichia coli* O157:H7 was first identified as a human pathogen after two outbreaks in Oregon and Michigan (Sewlikar & D'Souza, 2017; Riley, 2014). In this year, three outbreaks of hemorrhagic colitis (HC) caused by *E. coli* serotype O157:H7 occurred in north America, at fast-food (ground beef sandwiches) prepared at restaurants in Oregon and Michigan and a nursing home in Ontario, Canada, Two common-source outbreaks probably food related in nursing homes. In Canada in 1983 (31 cases) and in 1985 (73 cases) accounted for 66 cases of hemorrhagic colitis, 12 cases of hemolytic uremic syndrome (HUS), and 17 deaths (Carter *et al*., 1987). In central Scotland at the end of 1986 there was a report that 21 people died and more than 500 fell in ill due to an outbreak this was among one of the world's worst food -borne in terms of morbidity and mortality in humans. Approximately, 52% of recorded human disease outbreaks have been associated with cattle products (Griffin & Tauxe, 1991). Since then, *E. coli* O157:H7, and in more recent years also a number of other serotypes, have caused major human illness outbreaks worldwide with considerable morbidity and mortality (Constable *et al*., 2017).

2.1.2. E. coli O157: H7 as emerging food-borne

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

The new emerging food-borne *E. coli* O157:H7 infections are related to food handling practices with processing and packaging of food, or the importation of certain food from a new geographical area (Robinson *et al*., 2007). Its food-borne outbreaks occurred most commonly in communities such as restaurants and schools with ground beef being the most common vehicle among outbreak.

The development of antimicrobial resistance in *E. coli* O157:H7 is the matter of increase concern and generate new public health challenge (Newell *et al*., 2010). The use of antimicrobials in food cattle to the development of resistance pathogenic *E. coli* O157:H7 that can reach human's through the beef food chain. Misuse of antimicrobial agents for farming and therapeutic purpose in animals and humans is the main cause of transmission of antibiotic-resistant strains, which are very difficult to treat with commonly used antibiotics, to humans via the food supply (Akbar *et al*., 2014). Antimicrobial resistance is common in *E. coli* O157:H7, include multiple drug resistance to ampicillin, amoxicillin, ceftriaxone, chloramphenicol, ciprofloxacin, cotrimoxazole, methicillin, tetracycline and vancomycin (Constable *et al*., 2017; Naik and Desai, 2012; Vijayarani *et al*., 2010).

2.1.3. Epidemiology

Geographical Distribution: *E. coli* O157:H7 infections occur worldwide and this have been reported on every continent except Antarctica (CFSPH, 2009). *Escherichia coli* (STEC) are responsible for gastrointestinal diseases reported in numerous outbreaks around the world (Parsons *et al*., 2016). Since its recognition in 1982, it has become an important concern in North America, Europe, South Africa, Japan, South America, and Australia. Particularly, in North America, Japan, and the UK, *E. coli* O157:H7 is the serotype most commonly associated with clinical disease in people. High rates are present in regions of South America, especially Argentina, where HUS is endemic (Constable *et al*., 2017). The meta-analysis study on prevalence of *E. coli* O157:H7 globally, estimate that the prevalence of agent in cattle at the global level was 5.68% and also revealed the prevalence of world regions as shown in Table 1.

Table 1: Estimated pooled prevalence of E coli O157:H7 in cattle by world region

World region	No. of study	No. cattle sampled	No. of positive cattle	Pooled estimate (%)
Global estimate	140	220,427	12,683	5.68
Africa	4	626	118	31.20
Asia	22	14,916	937	4.69
Europe	53	88,643	5,425	5.15
Latin America and Caribbean	11	4,313	73	1.65
Northern America	46	110,641	6,059	7.35
Oceania	4	1,288	71	6.85

Source: Islam *et al*., 2014.

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

Reservoir of *E. coli* O157:H7: Livestock are the most important reservoir of *E. coli* O157:H7 with cattle being the principal sources (Tourret *et al*., 2016) so, ground beef and beef products are identified as major sources of food-borne transmission. Cattle are now considered to be the major source of *E. coli* O157:H7 causing human disease and transmission may occur through a variety of routes. In addition to the contamination of meat and dairy products, bovine feces can contaminate drinking water and crops intended for human consumption. Various outbreaks have been associated with vegetable products, such as radish and apple cider, presumably following contamination with animal wastes (CFSPH, 2009).

Source of Infection: The predominant carriers and shedders of EHEC are healthy domesticated ruminants, cattle in particular, and to a lesser extent sheep and possibly goats (Su *et al*., 2012; Varela-Hernaíndez *et al*., 2007). Cattle food products and fresh products contaminated with cattle faces waste are the most common sources for infections (Callaway *et al*., 2009). Beef carcass contamination is a direct result of pathogen transfer from cattle hides harboring enterohemorrhagic *E. coli*. Hide contamination occurs from direct and indirect fecal contamination in cattle production and lairage environments. Individual animals shedding the pathogens at high levels (>10^4 CFU per gram of feces (Arthur *et al*., 2010).

Transmission is via the fecal-oral route. The most frequent mode of transmission for *E. coli* O157:H7 infection is through consumption of contaminated food and water (Sodha *et al*., 2015). This primarily has been linked to undercooked meat. Human infections have been mostly associated with the consumption of contaminated and improperly cooked minced beef (Catford *et al*., 2014). However, acquisition of disease by direct contact with animals and manure at petting zoos and dairy farms are of increasing concern (Constable *et al*., 2017). It can also transmit direct from person to person or from infected animals. Birds and flies can also transmit mechanically as vectors. The habit of consuming raw and/or undercooked meat is one of the factors that exacerbate the transmission of food-borne *E. coli* O157:H7 (Hubaílek & Rudolf, 2010).

Cattle feces are the most important source of *E. coli* O157:H7. However; It also present in the feces of other animal species (goat, sheep, horse…etc) (Su *et al*., 2012; Gordillo *et al*., 2011; Hubaílek & Rudolf, 2010; Dontorou *et al*., 2003). Carcass contamination occurs through skin-to-carcass or fecal-to-carcass transfer of the pathogen .during slaughter process at processing plants and this is the major risk factor for human infection. Butcher houses and restaurants are frequently incriminated as sources of *E. coli* O157:H7 for human infections (Arthur *et al*., 2017; Fink *et al*., 2017). *E. coli* O157:H7 is highly virulent, with a low infectious dose: an inoculation of fewer than 10 to 100 CFU of *E. coli* O157:H7 is sufficient to cause infection, compared to over one-million CFU for other pathogenic *E. coli* strains (Greig *et al*., 2010).

Pathogenesis The virulence factors of *E. coli* O157:H7 are its ability to attach and efface the intestinal epithelium and its production of the cytolethal shiga toxin Stx1 and Sxt2. After the victim ingest the food contaminated with *E. coli* O157:H7, the organisms withstands the acidic environment of the human stomach and begins the process of infection (Robinson & McKillip, 2010). First, *E. coli* O157:H7 must initially adhere to the microvilli of the host epithelial cells (Mainil & Daube, 2005). The intimate attachment of the bacterial cell to the host epithelium is attributed to the adhesion intimin and translocated intimin receptor (Tir), a bacterial protein, which is inserted into the host membrane and serves as the response for intimin and mediate adhesion between mammalian cells and attaching and effacing (A/E) pathogens. The bacterial outer membrane adhesin, intimi, is necessary for the production of the A/E lesion and diarrhea (Constable *et al*., 2017).

The exact means by which *E. coli* O157:H7 establishes and sustains colonization in the host remains elusive. Once it has successfully colonized and established itself within the host, *E. coli* O157:H7 produces and releases its Stxs in the intestinal lumen. Shiga toxins act to inhibit protein synthesis within target cells (Mainil & Daube, 2005). The Stxs can translocate from intestinal epithelial cells into the bloodstream. Here, the Stxs bind to the Gb3 receptors on glomerular endothelial cells. The Stxs injure the glomerular cells and cause platelets and fibrin to deposit within the glomeruli. Eventually, the deposits decrease renal filtration and lead to the acute kidney damage characteristic of HUS (Welinder-Olsson & Kaijser, 2005).

Disease pattern: The acute disease associated with this organism is named hemorrhagic colitis in humans. The symptoms characteristic to this disease are watery and/or bloody diarrhea, fever, nausea, severe abdominal cramping, and vomiting (Walker *et al*., 2012). From the point of ingestion, the incubation period of *E. coli* O157:H7 ranges from 8 hours to 16 days, but the typical incubation period is three to four days (Robinson & McKillip, 2010) and the illness usually lasts 5–10 days. Life threatening complications, some victims, particularly the very young, may develop hemolytic uremic syndrome (HUS) (Martorelli *et al*., 2017). HUS, which is characterized by renal failure and hemolytic anemia, occurs in up to 15% of hemorrhagic colitis victims and can lead to permanent loss of kidney function. People of all ages are susceptible to infection with STEC. However, the young and the elderly are more susceptible and are more likely to develop more serious symptoms (FDA, 2012). In the elderly, the combination of HUS with fever and neurologic dysfunction is characteristic of thrombotic thrombocytopenic purpura (TTP) (Sewlikar & D'Souza, 2017; Chekabab *et al.*, 2013). Haemolyticuraemic syndrome (HUS) consists of the triad micro-angio-pathichaemolytic anemia, acute uraemia and thrombocytopenia. HUS leads to significant morbidity and mortality during the acute phase and it is the most common cause of acute renal failure in children (Bayat *et al.*, 2012). In clinical cases, in human the mortality rate varies with the syndrome. Hemorrhagic colitis alone is usually self–limiting, although deaths can occur. Complications and fatalities are particularly common among children, the elderly, and those who are immunosuppressed or have debilitating illnesses. Infection associated to HUS is estimated to be fatal in 1-10% of children and up to 50% of the elderly. In European surveillance, the case fatality rate in all reported EHEC infections was < 0.5% (CDC, 2016).

2.1.4. Role of cattle in human E. coli O157:H7 infection

Cattle play an essential role in epidemiology of human *E coli* O157:H7 infection and cattle feces considered as primary source which the beef food-become contaminated with this pathogen. The first identified human outbreaks of *E. coli* O157:H7 in 1982 was associated with consumption of ground beef, and the importance of cattle as a reservoir for *E. coli* O157:H7 became evident as more outbreaks were associated with undercooked beef and other bovine products such as unpasteurised milk (CDC, 2017). The association of *E. coli* O157:H7 with undercooked ground beef and raw rice led to investigations of the role of cattle as a reservoir of the pathogens (Pal & Mahendra, 2016).

Beef, particularly ground beef, continues to be the major source of *E. coli* O157:H7 outbreaks, likely because cattle are the main reservoir for *E. coli* O157:H7. The study conducted in United Stated during 2003–2012, state that there were 353 outbreaks, from those 20% transmission was through consumption of beef and beef product. The incidence of human cases of *E. coli* O157:H7 is positively related to cattle density and the cattle to human ration (Heiman *et al*., 2015). Colonization of *E. coli* O157:H7 in adult cattle are asymptomatic (Verstraete *et al*., 2014) because intestinal mucosal cells lack the Stx-specific globotriaosylceramide receptor (Constable *et al*., 2017). Some cattle shed 10^4 CFU *E. coli* O157:H7per one gram of feces, are called “Super Shedders”. Super shedders have prominent outcome for distribution of EHEC in cattle as it is the main reservoir and therefore increase the risk of human infection (Chase-Topping *et al*., 2008).

2.1.5. Diagnosis of E .coli O157: H7

Clinical cases can be diagnosed by finding the organisms in fecal samples, food and environmental samples may also be tested to determine the source of the infection. Many diagnostic laboratories can detect and identify *E. coli* O157:H7. There is no single technique that can be used to isolate all EHEC serotypes (CDC, 2016). Infection with this agent is associated with a broad spectrum of illness ranging from mild diarrhea and hemorrhagic colitis to the potentially fatal hemolytic uremic syndrome (HUS) (Rahal *et al*., 2012).These clinical symptoms used as one diagnosis technique.

Common sample are diarrheic feces in animals, predictable food item in both animal and human food, stool of infected individual in human with hemolytic-uremic syndrome and from food-borne outbreaks (Elhadidy *et al*., 2015).The most sensitive sampling method from animal for STEC O157:H7, is the rectal swab, because STEC specifically colonize the recto-anal junction of the intestinal mucosa that is directly sampled with the swab approach (Constable *et al*., 2017).

Immunoassays and polymerase chain reaction technology have led to more rapid detection of *E. coli* in stools, food, and water (Bavaro, 2009). Techniques included in this category are PCR and DNA-based techniques, immunomagnetic separation, and enzyme-linked immunosorbent assays (ELISAs). Molecular-based techniques are distinctly advantageous because of their sensitivity, selectivity, and their rapid results. However, molecular-based techniques are appreciably more expensive than traditional plating techniques and are also more novel and unfamiliar. Therefore, the integration of molecular-based approaches into quality control procedures depends on the overall needs and resources of the food processing plant (Robinson & McKillip, 2010).There are also Latex Agglutination Test for the rapid identification of *E. coli* O157:H7. The test is best used in conjunction with Sorbitol MacConkey Agar. A positive result is indicated by agglutination with the test reagent, whilst the control reagent should appear milky and smooth (Al-Dragy & Baqer, 2014).

2.1.6. Treatment, prevention and control

Treating *E. coli* O157:H7 infection with antimicrobial agents is associated with an increased risk of severe sequel such as HUS (Rahal *et al*., 2012), that may exacerbate the patient's condition by increasing either the release of preformed Shiga toxins (Stx) upon cell lysis. However, early administration using some antimicrobials is effective (Nassar *et al*., 2013).

Certain management practices optimize the likelihood of good outcomes, such as avoidance of antibiotics during the pre-hemolytic uremic syndrome phase, admission to hospital, (Davis *et al*., 2013) and the patients with complications may require in rigorous care including dialysis, transfusion and/ or platelet infusion besides kidney transplant (CFSPH, 2009).

Prevention of *E. coli* O157:H7 by frequently washing of hands after using the bathroom, before preparing or eating food, and contact with animals. Adequate sanitation and proper processing of foods is seriously important, Cook meats thoroughly at a temperature of at least 160°F/70˚C and avoid raw meat, milk, unpasteurized dairy products (Mathusa *et al*., 2010). Keeping cattle away from water supply, proper disposal of infected faces, good kitchen hygiene may reduce the incidence of *E. coli* O157:H7 human infection. And implementation of *E. coli* O157:H7.Testing contaminated material for and withholding that material, before releasing it to the market is one way of preventing human infection and illness (CFSPH, 2009). One Health approaches is the opportunity to implement control programmers that reduce the multiple impacts of zoonosis in both human and animal populations. Interventions that may control zoonotic infection in animal populations or prevent disease transmission from animals to people may offer more effective and economically viable approaches to disease management than those focusing on the human population alone (Halliday *et al*., 2015). Vaccines against EHEC O157:H7 for cattle may reduce shedding and have received full or conditional approval in some countries including the U.S. and Canada, but are not in wide use but there is no human vaccine against enterohemorrhagic *E. coli* (EHEC) infections (Smith, 2014).

2.1.7. Status of E. coli O157:H7 in Ethiopia

There are some studies which conducted concerning the EHEC strain *E. coli* O175: H7 at some areas on different types of samples in Ethiopia. Thus, Prevalence of *E. coli* O157:H7 in beef cattle at slaughter and beef carcasses at retail shops in Ethiopia (Abdissa *et al*., 2017), Prevalence and antimicrobial susceptibility of *E. coli* O157:H7 in beef at butcher shops and restaurants in central Ethiopia (Beyi *et al*., 2017), occurrence of *E. coli* O157:H7 and the multiple antibiotic resistance profiles in cattle slaughtered at municipal abattoir and retail meat sold at butcher shops in the Hawasa (Atnafie *et al*., 2017), Also (Tassew, 2015) and (Bekele *et al*., 2014) were studied on 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 and *E. coli* O157:H7 in Raw Meat in Addis Ababa, Ethiopia: Prevalence at an Abattoir and Retailers and Antimicrobial Susceptibility respectively. The prevalence of *E. coli* O157:H7 from the children under 5 year at Behir Dar, Ethiopia also studied by (Adugna *et al*., 2015).

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

Study Area	**Sample Unit**	**Sample Type**	**Prevalence**	**References**
Hawasa	Cattles Butcher shop Meat hander Knifes	Swab (knife Close of meat transporter),	2.4%	(Atnafie *et al*.,2017)
Addis Ababa DebreBerhan	Cattles	Fecal	2%	(Abdissa *et al*.,201)
	Butcher shop	Skin swab	0.5%	
		Intestinal mucosal swabs	0.8%	
		Internal carcass swabs	0.5%	
	Human	Stool	0%	
Dire Dawa	Cattles	Raw meat	2.06%	(Edget *et al*., 2017)
Jimma	Cattle	Carcass swab	9.3%	(Feleke *et al*., 2017)
		Cecal content	7.3%	
DebreZeit Addis Ababa		Carcass swab	5.5%	(Tassew, 2015)
Bahir Dar	Human	Stool	48.3%	(Adugna *et al*.,2015)
Addis Ababa	Cattles	Beef	10.2%	(Bekele *et al*.,2014)
Addis Ababa	Cattle	Carcass swab	0.72%	(Haile, 2014)
Haramaya	Cattle	Carcass swab	2.65%	(Taye *et al*., 2013)
Modjo	Cattles	Raw meat	4.2%	(Hiko *et al*., 2008)

2.2. Non-Typhoidal *Salmonella*

Non-typhoidal *Salmonella* is a zoonotic food-borne pathogen causing salmonellosis. It is comprised of two species, *Salmonella bongori* and *Salmonella enterica*, and more than 2600 recognized serovars (Guibourdenche *et al*., 2010). However, most human salmonellosis cases are caused by relatively few serovars within *S. enterica* (Jones *et al*., 2008). *Salmonella* serovars have different hosts and reservoirs ranging from cold-blooded to warm-blooded animals (Hoelzer *et al*., 2011). Furthermore, *Salmonella* can survive in farm and other environments for prolonged periods of time (Holley *et al*., 2006; Cummings *et al*., 2010).

Salmonella has been recognized as a major and important food-borne pathogen resulting in high medical and economical cost (Lee *et al*., 2015). *Salmonella* is a global food-borne pathogen with reported case of 86% (80.3 million/93.8 million) gastroenteritis caused by *Salmonella* and annually 155,000 deaths globally (Majowicz *et al*., 2010). It is responsible for approximately 30% of food-borne outbreaks in the United States and 23% in the European Union (Gould *et al*., 2013; EFSA, 2015). One million non-typhoidal *Salmonella* illnesses, 20,000 hospitalizations and 370 deaths occur through food-borne transmission in the United States each year (Scallan *et al*., 2011). Furthermore, the mortality rate of *Salmonella* infection in developing countries is 24% higher than in the developed countries (Chimalizeni *et al*., 2010).

Animals are known to play a major role as a source of a variety of zoonotic *Salmonella* serotypes, which are often asymptomatically carried by them. Cattle are among the known reservoirs of *Salmonella*, and ground beef has been implicated as one mode of transmission in food-borne outbreaks (CDC, 2006).

Foods of animal origin have been implicated as major vehicles associated with illness caused by *Salmonella* species. Much of the published reports indicated that the number of salmonellosis has increased mainly associated with the consumption of raw or undercooked poultry, meat or dairy products (Braden, 2006). Of particular concern, contaminated raw or undercooked poultry and red meat are important in transmitting *Salmonella* species. Since this pathogen is transmitted primarily through contaminated food or water, the presence of *Salmonella* spp. in food animals and ultimately in raw meat products has important public-health implications (CDC, 2006).

Contamination of meat with *Salmonella* may occur during slaughtering processes where such pathogen can be easily transferred to the carcass during hide removal or during evisceration. Also, cross contamination from meat handlers during the processes of manufacturing, packing and marketing, may also contribute to the prevalence of salmonellosis (Al-Mutairi, 2011).

The prevalence of *Salmonella* in beef and their products as well as in humans along with the economic impacts is important to lead to the importance of detection. The detection of *Salmonella* is the key to the prevention and identification of problems related to health and safety. The three areas of application accounting for over two thirds of all research in the field of *Salmonella* detection are the food industry, water and environment quality control and clinical diagnosis (Lazcka *et al*., 2007). To ensure faster release, rapid methods intended for use at food production chain must be sensitive, robust, non-complex, low-cost, and, not least, amendable to high throughput to prevent *Salmonella* contamination of food (Eijkelkamp *et al*., 2008; Postollec *et al*., 2011).

Many efforts have been made to develop and improve detection technologies for this organism because to prevent the devastating food-borne illness effect of the pathogen. *Salmonella* detection methods are desirable to have sensitivity enough to detect one cell in a defined sample. The analysis time of conventional and rapid methods can vary with cell enrichment steps to reach minimal cell concentration enough for *Salmonella* detection. The cell enrichment process is typically lengthy in a conventional method whereas the rapid detection method generally requires at least 10^4 cells in 1ml of *Salmonella* concentration for detection (Lee *et al*., 2015).

Conventional methods for *Salmonella* detection include propagation and isolation from culture. These methods are effective and sensitive but tend to be costly, labor intensive and time consuming (typically results are available in 2–10 days). Alternative molecular methods based on polymerase chain reaction (PCR), real time PCR (RT-PCR) are more specific, sensitive and take less time, but they need isolated genetic materials, manipulation with special care and necessitate sophisticated equipment, and, thus, they are hardly to be applied for on-site monitoring. Consequently, development of a valid diagnostic assay for swift pathogen detection and identification, with high sensitivity and selectivity is a challenge for researchers all over the world (Vidic *et al*., 2017).

Convenient and sensitive point-of-care rapid diagnostic tests for food-borne pathogens have been a long felt need of clinicians (Fang *et al*., 2014). *Salmonella* surveillance and monitoring should be based on reliable and efficient detection methods, which should help improve the food safety. It is essential that surveillance and monitoring should cover the entire food chain, preferably starting from investigation of feed and feed ingredients for *Salmonella* contamination (Rodríguez-Lazaro *et al*., 2007).

2.2.1. Etiology

Salmonella is named after an American Veterinary Bacteriologist, D.E. Salmon who first isolated *Salmonella cholerasuis* from porcine intestine in 1884 (Rabsch *et al*., 2003). The classification and nomenclature of *Salmonella* has been controversial for many years. According to the latest nomenclature, which reflects the recent advances in taxonomy, the genus *Salmonella* consists of two species, *S. enterica*, the type species and *S. bongori*, the former sub species V. *S. enterica* in turn is further divided into six subspecies which are referred to by Roman numeral and/or name. These are *S. Enteric sub spp. Enterica* (I), *S. Enteric subsp. Salamae* (II),*S. Enteric subsp. Arizonae* (IIIa), *S. Enteric subsp. Diarizonae* (IIIb), *S. Enteric subsp. Houtenae* (IV) and *S. Enteric subsp. Indica* (VI) (Shelobolina *et al*., 2004; Grimont and Weill, 2007).

Salmonella species are further classified into serotypes using the Kauffman-White scheme, which is defined and maintained by the WHO collaborating center for reference and research on *Salmonella* at the Pasteur Institute, Paris, France. The classification is based on the basis of extensive diversity of lipopolysaccharide antigen (O antigen), flagellar protein antigen **(**H antigen) and sometimes the capsular (VI) antigens. Currently, there are above 2500 serotypes of *Salmonella* and new serotypes are listed on annual updates of the Kauffman-White scheme (Grimont and Weill, 2007).

The *Salmonellae* are small, gram-negative, non-sporing rods, facultative anaerobic bacilli, and 2 to 3 by 0.4 to 0.6 μm in size. Like other members of the family Enterobacteriaceae, they produce acid on glucose fermentation; reduce nitrates to nitrite, and don't produce cytochrome oxidase (Rabsch *et al*., 2003). Most organisms, except *S. gallinarum* and *S. pullorum* are motile by peritichous flagella (Chaubal and Holt, 1999). The differential metabolism of sugars can be used to distinguish some *Salmonella* serotypes, for instance most don't ferment lactose. *S. Typhi* is the only organism that does not produce gas in sugar fermentation. *Salmonella* are non-capsulated except *S. Typhi*, *S. Paratyphi C* and some strain of *S. Dublin*. *Salmonella* grows between 8 ^{O}C and 45 ^{O}C (optimally at 37 ^{O}C) and at a pH of 4 to 9. A temperature higher than 70 ^{O}C rapidly kills them. Pasteurization at 71.1oC for 15 seconds is sufficient to destroy *Salmonella* in milk. These bacteria can resist dehydration for a very long time ($Aw \geq 0.93$), both in faeces and in foods for human and animal consumption. In addition, they can survive for several months in salt water with 20% salinity, particularly in products with a high protein or fat content, such as salted sausages; they also resist smoking. *Salmonellae* have several virulence factors that contribute to causing diarrhoea, bacteremia, and septicemia. These factors include the lipopolysaccharide of the outer wall, pili, flagella, cytotoxin, and enterotoxin (Quinn *et al*., 2002).

2.2.2. Epidemiology

The epidemiology of salmonellosis is complex largely because there are more than 2,500 distinct serotypes (serovars) with different reservoirs and diverse geographic incidences. Changes in food consumption, production and distribution have led to an increasing frequency of multistate outbreaks associated with fresh produced and processed foods. All livestock species can be affected by salmonellosis with young, debilitated and parturient animals most susceptible to clinical disease and all age group of humans also affected (Rounds *et al*., 2010). Unlike *Salmonella Typhi* and *Salmonella Paratyphi*, which have host specificity for humans, NTS can be acquired from both animal and humans (Braden, 2006; Hohmann, 2001).

Salmonella is one of the leading causes of bacterial food-borne disease in industrialized as well as developing countries even though the incidence seems to vary between countries (Molla *et al*., 2003) Chiu *et al*., 2004). Significant outbreaks of Salmonellosis occurred around the world at different times. For instance; in the United States, 164,044 (approximately 32,000 annually) during 1998 - 2002 (Lynch *et al*., 2006). In China, approximately 70% to 80% and during 1992- 2005 (Wang *et al*., 2007; Chen *et al*., 2008; Liu *et al*., 2008), in Germany, a total of 42,851 (Robert Koch Institute, 2008) (EFSA, 2009). In 2006, a total of 160,649 confirmed cases of human salmonellosis were reported in the EU (Liu, 2010). In many countries, incidence of human *Salmonella* infection has increased drastically over the years. Salmonellosis is an important global public health problem causing substantial morbidity and mortality (CDC, 2009).

Globally, an estimated 93.8 million cases of gastro enteritis and 155,000 deaths are caused by non-typhoidal *Salmonella* spp. annually. Under reporting is a problem worldwide. In the U.S., the incidence of salmonellosis has remained relatively stable in recent years. About 16 cases were reported per 100,000 people in 2012; however, for every reported case an estimated 29 undiagnosed cases occur. In the EU, reported cases of salmonellosis have been decreasing. In 2011, about 20 cases were reported per 100, 000 people (CDC, 2014). Surveillance data are often not available in developing countries. In Southeast Asia, it is estimated that 22.8 cases of salmonellosis occur each year. The overall mortality rate for most forms of salmonellosis is less than 1%. In hospital or nursing home outbreaks, the mortality rate can be up to70 times higher. *Salmonella* gastroenteritis is rarely fatal in healthy people (CDC, 2014). Besides the importance of this microorganism in public health, another aspect is the cost incurred by human Salmonellosis. With the increasing population in the developing world, there is an increasing demand for meat and meat products which will force the present resource-driven system of livestock production to a demand-driven system (Zessin, 2006) which will increase the disease transmission risks. There is a multifactorial risk of food-borne hazards including salmonellosis in the developing countries due to poor sanitation and inadequate access to potable water (Henson, 2003).

GIT tracts such as rumen (Anderson *et al*., 2000), rectum (Rose *et al*., 2002), caecum and colon (Galland *et al*., 2001) contain high concentration of *Salmonella*. People are often infected when they eat contaminated foods of animal origin such as meat, eggs, milk, vegetables and fruits. They can also be infected by ingesting organisms in animal feces, either directly or in contaminated food or water (OIE, 2005).

Transmission of *Salmonella* is cyclic between humans, animals, food, and environmental sources. Usually, non-typhoidal *Salmonellae* spread along the food chain. Animals can become infected from contaminated feed, drinking water or close contact with infected animals. In farm livestock animal feed and high levels of fecal shedding of infected animals has been recognized as important entry site in the food chain. Furthermore, another source of contamination is the slaughtering of the animals (Liu, 2010).

2.2.3. Pathogenesis

Infections with *Salmonella* are an important cause of diarrhea and mucosal inflammation and can lead to severe systemic disease. Infection is usually initiated by the ingestion of contaminated food (Dougan *et al*., 2011).The nature and severity of

Salmonella infections in humans vary enormously and are influenced infecting *Salmonella* serovar, strain virulence, infecting dose, age, and immune status of the host. Usually*, Salmonellae* colonize the intestine by the adhesion of the bacteria to the epithelial cells using fimbrial antigens. The cells invade the intestinal mucosa and multiply in the gut-associated lymphoid tissue (GALT) (Lim *et al*., 2014; MacLennan *et al*., 2008).

From the infected tissues the pathogens spread to the regional lymph nodes, where macrophages form a first effective barrier to prevent a further spread (Radostits *et al*., 2007). If the macrophages are unable to avoid the spread, systemic disease can occur. During the systemic disease the bacteria spread from the GALT via the efferent lymphatics and the thoracic duct into the vena cava from where it spreads out through the body (Lim *et al*., 2014; MacLennan *et al*., 2008). The bacteria multiply in spleen, liver and released in large numbers to the blood stream infecting other organs. *Salmonellae* are able to survive and multiply inside host cells (Liu, 2010).

Salmonella harbors large clusters of virulence genes that act together in a complex virulence function for different outcomes of *Salmonella* infections (Radostits *et al*., 2007). They have been chromosomally acquired by horizontal gene transfer and are called pathogenicity islands. These islands contain genes required for the different roles in gastrointestinal and systemic pathogenesis (Bäumler, *et al*., 2000). Some of the pathogenicity islands encode type III secretion systems (TTSS) for the contact dependent translocation of substrate proteins into eukaryotic host cells or are responsible for the survival of *Salmonellae* in macrophages (Kingsley and Bäumler, 2002). Furthermore, *Salmonella* possess adherence fimbriae enables it to attach and adhere easily to cell surfaces, particularly mucous membranes (Cheesbrough, 2006).

2.2.4. Clinical signs and Symptoms

Two to eight percent of NTS infections are associated with bacteremia, and are not always preceded by gastroenteritis. Risk factors for NTS bacteremia include immune-compromise (including HIV, malignancy, chemotherapy, steroid therapy) and extremes of age (< 3 month and greater than 50 years old). Risk factors are not apparent in up to one third of cases of NTS bacteremia. Extra intestinal focal infections (eg. arthritis, meningitis, pneumonia) occur in 5-10% of those with bacteremia (Matheson, 2010).

Acute gastroenteritis is the most common presentation of NTS infection. Typical symptoms include non-bloody diarrhea, nausea, and/ or vomiting. Fever, abdominal cramps, bloody diarrhea may also be reported. Asymptomatic carriage can occur in as many as 4.7% of healthy hosts (Sirinavin, 2004). These symptoms of gastroenteritis develop within six to seventy two hours after ingestion of the bacteria and are usually self-limiting and typically resolve within two to seven days (CDC, 2001; Pegues *et al*., 2005).

2.2.5. Detection of non-typhoidal Salmonella from animals and foods of animal origin

Conventional method: Diagnosis is based on isolation of non-typhoidal *Salmonella* organisms from feces, food items or in cases of disseminated disease, from the blood by culture. Isolates of non-typhoidal *Salmonellae* are needed for serotyping and antimicrobial susceptibility testing (Acha and Szyfres, 2003). Conventional bacterial identification methods usually include a morphological evaluation of the microorganism as well as tests for the organism’s ability to grow in various media under a variety of conditions. These methods are very sensitive, inexpensive and can give both qualitative and quantitative information on the number and the nature of microorganisms present in the food sample (Madsen *et al*., 2001). Although standard microbiological techniques allow the detection of single bacteria, amplification of the signal is required through growth of a single cell into a colony. Isolation of non-typhoidal *Salmonella* by culture based methods requires the prolonged enrichment steps and is still the most widely used detection techniques and remains the gold standard for the detection of non-typhoidal *Salmonella* due to their selectivity and sensitivity (Lee *et al*., 2015). Due to their widespread use, numerous and varied bacteriological media (selective enrichment broths and selective agar plates) are applied to best monitor for non-typhoidal *Salmonella* in food and food ingredients. The media may contain inhibitors in order to stop or delay the growth of non-target organisms, or particular substrates that only the target bacteria can degrade, or that confer a particular color to the growing colonies (Manafi, 2000).

Cultural methods of non-typhoidal *Salmonella* typically involve the enrichment of a portion of food sample to recover sub-lethally injured cells due to heat, cold, acid, or osmotic shock (Gracias and McKillip 2004) in a non-selective pre-enrichment media, such as Buffered Peptone Water (BPW) in a ratio of 1g to 9ml (1:9) to increase the number of target cells as these are generally not uniformly distributed in foods, typically occur in low numbers, and may be present in a mixed microbial population. Next, primary enrichment cultures are typically inoculated into secondary selective enrichment broths, such as Selenite Cystine broth (SC), Rappaport Vasiliadis Soy broth (RVS), Tetrathionate Broth (TT), or Muller Kauffmann Tetrathionate-Novobiocin broth (MKTTn) and incubated at elevated temperatures (37°C or 42°C for 18-24 hours) before being struck onto selective agars such as Xylose Lysine Deoxycholate agar (XLD agar), Bismuth Sulphite agar (BIS), Brilliant Green agar (BG) with or without the addition of sulfadiazine or sulfapyridine (BGS), modified semisolid Rappaport Vasiliadis (MSRV), non-typhoidal *Salmonella Shigella* Agar, or Hektoen Enteric agar (ISO 657: 2002).

The conventional microbiological methods serve as the basis for analysis in many food safety and public health laboratories due to the ease of use, reliability of results, high sensitivity and specificity and lower cost compared to emerging molecular-based technologies (Gracias and McKillip, 2004; Maciorowski *et al*., 2006). However, these procedures need to prepare multiple subcultures required for several identification steps, taking more than 5 days for complete isolation and confirmation. In addition, false positive results may occur due to competitive flora (e.g. proteus) (Naravaneni and Jamil, 2005).

Under circumstances in which high throughput screening is required for a large number of samples, the laborious and time consuming culture-based techniques may not properly address such a requirement. Some modification of conventional method such as chromogenic and fluorogenic growth media (SM-ID agar, Rambach agar, and BBL CHROMagar *Salmonella*) are used for detection, enumeration, and identification directly on the isolation plate have shown to be convenient, reliable, and more specific and selective than traditional conventional media. The test result using these selective media is typically available 1 day earlier than conventional methods but is not fast enough to respond to bioterrorism events, non-typhoidal *Salmonella* outbreak, and product recall (Perry and Freydiere, 2007; Alakomi and Saarela, 2009).

Rapid detection methods of *Salmonella*: Conventional pathogen detection methods largely rely on microbiological and biochemical analysis, which are highly accurate but overly time consuming, cost-ineffective and non-amenable to integration for on-site diagnosis. Besides, successful execution of pathogen identification and detection by conventional methods require extensive training and experience. Alternative rapid but accurate methods for pathogen detection have therefore been sought to overcome these limitations. Advances in immunological methods such as enzyme-linked immunosorbent assay (ELISA) have paved the way towards development of easier and quicker pathogen detection methods, relying on the recognition specificity of antibodies (Abs). Immunological methods however suffer from cross-reactivity of polyclonal Abs, high production cost of monoclonal Abs, need for sample pre-processing and pre-enrichment due to low processing sample volume and lower limit of detection. Polymerase chain reaction (PCR) is yet another method that leverages the nucleic acid complementarity-based specificity of pathogen detection (Lee *et al*., 2015).

Enzyme-linked immunosorbent assay (ELISA): Of immunology-based assays, ELISA is the most commonly used assay for the detection of antigens or products of non-typhoidal *Salmonella* spp. The different ELISA systems have been developed and commercially available in kit form. In the ELISA assay, an antigen specific to non-typhoidal *Salmonella* spp. is bound to the appropriate antibody linked to a solid matrix. After forming the antigen antibody complex, the concentration of the antigen and the presence of non-typhoidal *Salmonella* can be measured through the change in color caused by the enzymatic cleavage of a chromogenic substrate (Tietjen and Fung, 1995; Blivet *et al*., 1998). Alternatively, the presence of antibodies in samples infected with non-typhoidal *Salmonella* spp. can be detected using antigens coupled to the solid phase of ELISA (Wiuff *et al*., 2000). ELISAs have also been used to detect antibodies for development of vaccines against *Salmonella* infections (Meenakshi *et al*., 1999). Kuhn *et al*. (2012) recently reviewed ELISAs for non-typhoidal *Salmonella* infections, which were based on *Salmonella enteritidis* and *Salmonella typhimurium* Lipopolysaccharides (LPS) and found most sensitivities in the 70–95% range, with specificities >90%, though most studies were small and many did not report specificity.

Latex agglutination test: The agglutination technique employs latex particles coated with antibodies which react with antigens on the surface of *Salmonella* cells to form visible aggregates for identification of *Salmonella* positive samples (Tietjen and Fung, 1995). The assays are specific, uncomplicated, and reliable so that generally, they have been used as a confirmatory analysis technique, rather screening test for *Salmonella* organisms (Love and Sobsey, 2007; Eijkelkamp *et al*., 2008).

Immuno diffusion assays: Before inoculation into the system unit which consists of two connected chambers, the sample is pre enriched for 24 hr. The enriched sample is inoculated to a tetrathionate brilliant green broth in the inoculation chamber. *Salmonella* then moves out of the inoculation chamber into the mobility chamber in which antibody has been added onto a distal surface of a semisolid medium. *Salmonella* in the mobility chamber is immobilized by forming an antigen antibody complex. After incubation for 14hr, the readable three-dimensional immunodiffusion band is produced. Modifications in an enrichment step before inoculation and an increase of incubation time improved the effectiveness of detection of *Salmonella* spp. (Nath *et al*., 1989).

Immunochromatography (Dip stick) method: Shukla *et al* (2011) has developed a rapid and easy immunochromatographic strip assay for the detection of *S. Typhimurium* based on competitive immunoassay using anti-*Salmonella* IgG-tagged liposomes. Bautista *et al* (2002) conducted study to evaluate the sensitivity and specificity of an immunochromatography-based diagnostic kit for *Salmonella*. Accordingly, the analytical sensitivity of the test when using pure colonies of different *Salmonella* species was in the range of 1×10^4 to 1×10^5 colony-forming units per milliliter. Moongkarndi *et al*. (2011) has developed immunochromatographic assay to detect *Salmonella enterica serovars Typhimurium* and *Enteritidis* in a single strip. The assay was constructed in the form of a sandwich, using 2 specific anti–*S. Typhimurium* and anti–*S. Enteritidis* antibodies immobilized on a nitrocellulose membrane at separated test lines, while the other specific antibody to *Salmonella* spp. was conjugated with gold nanoparticles.

In general for serological/immunological tests, there is a lack of standardization of ELISA assays for non-typhoidal salmonellosis. A challenge with developing serologic tests for invasive non-typhoidal salmonellosis (iNTS) is the diversity of non-typhoidal serovars globally, which will require empirically informed and perhaps locally targeted, selection of LPS antigens to achieve adequate sensitivity. Additionally, it will be difficult for serologic diagnostics using current technologies to distinguish between the two syndromes iNTS can cause: self-limiting gastroenteritis and invasive systemic infection (Andrews and Ryan, 2015).

Nucleic acid-based assays: Within each species of microorganism there exist unique nucleic acid signature sequences that can be exploited to determine the presence of that specific microorganism. Nucleic acid-based diagnostics (NADs) refers to the use of these specific sequences of nucleic acid (either DNA or RNA) to detect the presence of a pathogenic microorganism in a clinical sample (O'Connor and Glynn, 2010).

Chaney *et al*. (2017) validated the Atlas *Salmonella* Detection Assay (SEN), a nucleic acid amplification technology that targets *Salmonella* rRNA, for the qualitative detection of *S. enterica* with sample enrichment using immunomagnetic separation as a reference test, and they further evaluated its accuracy to predict pathogen load using SEN signal-to-cutoff (SCO) values from unenriched samples to classify animals as high or non-high shedders. Accordingly, SEN assay was a rapid, sensitive, and specific molecular test, both for the detection of *Salmonella* and the categorization of shedding load in samples from beef cattle.

The nucleic acid-based assays of *Salmonella* detection tests utilize specific nucleic acid target sequence within the organism. The assays have been most intensively explored and developed for the past decade among *Salmonella* detection methods because they offer some advantages of sensitivity, specificity, and inclusivity over other methods, rapidly identifying *Salmonella* without obtaining pure cultures (Glynn *et al*., 2006; Mozola, 2007). Two major techniques of the assays are direct hybridization (DNA probe) and amplification (PCR) methods. The great progress of the assays allows the detection of very low numbers of organisms in the sample and high throughput of a large number of samples for routine analysis (Mozola, 2007).

Polymerase chain reaction (PCR): Conventional methods for detection of *Salmonella* serovars in foods are generally time consuming and labor intensive. A real-time PCR method has been developed with custom designed primers and a TaqMan probe to detect the presence of a 262-bp fragment of the *Salmonella*-specific invA gene (Cheng *et al.,* 2008). Several *Salmonella* specific target genes such as oriC, fimA, himA, hilA, and stn have been identified (Chen *et al*., 2000; Sanchez *et al*., 2004; Moore *et al*., 2007). However, none of the primer sets for these genes has been shown to be 100% accurate. This observation may be due to either the lack of species specificity of these genetic markers or to the uneven distribution of these markers with-in the *Salmonella* population. The *Salmonella* invasion gene, invA, has been shown to be involved in internalization of *Salmonella Typhimurium* in mammalian epithelial cells. This gene is unique to *Salmonella* and the DNA sequence is highly conserved among *Salmonella* spp. These properties suggested that the invA gene could serve as a reliable and accurate target gene for detection of *Salmonella* by PCR methods (Galan *et al*., 1991; Swamy *et al*., 1996).

Nikbakht and Sani, (2016) confirms positive *Salmonella* isolates from bovine and poultry origin by using multiplex PCR to amplify the pathogenic genes of *S.*

typhimurium (STM), *S. enteritidis* (SDF) and *S. infantis* (S1) by using specific primers for these genes.

Although PCR is a powerful technology, the reactions can be dramatically affected by the presence of inhibitory compounds in foods and selective microbiological media like bile salts and acriflavin. A problem to routine use of PCR in food testing lab is that the procedures are rather complicated and very clean environment is needed to perform the tests. Further, PCR cannot distinguish between live and dead cells and hence providing more false positive results (Biswas *et al*., 2008). The system is also requires testing of multiple primer sets, and thus are still time-consuming (Yang *et al*., 2015).

Biosensors: Biosensors are defined as an analytical device that integrates a biologically derived molecular recognition molecule such as antibodies, phages, aptamers or single-stranded DNA, with a suitable physicochemical transducing mechanism. Common transducing elements are optical, electrochemical, thermometric, piezoelectric, magnetic and others (Iqbal *et al*., 2000). Biosensors produce an electronic or optical signal proportional to the specific interaction between the analyte and the recognition molecule present on the biosensor (Turner, 2000).

Biosensors can detect a wide range of targets from small protein molecules to large pathogens. Compared to the conventional methods, a biosensor is a device for the detection of pathogenic antigens and does not require highly trained personnel for using it. Further, if a biosensor is highly sensitive and selective it can provide results more rapidly than culture-based methods making them ideal for practical and field applications (Sharma and Mutharasan, 2013).

Enzyme bioreceptors: Biosensors that make use of enzymes as the biorecognition elements are a well-developed and widely studied area. Enzymes are chosen based on their specific binding capability and their catalytic activity and the chosen enzyme with a suitable substrate should provide sufficient electron transfer to the working electrode (Vo-Dinh and Cullum, 2000).

In the field of pathogen detection, using enzymes as bioreceptors not only provides biosensors with a high degree of specificity, but their catalytic activity can amplify the pathogenic bacteria being detected and measured, allowing for sensitive analyses. In most of the cases enzymes are used to function as labels than the actual bioreceptor. Owing to the improvements in enzyme-labeling methods during the past decades, enzyme-labeled antigens and antibodies have been increasingly used. But there are some disadvantages found when using enzymes as labels, which include multiple assay steps and the possibility of interference from endogenous enzymes (Velusamy *et al*., 2010).

Nucleic acid bioreceptors: Recent advances in nucleic acid recognition have enhanced the power of DNA (deoxyribonucleic acid) biosensors and biochips. In the case of nucleic acid bioreceptors for pathogen detection, the identification of a target analyte's nucleic acid is achieved by matching the complementary base pairs that are often the genetic components of an organism. Since each organism has unique DNA sequences, any self-replicating microorganism can be easily identified. Biosensors based on nucleic acid as biorecognition element are simple, rapid, and inexpensive and hence it is widely used in pathogen detection. In contrast to enzyme or antibodies bioreceptors, nucleic acid recognition layers can be readily synthesized and regenerated. DNA damage is one of the most important factors to be considered when nucleic acid bioreceptor are used. Hundreds of compounds bind and interact with DNA. Detection of chemicals may cause irreversible damage to DNA by changing the structure of DNA and the base sequence, which in turn disturbs the DNA replication (Velusamy *et al*., 2010). Lermo *et al*., 2007) has reported nucleic acid based biosensor detection of *Salmone*lla spp. based on the specific IS200 element.

Using single-stranded nucleic acid aptamer, Kim invented a rapid method for specific identification and quantification of foodborne pathogens. The single-stranded nucleic acid aptamer specifically binding to a food-borne pathogen had a nucleotide selected sequence where in the food-borne pathogens were *E. coli*, *Salmonella* spp., *Listeria* spp. and *Staphylococcus* species (Kim, 2007).

Recent advances in nucleic acid recognition, like the introduction of peptide nucleic acid (PNA) has opened up exciting opportunities for DNA biosensors. PNA is a synthesized DNA in which the sugar–phosphate backbone is replaced with a pseudopeptide. PNA as a probe molecule has several advantages like superior hybridization characteristics, detection of single-base mismatches and improved chemical and enzymatic stability relative to nucleic acids. In addition, its different molecular structure enables new modes of label-free detection contributing significantly towards the establishment of rapid, stable and more reliable analytical processes. PNA based nucleic acid recognition have been reported by many researchers (Briones *et al*., 2004; Fan *et al*., 2007; Steichen *et al*., 2007).

Antibody bioreceptors: Antibodies are common bioreceptors used in biosensors. Antibodies may be polyclonal, monoclonal or recombinant, depending on their selective properties and the way they are synthesized. In any case, they are generally immobilized on a substrate, which can be the detector surface, its vicinity, or a carrier. The way in which an antigen and an antigen-specific antibody interact is similar to a lock and key fit (Vo-Dinh and Cullum, 2000).

An antigen-specific antibody fits its unique antigen in a highly specific manner, so that the three-dimensional structures of antigen and antibody molecules are matching. Due to this three-dimensional shape fitting, and the diversity inherent in individual antibody make-up, it is possible to find an antibody that can recognize and bind to any one of a large variety of molecular shapes. This unique property of antibodies is the key that makes the immunosensors a powerful analytical tool and their ability to recognize molecular structures allows one to develop antibodies that bind specifically to chemicals, biomolecules, microorganisms, etc. One can then use such antibodies as specific probes to recognize and bind to an analyte of interest that is present, even in extremely small amounts, within a large number of other chemical substances (Lazcka *et al*., 2007).

2.2.6. Treatment

Gastroenteritis caused by *Salmonella* is usually a self-limiting disease and diarrhea resolves within three to seven days and fever within seventy two hours (Braunwald *et al.,* 2005). Accordingly therapy should be directed primarily to the replacement of fluid and electrolyte losses. Therefore, antimicrobials should not be used routinely to treat uncomplicated NTS gastroenteritis or to reduce convalescent stool excretion. However, antimicrobial therapy should be considered for any systemic infection (Rupali *et al*., 2002). Antibiotic treatment usually is not recommended and in some studies has prolonged carriage of *Salmonella*. Neonates, the elderly, and the immune suppressed (e.g., HIV infected patients) with NTS gastroenteritis are especially susceptible to dehydration and dissemination and may require hospitalization and antibiotic therapy (Fuaci and Jameson, 2005).

Because of the increasing prevalence of antimicrobial resistance, empirical therapy for life threatening bacteremia or local infection suspected to be caused by NTS should include a third generation cephalosporin and a quinolone until susceptibility patterns are known. Amoxicillin and trimethoprim sulfamethoxazole are effective in eradication of long-term carriage. The high concentration of amoxicillin and quinolone in bile and the superior intracellular penetration of quinolone are theoretical advantages over trimethoprim-sulfamethoxazole (WHO, 2012).

2.2.7. Prevention and control

A periodic surveillance of the level of *Salmonella* contamination in the different food animals, food products and environment is necessary to control the spread of the pathogen and infection of man (Norrung and Buncic, 2009). The most serious meat safety issues affecting consumer health and triggering product recalls involve microbial and particularly bacterial pathogens (Sofos, 2008). Control of these pathogens at all stages of the farm-to-fork chain is vital to minimize the occurrence of food-borne disease in the human population (Norrung and Buncic, 2009).

Salmonellosis is the most wide spread foodborne and zoonotic problem throughout the world. Reducing *Salmonella* prevalence requires comprehensive control strategy in animals and animal food stuffs with restrictions on the infected flocks until they have been cleaned up from infections. In addition, mandatory testing before slaughter should be conducted like the one being implemented in Sweden (Boqvist and Vagsholm, 2005). The prevention of paratyphoid *Salmonella* infection which has greater public health consequences requires a comprehensive control strategy including regular monitoring, strict biosecurity and vaccination (Breytenbach *et al*., 2004).

Reservoirs for NTS organisms include a wide range of domestic and wild animals, such as cattle, poultry, swine, rodents, and pets like iguanas, turtles, dogs, cats, chicks, and ducklings. In humans infected with *Salmonella*, the excretion of bacteria can last throughout the course of infection and as a temporary carrier state for months. The mode of transmission may include ingestion of the organisms in food derived from infected animals or contaminated by feces of an infected animal or person. The source may be contaminated meat, poultry, eggs, milk, and their products, as well as water, fruits and vegetables. Preventive measures therefore should include the education of food handlers about hand hygiene, refrigerating foods in small portions, thoroughly cooking all foodstuffs, avoiding recontamination of cooked food, and maintaining a sanitary kitchen to prevent from contamination by rodents and insects (Varma *et al*., 2005). Safe food production requires knowledge on the nature and origin of the animals, animal feed, the health status of animals at the farm. It also needs knowledge on the use of veterinary medicinal products, the results of any analysis of the samples taken at the farm and slaughter data regarding ante-mortem and post-mortem findings and the risks associated with post-harvest production stages (Snijders and Knapen, 2002). No part of the food chain can be regarded alone but has to be seen as part of the whole. It must also include the consumers. Additional measures to control secondary contamination could be prevention of contamination by cleaning and disinfection, hygiene of personnel and proper processing (Nowak *et al*., 2006). Growth of microorganisms in meat and poultry products can be controlled by maintaining a cold temperature at 100C, especially for *Salmonella* during transport and storage (Coleman *et al*., 2003).

2.2.8. Antimicrobial Resistance of Salmonella

Antibiotic-resistant *Salmonella* infections of both human and animal are universal concerns, particularly in developing countries where the risk of infection is high because of unhygienic living conditions, close contact and sharing of houses between animals and humans and the traditions of consumption of raw or undercooked animal-origin food items (Feasey *et al*.,2012). There is an increasing concern with this pathogen due to the emergence and spread of antibiotic-resistant and potentially more pathogenic strains. If resistant microorganisms are common in animals, the chance that they will be transmitted to human beings is more likely (WHO, 2014).

Antimicrobial-resistant *Salmonella* spp. have been isolated from different foods of animal origin around the world, which is attributed to the inappropriate use of antimicrobials as therapeutic or prophylactic agents in human and veterinary medicine, as well as the use of growth promoters in animal production (WHO,2012). Antimicrobial resistance among NTS serotypes has been a serious problem worldwide. Other possibility for the emergence and spread of *Salmonella* strains resistant to antibiotics commonly used for treatment is, because this infection can be invasive and difficult to treat by the drugs of choice for invasive *Salmonella* disease (Mineau *et al*., 2018).

Salmonella strains resistant to various antimicrobial agents, particularly resistant to fluoroquinolones and third-generation cephalosporins, are considered as an emerging problem worldwide (WHO, 2014), resulting in higher morbidity and mortality rates and higher overall treatment costs. This may represent a public health risk by transfer of resistant *Salmonella* strains to human’s through the consumption of contaminated food and food products. However, the sources and transmission routes of *Salmonella* in developing countries are poorly understood due to the lack of coordinated national epidemiological surveillance systems (Käferstein, 2003).

2.2.9. Status of Salmonella in Ethiopia

Food-borne diseases are common in developing countries including Ethiopia because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment, and lack of education for food handlers (WHO, 2004). National Hygiene and Sanitation Strategy program (MoH, 2005) reported that in Ethiopia more than 250,000 children die every year from sanitation and hygiene related diseases and about 60% of the disease burden was related to poor hygiene and sanitation in Ethiopia. Unsafe sources, contaminated raw food items, improper food storage, poor personal hygiene during food preparation, inadequate cooling and reheating of food items and a prolonged time lapse between preparing and consuming food items were mentioned as contributing factors for outbreak of food-borne diseases (Linda du and Irma, 2005).

Studies conducted in different parts of the country showed the poor sanitary conditions of catering establishments and presence of pathogenic organisms like *Campylobacter*, *Salmonella*, *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* (Bayleyegn *et al*., 2003; Abera *et al*., 2008; Knife and Abera, 2007; Tefera *et al*., 2009 and Mekonnen *et al*., 2013). The incidence of foodborne *Salmonella* infections has increased dramatically in Ethiopia during the past few years. Studies conducted in different parts of the country have demonstrated the presence of *Salmonella* in human beings (Tadesse, 2014; Nyeleti *et al*., 2000) and in different food animals and food products (Nyeleti *et al*., 2000; Molla *et al*., 2003).

Of the foods intended for humans, those of animal origin tend to be most hazardous unless the principles of food hygiene are employed. Animal products such as meats, fish and their products are generally regarded as high-risk commodity in respect of pathogen contents, natural toxins and other possible contaminants and adulterants (Yousuf *et al*., 2008). Bacterial contamination of meat products is an unavoidable consequence of meat processing (Jones *et al*., 2008).

In Ethiopia, several factors including under and mal-nutrition, HIV-AIDS, the unhygienic living circumstances and the close relations between humans and animals may substantially contribute to the occurrence of Salmonellosis. Although surveillance and monitoring systems are not in place and its epidemiology is not described, qualitative and quantitative syntheses of previous studies could shed light on the occurrence of the disease and the major serotypes that frequently cause infections (Tadesse, 2014; Nyeleti *et al*., 2000). Even if data regarding meat borne diseases in Ethiopia are extremely scarce, a few studies conducted in different parts of the country have shown the public health importance of several bacterial pathogens associated with foods of animal origin (Bayleyegn *et al*., 2003; Ejeta *et al*., 2004: Hiko *et al*., 2008; Kumar *et al*., 2010 ; Tefera *et al*., 2009). NTS remains a leading etiological agent in bacterial foodborne diseases (Jones *et al*., 2008).

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted in Bishoftu Towns, Oromia Regional State, from November, 2018 to May, 2019. The Town is located at 45kms south east of the capital of Ethiopia, Addis Ababa. It is situated at latitude of about 9° north and a longitude of 4° east. The city lies at an altitude of 1850 meters above sea level in central high land of Ethiopia (Figure 1). The areas have three distinct seasons, namely main rain, short rain and dry seasons. Based on weather data, the mean annual rainfall of the area is 866 ml with mean minimum and maximum temperatures of 14^0C and 26^0C, respectively and with mean relatively humidity of 61.3%. Farmers in the vicinity of Bishoftu Town use a mixed crop and livestock farming system. Moreover, Bishoftu and its surroundings have variable and yet representative agro-ecologies of the country (NMSA, 2008).

According to the world population review of 2018, the population of Bishoftu town was 104,215. The Town is found in Ada'a District and surrounded by some Ada'a District peasant associations. Currently the woreda has an estimated 114,250 of cattle population (AWLF, 2018). The livestock production system in the area is both intensive and extensive type (CSA 2015).

Bishoftu Town has two abattoirs one owned by the Municipal and other owned by private company that directly supply beef to the Town. Private abattoir has intensive type of fattening farms and supply meat additionally to Addis Ababa, the capital city of the country. The abattoirs are slaughtering on average 15 heads of cattle per day from Monday to Friday. The numbers of cattle slaughtered at abattoirs could be increased by double during festivals or holidays. The sources of the cattle for abattoirs were from the surrounding local market of the Bishoftu Town, Adama Town and Arsi Zone. Rarely, cattle purchased from Borena pastoralist areas particularly for the private abattoirs for fattening farm based on abattoir office personal information. Cattle transported from market to abattoirs by vehicles from pastoralist areas and Adama cattle market.

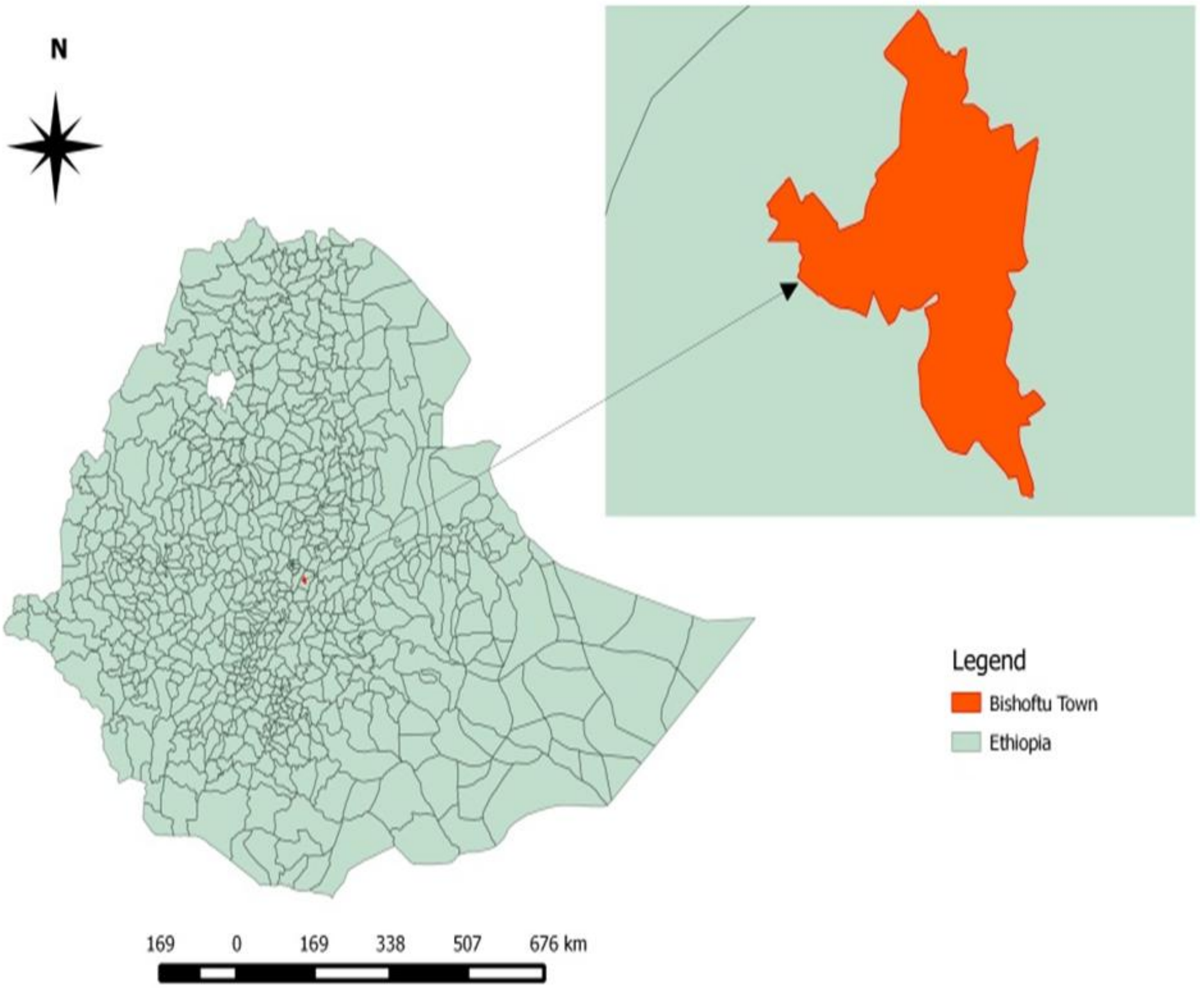

Figure 1: Map of the study area

3.2. Study design and study population

A cross-sectional study design was employed to assess the occurrence and determine antimicrobial resistance profile of *E. coli* O157 and NTS. Composite hide swabs, beef carcass swabs and feces samples were collected. The study population included apparently healthy beef cattle slaughtered at both abattoirs.

3.3. Sample collection and Transportation

Overall, the study conducted on a total of 70 apparently healthy slaughtered cattle from private and municipal abattoirs consisting 35 animals from each abattoirs. Totally 700 samples were examined consisting of composite hide swabs (n=140), carcass swab (n=420) and feces (n=140) samples.

During the entire study periods, a total of 14 visit 7 municipal and 7 private abattoirs were done one visit per week. On each visit, five (5) animals were conveniently selected, labeled and five (5) feces samples, five (5) composite hide samples and fifteen (15) separate beef carcass swab samples from medial parts of forelimbs, hind limbs and from brisket were collected for *E .coli* O157 and NTS isolation and recorded align with sample record sheet (Annex 1).

Composite hide swab samples were collected from medial parts of forelimbs, hind limbs and brisket of animals immediately after stunning from area of approximately (20 cm x20 cm) 400cm2. A sterile cotton swab fitted with forceps was soaked in 10 ml of buffered peptone water (Hamida Ltd., India) rubbed first horizontally and then vertically several times on the hide surface before skinning.

The carcass was tagged during hide removal so that the samples were matched from the hide through all the other processing steps. After skinning, beef carcass swab samples were collected aseptically from forelimbs brisket and hind limbs from the area of 400cm2 before evisceration using sterile cotton swab fitted with forceps socked in 10ml buffered peptone water (BPW) (Hamida Ltd., India) for hydration and rubbed as above.

Approximately 50 grams of fecal samples were collected using sterile arm length from the distal parts of gastrointestinal tracts (rectum) of slaughtered animals per each visit. The samples were labeled and transported using icebox containing ice pack to College of Veterinary Medicine, Addis Ababa University. Upon arrival, the samples were stored in a refrigerator at 4 °C until processing.

3.4. Isolation and identification

3.4.1. Isolation and identification of E. coli O157

Culturing: Isolation and identification of *E.coli* O157 was carried out in line with the guidelines of the International Organization for Standardization (ISO 6579: 2002). For isolation of *E. coli* O157 twenty five (25gm) of feces was weighed and transferred into a sterile stomacher bag which contains 225 ml of Modified trypton Soya broth (MTSB) (Oxoid Ltd., Basingstoke,UK) supplemented with 20 mg/l novobiocin. The mixture homogenized using stomacher (Seward) for 2 minutes. For processing of hide and carcass swabs 10 ml extra buffered peptone water were added in the laboratory on top of 10 ml transport media in to sterilized plastic bag that reaches to final volume of 20 ml BPW (Hamida Ltd., India). In addition 20 ml double concentration of modified trypton soy broth (DMTS) (Oxoid Ltd., Hampshire, UK) was added. Both pre enriched fecal, hide and carcass swab mixture was incubated at 41.5^0C for 24 hours.

One (1) ml pre enriched overnight mixture from each sample was taken and transferred to epindroff tube containing 20µl Dynabeads TM anti-*E. Coli* O157 (Thermo Fisher, Scientific), mixed and subjected to immunomagnetic separation (IMS) for 30 minutes. The mixed samples were washed 3 times using sterile saline water. 100µl washed samples were taken and inoculated onto Sorbitol MacConkey agar (Oxoid Ltd., Hampshire, UK) containing 0.05 mg/l cefixime and 2.5 mg /l potassium tellurite (CTSMAC; Dynal Biotech ASA) (Figure 5).

All the inoculated plates were incubated at 37 °C for 24 hours and the plates were examined for the presence of non-sorbitol fermenting pale colonies. The suspected colonies were picked, sub-cultured and subjected for further confirmation such as biochemical test and latex agglutination test.

Biochemical tests: For the confirmation of bacteria by biochemical tests, suspected colonies of *E. coli* O157 were selected from the selective plating media, streaked onto the surface of tryptone soaya agar (TSA) (Accumix Microxpress, Ltd., India) plates and incubated at 37 °C for 24 hours. *E. coli* O157 suspected colonies picked from tryptosaya agar and inoculated to killgler iron (KIA) agar slant (Hamida Ltd., India) and tryptophan broth (indole test) was incubated for 24 hours at 37 °C. The colony considered positive if acid butt and acid slant (yellow) on killgler iron (KIA) agar and production of indole in tryptophan broth indicate the presence of *E. coli* O157 with formation of pink red rings up on addition of Kovac regents (Hamida Ltd., India). The KIA and indole tests were conducted according to Quinn *et al* (2002). The bacteriological media used for the study were prepared following the instructions of the manufacturers (Annex 5).

Latex Agglutination test: Rapid Latex Test kit is a rapid latex agglutination test intended for confirmatory identification of *E. coli* serogroup O157 (Non-Sorbiotl Fermenting isolates). This test allows the rapid differentiation of *E. coli* O157 from other *E. coli* serotypes (OXOID, Hampshire, UK). The test was conducted by adding one drop of latex suspension and dispensing near the edge of the circle on the reaction card. Then a portion of a typical 2 to 5 colony to be tested was emulsified using a loop in a drop of sterile saline solution near the drop of test latex on the test card. After ensuring a smooth suspension of the bacteria and saline, the test latex was mixed with the suspension and spread to cover the reaction area over the loop. Then, the card was rocked in a circular motion for one minute and examined for agglutination by naked eye. Agglutination of the test latex within one minute was considered as positive result (Figure 7).

3.4.2. Isolation and identification of Non-Typhoidal Salmonella

Culturing (Primary enrichment): NTS isolation and identification from food also carried out in line with the guide lines of the International Organization for Standardization (ISO 6579: 2002). Twenty five (25gm) of fecal samples were weighed and transferred to sterile stomacher bags and mixed with 225 ml nonselective pre-enrichment buffered peptone water (Hamida Ltd., India) at 1:9 ratio. The mixture was homogenized using a Stomacher (Seward) for 2 minutes. For processing of hide and carcass swabs 30 ml extra buffered peptone water were added in the laboratory on top of 10 ml transport media in to sterilized plastic bag that reaches to final volume of 40 ml BPW (Hamida Ltd., India). Both pre enriched fecal, hide and carcass swab mixture was incubated at 37^0C for 24 hours.

Secondary enrichment: From overnight incubated buffered peptone water, 0.1ml mixture from feces, hide and carcass swabs were taken and again further enriched in to secondary selective enrichment semi solid Modified Rappaport Vasilidus (MSRV; Oxoid, Basingstoke, UK) and incubated at 41.5^0C for 24 hours. If there is color change on the media from green to grey color it is suspected as NTS positive and further inoculated on selective plating media (Figure 3).

After 24 hours diffused grey colors on a semi solid Modified Rappaport Vasilidus a plate were examined and selected for sub culturing. A loop full of bacteria taken from the edge of diffused zone and streaked onto Xylose Lysine Deoxycholate agar plate (XLD) (Accumi, Microxpress, Ltd., India).The plate were incubated at 37 ^{0}C for 24 hours and examined for the presence of red colonies with black centers on XLD agar that indicates growth of NTS. The suspected colony sub cultured and kept for further confirmation (Figure 3).

Biochemical tests: Colonies suspected to be NTS were picked from tryptosaya agar (TSA) and inoculated to triple sugar iron (TSI) agar slants (OXOID, Basingstoke, England), L-lysine decarboxylation medium (DIFCO, Becton, Dicknson, USA) and tryptophan broth and incubated for 24 hours at 37 °C. A Colony was considered NTS if an alkaline slant (Red), with acid butt (yellow) on TSI with hydrogen sulfide production, positive for lysine (purple) color formation, negative for tryptophan utilization or indole production (yellow-brown ring) up on addition of Kovac reagents. TSI test, L-lysine decarboxylase test, and indole test were conducted according to (ISO 6579: 2002) (Annex 7).

3.5. Antimicrobial susceptibility

The antimicrobial susceptibility testing of the isolates of *E. coli* O157:H7 and NTS was done for 12 antimicrobial agents (Oxoid OR TM media, New Delhi, India) using the disc diffusion method according to the criteria of the National Committee for Clinical Laboratory Standards (NCCLS) (NCCLS, 2013). Pure bacterial colonies were inoculated in to non-selective trypton soya agar (Accumix Microxpress, Ltd., India) and incubated at 37^{O}C overnight. Colonies from the trypton soya agar were transferred into tubes containing saline suspension and inoculum visually compared with 0.5 McFarland turbidity standards. A sterile cotton swab was used to inoculum uniformly over the surface of Muller Hinton agar plate (HiMedia, Pvt. Ltd., India). The plates were held at room temperature for 30 min to allow drying with subsequent application of antibiotic disc and incubation at 37^{O}C for overnight. Clear zones of bacterial growth inhibition were measured in mm using a measuring caliper (Figure 8).

The zone of inhibition results were interpreted according to Clinical and Laboratory standards Institutes (CLIS, 2018) guideline. The list of panel of antimicrobial utilized, their symbols and concentrations and break points are shown in (Annex 4).

3.6. Data management and analysis

Data entry for describing the presence of NTS and *E. coli* O157 were done using Microsoft Excel® 2007. The data was analyzed using STATA version 12 computer software. Descriptive statistics such as percentages and frequency distribution were used to describe/present bacterial isolates and antimicrobial susceptibility. Chi square test (χ^2) was used to assess the difference in isolation rate or occurrences between sample types and abattoirs. The significance level was set at 0.05 level and Occurrence of NTS and *E. coli* O157:H7 were calculated as a percentage of NTS and *E .coli* O157:H7 culture-positive among the total number of samples examined.

4. RESULTS

4.1. Occurrences of non-typhoidal *Salmonella* and *E. coli* O157

Of the total samples (n=700) examined *E. coli* O157 and NTS were found in an overall occurrences of 4.29% and 4.57% of the samples, respectively. Table (3) summarized the occurrences of *E. coli* O157 and NTS species in different samples types and sources.

Table 3: Occurrence of *E. coli* O157 and NTS in cattle feces, hide and carcass

Slaughter house	**Sample type**	**Number of sample**	***E. coli* Positive**	**%**	***Salmonella* Positive**	**%**
Private Abattoir	Hide swab	70	1	2.85	4	11.4
	Carcass swab	210	3	2.85	3	2.85
	Feces	70	5	14.28	1	2.85
Total		**350**	**9**	**5.14**	**8**	**4.57**
Municipal Abattoir	Hide swab	70	2	5.71	1	2.85
	Carcass swab	210	2	1.9	3	2.9
	Feces	70	2	5.71	4	14.3
Total		**350**	**6**	**3.42**	**8**	**4.57**
Overall Total		**700**	**15**	**4.29**	**16**	**4.57**

The study indicated that there was no statically significant difference in occurrences of *E. coli* O157:H7 and NTS (χ^2= 11.0282, P= 0.051) and (χ^2 = 9.4311, P= 0.093) between three samples types as shown in Table (4).

Table 4: Occurrence and difference of NTS and *E. coli* O157 between sample types

	Non-typhoidal *Salmonella*				***E. coli* O157**			
Sample Types	Observation	Positive	Chi2 value χ^2	P-value	Observation	Positive	Chi2 value χ^2	P-value
Feces	70	6			70	7		
Hide swab	70	4	9.43	0.093	70	3	11.02	0.051
Beef carcasses	210	6			210	5		
Total	**350**	**16**			**350**	**15**		

There was no significant difference in the frequency of occurrence of NTS (χ^2 =0.63; P=0.43) as well as in the frequency of occurrence of *E. coli* O157 (χ^2 = 0.000; P= 1.000) between the private and municipal abattoirs as shown in Table 5.

Table 5: Frequency of NTS and *E. coli* O157 recovery between abattoirs

	Non-typhoidal *Salmonella*				***E.coli* O157**			
Slaughters Houses	Observation	Positive	Chi2 value χ^2	P-value	Observation	Positive	Chi2 value χ^2	P-value
Municipal	175	8	0.0000	1.000	175	6	0.6269	0.429
Private	175	8			175	9		
Total	**350**	**16**			**350**	**15**		

4.2. Antimicrobial susceptibility test

Sixteen (16) NTS and fifteen (15) *E. coli* O157 isolates were subjected to 12 commercially available antimicrobial agents as summarized in Table 6 and 7 using disk diffusion methods. The results were classified into resistant, intermediate, or susceptible. All isolates of NTS and *E. coli* O157 were resistant to Cephalothin (CEP), Cloxacillin (CX) and Ampicillin (AMP). All E. *coli* O157 and NTS were susceptible to Ciprofloxacin (CIP). Moreover; all NTS isolates were resistant to Streptomycin (S) while 93.3% of *E. coli* O157 isolates were resistant to Streptomycin.

Table 6:-Susceptibility pattern of all NTS isolates each antimicrobial

No	Sample Types	Antimicrobials used for testing isolates												Frequenc (%) resistance
		CEP	**CX**	**TE**	**TR**	**C**	**AMP**	**K**	**R**	**CIP**	**GEN**	**S**	**PB**	
1	Feces	R	R	R	S	R	R	R	R	S	I	R	R	9(75)
2	Feces	R	R	R	S	R	R	R	R	S	I	R	R	9(75)
3	Feces	R	R	R	S	R	R	I	R	S	S	R	R	8 (66.67)
4	Feces	R	R	R	S	R	R	I	R	S	S	R	R	8(66.67)
5	Hide	R	R	I	S	S	R	I	R	S	S	R	S	5(41.67)
6	Hide	R	R	S	S	S	R	I	R	S	I	R	R	6 (50)
7	Hide	R	R	S	S	R	R	I	R	S	S	R	R	7(58.33)
8	Hide	R	R	S	S	S	R	I	R	S	S	R	R	6 (50)
9	Feces	R	R	I	S	R	R	R	R	S	I	R	S	7(58.33)
10	carcass	R	R	R	S	S	R	S	R	S	S	R	R	7(58.33)
11	Carcass	R	R	R	S	R	R	R	R	S	I	R	R	9(75)
12	Carcass	R	R	R	S	R	R	I	R	S	S	R	R	8 (66.67)
13	Carcass	R	R	S	S	S	R	R	R	S	S	R	R	7(58.33)
14	Carcass	R	R	I	S	S	R	I	R	S	S	R	R	6 (50)
15	Hide	R	R	R	S	R	R	R	R	S	S	R	R	9(75)
16	Carcass	R	R	I	S	S	R	R	R	S	I	R	R	7(58.33)
Total Resistant		16	16	8	0	9	16	7	16	0	0	16	14	
% resistant		100	100	50	0	56.2	100	43.75	100	0	0	100	87.5	

CEP = Cephalothin, CX= Cloxacillin, TE= Tetracycline, TR= Trimethoprim, C= Chloramphenicol, AMP= Ampicillin, K= Kanamycin, R= Rifampicin, CIP= Ciprofloxacin, GEN= Gentamicin, S= Streptomycin, PB= Polymyxin-B

Table 7: Susceptibility pattern of all *E. coli* O157 to each antimicrobial tested

Code	**Sample source**	**Antimicrobials used for testing isolates**												**Frequenc (%) resistanc**
		CEP	**CX**	**TE**	**TR**	**C**	**AMP**	**K**	**R**	**CIP**	**GEN**	**S**	**PB**	
1	Hide	R	R	S	S	S	R	R	S	S	S	R	R	6 (50)
2	Hide	R	R	R	R	I	R	R	R	S	S	R	R	9 (75)
3	Feces	R	R	S	S	S	R	I	I	S	S	R	R	5 (41.67)
4	Feces	R	R	S	S	S	R	S	S	S	S	R	S	4(33.33)
5	Feces	R	R	S	R	S	R	S	S	S	S	R	R	6 (50)
6	Feces	R	R	S	S	S	R	I	S	S	S	R	R	5 (41.67)
7	Feces	R	R	R	R	I	R	R	R	S	S	R	S	8 (66.67)
8	Carcass	R	R	S	S	S	R	I	S	S	S	R	R	5 (41.67)
9	Carcass	R	R	S	S	S	R	I	S	S	S	R	R	5 (41.67)
10	Carcass	R	R	S	R	S	R	S	S	S	S	I	R	5 (41.67)
11	Hide	R	R	R	S	I	R	S	R	S	S	R	R	7 (58.33)
12	Feces	R	R	R	S	I	R	S	R	S	S	R	R	7 (58.33)
13	Carcass	R	R	R	S	R	R	R	R	S	S	R	R	9(75)
14	Feces	R	R	R	S	I	R	S	R	S	S	R	R	7 (58.33)
15	Carcass	R	R	I	S	I	R	S	R	S	S	R	S	5 (41.67)
Total Resistant		15	15	6	4	1	15	4	7	0	0	14	12	
% of resistance		100	100	40	26.7	6.7	100	26.7	46.7	0	0	93.3	80	

CEP = Cephalothin, CX= Cloxacillin, TE= Tetracycline, TR= Trimethoprim, C= Chloramphenicol, AMP= Ampicillin, K= Kanamycin, R= Rifampicin, CIP= Ciprofloxacin, GEN= Gentamicin, S= Streptomycin, PB= Polymyxin-B

According to the definition of Magiorakos *et al*. (2012) Multi-drug resistance (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. Moreover; in this study, multi-drug resistance isolated in (6.67%) 1/15, (33.33%) 5/15 and (40%) 6/15 for four, five and six or more antimicrobials drug for *E. Coli* O157, respectively. Similarly; (6.25%) 1/16, (18.75%) 3/16 and (43.5%) 7/16 for five, six and seven or more antimicrobials drug for NTS, respectively. The result was shown in Table 8**.**

Table 8: Multi drug resistance pattern of *E .coli* O157 and NTS

***E. coli* O157**		**Non-typhoidal *Salmonella***	
Antimicrobials tested (number of isolate)	**No of resistant isolates**	**Antimicrobials tested (number of isolate)**	**No of resistant isolates**
CEP, CX, S, AMP (4)	1	CEP, CX, AMP , R, S (5)	1
CEP, CX, AMP, S, PB (5)	3	CEP, CX, AMP , R, S, PB (6)	3
CEP, CX, AMP, TR, PB (5)	1	CEP,CX, AMP, K,R, S,PB (7)	2
CEP, CX, AMP, R, S (5)	1	CEP, CX, AMP, K, R, S, PB (7)	2
CEP, CX, TE, TR, AMP, S (6)	1	CEP,CX,TE,AMP,R,S , PB (7)	1
CEP, CX, TR, AMP, S, PB (6)	1	CEP, CX, AMP , C, R,S,PB (7)	1
CEP, CX, AMP, K, S, PB (6)	1	CEP, CX, AMP , C, K, R, S (7)	1
CEP, CX, TE, AMP, R, S (6)	3	CEP, CX, TE, C, AMP , R, S, PB (8)	3
CEP, CX, TE, TR, AMP, S, PB (7)	1	CEP, CX, TE, C, AMP , K, R, S, PB (9)	4
CEP, CX, TE, AMP, C, S, PB (7)	1		
CEP, CX, TE, TR, AMP, K, R, S, PB (9)	1		

5. DISCUSSION

5.1. Occurrences of *E .coli* O157 and Non -Typhoidal *Salmonella*

E. coli O157 and NTS are among the most significant food-borne pathogens. Human infections of *E. coli* O157 and NTS have mostly been recognized to be originated from animal foods. Cattle are the major reservoirs and sources of both bacteria for human (Barkocy-gallagher *et al.,* 2002; Lim, *et al.,* 2010). In Ethiopia consumption of raw meat or minced meat is the common habits serve means of transmission of the pathogens resulting public health problems (Ejeta *et al*., 2004; Biffa and Skjerve, 2010 ;Thomas *et al.,* 2015).

The current study revealed the occurrence of E. coli O157 and NTS in beef carcass swab, feces and hide swab samples of apparently healthy slaughtered animals. The overall occurrence of E. coli O157 and NTS was 4.29% (15/350) and 4.57% (16/350), respectively.

5.1.1. E. coli O157

In the present study there was occurrence of *E. coli* O157 in feces, hide and carcass swabs. Cattle have been identified as a major reservoir of E. coli in their feces (McEvoy et al., 2003). Higher (10%) prevalence was recovered in feces as compared to other studies reported, 4.7% by Atnafie *et al* (2017); 2% by Abdissa *et al* (2017); 7.26% by Nazareth (2017) and 5.9% by Barkocy-Gallagher *et al* (2003). This variation may be occurs due to sanitation differences among abattoirs, animal husbandry practices and ecology of area.

Studies conducted outside Ethiopia reported less prevalence by Ateba *et al* (2008) and Rhoades and Koutsoumanis (2009) from feces but, higher prevalence also reported by Iweriebor *et al* (2015). This may be due to large sample size, husbandry practice and technique used for detection. For this study, the type of samples taken: hide, fecal or meat also showed variations in prevalence with feces showing the higher rates followed by hide and carcass comparable with finding by (Barkocy-Gallagher *et al*., 2003).

The current study reveals that 4.29% (3/70) of *E. coli* O157 from hide swabs that indicated hide is the potential sources in contamination of beef carcass during removals of hide and slaughtering process. Hide of cattle is known to be a source for the microbial contamination of beef, where microorganisms transferred onto the carcass from the hide, during the slaughter and dressing processes (Reid *et al*., 2002). The finding shows high prevalence as compared to similar study reported 0.54% in Ethiopia by Abdissa *et al* (2017). Compared to studies reported from other countries, the present findings shows less occurrences of *E. coli* O157 from hide, reported by Narvaez-bravo *et al* (2012) from Mexico, Schmidt et al (2012) from USA; Bosilevac *et al* (2009), Barkocy-Gallagher *et al* (2003) and Rhoades and Koutsoumanis (2009) 11.7%, 15.8%, 3.6%, 60.6% and 7.3–76%, respectively. This variation in prevalence may occurs due to large number of sample size, abattoir management system, lairage related situations, state of animals and sessional variation in occurrences of bacteria. The swabbing area may also attribute for the variation in prevalence. In this study, brisket, for limbs and hind limbs were considered for hide swabbing as the area was prone to contamination and contacts with feces.

There was occurrence of *E. coli* O157 from carcass swab of hind limbs, brisket and fore limbs at different rates, with high recovery of bacteria from hind limb and followed by brisket and no isolation from for limbs. The high recovery of bacteria from hind limbs suggest, the carcass was contaminated by leftovers anal surfaces feces particularly around the hock joint, by knife and contaminated hands as the carcass was manually pushed in the slaughter process. This finding was partly agree with McEvoy *et al* (2000) that reported high total viable counts from hock and brisket of carcasses swabs. Similarly, 2.8% recovery of *E .coli* O157:H7 from meat and meat products was reported by Abong'o and Momba (2009) from South Africa that was slightly comparable with the present findings.

Less prevalence of *E. coli* O157 as compared to other studies reported in different parts of Ethiopia by different researchers such as by (Hiko, 2008; Taye *et al.,* 2013; Bekele *et al.,* 2014; Beyi *et al.*, 2017 and Bedasa *et al.*, 2018) with the prevalence of 8%, 2.7% 13.3%, 4.5% and 3.07% from carcass swabs, respectively. This variation might occurs due to different ecological area, different husbandry practice and abattoirs sanitation. In contrary to this, less prevalence was reported by Mengistu *et al* (2017) and Abdissa *et al* (2017) with prevalence of 2.06% and 0.54% respectively. This is due to the difference in sampling techniques carried by Abdissa *et al* (2017). For the case of Mengistu *et al* (2017) the variation may be due to laboratory procedure used for isolation of bacteria where Immuno magnetic separation (IMS) beads not used. In our case the carcass swabs taken from external parts where directly exposed to contacts by hands and knife. Different studies from African countries and others reported that higher prevalence *E. coli* O157:H7 as compared to the present findings, Gun *et al* (2003) reported 3.6% from beef carcass at abattoirs in Istambul and Ahmed and Shimamoto (2014) reported 3.4% from Egypt. Contrary to this, Less 1.2% prevalence also reported by Rhoades and Koutsoumanis (2009) from raw beef products. This variation may occur due to ecological difference and techniques used for identification of the bacteria.

Higher prevalence of *E. coli* O157:H7 reported from USA by Arthur *et al* (2008) and Barkocy-Gallagher *et al* (2003) from carcass before the pre evisceration wash where inconsistent with the present finding that may be due to large number of sample size. Moreover; higher (8.4%) prevalence also reported from Italy by Nobili *et al* (2017) from raw beef at retail market. This difference in the prevalence may occur due to contamination of meat during transportation starting from abattoirs to retail market.

Possible contamination of edible carcass tissue is the most significant challenge to food safety and the extent and nature of such contamination are related to *E. coli* O157:H7 status of the preslaughter animal, and any processes which distribute the organism within or between carcasses during dressing operations (McEvoy *et al.*, 2003). In this study findings, occurrence of *E. coli* O157 in beef carcasses leaving the abattoirs is observed low as compared to other types of samples investigated however, this should not be underestimated since this bacterium has been a cause of large food borne infections to human.

5.1.2. Non-Tyhoidal Salmonella

The current study revealed the occurrence of NTS in beef carcass swab, feces and hide swab samples of apparently healthy slaughtered animals. The overall occurrence of NTS was 4.57% (16/350). The results showed the presence of NTS was distributed in all samples types with various occurrences ranges. The contamination of beef carcasses with NTS causes human infections at low occurrences as raw or undercooked beef widely consumed in Ethiopia.

In the present study, occurrences of NTS in feces was slightly less than the study conducted by Beyene *et al* (2016) from Asella Ethiopia who was reported 8.5% prevalence of NTS from abattoirs. Less occurrences of NTS was reported by a few researchers in Ethiopia from feces by Kore *et al* (2017) and Eguale *et al* (2016) as compared to present study findings with prevalence of 2.7% and 2.3 %, respectively. Moreover; outside Ethiopia less prevalence of NTS was reported by Barkocy-Gallagher *et al* (2003) and Nazareth (2017) from feces of the slaughtered animals. This was due to ecological differences, abattoir management, animal's husbandry and confirmatory test. High occurrence of NTS reported from Ethiopia and others by Sibahat *et al* (2011), Addis *et al* (2011) and Narváez-Bravo *et al* (2013) from cattle feces. This high number of variation may occur due to high contamination of farms by NTS and different husbandry practices.

Low (7.14%) prevalence of NTS from hide recovered compared with Sibahat *et al* (2011) who reported 31%. This might be due to sample size. Moreover; from other regions outside Ethiopia indicated that high occurrences of NTS from hide of slaughter animals at abattoirs by Narvaez-bravo *et al* (2012), Nazareth (2017) and Madoroba *et al* (2016). The present finding was inconsistent may be due to techniques used for detection, geographic differences and husbandry practices. In contrary to this higher prevalence reported by from USA by Bacon *et al* (2002) Bosilevac *et al* (2009) and Schmidt *et al* (2012). . This variation may occur due to large number of size and technique used for detection. Hide of cattle is known to be a source for the microbial contamination of beef, with microorganisms transferred onto the carcass from the hide, during the slaughter and dressing processes (Reid, 2002; Barkocy-Gallagher *et al.*, 2003).

The occurrence of NTS from carcasses in the present study was found to be 2.86% (6/210). Occurrence of NTS isolate from different swabs area in the rate of 4.28 % (3/70) from hind leg, 2.86% (2/70) from brisket and 1.43% (1/70) from for limbs. The high recovery of bacteria from hind leg that suggest the carcass is contaminated by leftovers anal surfaces feces by knife and contaminated hands as the carcass was manually pushed in the slaughter process. This finding was partly agree with McEvoy *et al* (2000) that reported high total viable counts from hock and brisket of carcasses swabs.

The present finding result was lower than previously reported studies elsewhere in Ethiopia by different researchers (Molla *et al.*, 2003; Muluneh and Kibret, 2015; Tadesse and Gebremedhin 2015; Wabeto *et al.*, 2017) with the reported prevalence of 4.2%, 7.6% 4.53% and 12.5%, respectively. The lower prevalence may be as the result of different sampling regimes and techniques used in the various studies, differences in the hygiene of dressing operations or geographical variation in the incidence of NTS. Similar study in Ethiopia by Mengistu *et al* (2017) reported 2.75% NTS from Slaughter a house that was comparable with the present findings. But, Sibahat *et al* (2011) reported 2% of NTS occurrences from carcass a swab which was slightly lower than the present findings results this might be due to techniques used for isolation, method of sampling and geographical location.

NTS occurrence in carcass of slaughtered cattle observed in this study was generally lower when compared to previous reports from different regions of the county (McEvoy *et al.*, 2003; Small *et al.*, 2006; Arthur *et al.*, 2008; Bosilevac *et al.*, 2009; Rhoades and

Koutsoumanis, 2009; Narvaez-bravo *et al*., 2013; Ahmed and Shimamoto 2014; Igbinosa, 2015; Xie *et al*., 2015 Ibrahim, 2016). This was due to large sample size and extended period of study time. In the present finding the recovery proportion of NTS in beef cattle slaughtered in abattoirs was higher than 1.3% and 1.5 % reported by (Bacon *et al*., 2002) and (Terentjeva *et al*., 2017) from carcass surfaces and minced meat and meat preparations.

Previous studies show that transfer of NTS from hide to beef carcass during slaughtering process in abattoirs (Stolle 1981; Puyalto *et al*., 1997). Moreover; carcass contamination is not merely the results of feces or hide but also may be resulted from cross contamination from adjacent positive carcasses on the dressing line in abattoirs.

5.2. Antimicrobial resistance

E. coli O157 isolates were 100% resistance to Cloxacillin, Cephalothin and Ampicillin, where as 93.3% and 80 % was observed in Streptomycin and Polymyxin-B, respectively. Moreover; *E. coli* O157 isolates were highly sensitive to Ciprofloxacin, Chloramphenicol and Gentamicin from 15 isolates, 15(100%) were susceptible. This is in agreement with the previous study in Ethiopia by Atnafie *et al* (2017) and Bedasa *et al* (2018). The isolates recovered from carcass swab, hide swab and feces were susceptible to antimicrobials at different rates to Tetracycline, Trimethoprim, Chloramphenicol, Kanamycin, Rifampicin, Streptomycin and Polymyxin-B. This is in agreement with Bekele *et al* (2014).

Multi-drug resistance was observed in 75% of the isolates from different samples types for each antimicrobial tested. The highest 75% (9/12) prevalence of resistance from hide and carcasses observed. This may suggest that contamination of bacterial isolates from feces or hide to beef carcass. Antimicrobial resistance emerges from the use of antimicrobials in animals and human, and the subsequent transfer of resistance genes and bacteria among animals, humans, animal products and the environment (McEwen *et al*., 2002). In Ethiopia different study showed that drug resistance of *E .coli* O157 from animal's food products (Hiko *et al*., 2008; Bekele *et al*., 2104; Taye *et al.,* 2013).The current study showed that *E. coli* O157 isolates were highly sensitive to ciprofloxacin and Gentamicin and helps for suggesting the use of this antibiotics.

NTS isolates were (100%) resistance to Cloxacillin, Cephalothin Rifampicin, Ampicillin and Streptomycin whereas 87.5% resistance to Polymyxin-B which are in agreement with (MCevoy *et al*., 2003). This could be due to misuse and availability of the antimicrobial agents in the hands of nonprofessionals. Moreover; NTS isolates were highly sensitive to trimethoprim, ciprofloxacin and Gentamicin, from 16 isolates, 93% were susceptible to trimethoprim, ciprofloxacin and 63% were susceptible to Gentamicin which is in agreement with study reported from Bahir Dar Ethopia by Alemu and Zewde (2012). This might be due to recent introduction of the drug or might not widely used for treatments.

Multi-drug resistance was observed in 68.75% of the isolates of NTS. This Multidrug resistant isolated from different samples sources and types for each antimicrobial tested. The proportion is almost the same from different sources and types of origin for NTS isolates. The highest 75% (9/12) prevalence of antimicrobial resistance to different drug was observed among isolates from feces and carcass. In Ethiopia different study showed that drug resistance of NTS from animal's food products (Bedasa *et al*., 2018; Eguale *et al*., 2015, Tadesse, 2014; Addis *et al*., 2011). The result indicates that Ciprofloxacin, trimethoprim and Gentamicin are the drugs of choice for NTS treatment, as the entire drug shows no resistant. This finding helps for suggesting the use of these antibiotics. Cattle have been the known source of human infection through direct contact with livestock and livestock products, from which antibiotic resistant NTS and *E .coli* have been consistently isolated (Anthony, *et al*., 2010).

6. CONCLUSION AND RECOMMENDATION

Contamination of beef carcass by feces can occurs during slaughtering process at abattoirs; however an alternative source, such as the hide or adjacent carcasses on the line, may also contribute to carcass contamination.

The present study showed considerable occurrence of *E. coli* O157 and NTS species in both municipal and private abattoirs from slaughtered animals samples including of cattle feces, hide swabs and beef carcass swabs. The isolates that subjected to antibiotic sensitivity test also showed different degrees of resistance indicating development of antimicrobial resistance to commonly used drugs for both human and veterinary medicine. Contamination in beef carcass with organism's suggest, that consumption of meat could be a potential risk of meat borne diseases in the study area.

In line with the above conclusion, the following recommendations were forwarded:-

- Applying food safety procedures like Hazard analysis and critical control point (HACCP) to reduce introduction of pathogen in meat during slaughtering operation.
- Integrated control strategies to prevent contamination of carcass at abattoir should be implemented.
- Training of abattoir workers on hygienic handling of carcass in the slaughtering process.
- Rational use of drug encouraged to reduce antibiotic resistance like appropriate and enforced regulation, public education about medicines and establishment of a multidisciplinary national body to coordinate policies on medicine use.

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8. ANNEXES

Annex 1: Sample record sheet for *Salmonella* and *E. coli* O157:H7

No	**Samples Type**	**Collection site**	**Date on culture BPW/MTSB**	**Presumptive Results**	**Remarks**
1					
2					
3					
4					
5					
6					

Annex 2: Biochemical test Results Record sheet for Non-Typhiodal *Salmonella*

Code	**Tests**	**Glucose**	**Lactose/ Sucrose**	**Gas**	**H_2S**	**Indole**	**Lysine**	**Id**	**Remarks**
	***Salmonella* spp**	+	-	+	+	-	+	+	
1									
2									
3									

Annex 3: Biochemical test Results Record sheet for *E. coli* O157:H7

Code	**Tests**	**Glucose**	**Lactose**	**Gas**	**H_2S**	**Indole**	**Lysine**	**Id**	**Remarks**
	***E. Coli* O157:H7 spp**	+	-	+	-	+	+	+	
1									
2									
3									
4									

Annex 4 : CLSI breakpoints for Enterobacteriaceae available for these antimicrobial

№ Antibiotic disks	Disc code	Concentration in (µg)	Diameter of zone of inhibition in millimeter (mm)		
			Resistant ≤	**Intermediate**	**Susceptible ≥**
Gentamicin	GEN	10	12	13-14	15
Tetracycline	TET	30	11	12-14	15
Streptomycin	S	10	11	12-14	15
Vancomycin	VA	30	9	10-11	12
Ciprofloxacin	CIP	5	15	16-20	21
Cloxacillin	CX	5	-	-	-
Ampicillin	AMP	10	13	14-16	17
Kanamycin	K	30	13	14-17	18
Trimethoprim	TR	5	10	11-15	16
Rifampicin	R	5	14		14
Chloramphenicol	C	30	12	13-17	18
Polymyxin-B	PB	300	15	-	18
Cephalothin	GEP	30	14	15-17	18

Annex 5: Types and preparation of media used for isolation, biochemical test and antimicrobial test.

1. **Buffered peptone water (M14941-500G, LBS Mumbai, India**

Preparation: suspend 20grms of composition in 1000 ml of distilled water, mix well and distribute in to universal bottle of suitable capacity to obtain the portion necessary for the test and sterilize in in the autoclave at 1210C for 15 mints. Final PH is 7 + 0.2 at 250C.

Composition (g/l): Enzymztic digest of casein 10.00g; sodium chloride 5g; Disodium hydrogen phosphate.12 H_2O 9g and potassium dihydrogen phosphate 1.5g.

2. **Modified Tryptone Soya Broth (CM0989-500G) (oxoid)**

Preparation: suspend 16.5 grams of hydrate medium in to 500ml distilled water. Mix well and sterilize by autoclaving at 121 0C for 15 minutes. Cool to approximately 50^0C and aseptically add one vial of Novobiocine supplement (SR181E) reconstituted as directed. Mix well and aseptically distribute in to sterile containers.
Composition (g/l): pancreatic digest of casein 17.0g; papaic digest of soya meal 3.0g; D (+)-glucose 2.5g; Bile salts; No. 3 1.5g; Sodium chloride 5.0g; Di-Potassium Hydrogen phosphate(K2 HPO4) 4.00g and Water; 1000ml.

3. **Modified Rappaport Vassilidus (semi-solid) *Salmonella* enrichment Broth (MH 491-500G, HAMED, Mumbai, India).**

Preparation: suspend 31.5 grams of hydrate medium in 1000ml distilled water. Allow to soak for 10 minutes. Then bring to boil with constant swirling. Cool to 47 o C and add to 2 vials or X 150. Pour in to sterile Petri dishes ensuring mixing between each dish. So the medium does not settle .once poured do not move whilst setting
Composition (g/l): Tryptone 2.3; Meat peptone 2.3; Acid Hydrolysed casin 4.65; Sodium chloride 7.34; Potassium dihydrogen phosphate 1.5; Magnesium chloride 10.9; Malachite green 0.037 and Agar No.1 2.5.

4. **Xylose Lysine Desoxycholate Agar (XLD) (CM 0469, OXOID, Basingstoke, England)**

Preparation: Preparation: suspend in 56.68grms in one liter of distilled water. Heat with frequent agitation until the medium boils. DO NOT OVER HEAT. Transfer immediately to water bath at 500C. Pour in to the plate as soon as the medium has cooled. It is important to preparing large volumes which will cause prolonged heating. PH: 7.4 +0.2 at 25^0C**.**

Composition (g/l): Yeast extracts 3.0; L-Lysine hydrochloric acid 5.0; Xylose 3.75; Lactose 7.5; Sucrose 7.5; Sodium chloride 5.0; Sodium thiosulphate 6.8; Ferric ammonium citrate 0.8; Phenol red 0.08 and Agar 15.0.

5. Soayabean Casein Digest Agar (Tryptone Soya Agar).

Preparation: Preparation: suspend in 40 g of powder in distilled water and mix thoroughly. Boil with frequent agitation to dissolve the powder completely.DO NOT OVER HEAT.sterilize by autoclaving at 1210C (15lbs pressure) for 15 minutes. Final PH: 7.3 + at 25 0C.

Composition (g/l): Tryptone 15; Soya peptone 5; Sodium chloride 5; Agar 15

6. Cefixime tellurite Sorbitol MacConkey Agar (CT- SMCA) (OXOID)

Preparation: 51.5g of the powder medium was suspended in one liter of distilled water and brought to the boil to dissolve completely. Then it was sterilized by autoclaving at 121°C for 15 minutes. Thereafter, it was allowed to cool to 50 °C and poured into sterile Petri dishes, and lastly allowed to solidify at room temperature, and stored upside down at 4 to 8°C, refrigerator, for subsequent use and finally adjusted at pH of 7.1±0.2 at 25°C

Composition (g/l): Enzymatic digest of casein 17.0g; Enzymatic digest of animal tissue 3.0g; Sorbitol 10.0g; Bile salts No.3 1.5g; Sodium chloride 5.0g; Natural Red 0.03g; Crystal Violet 0.001g; Agar 9 to 18 g and Water 1000ml.

7. Triple sugar Agar (CM 0277, OXOID, Basingstoke, England)

Preparation: Preparation: suspend 65grams in 1000ml distilled water. Bring to boil to dissolve completely. Mix well and distributes in to containers. Sterilize by autoclaving at 121 0C for 15 minutes. Allow the set as slope with 2.5cm butts. PH: 7.4, + 0.2 at 250C.

Composition (g/l): Yeast extracts 3.0; Peptone 20.0; Sodium chloride 5.0; Lactose 10.0; Sucrose 10.0; Glucose 1.0; Ferric citrate 0.3; Sodium thiosulphate 0.3; Phenol red 0.024 and Agar 12.0

8. Kligler Iron Agar (Oxoid)

Preparation: Suspend 52 g of the powder in 1 L of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Dispense and autoclave at 121°C for 15 minutes. Cool in a slanted position such that deep butts are formed. For best results, the medium should be used on the date of preparation or melted and resolidified before use.

Composition (g/l): Pancreatic Digest of Casein 10.0 g; Peptic Digest of Animal Tissue 10.0g; Lactose 10.0g; Dextrose 10.0g; Sodium Chloride 5.0g; Ferric Ammonium Citrate 0.5g; Sodium Thiosulfate 0.5g; Agar 15.0g and Phenol Red 25.0 mg.

9. Tryptone /Tryptophan Media (OXOID)

Preparation: 10g/1000ml, 5g/1000 and 3g/1000ml of tryptone, sodium chloride and DL_Tryptophan respectively were dissolved together by heating if necessary and dispense 5ml in to test tubes and sterilize at 121℃for 15 minutes. The broth was clear and yellow.

Composition (g/l):

Tryptone 10.0g; Sodium chloride 5.0g; DL-Tryptophan 1.0g and Water 1000ml

10. Kovac's Indole Reagents (OXOID)

Composition (g/l): 4-Dimethytaminobenzaldehde 5.0g; 2-Methylbutan-1-ol or pentan-1-ol 75.0 ml and Hydrochloric acid (p20 1.18g/ml to 1.19g/ml) 25 ml

11. Mueller-Hinton Agar (CM 0337, OXOID, Basingstike, England)

Preparation: suspend 38 grams in 1000ml of distilled water. Bring to boil to dissolve the medium completely. Sterilize by autoclaving at 1210C for 15 minutes. PH: 7.3: +0.1 at 250C.

Composition (g/l): Beef dehydrated infusion 300.0; Casein hydrolysate 17.5; Starch 1.5 and Agar 17.0.

12. McFarland Standards

Composition: 1.17% BaCl.H2O solution and 0.36N of 1% sulfuric acid (H2SO4)

Preparation: Add approximately 85 ml of 1% H2SO4 to 100ml of volumetric flask. Using a 0.5ml pipette add 0.5ml of 1.1% BaCl.H2O drop wise to the H2SO4 while constantly the flask. Bring to 100ml with 1% H2SO4. Place a magnetic stirring in the flask and place on the magnetic stirrer for approximately three to five minutes. Examine solution visually to make certain it appears homogeneous and free of visible clumps. Dispense three to seven ml, cub tightly and seal with paraffin and keep at dark and room temperature.

13. L- Lysine Decarboxylation Medium (DIFCO, Becton, Dicknson, USA)

Preparation: 5.25g/500ml and 5g/500ml decarboxylase base moller and L-Lysine mono hydrochloride respectively were dissolved together by heating if necessary and dispense 5ml in to test tubes and sterilize at 1210C for 10 minutes. The broth was clear and yellow tube to amber.

Composition (g/l): L-Lysine mono hydrochloride 5.0; Yeast extracts 3.0; Glucose 1.0 and Bromocresol purple 0.015

14. Tryptophan Medium

Preparation: Preparation: 10g/1000ml, 5g/1000 and 3g/1000ml of tryptone, sodium chloride and DL_Tryptophan respectively were dissolved together by heating if necessary and dispense 5ml in to test tubes and sterilize at 121℃for 15 minutes. The broth was clear and yellow

Composition (g/l): Tryptone10; Sodium chiloride 5 and DL- Tryptone 1

Annex 6: Principles and result characteristics of biochemical tests of *Salmonella*

	Principles	Result	
Media	Reactions/ Enzymes	Negative	Positive
TSI	Acid production from glucose fermentation and gas production	butt red	slant red, butt yellow or black
	gas production	no air bubble	air bubble
	H_2S production	no black color	black color
Tryptone broth	Indole production	no red ring on the top of the broth	red ring formation on top of the broth
Lysine decarboxylase			
MR-VP medium	Acetone production	media remains as it is	the whole broth is changed to red/ pink
	Mixed acid fermentation	media remains as it is	the broth is changed to red/ pink at the top half of the broth

Sources: Quinn *et al.,* 2002

Annex 7: Procedures and interpretation of biochemical tests

5.1 TSI Agar test: Streak the agar slant surface and stab the butt. Then incubate at 37℃ for 24 hours.

Interpretation:

A) Butt: yellow if glucose used, red/unchanged if glucose not used, black if hydrogen sulphide is formed and bubbles if gas is formed.

B) Slant surface: Yellow if lactose and/or sucrose used and red/unchanged if lactose and/or sucrose not used.

5.2 L-Lysine decarboxylase test: Inoculate the medium just below the surface of the liquid medium. Incubate at 37℃for 24hrs.

Interpretation: Turbidity and purple colour after incubation indicate a positive reaction. A yellow color indicates a negative reaction.

5.3 Indole test: inoculate a tube containing 5ml of the tryptone/tryptophan medium with the suspected colony. Then incubate at 37℃ for 24 hrs. After incubation, add 1ml of the kovacs reagent.

Interpretation: the formation of red ring indicates a positive reaction. A yellow-brown ring indicates a negative reaction.

Figure 2: Swabs samples collection at abattoir

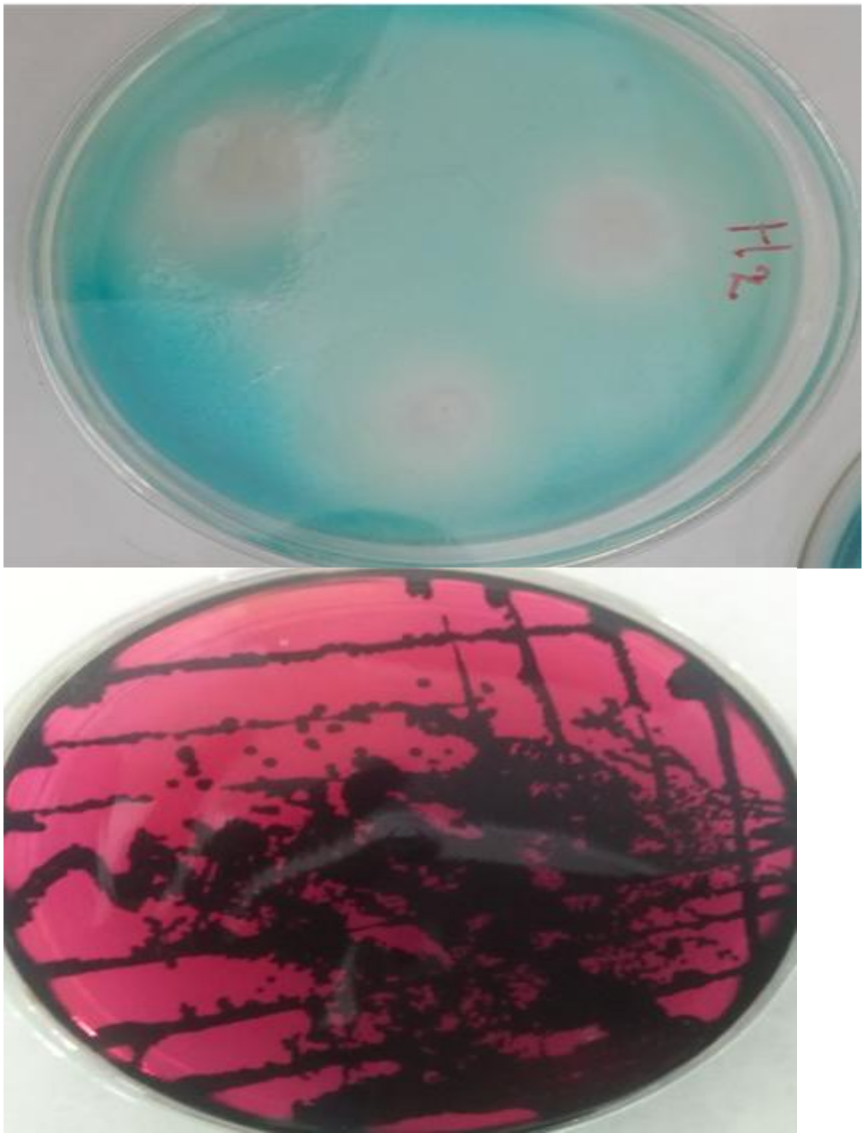

Figure 3: Appearance of *Salmonella* on Selective enrichment media (MRVM) on the top and the selective plating agar (XLD) the bottom one.

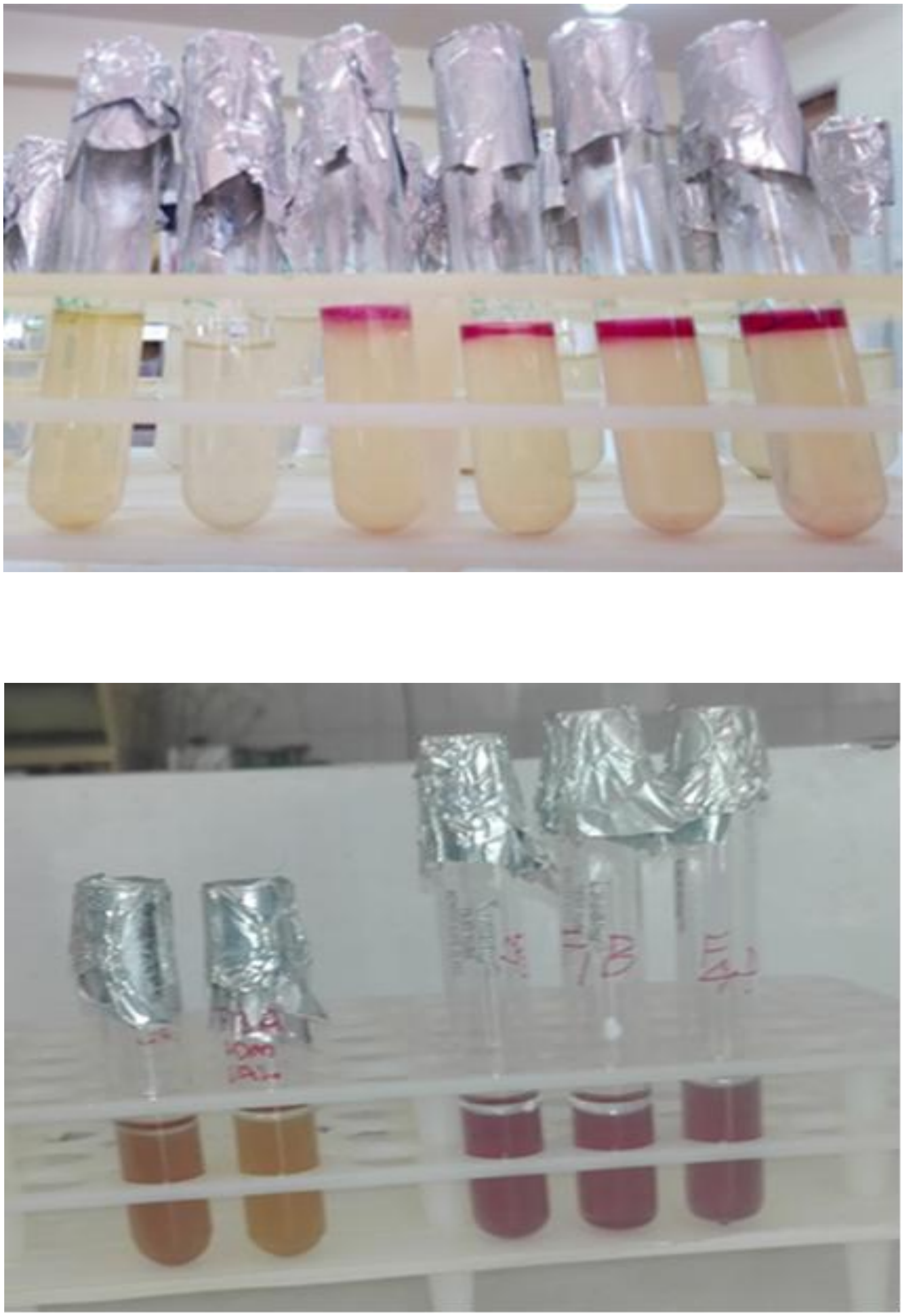

Figure 4: Biochemical test for non-typhoidal *Salmonella*

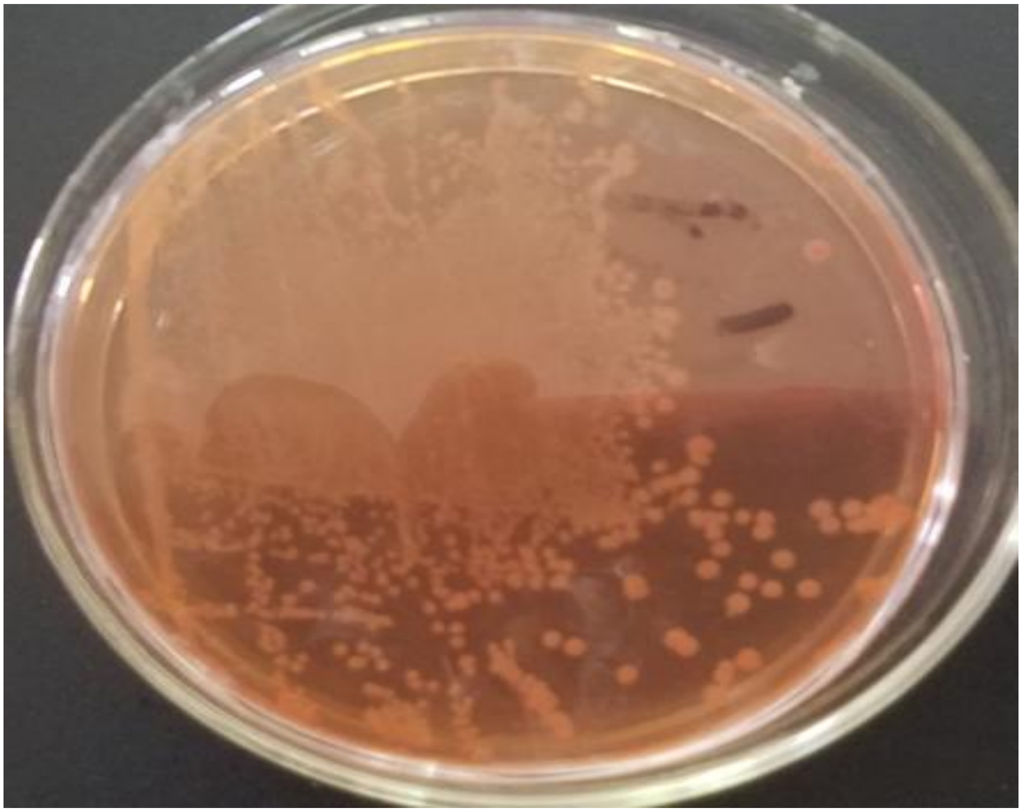

Figure 5 : Appearance of *E. coli* O157:H7 on Selective Sorbitol Mac Conkey Agar

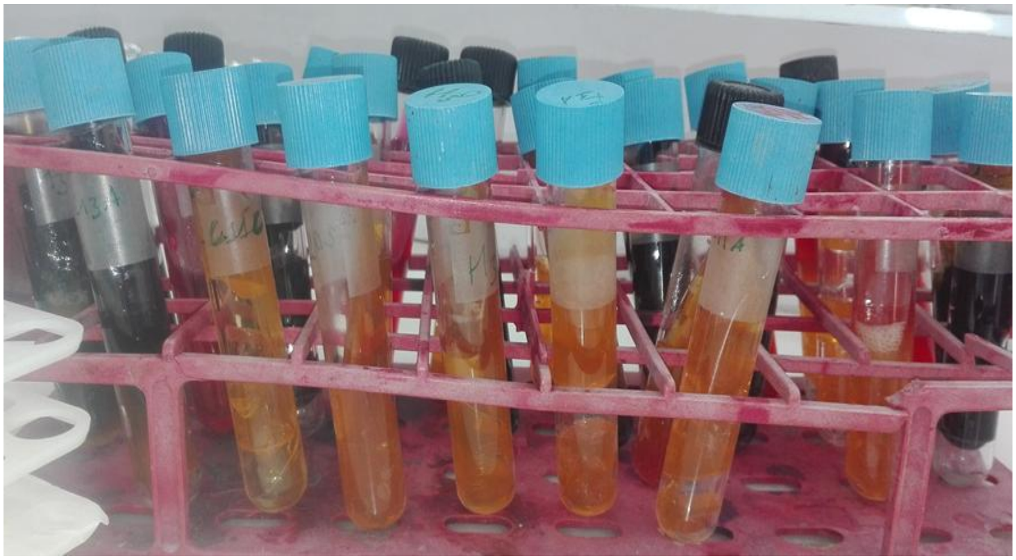

Figure 6 : Biochemical test for *E. coli*

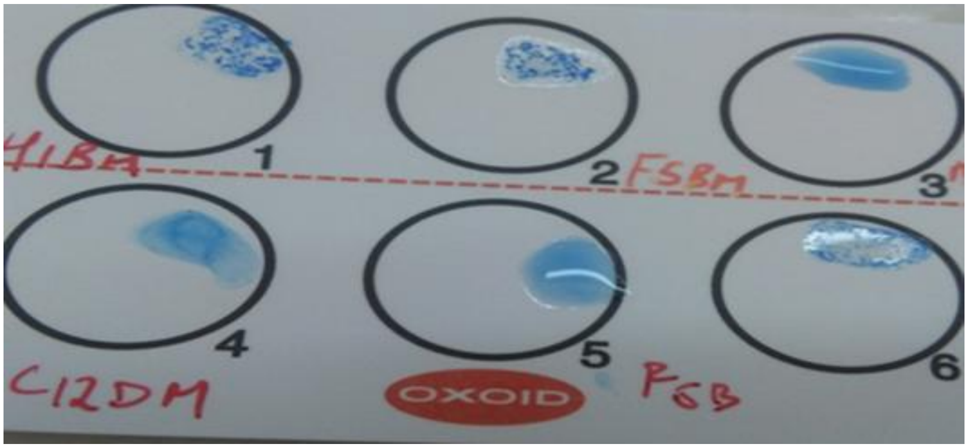

Figure 7 : Latex agglutination for *E. coli* O157

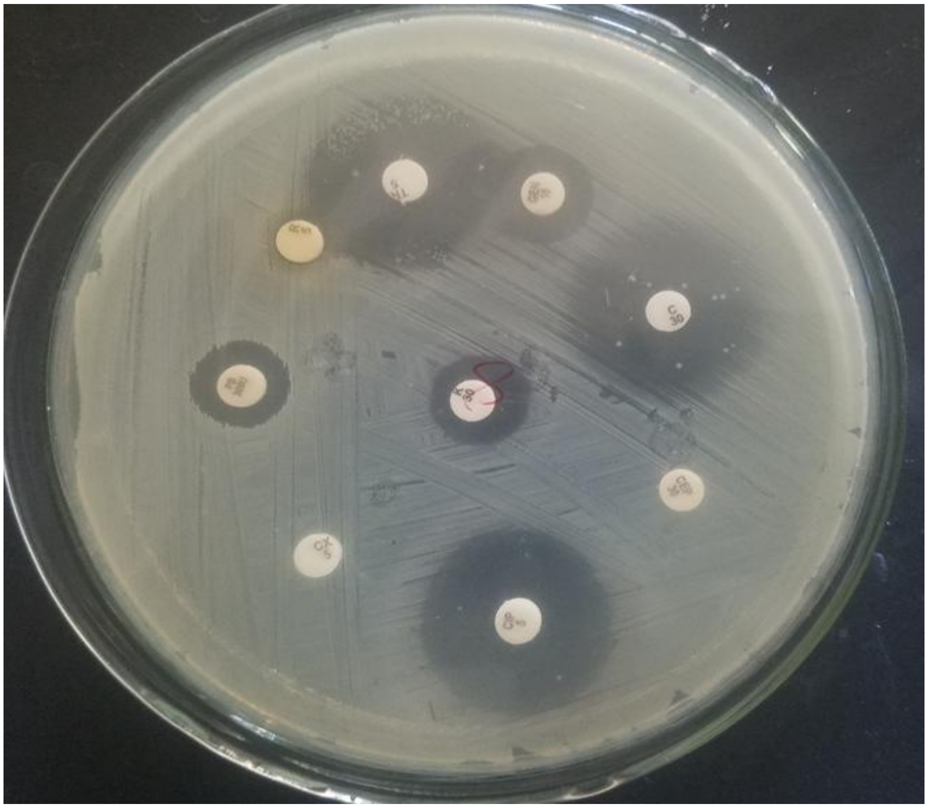

Figure 8 : Antimicrobials testing and disk diffusion appearance