

Thesis Ref. No. _____

ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE



**DETECTION AND ANTIMICROBIAL SUSCEPTIBILITY TEST OF
SALMONELLA SPECIES ALONG BEEF SUPPLY CHAIN IN BISHOFTU TOWN**

BY

OLANA MERERA GOBOSHO

JUNE, 2018
BISHOFTU, ETHIOPIA

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SALMONELLA SPECIES ALONG BEEF SUPPLY CHAIN IN BISHOFTU TOWN**

**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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**DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND VETERINARY
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***SALMONELLA* SPECIES ALONG BEEF SUPPLY CHAIN IN BISHOFTU TOWN**

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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: Olana Merera entitled “**Detection and antimicrobial susceptibility test of salmonella species along beef supply chain in Bishoftu town**” 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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ACKNOWLEDGEMENTS

This thesis was done partially by the fund for research grant allocated by College of Veterinary Medicine (CVM), Addis Ababa University post graduate program. I am grateful to the University, in particular, and the government of Ethiopia, in general, for providing me the research fund. Samara University is also highly acknowledged for its financial, material and economical support throughout the study period. A great deal of information incorporated in the literature review of this paper was the result of many writers' books and journals; without those contribution I can't find my research work gap and generally this work would not have been possible.

I am particularly indebted to my Advisor Dr. Fanta Desissa for his constant supervision throughout the work, provision of necessary materials and patiently and skillfully going through the manuscript.

This research thesis is an ongoing project work funded by Ghent University, Belgium and I would like to acknowledge them for their laboratory media supply and material fund.

By using this opportunity, I want to express my heartfelt gratitude to all Zelalem Moges slaughterhouse workers, Bishoftu municipal abattoir workers and Bishoftu general hospital Stool examination and Urine analysis department workers in particular for their friendly approach and kind support in any direction of this research work. Alongside of my research work I have created many best and cooperating friends through my course of sample collection and laboratory work. I want to thank all these cooperating friends.

I take this opportunity to pass my special gratitude to my beloved wife (Tolashe Girma) for her unreserved psychological and moral support she gave me throughout two years staying in Addis Ababa University.

My final gratitude is reserved to my mom Gete Workessa and all my family members for their invaluable financial and moral support in all facets of my life.

LIST OF ABBREVIATIONS

Abs	Antibodies
ADP	Adenosine DiPhosphate
A _w	Water Activity
CLSI	Clinical Laboratory Standard Institute
Cfu	Colony forming unit
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agricultural Organization
hr	Hour
iNTS	Invasive Non-typhoidal Salmonellosis
invA	Invasive gene A
ISO	International Organization for standardization
LPS	Lipo polysaccharides
ml	Mililitre
NAD	Nucleic Acid Based Diagnostic
NSAID	Non-Steroidal Anti Inflammatory Drugs
NCCLS	National Committee for Clinical Laboratory Standard
PCR	Polymerase Chain Reaction
PNA	Peptide Nucleic Acid
RNA	Ribonucleic Acid
Spp	Species
T3SS	Type Three Secretion System
TLR	Toll Like Receptors
WHO	World Health Organization

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ABSTRACT

Salmonella is one of the major and important foodborne pathogen of humans and animals which have great medical and economical cost with an increasing concern for the emergence and spread of antimicrobial-resistance. The present study was conducted to determine the occurrence, serotype distribution and antimicrobial resistance profile of *Salmonella* species in slaughtered cattle, diarrheic patients and beef retailer shops using standard bacteriological method. For this purpose cross-sectional study for cattle ready to be slaughtered and beef retailer shop and case study design on human diarrheic patients were conducted from October 2017 up to May 2018. A total of 583 samples constituting 240 fecal samples, 127 meat samples from retailer and 216 diarrheic stool samples from hospital were analyzed. The overall prevalence of 22/583 (3.77%, CI 2.22-5.32) was recorded. At individual sample type level *Salmonella* isolates were detected in 11/127 (8.66%, CI 3.7-13.62) of raw meat, 6/240 (2.5%, CI 0.29-4.33) of fecal samples of ready to be slaughtered cattle and from 5/216 (2.3%, CI 0.5-4.4) of diarrheic patients. 8 of isolates which were serotyped were classified under two major serotypes, *S. typhimurium* (4) and *S. eastbourne* (4). Furthermore, antimicrobial susceptibility test was done and identify 14 different pattern of resistance to two up to seven different antimicrobial drugs. All isolates were 100% susceptible to Ciprofloxacin and Gentamycin antimicrobials. Among all isolates tested, 100%, 100%, and 95.5% were found to be resistant to tetracycline, Ampicillin and Cephalothin, respectively while 9.1%, 27.3% and 36.4% were resistant to chloramphenicol, Kanamycin and Nalidixic Acid respectively. The findings of the present study shows that *Salmonella* were present in feces of live animal, diarrheic human patient and significant number in retailer meat samples and all have showed resistance for routinely prescribed antimicrobial drugs both in veterinary and public health sectors. This brings substantial health threats to the consumers unless adequate heat treatment of meat, improvement of standards of hygiene of retailer shops and abattoirs, development of judicious antimicrobial usage and enforcement of appropriate regulation which safeguard consumers are immediately initiated.

Key words: *Antimicrobial susceptibility, Beef supply chain, Isolation, Salmonella, Serotype*

1. INTRODUCTION

Salmonella infection remains a major public health concern worldwide, contributing to the economic burden of both industrialized and under developed countries through the costs associated with surveillance, prevention and treatment of disease (Crump *et al.*, 2004; Lee *et al.*, 2015). It is responsible for approximately 30% of foodborne outbreaks in the United States (Gould *et al.*, 2011) and 23% in the European Union (EFSA, 2015). Majowicz *et al.* (2010) reported about 86% (80.3 million/93.8 million) of foodborne infectious gastroenteritis is caused by *Salmonella* and annually cause 155,000 deaths globally. Mortality rate of *Salmonella* infection in developing countries is 24% higher than in the developed countries (Chimalizeni *et al.*, 2010).

Salmonella is comprised of two species, *Salmonella bongori* and *Salmonella enterica*, and more than 2500 recognized serovars (Guibourdenche *et al.*, 2010). Among all the subspecies of *Salmonella*, *S. enterica* subsp. *enterica* (I) is found predominantly in mammals and contributes approximately 99% of *Salmonella* infections in humans and warm-blooded animals. In contrast, the other five *Salmonella* subspecies and *S. bongori* are found mainly in the environment and also in cold-blooded animals, and hence are rare in humans (Brenner *et al.*, 2000; Hoelzer *et al.*, 2011). *Salmonella* can also survive in farm and other environments for prolonged periods of time (Holley *et al.*, 2006; Cummings *et al.*, 2010).

Cattles are known to play a major role as a source of a variety of zoonotic *Salmonella* serotypes. *Salmonella enterica* subspecies *enterica* serotype Dublin (*S. dublin*) and *Salmonella enterica* subspecies *enterica* serotype *Typhimurium* (*S. typhimurium*) are the most common *Salmonella* serotypes associated with bovine. Bovine salmonellosis usually manifested clinically as a syndrome of septicemia, acute or chronic enteritis and abortion (Radiostits *et al.*, 2007). The presence of *S. typhimurium* in cattle feces and the cross contamination of beef carcass tissue during hide removal and evisceration are the most common cause of *Salmonella* infection in developed countries (Cummings *et al.*, 2010).

Cross contamination from meat handlers during the processes of manufacturing, packing and marketing, may also contribute to the prevalence of salmonellosis (Al-Mutairi, 2011).

Ingestion of the contaminated products is the main sources of infection. Within Ethiopia, one of the major sources of foodborne diseases was the widespread habit of raw beef consumption (Seleshe and Lee, 2014). After ingestion, salmonellosis in humans appear 12 to 72 hours shows clinical sign such as diarrhea, fever, abdominal cramps, nausea, and sometimes vomiting but asymptomatic infections may also occur (Pal, 2007). According to a demographic and healthy survey held in Ethiopia in 2011, the prevalence of diarrhea in under-five aged children is as high as to 25% and cause of mortality of children aged under-five (88 per 1000 live births). It is the second most important disease (EDHS, 2011). Some studies in Ethiopia reports on typhoidal Salmonellosis from diarrheal patients commonly from under-five children (Beyene *et al.*, 2008; Getamesay *et al.*, 2014; Mamuye *et al.*, 2015).

Meat available at retail outlets comes through a long chain of slaughtering and transportation, where each step may pose a risk of microbial contamination including *Salmonellae*. The sanitary conditions of abattoir and meat shop environments play important roles in the spreading of *Salmonella* contamination (Mannion *et al.*, 2012; Garedew *et al.*, 2015).

The detection of *Salmonella* is the key to the prevention and identification of problems related to health and safety. *Salmonella* detection method includes culture based, immunological based, nucleic acid based and biosensors. But, surveillance and monitoring should be based on reliable and efficient detection methods, which should help improve the food safety (Rodríguez-Lazaro *et al.*, 2007). 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 (Pires and Hald, 2010).

By amplifying *Salmonella* DNA using *invA* primers, selective for *Salmonella* was possible, in less than 3hr, to classify the isolates as *Salmonella* spp., confirming the results of microbiological outcomes (Carraturo *et al.*, 2016).

An increasing antimicrobial resistant *Salmonella* isolates are common both in veterinary and public health sectors (Kemal, 2014). The increasing proportion of single and multiple antibiotic-resistant *Salmonella* strains isolated from human salmonellosis cases has been associated with the widespread use of antibiotics in food animals (Alexander *et al.*, 2009). Antibiotics excessively used in Ethiopia and other African countries include tetracyclines, β -lactams, chloramphenicol, quinolones, nitrofurans, and macrolides. Tetracycline levels have been found to be especially high in meat and kidney samples from several abattoirs in Ethiopia, exceeding the WHO limits (Darwish *et al.*, 2013).

Nontyphoid *Salmonella* strains are the leading causes of foodborne illnesses worldwide; however, there is very limited information on the presence and characteristics of *Salmonella* in the beef supply chain in developing countries. In Ethiopia, raw meat is available in open-air local retail shops without appropriate temperature control where consumers either purchase for home consumption or consume at the butchery shop. Minced meat, traditionally named as “Kitfo”, is also served in restaurants as raw, slightly-cooked or well-cooked throughout the country (Avery, 2004). However, there is no information regarding the association of eating raw meat in causing human diarrhea. There is also a gap regarding the detection and antimicrobial resistance status of *salmonella* from beef supply chain (cattle, beef meat and human) and its involvement for human diarrhea in Ethiopia.

Therefore the objectives of this research paper are:

- ✓ To isolate and identify *Salmonella* species from cattle feces, meat retailer shops and human diarrheic patients.
- ✓ To characterize the human and animal isolates based on their antimicrobial susceptibility profile

2. LITERATURE REVIEW ON SALMONELLA AND SALMONELLOSIS

2.1. Taxonomy and nomenclature

Salmonella is named after an American veterinary bacteriologist, D.E. Salmon who first isolated *Salmonella choleraesuis* 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*. *S. enterica* in turn is further divided into six subspecies which are referred 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).

2.2. Physiological and cultural characteristics

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 peritrichous flagella (Getnet *et al.*, 2008). 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°C and 45°C (optimally at 37°C) and at a pH of 4 to 9. A temperature higher than 70°C rapidly kills them. These bacteria can resist dehydration for a very long time ($A_w \geq 0.93$), both in faeces and in foods for human and animal consumption. In addition, they can survive for several months in saltwater 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, bacteraemia, and septicaemia. These factors include the lipopolysaccharide of the outer wall, pili, flagella, cytotoxin and enterotoxin (Quinn *et al.*, 2002).

2.3 Virulence factors

Whether an infection with *Salmonella* spp. leads to a disease largely depends on the virulence of the strain and the constitution of the host. These virulence factors such as virulence-plasmids, toxins, fimbriae and flagella are therefore referred to as “classic” virulence factors (Asten and Dijik, 2005). Intracellular survival and replication are important virulence determinants and the bacteria can be found in a variety of phagocytic and non-phagocytic cells *in vivo*. Invasion of host cells and intracellular survival are dependent on two type III secretion systems, T3SS1 and T3SS2, each of which translocates a distinct set of effector proteins. However, other virulence factors including ion transporters, superoxide dismutase, flagella and fimbriae are also involved in accessing and utilizing the intracellular niche (Ibarra and Steele-Mortimer, 2009).

The *Salmonella* invasion gene, *invA*, has been shown to be involved in internalization of *Salmonella* 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 (Swamy *et al.*, 1996).

2.3.1 Fimbriae

Salmonella have 13 predicted fimbrial loci, many of which are induced *in vivo* and are required for biofilm formation, attachment to host cells and colonization but not intracellular survival (Humphries *et al.*, 2001). The type 1 fimbrial adhesin FimH mediates T3SS1-independent uptake in murine dendritic cells (Guo *et al.*, 2007).

2.3.2 Flagella and flagellin

Flagellar-based motility can increase the invasiveness of *Salmonella* (Schmitt *et al.*, 2001), although this remains somewhat controversial especially since flagellin monomers are potent inducers of innate immunity (Miao *et al.*, 2006). In *Salmonella*-infected macrophages flagellin is translocated into the cytosol by T3SS1 resulting in activation of the inflammasome and caspase-1-mediated cell death (pyroptosis) (Miao *et al.*, 2007; Sun *et al.*, 2007). In the intestinal epithelium flagellin induces inflammation while *inhibiting* apoptosis also via TLR5, but the flagellin must be translocated to the basolateral side of the epithelial cells, since TLR5 is not expressed on the apical surface (Vijay-Kumar *et al.*, 2006). Flagella are usually downregulated inside the host, although inside macrophages it has been suggested they may be induced with T3SS1 and used for escape (Sano *et al.*, 2007).

2.3.4 Virulence plasmid

Two genes *spvB* and *spvC* encode the principal factor for plasmid-mediated virulence of *Salmonella typhimurium* (Matsui *et al.*, 2001). Both are translocated via the T3SS2 into host cells (Browne *et al.*, 2008; Mazurkiewicz *et al.*, 2008). SpvB ADP-ribosylates actin, destabilizes the cytoskeleton and is associated with host cell cytotoxicity (Browne *et al.*, 2008). SpvC has phosphothreonine lyase activity and can inactivate the mitogen-activated

protein kinases and protein 38 (p38) in mammalian cells (Li *et al.*, 2007; Mazurkiewicz *et al.*, 2008).

2.3.5 Superoxide dismutase

Many host cells produce reactive oxygen species, largely through the activity of the phagosome NADPH oxidase (NOX2) that are required for killing of intracellular pathogens. To counteract this activity *Salmonella* uses a superoxide dismutase, SodCI, to confer protection from extracellular reactive oxygen species. SodCI is ‘tethered’ within the periplasm and protease resistance may be a critical property that allows this enzyme to function in the harsh environment of the phagosome (Krishnakumar *et al.*, 2007; Pacello *et al.*, 2008).

2.4 Pathogenesis

The severity of *Salmonella* infections varies depending on the serotype involved and the health status of the host. Children below the age of 5 year, elderly people and patients with immunosuppression are more susceptible to *Salmonella* infection than healthy individuals. Almost all strains of *Salmonella* are pathogenic as they have the ability to invade, replicate and survive in human host cells, resulting in potentially fatal disease (Eng *et al.*, 2015).

Salmonella displays a remarkable characteristic during its invasion of non-phagocytic host cells (Hansen-Wester *et al.*, 2002) whereby it actually induces its own phagocytosis in order to gain access to the host cell. The remarkable genetics underlying this ingenious strategy is found in *Salmonella* pathogenicity islands (SPIs), gene clusters located at the large chromosomal DNA region and encoding for the structures involved in the invasion process (Grassl and Finlay, 2008).

When the bacteria enter the digestive tract via contaminated water or food, they tend to penetrate the epithelial cells lining the intestinal wall. SPIs encode for type III secretion

systems, multi-channel proteins that allow *Salmonella* to inject its effectors across the intestinal epithelial cell membrane into the cytoplasm. The bacterial effectors then activate the signal transduction pathway and trigger reconstruction of the actin cytoskeleton of the host cell, resulting in the outward extension or ruffle of the epithelial cell membrane to engulf the bacteria. The morphology of the membrane ruffle resembles the process of phagocytosis (Takaya *et al.* 2003). The ability of *Salmonella* strains to persist in the host cell is crucial for pathogenesis, as strains lacking this ability are non-virulent (Bakowski *et al.*, 2008).

Following the engulfment of *Salmonella* into the host cell, the bacterium is encased in a membrane compartment called a vacuole, which is composed of the host cell membrane. Under normal circumstances, the presence of the bacterial foreign body would activate the host cell immune response, resulting in the fusion of the lysosomes and the secretion of digesting enzymes to degrade the intracellular bacteria. However, *Salmonella* uses the type III secretion system to inject other effector proteins into the vacuole, causing the alteration of the compartment structure. The re-modelled vacuole blocks the fusion of the lysosomes and this permits the intracellular survival and replication of the bacteria within the host cells. The capability of the bacteria to survive within macrophages allows them to be carried in the reticulo endothelial system (Monack *et al.*, 2004).

Nontyphoidal *Salmonella* serovars, exemplified by classical *S. enterica* serovars *typhimurium* strains, cause gastroenteritis by employing two type III secretion systems (T3SS) (Barthel *et al.*, 2003; Coburn *et al.*, 2005). The invasion-associated T3SS encoded by *Salmonella* pathogenicity island 1 (SPI-1) enables *S. Typhimurium* to enter the intestinal epithelium (Zhou, 2001), while the T3SS encoded by SPI-2 is subsequently used to promote survival within macrophages (Abrahams and Hensel, 2006). The SPI-1 and SPI-2 T3SS are structurally related to the archetypal flagellar T3SS, which has provided a model for understanding the intricate process involved in the self-assembly of such complex nano machines. Kelly Hughes, from the University of Utah, provided an update on the details of flagellar assembly, including the discovery that proton motive force, rather than ATP hydrolysis, is required for type III secretion (Hughes, 2009).

2.5 Epidemiology

Gastroenteritis is the most common *Salmonella* infection worldwide, accounting for 93.8 million cases which result in 155,000 deaths per year (Majowicz *et al.*, 2010). Based on data for 2001–2005 provided by SalmSurv, (a foodborne disease surveillance network supported by WHO), the most common isolated serotype responsible for NTS infections worldwide was *S. enteritidis* (65%). This was followed by *S. Typhimurium* and *S. Newport*, which contributed 12% and 4% of the clinical isolates, respectively (Galanis *et al.*, 2006). *Salmonella enteritidis* was the most common serotype in Asia, Latin America and Europe, accounting for 38%, 31% and 87% of the clinical isolates, respectively. In Africa, *S. Enteritidis* and *S. Typhimurium* were the two most common serotypes reported, occurring in 26% and 25% of the isolates, respectively. In contrast to the countries mentioned previously, *S. Typhimurium* (29%) was most frequently reported in clinical isolates in North America, followed by 21% *S. enteritidis* (Galanis *et al.*, 2006).

2.5.1 Reservoir host

Chickens, pigs, and cattle are key reservoirs of *Salmonella Enterica*, a foodborne pathogen of worldwide importance (Chaudhuri *et al.*, 2013). Schikora *et al.* (2012) findings show that *Salmonella Typhimurium* can use plants as alternative hosts to humans and other animals. *Salmonella* serogroup/serotype with their respective hosts and diseases they cause is reviewed under the table 1 below.

Table 1: Host specificity and disease spectrum of representative *Salmonella* serotypes.

<i>Salmonella</i> serogroup/serotype	Host	Disease
D/ <i>Typhi</i>	Humans	Septicemia, fever
A,B,C/ <i>Paratyphi</i>	Humans	Septicemia, fever
B/ <i>Typhimurium</i>	Humans, cattle, swine, horses, sheep, poultry, wild rodents	Gastroenteritis, septicemia, fever
D/ <i>Enteritidis</i>	Humans, poultry, wild rodents	Gastroenteritis, septicemia, fever
D/ <i>Dublin</i>	Cattle, swine, sheep	Gastroenteritis, septicemia, abortion, fever
B/ <i>Derby</i>	Birds, swine	Gastroenteritis, septicemia
D/ <i>Gallinarum</i>	Poultry	Gastroenteritis, septicemia
B/ <i>Abortus ovis</i>	Sheep	Septicemia, abortion
B/ <i>Abortus equi</i>	Horses	Septicemia, abortion
C/ <i>Cholerae suis</i>	Swine	Septicemia, fever

Source: Baumler *et al.*, 1998

2.5.2 Transmissions methods

Salmonella species are mainly transmitted by the faecal-oral route (Gay, 2003). They are carried asymptotically in the intestines or gall bladder of many animals, and are continuously or intermittently shed in the faeces. They can also be carried latently in the mesenteric lymph nodes; these bacteria are not shed, but can become reactivated after stress or immunosuppression. Fomites and mechanical vectors (insects) can also spread *Salmonella*. Vertical transmission occurs in birds, with contamination of the vitelline membrane, albumen and possibly the yolk of eggs. *Salmonella* spp. can also be transmitted

in utero in mammals. *S. Typhimurium* isolated from a pig, a calf, and a child on a farm was indicating animal-to-animal and animal-to-human transmission (Hendricksen *et al.*, 2010).

Animals can become infected from contaminated feed (including pastures), drinking water or close contact with an infected animal (including humans). Birds and rodents can spread *Salmonella* to livestock. Carnivores are also infected through meat, eggs and other animal products that are not thoroughly cooked. Cats sometimes acquire *S. typhimurium* after feeding on infected birds or spending time near bird feeders (Radiostits *et al.*, 2007).

According to, Gould and his colleagues (2011) commonly recognized vehicles or mechanisms of transmission of salmonellosis to human beings includes; inadequately cooked or raw meat, poultry, or eggs, others foods cross-contaminated with any of the above, contaminated products of fruits and vegetables (e.g., sprouts, cantaloupe, mangos), unpasteurized milk or milk products, contact with the feces of pets and other infected animals, contaminated and inadequately treated drinking water. Person-to-person spread can occur when an infected person fails to wash hands thoroughly after defecation, but is surprisingly uncommon (reflecting high infectious dose). It is more likely to occur when the infected person has diarrhea, rather than during the carrier state

Person-to-person spread is most commonly seen among preschool children in day care facilities or amongst home and neighbor-hood playmates. It may also occur in medical care settings where immunocompromised patients are at increased risk (Gould *et al.*, 2011). *Salmonella* is spread by the trade of live animals within and between countries. In Europe, the spread of infection with *S. Typhimurium* is typically seen as a result of trade in calves, and by parent and grandparent flocks in poultry production. Trade in contaminated animal feed products has also significantly contributed to the spread of *Salmonella*, and several large outbreaks in humans have been traced back to contaminated animal feed (EFSA, 2015).

2.5.3 Carriers and sources of infection

Because *Salmonellae* are facultative intracellular organisms that survive in the phagolysosome of macrophages, they can evade the bactericidal effects of antibody and complement. Thus, persistence of infection in animals and in the environment is an important epidemiological feature of salmonellosis (Radiostits *et al.*, 2007). Although *Salmonella* may survive for long periods in the environment (Hendricksen *et al.*, 2010), it is the carrier state that provides the major source of infection for animals and humans. The carrier state is characterized by the absence of evidence of disease in animals that are able to transmit infection to susceptible individuals. Certain stress factors have been shown to promote excretion of *Salmonella* by carriers and to lead to activation or reactivation of disease in carrier animals (Hendricksen *et al.*, 2010).

Human infections with *Salmonella* typically occur when contaminated food products are ingested, resulting in severe gastroenteritis. Contaminated pork, beef, milk products and poultry have been implicated as sources of human infections (Geimba *et al.*, 2004; Pal, 2007). As a zoonotic foodborne bacterium, salmonellosis has reservoirs in various animals. The most common domesticated animal hosts are chickens, pigs, and cattle; but many other domestic animals as well as a wide range of wild animals can also harbor this organism (Hendricksen *et al.*, 2010).

2.5.4 Clinical signs

The most common mode of *Salmonella* infection is acute gastroenteritis. The incubation period may vary from 4 hours to 72 hours after the ingestion of contaminated food or water. Symptoms are acute onset of fever and chills, nausea and vomiting, abdominal cramping, and diarrhea. If a fever is present, it generally subsides in 72 hours. Diarrhea is usually self-limiting, lasting 3-7 days, and may be grossly bloody. *Salmonella* is excreted in feces after infection, a process that may last for a median of 5 weeks. In young children, the excretion may be prolonged. In older children and adults *Salmonella* excretion that lasts >8 weeks after infection is uncommon. Bacteremia occurs in 5-10% of infected persons, and some of

them may progress to have focal infection, such as meningitis, and bone and joint infection. Immune compromised patient and those who have impaired cellular immunity, may have prolonged or recurrent *Salmonella* infection (Hammack, 2012; Chen *et al.*, 2013).

2.5.5 Global overview of salmonellosis

Despite the improvement in sanitation and hygiene, NTS illnesses continue to impose a significant burden on the human health in industrialized and underdeveloped countries. It is estimated that 93.8 million cases of gastroenteritis due *Salmonella* spp. occur worldwide leading to 155,000 deaths each year. According to SalmSurv (a World Health Organization (WHO) supported food- borne disease surveillance network) data from 2001 to 2005, *S. enteritidis* was the most common serotype world-wide (65% of the isolates), followed by *S. typhimurium* (12%) and *S. newport* (4%) (Galanis *et al.*, 2006; Majowicz *et al.*, 2010).

In Africa, *S. enteritidis* and *S. typhimurium* represented 26% and 25% of the isolates, respectively. In Asia, Europe and Latin America/Caribbean, *S. enteritidis* was the most frequent isolate (38%, 87% and 31%, respectively). In North America *S. Typhimurium* was the most frequented reported (29%) followed by *S. Enteritidis* (21%) and other *Salmonella* spp. (21%) (Majowicz *et al.*, 2010). Sub-Saharan Africa hospital-based studies reported blood stream *Salmonella* spp. infections more frequently associated to NTS, particularly *S. enteritidis* and *S. typhimurium*, than *S. typhi* or *S. paratyphi*. In this region, invasive NTS is endemic and has elevated morbidity and mortality in children less 3 years and adults with human immunodeficiency virus (HIV) infection (Morphet *et al.*, 2009; Crump *et al.*, 2011). The summary of non typhoidal salmonellosis in humans was summarized in table 2 below.

Table 2: Status of non typhoidal salmonellosis in different parts of the world with their incidence cases/100000 human population

Median iNTS cases/100,000 population		
Country (city)	(range)	Reference
Kenya (Kilifi)	8 (4–1,457)	Berkley <i>et al.</i> , 2005
Uganda	855 (617–1223)	Mayanja <i>et al.</i> , 2010
Tanzania	5 (0–82)	Mtove <i>et al.</i> 2011
Gahna	>600	Marks <i>et al.</i> , 2012
Mozambique	22 (1–388)	Sigaúque <i>et al.</i> , 2009
Malawi, South Africa	South Africa, 1.6 (0.3–7.2); Malawi, 84 (2–1,963)	Feasey <i>et al.</i> 2010
Gambia	17 (1–300)	Enwere <i>et al.</i> , 2006
China, India, Indonesia, Pakistan, Vietnam	Pakistan, 1.6 (1.2–7.2); Indonesia, 0.2 (0.2–1.0); India, 0.05 (0.03–1.8)	Khan <i>et al.</i> , 2010
Denmark	1.9 (0–9.6)	Gradel <i>et al.</i> , 2006
Finland, Australia, Denmark, Canada	Finland, 0.2 (0.1–7.6); Calgary, Canada, 0.2 (0.1–6.5); Denmark, 0.4 (0.3–1.9); Sherbrooke, Canada, 0.5 (0.4–2.2); Victoria, Canada, 0.07 (0.05–0.3); Australia, 0.1 (0.09–0.5)	Laupland <i>et al.</i> , 2010

Salmonella has also been reported from foods and the food production environment, with outbreaks. Beef products can become contaminated with these organisms through exposure to cattle feces or hides during processing, making pathogen-reduction interventions during the harvest process necessary in order to produce safer products. The summary of *Salmonella* occurrence in beef supply chain in different parts of the country has presented in table 3 below.

Table 3: Status of zoonotic *Salmonella* in different countries along beef supply chain

Country	Sampling area	Animal type	Total sample	Prevalence %	References
USA	Farm	Beef cattle	480	0.2	Rodriguez <i>et al.</i> , 2006
		Dairy cattle	480	0.4	
Canada	Abattoir	Yearling cattle	654	0.2	Van Donkersgoed <i>et al.</i> , 1999
		Cull dairy cows	593	0.0	
Ireland	Abattoir	steers and heifers	250	2.0	McEvoy <i>et al.</i> , 2003
Morocco	Slaughter house	Not stated	2122	3.53	Bouchrif <i>et al.</i> , 2009
Australia	Abattoir	Steers and heifers	310	6.8	Fegan <i>et al.</i> , 2004
Switzerl and	Abattoir	Calves, cattle and cow	2930	0	Al-saigh <i>et al.</i> , 2004
Burkina Faso	Abattoir	Cattle	320	52	Kagambèga <i>et al.</i> , 2014
Tunisia	Abattoir	Beef meat	300	5.7	Oueslati <i>et al.</i> , 2016
India	Not stated	Human, animal and meat	Not stated	37	Singh <i>et al.</i> , 2012
Senegal	Slaughter house and Retailers	Feces and meat	347	78	Stevens <i>et al.</i> , 2008

2.5.6 Ethiopian status of zoonotic salmonellosis

In Ethiopia, like other developing countries, it is difficult to evaluate the situation of salmonellosis. This is mainly because of the very limited scope of studies, lack of coordinated epidemiological surveillance system and inadequacy of laboratory facilities for culture. In addition, under reporting of cases and the presence of other diseases considered

to be of high priority may have over shadowed the problem of salmonellosis in some countries, including Ethiopia. Nonetheless, considering the high prevalence of HIV/AIDS malnutrition, aggravated by recurrent drought, the general poor sanitary conditions, home slaughtering of food animals in the absence of meat inspection, feeding habit of people (consumption of raw meat, undercooked and some raw internal organs), lack of chilling facilities and wide spread occurrence of *Salmonella* in animals, it is expected that the disease to be common in Ethiopia, particularly among young, elderly and immune compromised citizens (Misganaw and Williams, 2013).

A number of studies conducted by different individuals on various slaughtered beef animals and foods of beef origin and humans are showed the presence of a number of *Salmonella* serotypes as indicated in the Table 1 below.

Table 4: Serotype distribution of zoonotic *Salmonella* isolated from bovine and their products in different parts of Ethiopia

Study area	Source of samples	<i>Salmonella</i> Serotype detected	Overall prevalence	Reference
Addis Ababa	Beef carcass	<i>S. dublin</i> , <i>S. anatum</i> , <i>S. saintpaul</i> , <i>S. meleagridis</i> , <i>S. muenchen</i> , and <i>S. rough</i>	10.6%	Nyeleti <i>et al.</i> , 1981
Bishoftu	slaughter beef cattle, slaughterhouse environment and personnel	<i>S. newport</i> , <i>anatum</i> and <i>eastbourne</i>	20.7%	Sibhat <i>et al.</i> , 2011
Bishoftu	Cattle ready for slaughter	<i>Salmonella mishmarhaemek</i> , <i>S. typhimurium</i> , <i>S. enteritidis</i> , <i>S. guildford</i> and <i>S. dublin</i>	7.1%	Alemayehu <i>et al.</i> , 2003
Addis Ababa		<i>S. dublin</i> , <i>S. typhimurium</i> ,	4.64%	Kebede <i>et al.</i> , 2016
Jimma and Addis Ababa		<i>S. concord</i> , <i>S. typhimurium</i> , <i>S. paratyphi B</i> , <i>S. haifa</i> , <i>S. typhi</i> , <i>S. enteritidis</i> , <i>S. butantan</i> , <i>S. infantis</i> , <i>S. pomona</i>		
Tigray	Bovines	<i>S. typhimurium</i> , <i>S. enteritidis</i> , <i>S. new port</i>	19.27%	Abebe <i>et al.</i> , 2014
Bahir Dar	Slaughtered cattle	<i>Salmonella typhimurium</i> , <i>Salmonella newport</i> , <i>Salmonella haifa</i> , <i>Salmonella heidelberg</i> , <i>Salmonella infantis</i> , and <i>Salmonella Mishmarhaemek</i>	15.1%	Alemu and Zewude, 2012
Bahir Dar	Slaughtered cattle	<i>Salmonella</i> group A, <i>Salmonella arizonae</i> and <i>Salmonella typhi</i>	7.6%	Muluneh and Kibret, 2015
Addis Ababa and Bishoftu	Slaughtered cattle and meat processing plant	<i>S. Saintpaul</i> , <i>S. london</i> , <i>S. muenchen</i> , <i>S. saintpaul</i> , <i>S. muenchen</i> , <i>S. larochelle</i>	12.9%	Hiko <i>et al.</i> , 2016

Table 5: Serotype distribution of zoonotic *Salmonella* isolated from humans in different parts of Ethiopia.

Gondar	Stool from Hospital	<i>Salmonella typhi</i>	1.3%	Dagneu <i>et al.</i> , 2013
Harar	Stool from Hospital	<i>Salmonella</i> spps	11.5%	Reda <i>et al.</i> , 2011
Jimma	Blood from febrile humans	<i>Salmonella</i> spps	4.3%	Zenebe <i>et al.</i> , 2011
Addis Ababa	Stool sample of farm workers	<i>Salmonella</i> spps	13.6%	Addis <i>et al.</i> , 2011
Harar	Diarrheic stool	<i>Salmonella</i> spps	11.5	Ayalu <i>et al.</i> , 2011
Addis Ababa	Diarrheic sample	Serogroup C, B, D, A, E and <i>S. typhi</i>		Mache <i>et al.</i> , 1997
Gonder	Diarrheic sample	<i>Salmonella</i>	1.08	Andualem <i>et al.</i> , 2014
Jimma	Diarrheic patients	<i>Salmonella</i> spps	6.2	Beyene and Tasew, 2014
Hawassa	Diarrheic samples	Serogroup B and A	2.5	Mulatu <i>et al.</i> , 2014

2. 6 Diagnosis of *Salmonella*

2.6.1 Culture method

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 (Gracias and McKillip 2004). 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 *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 *Salmonella* due to their selectivity and sensitivity (Lee *et al.*, 2015).

Cultural methods of *Salmonella* typically involve the enrichment of a portion of the sample to recover sub-lethally injured bacterial 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), and 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 Cysteine 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 Iron Sulphate agar (BIS), Brilliant Green agar (BGA) with or without the addition of sulfadiazine or sulfapyridine (BGS), modified semisolid Rappaport Vasiliadis (MSRV), Salmonella Shigella Agar, or Hektoen Enteric agar. There are several published standard methods utilizing combinations of media such as the current ISO horizontal method, ISO 6579:2002 for the detection *Salmonella*.

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; Ricke *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. The conventional methods have been improved to reduce cost and labor and to offer faster detection and identification of *Salmonella*. For example, chromogenic and fluorogenic growth media used for detection, enumeration, and identification directly on the isolation plate have shown to be convenient, reliable, and more specific and selective than conventional media (Perry and Freydiere, 2007; Alakomi and Saarela, 2009).

Although some immunological rapid assays are available, these assays still require enrichment steps and give results in 18–48 hr. Enzyme-linked immunoassay (ELISA) (Hart *et al.*, 2011) and polymerase chain reaction (PCR) (Hossain *et al.*, 2012) are robust but require several lengthy steps, expensive laboratory instruments, and experienced operators.

2.6.2 Immunology-based assays

Enzyme-linked immunosorbent assay (ELISA): The different ELISA systems have been developed and commercially available in kit form. In the ELISA assay, an antigen specific to *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 *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 *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) reviewed ELISAs for non-typhoidal *Salmonella* infections, which was 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.

At present, there is a lack of standardization of ELISA assays for non-typhoidal salmonellosis. A challenge with developing serologic tests for 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).

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).

Immunodiffusion 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 14 hr, 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).

2.6.3. 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).

The nucleic acid-based assays are *Salmonella* detection tests that utilize a 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. 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 (Chorng-ming *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 within 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 (Swamy *et al.*, 1996).

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 negative results (Biswas *et al.*, 2008).

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*, *S. Enteritidis* and *S. infantis* by using specific primers for these genes. 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 since conventional PCR methods are generally time-consuming and labor intensive. The method was highly specific in detecting *Salmonella* in spiked chili powder and shrimp samples, with a sensitivity of 0.04 CFU/g (Cheng *et al.*, 2008).

2.6.4 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).

2.7. Treatment

Treatment of non-typhoidal *Salmonella* infection is different from typhoidal infection. In treatment of non-typhoidal *Salmonella* infection antibiotics should not be used routinely, as used in typhoid. Antibiotic should be only used if required as most infection with non-typhoidal *Salmonella* is self-limiting type and duration of diarrhea and fever are not much affected by use of antibiotics. Additionally antibiotic therapy can increase relapse of infection and also prolong the duration of gastrointestinal carrier states. The main treatment should be aimed at correcting dehydration that may arise due to prolonged diarrhea by fluid and electrolyte replacement (Jemal, 2014). In case of patient with bacteremia and other complication antimicrobials are used. Likewise the treatment of enteric fever necessitates the use of antimicrobial drugs with chloramphenicol, ampicillin, amoxicillin, trimethoprim sulfamethoxazole and newer fluoroquinolones being drug of choice against sensitive *Salmonella*. Proper management of fluid and electrolyte balance is important in all patients with *Salmonella* gastroenteritis but is crucial in young children and elder individuals (Mohler *et al.*, 2009).

In animal treatment supportive treatment with intravenous fluid is necessary for patients that have anorexia, depression, significant dehydration. Individual patient may be treated aggressively following acid base and electrolyte assessment. Oral fluid and electrolyte may be somewhat helpful and much cheaper than IV fluid for cattle demand to be mildly or moderately dehydrated. The effectiveness of oral fluid may be somewhat compromised by malabsorption and maldigestion in salmonellosis patient but still should be considered useful. Cattle that are willing to drink can have specific electrolyte added to drinking water to help correcting electrolyte. The implementation of broad prophylactic strategies that are efficacious for all *Salmonellae* may be required in order to overcome the diversity of *Salmonella* serovars present on farms, and the potential for different serovars to possess different virulence factors (Mohler *et al.*, 2009). Early treatment is essential for septicemic salmonellosis but there is controversy regarding the use of antimicrobial agent for intestinal salmonellosis. Oral antibiotic may alter the intestinal micro flora and interfere with competitive antagonism and prolong shading of the organism. There is also a concern that

antibiotic resistance strain of *Salmonella* selected by oral antibiotic may subsequently infect human. Antibiotic such as ampicillin or cephalosporin lead to lyses of bacteria with release of endotoxin. NSAID may be used to reduce the effect of endotoxemia (Davison, 2005).

2.8 Prevention and control

2.8.1 Prevention and control in animals

Condition that contribute to an increasing incidence of epidemic salmonellosis include large herd size, more intensive and crowded husbandry and the trend of free-stall barn with loose housing, which contribute to the faecal contamination of the entire premise. When salmonellosis has been confirmed in a herd, the following control measure should be considered; isolate obviously affected animals to one group if possible, treat severely affected animals, affected animals institute measure to minimize public health concern like (no raw milk and meat should be consumed) physically clean the environment and disinfect the premise following resolution of the outbreak or crises period. Prevention is best accompanied by maintaining a cross herd and culturing new feed additives and components before using the entire ration (Davison, 2005).

***Salmonella* vaccine:** Calves vaccinated at 1-3 weeks of age with a modified aromatic dependent *S. dublin* bacteria have detectable anti-lipopolysaccharide immunoglobulins after immunization. Safe live oral vaccine against *S. typhimurium* and *S. dublin* has been constructed and shown to control protection against experimental infection with virulent wide type strain of the organisms. A virulent *S. cholerasuis* vaccine is efficacies experimentally against salmonellosis due to *S. dublin* in calves to protect young calves. The best program is to vaccinate the cow during pregnancy which will give passive protection to calves for 6 weeks. Generally, both live and attenuated vaccines produced from rough strain in bacteria commercially. There is some evidence that inactivated bacterins can induce a lower level of protection. The veterinarian research institute produced vaccine against bovine salmonellosis in activated bacteria prepared from isolate of *S. dublin*, *S. typhimurium*

and *S. bovis* morbificans and a live attenuated vaccine containing a virulent rough mutant of *S. dublin* (Radiostits *et al.*, 2007).

2.8.2 Prevention and control in humans

Prevention is mainly depending on the proper cooking of foods of animal origin in order to limit the entrance of zoonotic salmonellosis in human food chain. Report from Malawi has highlighted the potential importance of antibody in both serum killing and intracellular oxidative killing of iNTS in African children (MacLennan *et al.*, 2008; Gondwe *et al.*, 2010). The virulence of NTS is dependent on its ability to grow within macrophages of the reticuloendothelial system (Fields *et al.*, 1986). Extracellular replication and bacteremic dissemination also occur. Resistance to complement killing, by way of long-chain lipopolysaccharide, is an important virulence trait. Both complement and specific antibodies together are required to kill *Salmonella* species in vitro. Although serum samples from healthy African adults were able to kill NTS, serum samples from children aged <16 months often did not contain specific antibody titers sufficient to kill effectively (MacLennan *et al.*, 2008).

2.9 Antimicrobial resistance

Antibiotics are usually applied for the treatment and control of diseases in humans and animals. As a result of antibiotic use in food animals, however, drug-resistant pathogens are increasing (Alexander *et al.*, 2009) and this limits therapeutic options both in veterinary and public health practices. Sub-therapeutic and/or prophylactic doses and indiscriminate use of antibiotics in veterinary medicine result in on-farm selection of resistant *Salmonella* which may then pass to humans (Hoelzer *et al.*, 2011; Pui *et al.*, 2011).

Salmonella have acquired various antimicrobial resistance properties over the years. The corresponding resistance genes are commonly located on plasmids, transposons, gene cassettes or variants of the *Salmonella* Genomic Islands SGI1 and SGI2 (Michael *et al.*,

2016). According to a comprehensive review about genes and mutations conferring antimicrobial resistance in *Salmonella* published in 2013, five tetracycline resistance genes- *tet(A)*, *tet(B)*, *tet(C)*, *tet(D)* and *tet(G)* - all of which code for efflux pumps have been identified in *Salmonella*. Among them, the transposon-borne resistance genes *tet(A)* and *tet(B)* were encountered most frequently and *tet(G)* was exclusively found within the *Salmonella* Genomic Island 1 (SGI1)- or 2 (SGI2)-associated multi-resistance gene clusters. Resistance to non-fluorinated phenicols is due to type A chloramphenicol acetyltransferase genes (*catAI* and *catA2*) or to cassette-borne type B chloramphenicol acetyltransferase genes (*catB2*, *catB3* or *catB8*). In addition, the chloramphenicol exporter genes *cmlAI* and *cmlA4* as well as the chloramphenicol/florfenicol exporter genes *cmlA9* and *floR* account for phenicol resistance in *Salmonella* (Michael *et al.*, 2013).

With regard to resistance to aminoglycosides, at least ten different *aadA* genes coding for aminoglycoside-3'-O-adenyltransferases that confer resistance to streptomycin and spectinomycin- *aadAI*, *aadA2*, *aadA5*, *aadA6*, *aadA7*, *aadA12*, *aadA21*, *aadA22*, *aadA23*, *aadA24*, *aadA26* and *aadA27* - are known to occur in *Salmonella*. All of these genes are located on gene cassettes in class 1 or class 2 integrons or are part of the SGI1 - or SGI2-associated multi resistance gene clusters. At least 17 different *dfrA* genes [*dfrA* 1, *dfrA3*, *dfrA5*, *dfrA7*, *dfrA8*, *dfrA10*, *dfrA12*, *dfrA13*, *dfrA* 14, *dfrA15b*, *dfrA16*, *dfrA17*, *dfrA19*, *dfrA21*, *dfrA23*, *dfrA25* and *dfrA32*] and one *dfrB* gene [*dfrB6*] for trimethoprim resistance have been identified in *Salmonella* isolates (Lopes *et al.*, 2016). All three sulphonamide resistance genes, *sul1*, *sul2* and *sul3*, so far known to occur in Enterobacteriaceae, have been detected in *Salmonella* isolates (Michael *et al.*, 2013).

Mutations in the genes *gyrA*, *gyrB*, *parC* and/or *parE*, accounting for resistance to quinolones/fluoroquinolones, have frequently been observed in *Salmonella* isolates. In addition, various plasmid-mediated quinolone resistance genes, such as the gene *qnrD* as well as *qnrA*, *qnrB* and *qnrS* variants all coding for DNA topoisomerase protecting proteins, the gene *qepA* coding for a quinolone-specific efflux pump, and the aforementioned gene *aac(6')-Ib-cr* are known to occur in *Salmonella* isolates (Hopkins *et al.*, 2005; Piddock , 2002).

Previous studies undertaken in Ethiopia indicated the presence of a high level of antimicrobial resistance in *Salmonella* isolated from humans (Addis *et al.*, 2011; Reda *et al.*, 2011; Dagneu *et al.*, 2013) and food animals and food products (Alemayehu *et al.*, 2003). Further antimicrobial resistance was also detected in *Salmonella* isolated from bovines (Sibhat *et al.*, 2011; Alemu and Zewude, 2012; Abebe *et al.*, 2014; Muluneh and Kibret, 2015; Kebede *et al.*, 2016) all have assessed antimicrobial resistance of *Salmonella* serovars recovered from bovine origin from different parts of Ethiopia. All were reported that all *Salmonella* isolates were resistant to one or more commonly used antimicrobials. But there is no information of genes accounting for antimicrobial resistance in *Salmonella*.

2.10 Beef production and consumption behavior in Ethiopia

Bovine meat includes beef, from adult or near-adult animals, and veal from calves. Animals that are raised specifically for beef production are generally beef breed calves produced within the beef herd or the offspring of dairy cows mated with beef bulls in order to maintain the cycle of pregnancy and lactation. In addition, mature dairy cows may also enter the beef production chain once they are no longer required for milk production (Rhoades *et al.*, 2009).

An estimate indicates that Ethiopia is a home for about 54 million cattle, 25.5 million sheep and 24.06 million goats. From the total cattle population 98.95% are local breeds and the remaining are hybrid and exotic breeds. 99.8% of the sheep and nearly all goat population of the country are local breeds. The country is believed to have the largest livestock population in Africa (CSA, 2013). The livestock subsector has an enormous contribution to Ethiopia's national economy and livelihoods of many Ethiopians, and still promising to rally round the economic development of the country. Livestock plays vital roles in generating income to farmers, creating job opportunities, ensuring food security, providing services, contributing to asset, social, cultural and environmental values, and sustain livelihoods. The subsector contributes about 16.5% of the national Gross Domestic Product (GDP) and 35.6% of the agricultural GDP (Metaferia *et al.*, 2011). It also contributes 15% of export earnings and 30% of agricultural employment (Behnke, 2010).

There was distinct pattern in urban and rural meat consumption in Ethiopia in 1996, 2000 and 2004. For a given level of income, urban households (~15 Kg/year) consumed more meat than rural households (~5Kg/year). One of the factors that limit meat consumption in rural Ethiopia has been the absence of meat retail market. In rural areas people often consume meat during holidays or special occasions and it is considered rather as luxury food than essential component of daily household nutrition. During these occasions, commonly, a group of 10 to 20 people buy a live animal, slaughter and divide the meat among them. Therefore, the change in household income alone can hardly change rural meat consumption unless accompanied by change in market arrangements and culture of food consumption (Betru and Kawashima, 2009).

Cows are sources of protein, essential amino acids and other micronutrients such as zinc, vitamin A and calcium source for young children thus prevents stunting of children (Hoddinott *et al.*, 2015). Unfortunately, infectious pathogens associated with cattle exploit such human-animal interactions to jump from animal to human and vice-versa for their transmission. Thus, many zoonotic bacterial pathogens can reach humans through consumption of contaminated foods and food products of animal origin and through close contact with the animals (Gumi *et al.*, 2012).

Meat and meat products serve as important source of proteins for humans. However, recently the emerging antibiotic resistant foodborne pathogens combined with the injudicious use of antibiotics in animal bears considerable public health threats worldwide (Kemal, 2014). Usually, meat and meat products gets contaminated by pathogens during animal slaughter and food processing. *Salmonella* is the frequently isolated foodborne pathogens from meat and meat products. Meat can be contaminated by *Salmonella* during animal slaughter due to unhygienic slaughter practices, through airborne, rodents, insects, and other animals. Consumption of meat contaminated by *Salmonella* causes a serious illness and even death to affected individuals (Morley *et al.*, 2006).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Study area

This cross sectional study was conducted from October, 2017 to May, 2018 in two abattoirs supplying meat for local consumption located in Bishoftu town and Bishoftu General Hospital. Bishoftu is located in East Shewa Zone, Oromia regional state, in the central highlands of Ethiopia and is about 47 km distance from Addis Ababa (NMSA, 2008). It has around 104,215 estimated populations according to global population review report 2018. The two abattoirs selected for study purpose were supplying meat for local consumption. One abattoir is private abattoir and one is government/municipal abattoir. The private abattoir is supplying meat for Bishoftu retailer shop and for Addis Ababa city. But the municipal abattoir is supplying meat for meat retailers found in Bishoftu town as well as it also supplies meat up on order when the customers have ceremony and festivals. The municipal abattoir has the capacity of slaughtering up to 40/day animals but it slaughters 5-15 animals per day on usual days except on the eve of the Easter. The private slaughter house slaughters from 10-30 animals on usual day and up to 40 animals on the holiday. Both abattoirs are slaughtering animals by hanging upside down. They have stunned animals by sharp knife before bleeding of the animal. Bishoftu General Hospital provides service for >100400 of the town population and also for the surrounding community.

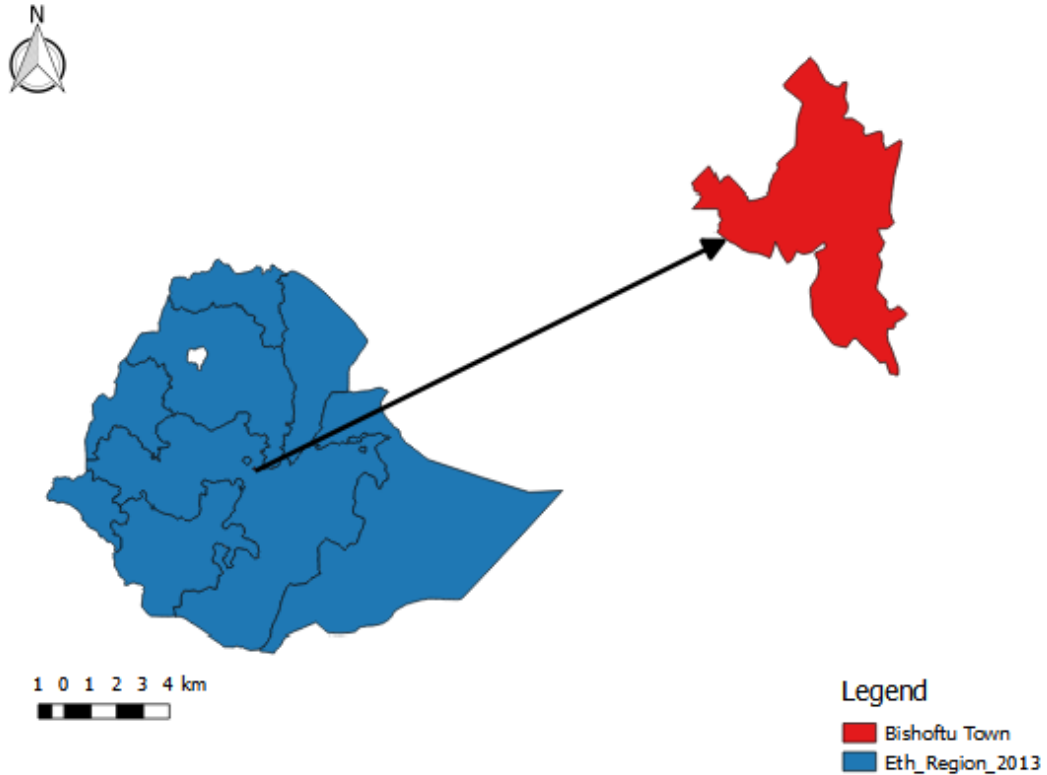


Figure 1: Map of study area

3.1.2 Study design and study population

A cross-sectional type study was used to collect feces from bovines ready to be slaughtered at one Municipal abattoir and one private slaughter house and meats from meat retailers in Bishoftu town. Case study design was employed for diarrheic human samples of human patients treated in Bishoftu General Hospital for *Salmonella* isolation purpose.

The study populations were apparently healthy cattle ready to be slaughtered at both abattoirs designed for local consumption. In addition, meat from meat retailers and stool from diarrheic patients at Bishoftu General Hospital were included in study population.

3.1.3 Sample size determination and sampling

The sample size for feces collection from ready to be slaughtered animals of this study was determined by the following formula given by Thrusfield (2005).

$$n = \frac{1.96^2 \times P_{exp} \times (1 - P_{exp})}{(d \times (1 - P_{exp}))^2}$$
 this formula is chosen because expected prevalence were

below ten (10).

Where 1.96 = the value of Z at 95% confidence interval

d = desired absolute precision

n = required sample size, and

P^{exp} = expected prevalence

The sample size required for this study was estimated based on the expected prevalence of *Salmonella* according to Thrusfield (2007). A previous meta-analysis study on prevalence of *Salmonella* in cattle reported a prevalence of 7.07% (Tadesse and Tesema, 2014). Therefore using the 7.07% expected prevalence, 95% confidence interval and 5% type I error, the number of animals required to demonstrate the prevalence of *Salmonella* representative of the slaughter beef cattle population was estimated to be at least 116. But to increase the precision 240 samples were collected. Animals were selected randomly using the animal's slaughter order if more than (20) animals were slaughtered, otherwise all animals were sampled.

In order to determine the desired sample size for human diarrheic patients, previous report of prevalence of *Salmonella* in Ethiopian diarrheic patient is 8.72% (Tadesse, 2014). Therefore according to the above formula at least 146 samples must be collected. However 216 stool samples were collected and analyzed for *Salmonella* isolation purpose from diarrheic human patients.

According to Tadesse and Gebremedin, (2015) prevalence of *Salmonella* in beef carcass by meta-analysis result is 4.32%. Taking this in to account and applying the above formula, at least 69 beef carcass/meat from retailer shop were collected and analyzed. But for this study purpose 127 meat/carcass from retailer shop were collected and analyzed for *Salmonella* isolation purpose.

During the study period, a total of 240 fecal samples were collected for 2 months and feces were collected twice a week and up to 20 samples per each visit from both abattoirs. Mostly 10 to 30 bovines were slaughtered in private slaughter house per day and from 5-20 animals were slaughtered in the Municipal abattoir depending on market demand/ customer order. Bovines slaughtered were at the age of above one year. These cattle came from its' own beef fattening farm for Private slaughter house or purchased from extensive or semi-intensive management systems in different parts of the country, either tracked or trucked to both municipal abattoir and private slaughter house for slaughtering.

3.2 Methodology

3.2.1 Sample collection

Study sites were visited to facilitate research collaboration prior to the sample collection. Subsequently, cooperation letter was sent to each study site and the study was implemented from November 2017 to May 2018. Private slaughter house and Municipal abattoir were selected for this study based on their supply to the local consumption of beef meat. The sampling days were randomly assigned to each abattoir. During the study period municipal abattoir and the private slaughter house were visited for 8 days and twice a week. During each visit up to 20 samples were collected from each abattoir depending on the animal supply to the abattoirs and a minimum of five animals per each visit. During study period 120 animals from each abattoir were sampled and used for *Salmonella* isolation purpose. The retailer meat shops were visited once and sampled on the visit day during study period. Stool samples were collected from Bishoftu General Hospital randomly from out-patients on the interval of 3-4 days for the period of 25 days. Mostly 5-15 diarrheic patients were committed to Bishoftu General Hospital and from these all diarrheic patients above one year old were sampled. The stool consistency was determined in the hospital laboratory immediately after samples were received according to the Bristol stool consistency scale (type 5, 6 and 7) defined as loose, mucoid and watery, respectively (Lewis and Heaton, 1997).

In general all samples were sampled aseptically. The meat samples were collected in a sterile polyethylene bags once a week from 127 all meat retailer shops found in town which were open during sample collection. The collected carcass sample was put in ice box containing ice pack to create cold chain environment. The fecal samples were collected from the rectum by using sterile plastic bags from all bovines ready to be slaughtered on the same day. The stool samples were collected purposively from diarrheic patients whom physician ordered for stool examination in collaboration with the laboratory personnel in the hospital. Samples were collected by using universal bottle tube filled with transport media (Buffered peptone water) (CONDA, Madrid, Spain) which was coded by sample number, date of sampling and age of the patient.

3.2.2 Sample Transportation

All samples were labeled legibly with permanent marker identifying type/source of sample, date of sampling and code of the abattoir/ retailer shop. The samples were then transported in ice box containing ice packs to the microbiology laboratory of college of veterinary medicine and agriculture, Addis Ababa University.

3.2.3 Isolation and identification

Salmonella isolation and identification was carried out in line with the guidelines of the International Organization for Standardization (ISO 6579: 2002) and World Health Organization (WHO, 2010) Global Foodborne Infections Network laboratory protocol. Steps that include primary enrichment in non-selective liquid medium (pre-enrichment), secondary enrichment in selective liquid media, plating out on selective and final confirmation by biochemical and molecular characterization will be employed. The bacteriological media used for the study were prepared following the instructions of the manufacturers (Annex 3A).

Primary enrichment in non-selective liquid medium (pre-enrichment): The chilled samples during transportation were left for 3 to 5 hr at 20 to 22°C before being processed if

the samples were kept at 4°C overnight. Twenty-five grams of minced beef and 10 to 25 g of faecal samples were transferred to sterile stomacher bag and mixed with buffered peptone water (BPW) (Conda, Madrid, Spain) in 1 to 9 ratios. The mixture was homogenized using a laboratory blender/stomacher (Thermoscientific, USA) at high speed for 2 min. For stool samples approximately, 1g of stool samples which were added to the 10 ml of buffered peptone water (BPW) (Conda, Madrid, Spain) at hospital were directly incubated aerobically at 37°C for 18 to 24 hrs.

Secondary enrichment in selective liquid media: 0.1ml of the samples incubated in the primary non selective enrichment media were inoculated on the Modified Rappaport-Vassiliadis medium (Himedia, Mumbai, India) semi solid media and incubated at 41.5°C for 24 hrs or 48 hrs to finally confirm the sample was negative for *Salmonella* species. If there is color change on the media from green to grey color it is suspected as *Salmonella* positive and further inoculated on plating out media (Annex 3A).

Plating out and identification: If growth was detected on secondary enrichment selective media, plating out was done on xylose lysine desoxycholate (XLD) agar (Oxoid, Basingstoke, England) plates. A loopful from enrichment semi solid cultures was streaked onto the plating out media. The plates were then incubated aerobically at 37°C for 18 to 24 hrs. Then the plates were examined for the presence of *Salmonella* colonies. Typical colonies of *Salmonella* grown on XLD medium are small red translucent and/or dome shaped colonies, which may have central black spot due to hydrogen sulphide production as seen on Annex 3A. For confirmation, five presumptive *Salmonella* colonies (or less depending on ability to grow) were selected from selective plating media. The selected colonies were streaked on the surface of pre-dried nutrient agar (OXOID, Basingstoke, England) plates in a manner that allow isolated colonies to develop and incubated at 37°C for 24 hours for further confirmation with biochemical tests.

Biochemical characterization: Colonies suspected to be *Salmonella* were further tested biochemically using triple sugar iron (TSI) agar slants (OXOID, Basingstoke, England), lysine decarboxylase test using lysine decarboxylase broth (DIFCO, Becton, Dickinson,

USA), indole test and urease test using urea broth (HIMEDIA, Mumbai, India). The TSI test; lysine decarboxylase test, urease test and citrate utilization test were conducted according to Quinn *et al.* (2002) (Annex 3B).

3.2.4 Serotyping of *Salmonella* isolates

Biochemically confirmed *Salmonella* isolates were sent for serotyping to Ghent University, Food Safety Laboratory, Belgium. However, only eight from 22 isolates were serotyped because these isolates were recovered at the beginning of study time and these were sent in advance when the research is ongoing. The left 14 isolates were planned to be sent. Generally for serotyping, the somatic (O) antigens were determined by slide agglutination tests (Ewing, 1986) and flagellar antigens were determined using a microplate agglutination technique (Shipp and Rowe, 1980). The antigenic formulae of Grimont and Weill (2007) were used to identify and assign the serotypes of the isolates.

3.2.5 Antimicrobial susceptibility test

The antimicrobial susceptibility testing was carried out in Veterinary Microbiology Laboratory, College of Veterinary Medicine, Addis Ababa University. All *Salmonella* isolates recovered and showing characteristics of *Salmonella* biochemically were tested for susceptibility against 10 (Table 5) antimicrobial agents (all from TM media, New Delhi, India) in accordance with the National Committee for Clinical Laboratory Standards (NCCLS, 2013) guidelines. Antimicrobial zone of inhibition results were interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2018) guideline. The list of a panel of antimicrobials utilized, their symbols, and concentrations and breakpoints are shown in Table 3 below.

Table 6: CLSI breakpoints for Enterobacteriaceae available for these antimicrobial

Antimicrobial	Concentration (μ g/disk)	Susceptible (mm)	Resistant (mm)	Intermediate (mm)
Cephalothin (CEP)	30	≥ 18	≤ 14	15-17
Ampicillin (AMP)	10	≥ 17	≤ 13	14-16
Chloramphenicol (CHL)	30	≥ 18	≤ 12	13-17
Ciprofloxacin (CIP)	5	≥ 21	≤ 15	16-20
Gentamicin (GEN)	120	≥ 15	≤ 12	13-14
Nalidixic Acid (NA)	30	≥ 18	≤ 13	14-18
Streptomycin (STR)	10	≥ 15	≤ 11	12-14
Rifampicin (R)	5	≥ 14	≤ 14	
Tetracycline (TET)	30	≥ 15	≤ 11	12-14
Kanamycin (K)	30	≥ 18	≤ 13	14-17

Source: CLSI (2018)

3.2.6 Questionnaire survey

As part of the routine investigation of foodborne *Salmonella* infections, structured interview was administered to each diarrheic patient from whom stool sample was received. The *Salmonella* questionnaire was designed by considering the bovine contact and consumption of bovine meat. The structured questionnaire was supplemented with an additional set of questions to ensure completeness of exposure data collection and to better align bovine risk for occurrence of diarrhea due to *Salmonella* transmission. Data collected includes clinical features and exposure history during the two weeks before disease onset. Exposure data included animal contacts, feeding history, and travel history (Annex 6).

3.2.7 Ethical clearance

Ethical clearance was obtained from animal research ethical review committee of Addis Ababa University College of Veterinary Medicine for study takes place on cattle. For study on human diarrheic patients in hospital, this research was approved by the Ethiopian Federal Democratic Government /Ministry of health Ethics Committee under the reference “XXXXXXXXXXXXX *“Investigating the association between cattle and diarrheal illness in humans due to zoonotic bacteria in Ethiopia”*”. Because this project was funded by Ghent University, Belgium for fulfillment of Doctoral dissertation (PhD) of my advisor and Ethical clearance was given for him. In addition to ethical clearance, during sample collection all diarrheic patients were informed as they are going to be sampled and only volunteers were used for sampling.

3.2.8 Data management and analysis

The data generated from the study was arranged, coded and entered to Excel spread sheet (Microsoft® office excels, 2007). Prevalence of *Salmonella* was calculated as a percentage of *Salmonella* culture-positive samples among the total number of samples examined. Chi-square test was done by STATA version 12 to study association between *Salmonella* isolates and risk factors (sample type, source of sample and study animal). The significance level was set at 0.05 and 95% confidence level. During data analysis of human diarrheic patient’s age was categorized according to Ethiopian age classification 2018 which categorize age in the interval of 14 years.

3.3 Limitations of study

The prevalence difference between this study and that of other researchers may emanate from small samples and limited health facility investigated in this study. Regarding the risk factor investigation the sample must be taken at each stage of beef production from farm to table. Due to this the risk factor analysis was not done deeply. The isolated *Salmonella*

serogroups were not characterized molecularly and it is better to conclude the linkage of *Salmonella* from beef supply to human diarrhea occurrence if this was done.

3.4 Constraints of study

This research was initially proposed to detect, molecularly characterizing the *Salmonella* and also aimed to look at the linkage between the isolates isolated in healthy slaughtered cattle, meat at retailer shop and human diarrheic patients, but this objective doesn't met due to different constraints. From this delaying release of research birr funded by Addis Ababa University post graduate coordinator beyond our proposal schedule, different conflicts in Ethiopia due to demonstration at various country side and as a result blockage of roads, shops and so on were great contributors for this research in order not to meet its original objective. Another constraint was that deficiency of foreign country (defilation of local currency) and absence (very expensiveness) of media's and reagents intended for isolation purpose.

For hospital investigation the great obstacle of the proposed plan was that of fasting due to Ethiopian Easter and short time fasting at different time makes. This makes the research to extend beyond the proposed time because there is no meat consumption at this time and this contradicts with our objective to look the relation of consumption of meat and meat products and occurrence of diarrhea. So the research was forced to wait until fasting was finished. In turn this prolongs the isolation procedure and the defense time was reached before the isolates are sent for molecular characterization. Due to all these reasons the objective on the initial proposal doesn't meet.

4. RESULTS

4.1 General occurrence of *Salmonella*

Out of the total 583 samples examined from different sources, 22 (3.77%, 95% CI: 2.22, 5.32) samples were found positive for *Salmonella*. Of the 240 fecal samples collected from ready to be slaughtered on the same day *Salmonella* was isolated from six (2.5%, 95% CI: 2.9, 4.3) samples. Eleven (11) (8.6%, 95% CI: 3.7, 13.62) of the 127 carcasses collected from retailer shops were found contaminated with *Salmonella*. *Salmonella* was recovered from five of the 216 diarrheic stool samples (2.3%, 95% CI: 0.29, 4.3). Table 7 below shows the prevalence of *Salmonella* species isolated from the different samples.

Table 7: Prevalence of *Salmonella* in different sample types

Sample type	Observation	No. of positive	Prevalence (95% Conf. Interval)
Meat	127	11	8.66 (3.7-13.62)
Stool	216	5	2.3 (0.5- 4.4)
Feces	240	6	2.5 (0.29 - 4.33)
Total	583	22	3.77 (2.22-5.32)

The prevalence of *Salmonella* recovery was statistically significant among the sample types along beef supply chain with Chi^2 10.69 and p-value 0.005 as shown in table 8.

Table 8: Prevalence and association of *Salmonella* recovery between sample types

Sample type	Total observation	Frequency of positivity	Prevalence	Chi^2 value	p-value
Meat	127	11	8.66%	10.69	0.005
Diarrheic stool	216	5	2.3%		
Feces	240	6	2.5%		

From 22 isolates 8 isolates isolated from meat at retailer shops were serotyped at Ghent University, Food Safety Laboratory, Belgium. From these, two serotypes comprising of four (4) *S. Typhimurium* serotype and four (4) *S. Eastbourne* serotypes were identified. The other isolates were planned to be sent to Ghent University for serotyping.

For questionnaire survey analysis a total of 216 respondents used, of which 93 (43.06%) and 123 (56.94%) were from female and male patients respectively. The mean age of the respondents was 27 years, with the majority of the patients age were between 24 and 35 years of age (43.06%) as presented in table 9. From a total of purposively sampled diarrheic patients from the hospital during study period five (5) *Salmonella* isolate were isolated. The association of *Salmonella* recovery in diarrheic patients with the possible risk factors was presented in table 9 below.

Table 9: Univariate analysis of the association and the risk factor of *Salmonella* among diarrheic out-patients attending Bishoftu General Hospital

Variables	Frequency (%) of <i>Salmonella</i> isolated	Crude Odds ratio	P-value
Having cattle farming			
Yes	4 (1.85)		
No	1 (0.46)		
Visit or contact of cattle feces			
Yes	4 (1.85)		
No	1 (0.46)		
Direct contact with cattle feces			
Yes	4 (1.85)		
No	1 (0.46)		
Consumption of raw meat in last two weeks			
Yes	3 (1.39)	2.89	0.35
No	2 (0.92)		
Contact with someone having diarrhea			
Yes	0 (0)		
No	5 (2.3)		
Consumption of food containing beef meat			
Yes	3 (1.39)	1.5	0.72
No	2 (0.92)		
Age			

0-14	0 (0%)		
15-29	2 (0.93%)		
30-44	2 (0.93%)		
45-60	0 (0%)		
>60	1 (0.46%)		
Consistency of diarrhea			
Watery	2 (0.93%)		
Mucoid	1 (0.46%)	1.67	0.68
Mixed	2 (0.93%)	1.11	0.92
Bloody	0 (0%)		
Sex			
Male	2 (0.93%)	0.49	0.45
Female	3 (1.39%)		
Duration of onset diarrhea			
1-3 days	3 (1.39%)		
4-6 days	2 (0.93%)	3.15	0.22
>6 days	0 (0%)		
Maximum episode of diarrhea			
1-3 times	0		
4-6 times	5 (2.3)		
>6 times	0		
Episode of diarrhea in the first/ two years			
Once	5 (2.3%)		
Twice	0		
>twice	0		
Travel Status in last 14 days			
Yes	1 (0.46%)	1.39	0.77
No	4 (1.85%)		
Consumption of beef outside home			
Yes	4 (1.85%)	1.5	0.72
No	1 (0.46%)		
Attendance of large gathering			
Yes	0		
No	5 (2.3%)		
Does somebody develops diarrhea in that gathering			
Yes	0		
No	5 (2.3%)		

4.2 Antimicrobial susceptibility test

Of the 22 *Salmonella* isolates subjected to disk diffusion antimicrobial susceptibility test using a panel of 10 different antimicrobials (Table 10), all of the isolates (100%) were resistant to Ampicillin and Tetracycline antimicrobials used. None of the *Salmonella* isolates showed resistance for the Gentamycin and Ciprofloxacin antimicrobials. All isolates were resistant at least to two (20%) antimicrobials and at most to seven (70%) antimicrobials tested. Nalidixic acid, Kanamycin and Cephalothin antimicrobials shows intermediate resistance to 4 (18.18%), 4 (18.18%) and 1 (4.5%) *Salmonella* isolates respectively as indicated in table 10 below.

Table 10: Susceptibility pattern of all isolates from different samples to each antimicrobial disk tested

Code	Sample source	Serogroup	Antimicrobials used for testing isolates										Frequency (%) resistance
			GEN	CEP	AMP	NA	TET	CIP	S	K	R	C	
F54	Fecal		S	R	R	I	R	S	R	I	R	S	5 (50)
F70	Fecal		S	R	R	R	R	S	R	R	S	S	6 (60)
F85	Fecal		S	I	R	S	R	S	S	S	S	S	2(20)
F89	Fecal		S	R	R	S	R	S	R	R	R	S	6 (60)
F212	Fecal		S	R	R	S	R	S	S	S	S	S	3 (30)
F220	Fecal		S	R	R	R	R	S	R	S	R	S	6 (60)
M8	Meat		S	R	R	R	R	S	R	R	R	S	7 (70)
M52	Meat		S	R	R	R	R	S	S	S	R	R	6 (60)
M75	Meat		S	R	R	S	R	S	R	S	S	S	4 (40)
M90	Meat		S	R	R	S	R	S	R	S	R	S	5 (50)
M91	Meat		S	R	R	I	R	S	R	R	R	S	6(60)
M92	Meat		S	R	R	S	R	S	R	R	R	R	7(70)
M93	Meat		S	R	R	S	R	S	R	I	R	S	5 (50)
M94	Meat		S	R	R	S	R	S	R	S	R	S	5 (50)
M95	Meat		S	R	R	I	R	S	R	I	R	S	5 (50)
M96	Meat		S	R	R	R	R	S	R	S	R	S	6 (60)
M97	Meat		S	R	R	S	R	S	R	S	R	S	5 (50)
S120	Stool		S	R	R	R	R	S	R	R	R	S	7 (70)
S150	Stool		S	R	R	R	R	S	R	R	R	S	7 (70)
S175	Stool		S	R	R	S	R	S	R	S	R	S	5 (50)
S181	Stool		S	R	R	I	R	S	R	I	R	S	5 (50)
S206	Stool		S	R	R	R	R	S	R	R	S	R	7 (70)
Total Resistant			0	21	22	8	22	0	20	6	18	2	
% resistant			0	95.5	100	36.4	100	0	91	27.3	82	9.1	

Key: AMP=Ampicillin, TET=Tetracycline, CEP=Cephalothin, S=Streptomycin, K=Kanamycin, NA= Nalidixic Acid, C=Chloramphenicol, R=Rifampicin, GEN=Gentamycin, C= Chloramphenicol

Table 11: Mono drug susceptibility profile of all isolates to the respective antimicrobials used

Type of drug	Susceptible (%)	Intermediate (%)	Resistant (%)
GEN	22 (100)	0 (0)	(0)
CEP	0 (0)	1 (4.5)	21 (95.5)
AMP	0	0	22 (100)
NA	10 (45.45)	4(18.18)	8 (36.37)
TET	0	0	22 (100)
CIP	22 (100)	0	0
S	2 (9.1)	0	20 (89.9)
K	12 (54.54)	4 (18.18)	6 (27.27)
R	3 (16.64)	0	19 (83.36)
C	20(90.9%)	0	2 (9.1%)

According to the definition of Magiorakos *et al.* (2012) for multi drug resistance, 21 (95.45%) isolates were multi drug resistant to at least three (3) different antimicrobials. Eight (8) of the isolates (36.36%) were resistant to five (5) different antimicrobials used and followed by 6 (27.27%) and 5 (22.72%) isolates which were resistant to 6 and 7 different antimicrobials tested respectively as shown in table 11. Three (3) isolates recovered from human diarrheic stool were resistant to seven (7) different antimicrobials used (table 12).

Table 12: Multi drug resistance pattern of isolates

Resistance pattern	Antimicrobials tested (number of isolate)	Total number of resistant isolates
2	AMP, TET (1)	1
3	CEP, AMP, TET (1)	1
4	CEP, AMP, TET, S (1)	1
5	CEP, AMP, TET, S, R (6)	8
	CEP, AMP, TET, K, R (2)	
6	CEP, AMP, TET, NA, S, R (2)	6
	CEP, AMP, TET, S, K, R (2)	
	CEP,AMP, TET, NA, S, R (2)	
	CEP, AMP, NA, TET, R, C(1)	
	CEP, AMP, NA, TET, S, K (1)	
7	CEP, AMP, NA, TET, S, K, R (2)	5
	CEP, AMP, TET, S, K, R, C (1)	
	CEP, AMP, NA, TET, S, R, C (1)	
	CEP, AMP, NA, TET, S, K, R (1)	

Nineteen (86.36%) isolates were resistant to at least five (5) tested antimicrobial agents. Regarding to the tested antimicrobials, Ampicillin and Tetracycline shows complete resistance (100%) to all tested isolates, followed by Cephalothin, Streptomycin and Rifampicin which shows 95.5%, 91% and 82% resistance to all tested isolates respectively. However, all isolates were susceptible to Ciprofloxacin and Gentamycin antimicrobials. Only two isolates (9.1%) were resistant to chloramphenicol antimicrobial tested.

5. DISCUSSIONS

5.1 Prevalence and distribution

Prevalence of *Salmonella* was consistently lower from samples collected from diarrheic patients in comparison to samples collected from feces from ready to be slaughtered animals and meat samples collected from the retailer market. The prevalence of *Salmonella* was low in fecal samples and diarrheic human patient samples. In humans, the prevalence of *Salmonella* in diarrheic patient was relatively consistent, regardless of exposure to livestock which agrees with the finding of Padungtod and Kaneene (2006). The possible explanation for low prevalence of non typhoidal *Salmonella* in diarrheic patients at Bishoftu General Hospital could be that patients were first visit health center and they were referred to this hospital if they weren't cured. Another most probable explanation is that most of diarrheic patients attending the hospital were infested by different parasites and protozoans rather than bacteria according to the annual report from the hospital and reports from different parts of the country such as (Edeget *et al.*, 2014; Eguale *et al.*, 2015; Beyene and Tasew, 2014).

5.1.1 Slaughtered animal feces

Salmonella occurrence in slaughtered animals, tissues and carcasses may be affected by hygienic conditions of holding pens, transportation stress, and length of stay in lairage, hygienic status of the slaughterhouse environment and even the prevalence of *Salmonella* in beef cattle population supplying the slaughterhouses (Mannone *et al.*, 2012). The occurrence of *Salmonella* in feces of slaughtered cattle observed in this study was generally lower when compared to previous reports from different regions of the county (Nyeleti *et al.*, 2000; Sibhat *et al.*, 2011; Alemu and Zewde, 2012; Kebede *et al.*, 2016) who reported 10.6%, 7.1%, 7% and 4.64% respectively. This may be due to that, most of the animals were came from beef fattening farms and these farms are using probiotics for prevention of bacterial infection and for fattening purpose (Kemal, 2014). This helps to reduce fecal shedding of *Salmonella* species in beef cattle. Another most probable explanation was that

the ready to be slaughtered animal doesn't stay more than 6 hours in ante mortem ranch. This means the animal doesn't stressed and the bacteria doesn't shed in feces as described by (Mannone *et al.*, 2012).

The recovery proportion of *Salmonella* in feces of ready to be slaughtered cattle in our study was higher than 0.6%, 1.3% 1.8% which were reported by Alemayehu *et al.*, 2003; Oloya *et al.*, 2007 Bekele and Ashenafi, 2009 respectively. But the present finding slightly agrees with the findings of Kore *et al.* (2017) who have reported 2% from ready to be slaughtered animals in Hawassa, Ethiopia.

5.1.2 Retailer shop meat

The prevalence of *Salmonella* in beef meat retailer shop was found to be 8.66 % (11/127) with 95% CI of 3.7-13.62. Factors, which account for increased prevalence, could be the knives, sows, hoes and storage utensils, which are not properly cleaned and disinfected, serve as means of cross-contamination for *Salmonella* from contaminated meat to clean meats. This is especially true when fat embedded infected lymph nodes, which contain higher number of *Salmonella*, are cut and same knife is used on other meats (Jay, 2000). The contamination of carcasses and beef with *Salmonella* known to cause human infections, even at a low level, is a significant public health risk in Ethiopia where a considerable proportion of the population consumes raw or undercooked beef.

The present finding 8.66% from meat retailer was high when compared with report elsewhere in the world such as, 5.2% from Karachi, Pakistan (Ali *et al.*, 2010) and comparable with the report from Hawassa, Ethiopia which was 9 % reported by Ashenafi (1994). But this finding is lower than 42% in raw and minced meat in Hawassa, Ethiopia, 14.4 % report from minced meat in Addis Ababa, 14.8 % observed in retail or butcher shops in India, report from raw retailed meat in Yucatan, Mexico 17% from retail meats of marketplace in Shaanxi and China and 54 % and (Ashenafi, 1994; Getahun *et al.*, 2004; Bhandare *et al.*, 2007; Zaidi *et al.*, 2006; Yang *et al.*, 2010) respectively. This difference in the prevalence of *Salmonella* could be due to differences in the sanitation of butcher shops,

hygienic standards of abattoir premises and meat handlers in the present study area was better than the previous ones.

5.1.3 Human diarrheic stool

A total 5 out of 216, 2.5 (95% CI=0.5-4.4) (5/216) was recorded from stool samples in the present finding. This finding is slightly lower than report of non typhoidal *Salmonella* from diarrheic outpatient children from Jimma which was 5.3% (Getnet *et al.*, 2011) and from patients with diarrhea and other gastrointestinal complaints in Addis Ababa, Ethiopia which was 6.2 % (Egualle *et al.*, 2015). This finding is also lower than the reports from elsewhere in the world such as Zaidi *et al.*, 2006; Lien-Khai *et al.*, 2010; Ke *et al.*, 2014 who have reported the prevalence of 18.7%, 4.9 and 4.11%, respectively. But this finding is slightly higher than the report of Zenebe *et al.* (2011) who has reported 0.8% prevalence from Jimma University specialized hospital, Jimma, Southwest Ethiopia.

Even though the present study doesn't characterize the isolates recovered at each study point, a correlation might be expected between the *Salmonella* species isolated from animals at the time of slaughter, meat at retailer and that of isolates recovered in humans, since the primary point of entry for *Salmonella* species into human populations was through consumption of meat and poultry as indicated by Gould *et al.* (2011).

The questionnaire analysis of the present study showed that, having cattle farming, visiting or contact of cattle feces, direct contact with cattle feces, contact with someone having diarrhea, attendance of large gathering, somebody having diarrhea in the gathering are risk factor for occurrence of diarrhea as caused by *Salmonella* species since the odds value of these factors are >1 even though there is non-significant p-value (>0.05). This may happened most probably because of most of the patients attending the Bishoftu General Hospital was from the town. However if the diarrheic patients were from both residential area (urban and rural) these factors may be a risk factor for the occurrence of diarrhea as it is stated by different authors (Chimalizeni *et al.*, 2010; Hendrickson *et al.*, 2010; Addis *et al.*, 2014). Contact with someone having diarrhea is a risk factor for isolation of *Salmonella*

with odds value of 10.1 and p-value of 0.000. Other factors listed in table 8 above were considered as a risk factor (odds ratio >1) even though their p-values are above 0.05.

5.2 Serotype distribution

From 22 *Salmonella* isolates 8 of them were serotyped and two dominant serotypes were identified. From these four (4) of them was *S. Typhimurium* while four of them were *S. eastbourne*. These two serotypes are the dominant isolates isolated from the bovine origin (Sibhat *et al.*, 2011; Tadesse and Tesemma, 2014; Kebede *et al.*, 2016) and have a significant public health, especially the *S. typhimurium*. *S. eastbourne* was reported as a zoonotic non-typhoidal salmonellosis in children playing with reptiles in Germany (Pees *et al.*, 2013). This serotype was also isolated both in cattle and in human in Nigeria (Fashae *et al.*, 2018) and from human diarrheic patient in Ghana (Andoh *et al.*, 2017). *S. eastbourne* was the dominant isolate isolated from cattle (Tadesse and Tesemma, 2014; Sibhat *et al.*, 2011; Hiko *et al.*, 2016) and from pigs (Aragaw *et al.*, 2007) from pigs in Ethiopia and elsewhere in the world (Mannion *et al.*, 2012; Carraturo *et al.*, 2016).

S. Typhimurium has a well-characterized ability to infect various species (Rabsch *et al.*, 2002) and can survive for a long time in the environment (Baudart *et al.*, 2000), enhancing its ability to be one of the most common causes of salmonellosis. *S. typhimurium* is the most dominant *Salmonella* serotype having zoonotic importance and isolated from human beings in different parts of the world (Ceysens *et al.*, 2015).

5.3 Antimicrobial resistance

The current study indicated the highest number 21 (95.45%) of multidrug resistant isolates. From these, 5 (22.73%) isolates were resistant to seven (70%) of different antimicrobials tested. The isolates being resistant to seven different antimicrobials were recovered from one meat sample and three of the stool samples as shown in the table 12 below. Three

isolates, one isolate from meat and two isolate from human diarrheic stool, shows similar resistance to seven different antimicrobials. This may show that there is a *Salmonella* species shared between meat from cattle and human diarrheic patient.

The highest resistance 100%, 100%, 95.5%, 91% and 82% was observed to Ampicillin, Tetracycline, Cephalothin, Streptomycin and Rifampicin in the present study respectively. This could be due to uncontrolled availability of the antimicrobial agents in drug vendors, which leads to misuse. Thus, this might exert greater selection pressure for the resistant strains thereby making them resistant to antimicrobials. The presence of antimicrobial resistance have the potential to adversely affect human health by causing illness that is more difficult to treat because of the resistance profile of the microorganism. The current study reported almost multidrug resistant isolates from different samples for each antimicrobial tested. But, there is a bit higher resistance observed among isolates from stool samples to maximum of seven (70%) different antimicrobial and minimum of five (50%) antimicrobials. This high resistance profile of *Salmonella* isolates to those antimicrobials might be attributed to high level of utilization of this drug both in veterinary and human medicines due to its relatively cheaper price and ready availability to the local community in the current study area.

Resistance for three or more of antimicrobials (95.45%) which was observed in this study was higher than other studies conducted in Ethiopia (Alemayehu *et al.*, 2003; Molla *et al.*, 2006; Zewdu and Cornelius, 2009; Sibhat *et al.*, 2011) and elsewhere in the world (Bouchrif *et al.*, 2009; Dallal *et al.*, 2009; Ali *et al.*, 2010; Hyeoy *et al.*, 2011; Michael and Schwarz, 2016). This difference may be due to the increasing rate of inappropriate utilization of antibiotics in the beef farms and livestock husbandry which favors selection pressure that increased the advantage of maintaining resistance genes in bacteria (Kemal, 2014). What is surprising from this research was that 90.1% (20/22) isolates were susceptible to Chloramphenicol in contrary to different reports (Beyene *et al.*, 2011) who reported 81.4%. But this finding agrees that the report of resistant salmonella isolates to Chloramphenicol drug decreases by 50% between 2005-2013 years ().

In the present study, all of the *Salmonella* isolates from beef meat were susceptible to Gentamycin and Ciprofloxacin which is consistent with other studies in Iran and Ethiopia (Dallal *et al.*, 2009; Alemu and Zewdu, 2012) respectively. Very good (90.9 %) efficacy was observed for Chloramphenicol. In contrary to 36.4% resistance of this study, 75 % to 96 % resistance to Nalidixic acid was reported from Modjo and Bahir Dar, Ethiopia (Alemu and Zewdu, 2012). But Thong and Modarressi, (2011) from Malaysia and Abebe *et al.* (2014) from Mekelle, Ethiopia reported resistance of *Salmonella* isolates from retailer meat to the Ciprofloxacin and Gentamycin. Resistance to Tetracycline, Ampicillin and kanamycin agrees with the finding of the Thong and Modarressi, 2012 and Alemu and Zewdu, 2012. Resistance to Cephalothin in this study agreed with the findings of Abebe *et al.*, 2014.

100% resistance of *Salmonella* isolates from human diarrheic stool to Ampicillin and Tetracycline agreed with the finding of Addis *et al.* (2011). In the present study, all isolates from human diarrheic patients were 100% susceptible to Ciprofloxacin and Gentamycin. Contrary to this finding 15% of *Salmonella* were resistant and 5% were intermediate resistant to Gentamycin and Ciprofloxacin as reported by Addis *et al.* (2011). Beyene *et al.*, 2011 reported the Ciprofloxacin resistance of *Salmonella* isolated from humans in Addis Ababa and Jimma was in disagreement to the present finding.

The antimicrobial susceptibility test result of the isolates recovered from feces of ready to be slaughtered animal agrees with the findings of Kebede *et al.*, 2016 who reported the 100% resistance to Streptomycin drug and almost all isolates were susceptible to Gentamycin drug which agrees with the present finding.

Results from this study indicate that Ciprofloxacin and Gentamicin are the drugs of choice for *Salmonella* treatment, since none of the isolates was resistant to these drugs. This finding helps for suggesting the use of these antibiotics.

6. CONCLUSION AND RECOMMENDATIONS

The results of this study indicated the presence of 2.5% *Salmonella* spp in cattle ready to be slaughtered at municipal abattoir and one private slaughter house in Bishoftu and 8.66% beef meat sold in retailer meat shops in the town by bacterial culture and biochemical characteristics test method. In addition to this, 2.3% *Salmonella* was recovered from 216 diarrheic patients treated in Bishoftu General Hospital during study period. The study also demonstrated that isolates are characterized by multiple drug resistance from all sample sources to commonly prescribed drugs both in veterinary and human pharmacies. The prevalence of *Salmonella* in healthy cattle needs attention since the daily life of most Ethiopian population was integrated with the husbandry of cattle. Furthermore, the contamination of beef meat with *Salmonella* serotypes known to cause human infections, although at a low level, is a significant public health risk in Ethiopia where a considerable proportion of the population consumes raw or undercooked beef. This study contributes to give information on the occurrence and antibiogram of *Salmonella* in general. Based up on the above conclusion the following recommendations were forwarded.

- ☒ Improve method and hygiene of meat transport from slaughterhouse to retail markets such as use of refrigerated transport vehicle.
- ☒ There is a need to educate consumers, meat handlers and all others who have access to beef supply chain about the importance of hygiene, cooling system, etc. in preventing *Salmonella* infection
- ☒ There is a need to gather sufficient information on the occurrence and distribution of *Salmonella* serotypes starting from farm level in order to control beef contamination and subsequent infection of human beings.
- ☒ Encouraging prudent and judicious use of antimicrobial drugs in veterinary and public health sectors.
- ☒ Continuous monitoring of antimicrobial resistance pattern and gene of isolates assists in tracing origin of strains causing human salmonellosis thereby limiting prevalence.

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Annexes 3: Type and preparation of media used for isolation, biochemical test and antimicrobial susceptibility test

1. Buffered Peptone Water (Cat. 1402.00, CONDA, Madrid, Spain)

Preparation: suspend 20 grams of components in 1000ml of distilled water. Mix well and distribute into universal bottle of suitable capacity to obtain the portions necessary for the test and sterilize in autoclave at 121 °C for 12 minutes. Final PH is 7.0 ± 0.2 at 25°C.

Composition (g/l): Enzymatic digest of casein 10.0; Sodium chloride 5.0; disodium phosphate dodecahydrate 9.0 and potassium dihydrogen phosphate 9.0

2. Modified Rappaport Vassiliadis Salmonella enrichment Broth (MH 491-500G, HIMEDIA, Mumbai, India)

Preparation: suspend 27.11 grams of hydrated medium in 1000ml distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired in to tubes and sterilize by autoclaving at 115 °C for 15 minutes. PH after sterilization: 5.2 ± 0.2

Composition (g/l): soya peptone 4.5; sodium chloride 8.00; potassium dihydrogen phosphate 0.60; dipotassium phosphate 0.40; magnesium chloride, hexahydrate 29.00; malachite green 0.036.

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

Preparation: Suspend 53grams in one liter of distilled water. Heat with frequent agitation until the medium boils. DO NOT OVER HEAT. Transfer immediately to a water bath at 50°C. pour in to plates 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 °C

Composition (g/l): yeast extracts 3.0; l-lysine hydrochloric acid 5.0; xylose 3.75; lactose 7.5; sucrose 7.5; L-Lysine hydrochloride 5.0; sodium chloride 5.0; sodium thiosulphate 6.8; ferric ammonium citrate 0.8; phenol red 0.08; agar 15.0.

4. Nutrient Agar (CM 0003, OXOID, Basingstoke, England)

Preparation: suspend 28 grams in 100ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure (121°C) for 15 minutes. Mix well and pour in to sterile petridishes. Final PH (at 25°C): 7.4 ± 0.2 .

Composition (g/l): peptic digest of animal tissue 5.00; sodium chloride 5.00; beef extract 1.5; yeast extract 1.5; agar 15.

5. Triple sugar agar (CM 0277, OXOID, Basingstoke, England)

Preparation: suspend 65 grams in 1000ml of distilled water. Bring to boil to dissolve completely. Mix well and distribute in to containers. Sterilize by autoclaving at 121°C for 25 minutes. Allow the set as slope with 2.5 cm butts. PH: 7.4 + 0.2 at 25°C.

Composition (g/l): 'meat extract 3.0; yeast extract 3.0g; peptone 20.0; sodium chloride 5.0; lactose 10.0; sucrose 10.0; glucose 1.0; ferric citrate 0.3; sodium thiosulfate 0.3; phenol red 0.024; agar 12.0

7. MR-VP Medium (M 070-500g, HIMEDIA, Mumbai, India)

Preparation: suspend 17.0 gram in 1000ml distilled water. Heat if necessary to dissolve the medium completely. Distribute in to test tubes 10ml amounts and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Composition (g/l): buffered peptone 7.00; dextrose 5.00; dipotassium phosphate 5.00

Reagent required for voges- proskauer reaction

- ✓ α -Naphtanol, ethanolic solution

Preparation: dissolve α -Naphtanol in ethanol

Composition (g/l): α -Naphtanol 6 grams; ethanol 96 % (volume fraction) 100ml.

- ✓ Potassium hydroxide solution

Preparation: dissolve potassium hydroxide in distilled water

Composition (g/l): potassium hydroxide 40 grams; distilled water 100ml

8. Mueller-Hinton Agar (CM 0337, OXOID, Basingstoke, England)

Preparation: suspend 38 grams in 1000ml of distilled water. Bring to boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes. PH: 7.3 + 0.1 at 25°C

Composition (g/l): beef, dehydrated infusion 300.00; casein hydrolysate 17.5; starch 1.5; agar 17.00

9: 0.5 McFarland standards

Composition: 1.17% BaCl₂H₂O solution and 0.36N of 1% sulfuric acid (H₂SO₄).

Preparation: Add approximately 85ml of 1% H₂SO₄ to a 100ml of volumetric flask, using a 0.5ml pipette add 0.5ml of 1.17% BaCl₂H₂O drop wise to the H₂SO₄ while constantly swirling 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 tube tightly and seal with paraffin and keep at dark and room temperature.

10. Urea broth (M 111-500g, HIMEDIA, Mumbai, India)

Preparation: 38.7g/l was dissolved in Sterilize by filtration or dispense aliquots of approximately 3 ml into test tubes and sterilize for 5 minutes in a current of steam under mild conditions. Don't autoclaved. PH = 6.8.±0.1. The broth was clear and yellow-orange.

Composition (g/l): Yeast extract 0.1, Potassium dihydrogen phosphate 9.1, Disodium hydrogen phosphate 9.5, Urea 20.0, Phenol red 0.01

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

Preparation: 5.25 g/500ml and 5g/500ml decarboxylase base moller and L-Lysine monohydrochloride respectively were dissolved together by heating if necessary and dispense 5ml in to test tubes and sterilize at 121°Cfor 10 minutes. The broth were clear and yellow to amber.

Composition (g/l): L-Lysine monohydrochloride 5.0, yeast extract 3.0, glucose 1.0, bromocresol purple 0.015.

12. Tryptone broth

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°Cfor 15 minutes. The broth was clear and yellow.

Composition (g/L): tryptone 10.0, Sodium Chloride 5.0, DL-Tryptophan 1.0.

Annexes 4: Principles and result characteristics of biochemical tests of *Salmonella*

Media	Principles Reactions/ Enzymes	Result	
		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
Tryptone broth	H ₂ S production	no black color	black color
	Indole production	no red ring on the top of the broth	red ring formation on top of the broth
	Formation of ammonia and carbonic acid	media retains its original color	the broth is changed to red/ pink
Urea broth			
Lysine decarboxylase			

Source: Quinn *et al.*, 2002

Annexes 5: Procedures and interpretation of biochemical tests

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

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

5.3 Voges-Proskauer test: suspend a loopful of the suspected colony in a sterile tube containing 3ml of the VP medium. Incubate at 37°C for 24hrs.

After incubation add two drops of the creatine solution, three drops of ethanolic solution of 1-alphanaphtanol and then two drops of potassium hydroxide solution, shake after addition of each reagent.

Interpretation: the formation of a pink to bright red color within 15minutes indicates positive reaction

5.4 Indole test: inoculate a tube containing 5ml of the tryptone/tryptophan medium with the suspected colony. Then incubate at 37oc 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: Appearance of salmonella on biochemical test

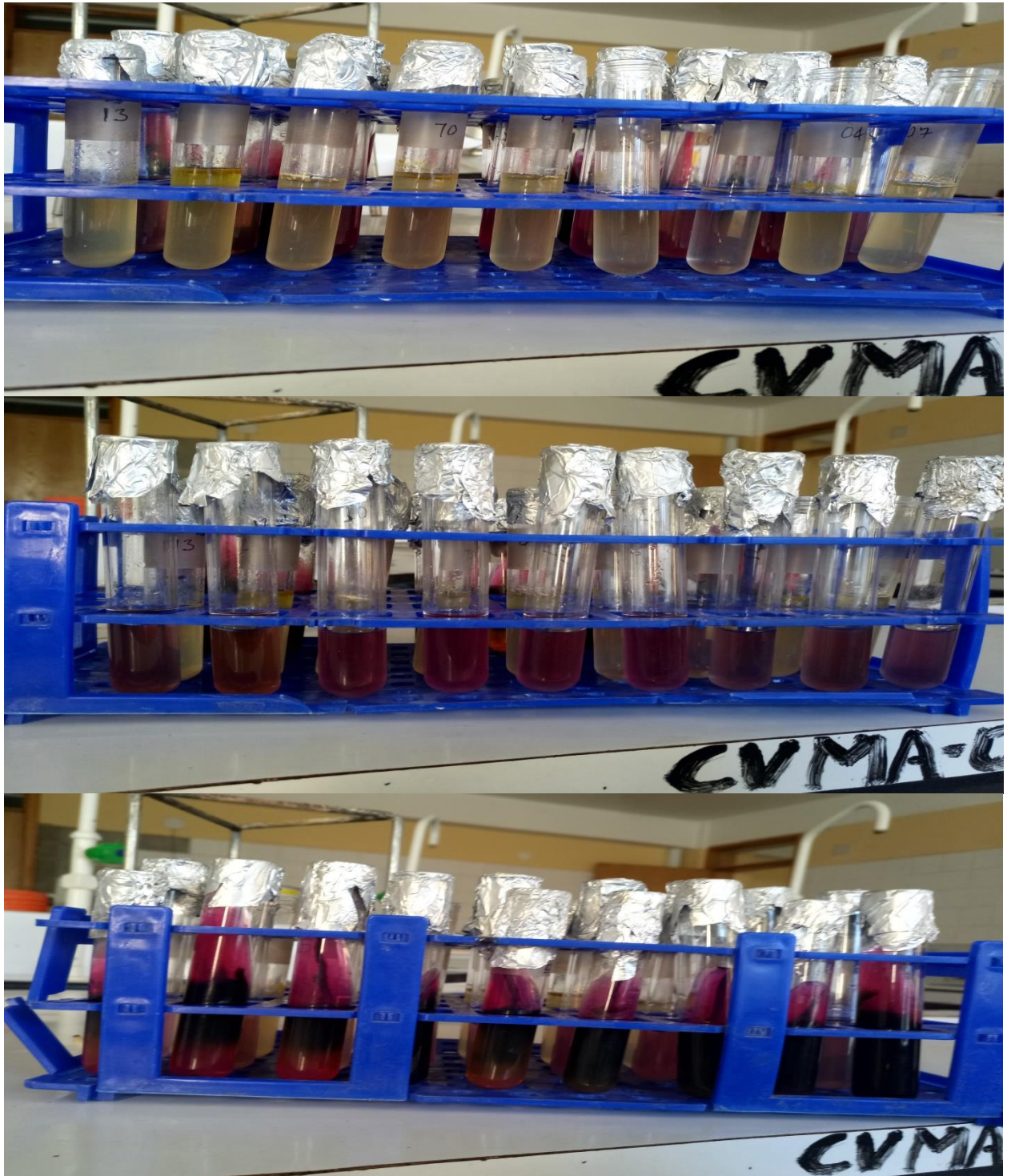


Figure 3: Appearance of *Salmonella* on Selective enrichment media (MRVM) on the top and the selective plating agar (XLD) the bottom one.

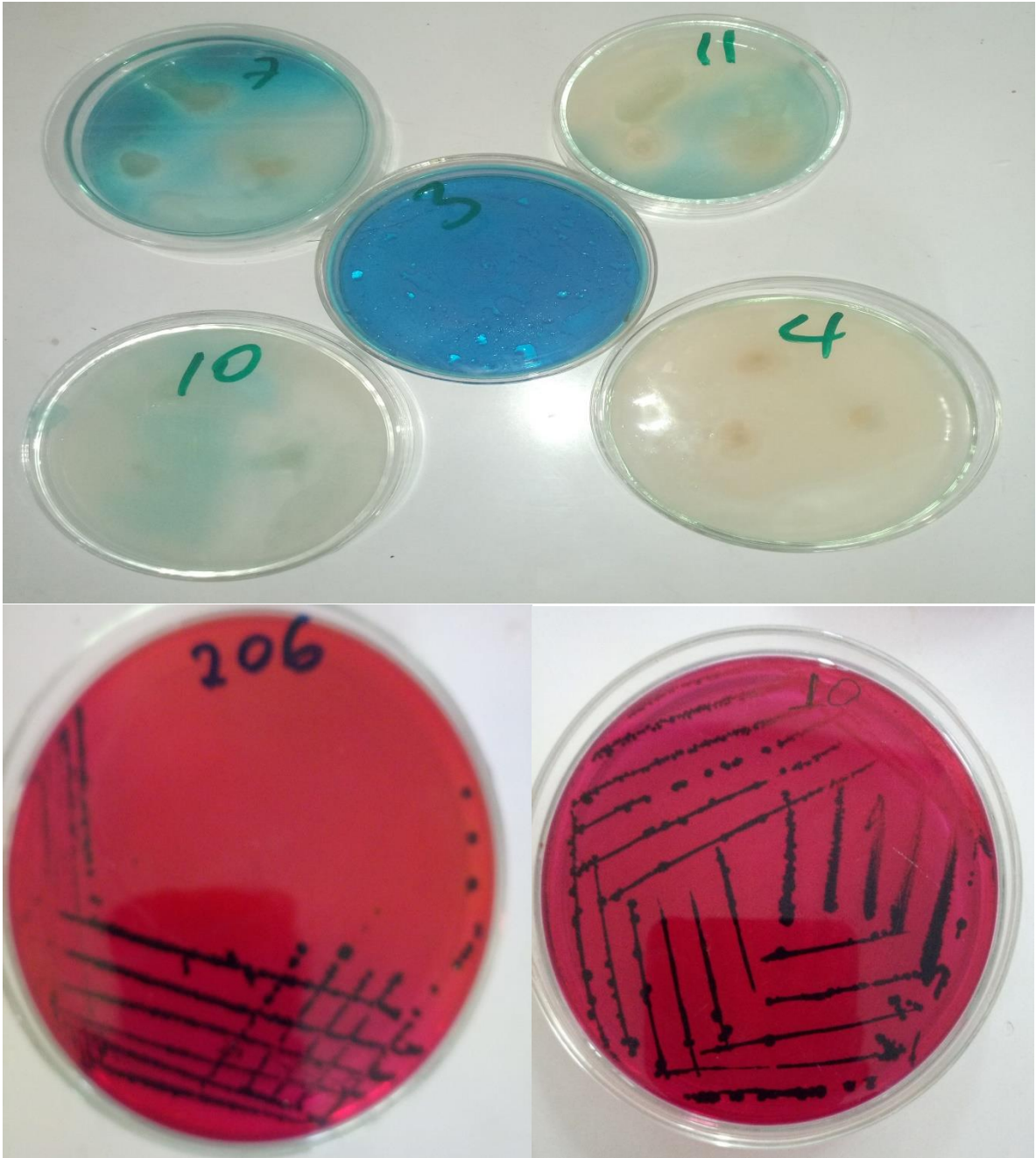


Figure 4: Antimicrobials testing procedure and disk diffusion appearance



Annexes 6: Questionnaire for diarrheic patients

Introduction: Hello? My name is Olana Merera and I am conducting a research work entitled “**Detection, serotype distribution and antimicrobial susceptibility test of *Salmonella* along beef supply chain and out patients from Bishoftu General Hospital**”. I’m from Addis Ababa University. I would like to ask you some questions about your socio demography, illness, having cattle and foods that you ate before becoming ill, that will help us in this work. The results of the study and related information will only be used for the purpose of this study. Your name will not be used on the sample and/or any report that might result from the study. We will use codes specific to the study. This will take about 15-20 minutes. Can we go ahead?

A. Basic information

1. Date _____
2. Questionnaire Code _____

B. Socio demographic characteristics of the patient

1. Sex: Male Female
2. Age _____ years (completed)
3. Level of Education: Illiterate Informal Education Primary Education Secondary Education Other (Specify).....
4. Ethnicity Oromo Amhara Tigire Gurage Others (specify) _____
5. Religion Orthodox Muslim Protestant Catholic Others (specify)
6. Residence Urban Rural
7. Occupation _____

C. Clinical information (*Taking into account the incubation period: E. coli O157:H7: 2-7 days, Campylobacter: 1-10 days, Salmonella: 0-5 days*)

1. Duration of diarrhea since onset _____
2. Maximum number of episode of diarrhea in last 24-hour period _____
3. Consistency of diarrhea watery mucoid bloody mixed
4. Episode of diarrhea in the last one to two years one two three four and more than four

D. Other potential risk factors (Exposure assessment)

1. Do you have cattle for farming? Yes No
2. Have you had visit or contact with farm animals (cattle) in the last two weeks before illness Yes No
3. Have you had direct contact with cattle feces before illness Yes No
4. Beef consumption behavior Raw heat treated semi-heat treated All forms
5. Did you consume raw meat in the last two weeks before illness? Yes No
6. Did you travel anywhere during the last two weeks before illness? Yes No
7. If yes, did you eat food containing beef Yes No
8. Did you eat beef outside home (retail shop, restaurants etc.) during the last two weeks before illness Yes No
9. Had contact with someone with diarrhea illness before becoming ill? Yes No
10. Did you attend a large gathering like wedding ceremony the week before your illness? Yes No
11. If yes, did others develop similar illness? Yes No I do not know

Thank you!

Annexes 7: Ethical clearance