JIMMA UNIVERSITY

COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE

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ISOLATION, IDENTIFICATION, ANTIMICROBIAL RESISTANCE PATTERN OF*SALMONELLA*ANDPUBLIC AWARENESS ON MEAT HYGIENE AT MIZAN MUNICIPAL ABATTOIR AND BUTCHER HOUSES, SOUTHWEST ETHIOPIA

MSc THESIS

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Isolation, Identification, Antimicrobial Resistance Pattern of *Salmonella* and Public Awareness on Meat Hygiene at Mizan Municipal Abattoir and Butcher Houses, Southwest Ethiopia

A Thesis

Submitted to the school of Veterinary medicine, Jimma University College of Agriculture and Veterinary Medicine in partial fulfillment of the requirements for the Degree of Master of science in Veterinary Public Health

By

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I have incorporated the suggestion and modification given during the internal thesis defense and got the approval of my advisors. Hence, I hereby kindly request the department to allow me to submit my thesis for external thesis defense.

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DEDICATION

I dedicate this thesis to my daughter Harona Aregahegn. She missed paternity care and love for two years when I left home for MSc studies after years of her birth.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my own work and that all sources of material used for this thesis have been duly acknowledged.

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BIOGRAPHICAL SKETCH

Aregahegn was born in Andiracha woreda, Sheka Zone, South nation nationality and people regional State on May 23, 1981 E.C from his Mather Birhane Geremew and from his father Alemu Yebereta. He attended his primary school at Andiracha Primary and Junior Secondary School and secondary school education at Andiracha Comprehensive Secondary School. In 1998 he joined Alage ATVET College and graduated with diploma in animal health. Soon after graduation he hired by the Masha Livestock and Fisheries Office as an animal health assistant. In 2005, he joined Wolaita Sodo University and graduated with a Bachelor degree in Animal health in 2009. He again has joined graduate program at Jimma University to peruse his career in Veterinary Public Health.

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ACRONYMS AND ABBREVIATIONS

AAP	American Academy of Pediatrics
AST	Antimicrobial Susceptibility Testing
BGA	Brilliant Green Agar
BPW	Buffered Peptone Water
CDC	Center for disease control and prevention
CFU	Colony Forming Unit
CLS	Clinical and Laboratory Standards Institute
EU	European Union
H2S	Hydrogen Sulphide
ISO	International Organization for Standardization
LIA	Lysine Iron agar
MDR	Multi-Drug Resistant
MKTTn	Muller-Kauffmann Tetrathionet with novobiocinnin
NCCLS	National Committee for Clinical Laboratory Standards
NHS	National Health Services for Wales
NMK	Nordic Committee on Food Analysis
OIE	Office International des Epizooties
RV	Rappaport Vassiliadis broth
SC	Selenite Cysteine broth
Spp.	Species
Sub sp.	Subspecies
TSI	Triple Sugar Iron agar
VLA	Veterinary Laboratories Agency
VP	Voges-Proskauer
WHO	World Health Organization
WTO	World Trade Organization
XLD	Xylose lysine desoxycholate
µg	Microgram

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ABSTRACT

Foodborne diseases are among the most widespread global public health problems and their implication for economy is increasingly recognized. *Salmonella* is the leading cause of foodborne diseases worldwide. A cross-sectional study was conducted from March to September 2019 at municipal abattoir and butcher houses of Mizan town, Ethiopia with the objectives to assess the occurrence of risk factors, antimicrobial resistance pattern of *Salmonella*isolates and awareness status of meat handlers on meat hygiene and safety. A total of 320 samples consisting of 240 from abattoir (beef carcass swab = 175, abattoir personnel hand swab =25, abattoir material swab =40) and 80 from butcher houses (butcher hand swab =30, butcher material swab =50) were collected and examined for the presence of *Salmonella* using the standard techniques and procedures outlined by the International Organization for Standardization. The overall prevalence of *Salmonella* was found to be 13.4% (43/320). Out of a total isolates, 30/240 (12.5%) were isolated from abattoir source; of which 21/175 (12%) from carcass swab, 4/25 (16%) from abattoir personnel hand swab and 5/40 (12.5%) from abattoir materials swab while 13/80 (16.2%) from butcher houses source; of which 5/30 (16.6%) from butcher personnel hand swab and 8/50 (16%) from butcher materials swab. However, there was no statistically significant difference observed in the prevalence of *salmonella* among sample source and type ($p > 0.05$). Out of the total 43 isolates subjected to a panel of 8 antimicrobials, 42 (97.67%) were multiple antimicrobial resistant and the highest level of resistance was observed for erythromycin (100%), ampicillin (83.7%), oxacilin (72.09%) and neomycin (67.44%).However, all isolates were susceptible to gentamycin. Multivariable logistic regression result showed that, materials which were not cleaned (OR=12.56; 95% CI: 0.986-160; P=0.048) and people who didn't knew contamination as risk (OR=11.5; 95% CI: 1.65-80; P=0.014) were the major risk factors for the occurrence of *Salmonella* among abattoir and butcher houses in the study area.Besides, the knowledge, attitude and practices of beef meat handlers (abattoir workers and butchers) were founded to be poor. Thus, urgent intervention program to minimize the risks associated for contamination of meat with *Salmonella* and prudent use of antimicrobialswere recommended.

***Key Words**: Abattoir, Antimicrobial Resistance, Butchers, Beef Meat, Prevalence, Salmonella, Mizan*

1. INTRODUCTION

1.1. Background Justification

Foodborne diseases are among the most widespread global public health problems of recent times, and their implication for health and economy is increasingly recognized (Hendriksen *et al.*, 2011 and Majowicz *et al.*, 2010). According to reports, every year, a huge number of people suffer from foodborne diseases worldwide due to contaminated food and water consumption (Majowicz *et al.*, 2010 and Käferstein, 2003).

There are many and varied sources of organisms causing food poisoning. Most cases of food poisoning are caused by bacteria which arise from animal, human or environmental sources (Gracey *et al.*, 1999). Contaminated raw meat is one of the main sources of foodborne illnesses (Bhandare *et al.*, 2007). Specific sources that contribute microbial contamination to animal carcasses and to fresh meat during slaughter and dressing include the faeces, the hide, water, air, intestinal contents, lymph nodes, processing equipment, and humans (Sofos, 2005), and can be transferred to the carcass during skin removal and evisceration (Reid *et al.*, 2002 and Hansson *et al.*, 2000).

There are four major pathogens that have frequently been associated with meat and meat products including *Salmonella* s*pecies*, *Campylobacter species*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7. These organisms have been linked to a number of cases of human illness (Mershal *et al.*, 2010). Among these, *Salmonella* is considered the most prevalent foodborne pathogen worldwide (Carrasco *et al.*, 2012 and Sánchez-Vargas *et al.*, 2011) and has long been recognized as an important zoonotic microorganism of economic significance in animals and humans (Carrasco *et al.*, 2012), predominantly in the developing countries.

Consumption of raw or unsafe food, cross-contamination, improper food storage, poor personal hygiene practices, inadequate cooling and reheating of food items, and a prolonged time lapse between preparing and consuming food items were mentioned as contributing factors to an outbreak of salmonellosis in humans (Carrasco *et al.*,2012 and Käferstein,2003).The ubiquity of *Salmonella* isolates creates a persistent contamination hazard in all raw foods (Carrasco *et al.*,2012) and also in animal-origin food products, which

are often implicated in sporadic cases and outbreaks of human salmonellosis(Tadesse ,2014 and Majowicz *et al.*,2010).

Antibiotic-resistant *Salmonella* infections of both human and animal are universal concerns, particularly in developing countries where the risk of infection is high because of unhygienic living conditions, close contact and sharing of houses between animals and humans (Feasey *et al.*,2012), and the traditions of consumption of raw or undercooked animal-origin food items. There is an increasing concern with this pathogen due to the emergence and spread of antibiotic-resistant and potentially more pathogenic strains. Moreover, an increase in the resistance of *Salmonella* to commonly used antimicrobials has been also noted in both public health and veterinary sectors in Ethiopia (Asrat, 2008; Molla *et al.,* 2003 and Molla *et al.,* 1999).

1.2. Statement of the problem

Salmonellosis is a perilous threat to livestock and public health, caused by *Salmonella* spp. These organisms are motile (exclude *S. pullorum* and *S. gallinarum*), gram-negative, rod-shaped, non-spore forming, non-capsulated, facultative anaerobic bacteria belongs to family *Entero-bacteriaceae* (Agbaje *et al.,* 2011). Being that, *Salmonella* is consistently among the leading birthplace of food-borne illness throughout the world. More than 36,000 serotypes were described and named are considered potentially pathogenic (Jackson *et al.,* 2013). Although this may be true that some serotypes are host-specific, but the majority can affect multitudinous hosts (Uzzau *et al.,* 2000). The primary reservoir of *Salmonella* is the intestinal tract of humans and animals, particularly in poultry and swine. Contaminated meats, mainly from avian and livestock origins are the prospective source of human salmonellosis therefore the most important fountainhead of meat-borne public health hazard (Buncic *et al.,* 2014 and Kabir, 2010).

Studies conducted in different parts of Ethiopia have demonstrated that, the incidence of foodborne *Salmonella* infections has increased dramatically over the past few years. Fore stance the presence of *Salmonella i*n human beings (Garedew-Kifelew *et al.,* 2014; Tadesse, 2014 and Nyeleti *et al.,* 2000) and in different food animals and food products (Garedew *et al.,*2012; Molla *et al.,* 2003 and Nyeleti *et al.,*2000) have been reported. At Mizan municipal

abattoir, there is no clear division of the slaughtering process into stunning, bleeding, skinning, evisceration, chilling, cutting, or frozen delivery. Bleeding and evisceration has been conducted on a horizontal position on the floor by incising the hide at the bottom of the abdomen without flying the skin. Workers hoisted the carcass manually using a chained pulley system after flying the skin and evisceration on the floor. There is no knife and axe sharpening machines. There is no means of sterilizing equipment. Carcasses are manually quartered using axes that increases the cross contamination of the carcasses in the abattoir. Further more there is much less information on the knowledge, attitudes and practices (KAP) around meat safety; the gender and social determinants of meat safety; or the relation between hazards in meat and health outcomes in consumers of meat in the country. In line with the aforementioned limitations in the study area the prevalence of *Salmonella* species andtheirantimicrobial resistance patternas well as the knowledge, attitude and practice of the community was not yet known. Therefore, this study was designed with the following general and specific objectives.

1.3. Objectives

1.3.1. General objective

To assess the occurrence of risk factors, antimicrobial resistance pattern of *Salmonella*isolates and public awareness (abattoir and butcher house workers) on meat hygiene in the study area.

1.3.2. Specific objectives

- To determine the prevalence and antimicrobial resistance pattern of *Salmonella* isolates from slaughtered cattle, personnel and materials in the abattoir and butcher houses.
- To determine risk factors associated for the occurrence of *salmonella*.
- To assess the knowledge, attitude and practice of meat handlers: abattoir workers and butchers on meat hygiene and safety.

1.4. Research Questions

- What is the prevalence and antimicrobial resistance pattern of *Salmonella* in apparently healthy slaughtered cattle, personnel and materials in the abattoir and butcher houses of the study area?

- What factors are associated with the occurrence of *salmonella* among personnel and materials in Abattoir, Butcher houses?
- What are the knowledge, attitude and practice of abattoir workers and butchers on meat hygiene and food safety?

2. LITERATURE REVIEW

2.1. Overview of *Salmonella*

2.1.1. Historical background

The *Salmonella* bacterium was first described by Theobald Smith (1859-1934) and then in 1885, two American veterinarians, Salmon and Smith isolated the bacterium causing hog cholera from infected pigs (Salmon and Smith, 1886).The name *Salmonella* was consequently adopted in honor of Dr. Salmon. Over the decades following the innovative work of Salmon and Smith, many other *Salmonella* were isolated from both animals and humans (Getenet, 2008 and Widal, 1896). The antigenic classification or serotyping of *Salmonella* used today is an outcome of years of study of antibody interfaces with bacterial surface antigens by Kauffman and White in the 1920s to 1940s (Kauffmann, 1950). According to this Kauffmann-White scheme, each *Salmonella* serotype is known by its possession of a particular lipopolysaccharide (LPS) or O antigen and a flagellar or H antigen. This led to the description of more than 2500 serotypes at present (Popoff *et al.*, 2004; Brenner *et al.*, 2000 and Popoff *et al.*, 1998).

2.1.2. Classification and nomenclature

Historically the name *Salmonella* had given grounded on the original places of isolation such as *Salmonella* London and *Salmonella* Indiana. This nomenclature system was changed by the classification based on the susceptibility of isolates to different selected bacteriophages which is also known as phage typing. Phage typing is generally employed when the origin and characteristic of an outbreak must be determined by differentiating the isolates of the same serotype. It is very reproducible when international standard sets of typing phages are used more than 200 definitive phage types (DT) have been reported so far. For instance, *S. Typhimurium* DT104 designates a particular phage type for Typhimurium isolates (Pui *et al.*, 2011; Andrews and Baumler, 2005 and Hanes, 2003).

Epidemiologic classification of *Salmonella* is based on the host preferences. The first group includes host-restricted serotypes that infect only humans such as *S. Typhi*. The second group includes host-adapted serotypes which are related with one host species but can cause disease

in other hosts serotypes such as *S. Pullorum* in avian. The third group includes the remaining serotypes. Typically, *SalmonellaEnteritidis,SalmonellaTyphimurium* and *SalmonellaHeidelberg* are the three most frequent serotypes recovered from humans each year (Boyen *et al.*, 2008 and Gray and Fedorka-Cray, 2002).

The genus consists of two species: the first is *S. enterica* which is divided into six subspecies (Figure 1*): S. enterica* subsp. *enterica, S. enterica* subsp. *salamae, S. enterica* subsp. *arizonae, S. enterica* subsp. *diarizonae, S. enterica* subsp. *houtenae* and *S. enterica* subsp. indica; and the second is *S. bongori* (formerly called *S. enterica* subsp. *bongori*) (WHO, 2003c). *Salmonella enterica* subspecies I is principally isolated from warm-blooded animals and accounts for more than 99% of clinical isolates while remaining subspecies and *S. bongori* are mainly isolated from cold-blooded animals and account for less than 1% of clinical isolates. As an example, the Kauffmann species *Salmonella* Typhimurium is now nominated as *Salmonellaenterica* subspecies I serotype Typhimurium. Under the modern nomenclature system, the subspecies information is often lost and culture is called *S. enterica* serotype Typhimurium and in subsequent form, it is written as *S. Typhimurium*. This system of nomenclature is used currently to bring consistency in reporting (Parry, 2006 and Andrews and Baumler, 2005).

Kauffmann-White scheme classifies *Salmonella* according to three major antigenic determining factor composed of flagellar H antigens, somatic O antigens and virulence (Vi) capsular K antigens. This was accepted by the International Association of Microbiologists in 1934. Agglutination by antibodies specific for the various O antigens is employed to group *Salmonella*e into the 6 sero groups: A, B, C1, C2, D and E. For example, S. Paratyphi A, B, C and *S. Typhi* express O antigens of sero groups A, B, C1 and D, respectively. More than 99% of *Salmonella* strains causing human infections belong to *Salmonellaenteric* subspecies *enterica*. Even though not common, cross-reactivity between O antigens of *Salmonella* and other genera of *Enterobacteriaceae* do occur (Pui *et al.*, 2011).

As a result, further classification of serotypes is based on the antigenicity of the flagellar H antigens which are highly specific for *Salmonella* (Scherer and Miller, 2001). In brief, O antigens are lipopolysaccharide (LPS) of the outer bacterial membrane. They are heat stable,

resistant to alcohol and dilute acids. H antigens are heat-labile proteins associated with the peritrichous flagella and can be expressed in one of two stages. The stage 1 H antigens are specific and associated with the immunological identity of the specific serovars while stage 2 antigens are non-specific antigens having different antigenic subunit proteins which can be shared by many serovars. K antigens which are heat- sensitive carbohydrates are formed by *Salmonella* serovars that express a surface-bound polysaccharide capsular antigen (Hu and Kopecko, 2003; Yousef and Carlstrom, 2003).

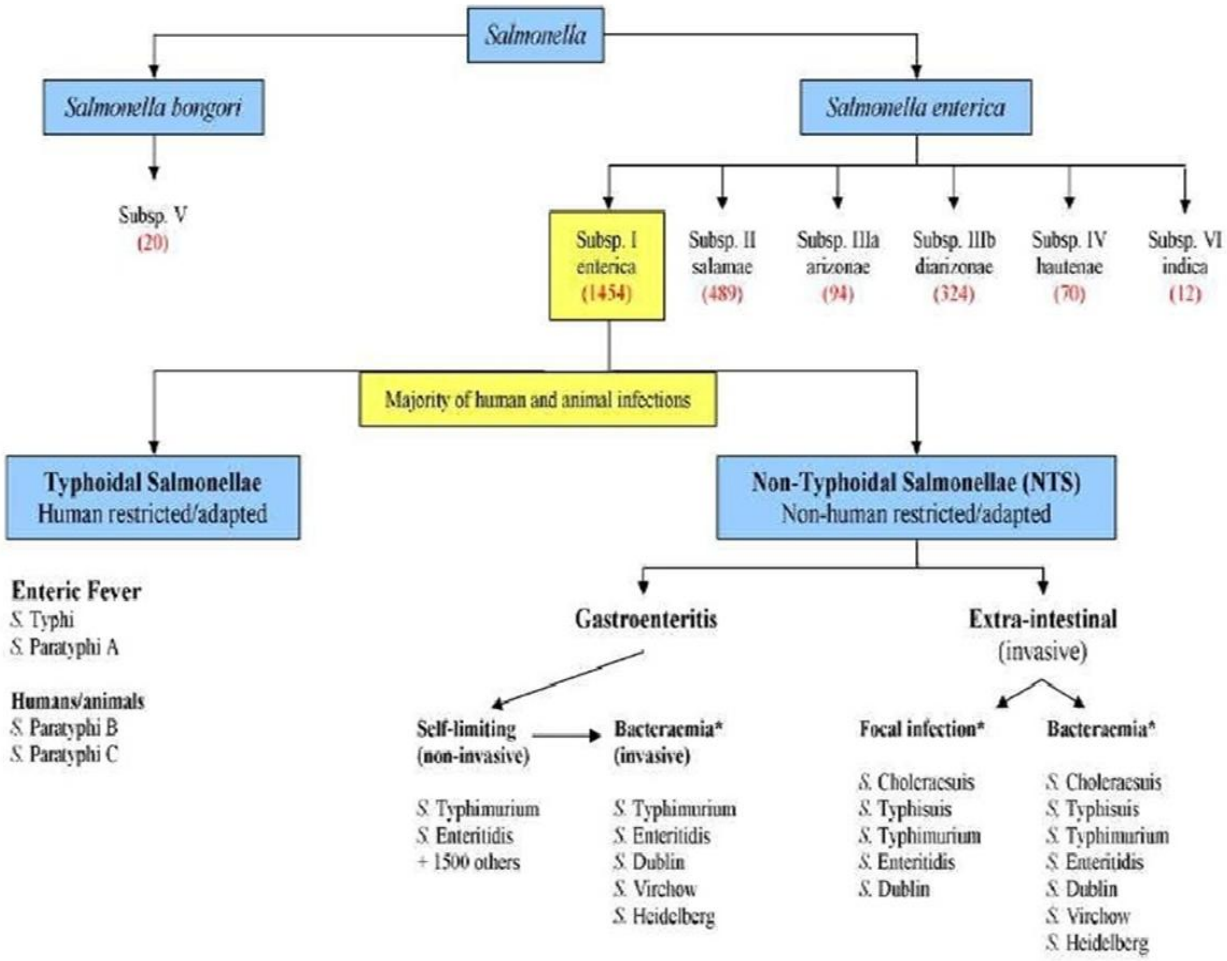

Figure 1: Classification and nomenclature

Source: Langridge *et al.*, (2008).

Note: Numbers in brackets designate the total number of serotypes comprised in each subspecies.
* Common serotypes are enumerated but other serotypes may cause bacteremia or focal infection; subsp = subspecies

2.1.3. Geographic distribution and host range

Salmonella is one of the leading causes of bacterial foodborne disease in industrialized as well as developing countries although the occurrence looks to differ between countries (Chiu *et al.,* 2004; Molla *et al.,* 2003; D‟Aoust, 1997 and Radostits *et al.,* 1994). The extensive differences in the country incidence of Salmonellosis possibly arise from narrow scope of studies and deficiency of synchronized epidemiological investigation systems, under-reporting of cases and the presence of other diseases considered being of main concern (Molla *et al.,* 2003 and Radostits *et al.,* 1994).

The epidemiology of salmonellosis is complex largely because there are more than 2,500 distinct serotypes (serovars) with different reservoirs and various geographic incidences. Variations in food consumption, production, and supply have directed to an increasing rate of multistate epidemics linked with fresh produced and processed foods (Rounds *et al.,* 2010).

According to the WHO Global Salm-Surv, during 2000-2002, *S. Enteritidis* was by far the most common serotype described from humans worldwide. In 2002, it accounted for 65% of all isolates, followed by *S. Typhimurium* at (12%) and *S.Newport* at (4%). Amongst non-human isolates, *S. Typhimurium* was the most commonly reported serotype in all the three years, accounting for (17%) of isolates in 2002 followed by S. Heidelberg (11%) and *S. Enteritidis* (9%). *SalmonellaEnteritidis, S. Typhimurium* and *S. Typhi* were ranked among the fifteen most common human serotypes in all regions of the world all over the three year study period. *Salmonella Agona, S. Infantis, S. Montevideo, S.Saintpaul, S. Hadar,S.Mbandaka,S. Newport, S. Thompson, S. Heidelberg* and *S.Virchow* were also widespread. In Africa by 2002, *S.* Enteritidis and *S.* Typhimurium were each reported from approximately one fourth of isolates from humans (Galanis *et al.,* 2006 and Swaminathan *et al.,* 2006).

2.1.4. Reservoir host and source of infection

Salmonellosis is the most common foodborne disease in both developing and developed countries, although incidence rates vary according to the country (Stevens *et al.,* 2006).The fecal wastes from infected animals and humans are significant bases of bacterial

contamination of the environment and the food chain (Ponce *et al.,* 2008). Members' of *Salmonellaenterica* subspecies *enterica* are commonly dispersed in the environment and in the intestinal tracts of animals (Anjum *et al.,* 2011). People can become infected resulting failure of personal hygiene after contact with infected animals and or other infected people. Environmental contamination, especially untreated water is also important (Gracey *et al.*, 1999). Most human infections are acquired through consumption of contaminated food of animal origin (Anjum *et al.,* 2011 and Gracey *et al.,* 1999).

Foods of animal origin, principally meat, poultry, and, in some instances, unpasteurized egg products are considered to be the primary sources of human salmonellosis (Acha and Szyfres, 2001; White *et al.,* 2001; Wray and Davies, 2000; Nielsen *et al.,* 1995 and Tauxe, 1991). It has been reported that livestock and their products can contribute to as much as 96% of the total *Salmonella* infection in humans (Dahal, 2007). Most of these food products, e.g. beef, mutton and poultry, become contaminated through slaughter and processing, from the gut contents of healthy excreting animals.

Similarly, all food that is produced or processed in a contaminated environment may become contaminated with *Salmonellae* and be responsible for outbreaks or separate cases of disease as a result of mistakes in transport, storage, or preparation (D"Aoust, 1997). Contrasting *S. typhi* and *S. paratyphi*, whose only reservoir is humans; non-typhoidal salmonellosis is acquired from multiple animal reservoirs (Fuaci and Jameson, 2005).

A less common source of non-typhoidal *Salmonella* infections is contact to pets, particularly reptiles. Fecal carriage rates in reptiles can be more than 90%. It is expected that nearly 74,000 infections with *Salmonella* result from exposure to reptiles and amphibians in the United States each year (AAP, 2013). Since 1986, an increase in the status of non-banned reptiles, including iguanas, has been followed by increases in degrees of *Salmonella* infections. Other pets, including African hedgehogs, snakes, birds, rodents, baby chicks, ducklings, dogs, and cats, can also serve as potential vectors (Fuaciand Jameson, 2005).

2.1.5. Mode of transmission

Salmonella infection seems to be one of the most common examples of an enteric disease that is spreaded from animals to humans. The transmission occurs both through food products, such as meat, dairy products, and eggs, and by direct contact between animals and humans through the fecal-oral route (Olsvik, *et al*., 1985).

Foodborne salmonellosis often follows consumption of contaminated animal products such as raw meat, poultry and eggs. Not washing fresh fruits and vegetables before eating them, as well as not thoroughly cleaning work surfaces used to prepare raw meat and other foods in the kitchen can also be source of *Salmonella*. Food can also be contaminated by food handlers who do not carefully wash their hands with soap after handling raw meat or after using the bathroom (WHO, 1989). *Salmonella* infections are primarily of foodborne origin but can also occur by contact with infected animals, humans, other feces (Rounds *et al.*, 2010).

The chief mode of transmission is from food products contaminated with animal products or waste most commonly eggs and poultry but also undercooked meat, unpasteurized dairy products, seafood, and fresh produced. *S. enteritidis* associated with chicken eggs is emerging as a major cause of foodborne disease. Approximately 1 in 20,000 eggs is thought to be infected with *S. enteritidis.* Between 1974 and 1994, there was a fivefold increase (from 5% to 25%) in the isolation of *S. enteritidis* from eggs in the United States; in 1998, the U.S. Department of Agriculture estimated that 80% of all salmonellosis cases were caused by infected eggs (Fuaci and Jameson, 2005).

2.1.6. Carrier states and susceptibility

Stool cultures remain positive for four to five weeks after infection. Morbidity and mortality associated with salmonellosis are highest among the elderly, infants, and immune compromised individuals, including those with hemoglobinopathies and those infected with HIV or with pathogens that cause blockade of the reticulo endothelial system (e.g., patients with bartonellosis, malaria, schistosomiasis, or histoplasmosis) (Fuaci and Jameson, 2005).

Conditions that decrease stomach acidity like an age of less than one year, antacid ingestion, or achlorhydric disease or conditions that decrease intestinal integrity (inflammatory bowel disease, history of gastrointestinal surgery, or alteration of the intestinal flora by antibiotic administration) increase susceptibility to *Salmonella* infection (Fuaci and Jameson, 2005).

2.1.7. Virulence factors

The outcome of a *Salmonella* infection is determined by the status of the host and status of the bacterium. The status of the bacterium is determined by the so called virulence factors which is described as follows (Van Asten and van Dijk, 2005).

***Salmonella* Pathogenicity Islands (SPIs)** -The majority of virulence genes of *Salmonella* are clustered in regions distributed over the chromosome called *Salmonella* pathogenicity islands (McClelland *et al.,* 2001). The SPIs are of major importance for the virulence of *S. enterica*. Hallmarks of *Salmonella* virulence, such as cell invasion, intracellular survival and the production of VI antigens capsule are encoded by SPIs. Until recently more than 10 SPIs have been identified on the *Salmonella* chromosome, but SPI-1 and SPI-2 is the central for pathogenesis of *Salmonella* infections (Hansen-Wester and Hensel, 2001).

All types of *S.enterica* have two large clusters of genes known as *Salmonella* Pathogenicity Island one and two. *Salmonella* Pathogenicity Island one encodes genes necessary for invasion of intestinal epithelial cells and induction of intestinal secretory and inflammatory response (Galyov *et al.,* 1997). *Salmonella* lacking a functional SPI-1 Type three secretion system are unable to invade epithelia cells and induce cytokine synthesis (Hobbie *et al.,*1997).*Salmonella* Pathogenicity Island 2 encodes genes essential for intracellular replication and necessary for establishment of systemic infection beyond the intestinal epithelium (Hensel, 2006). The function of the SPI-2 encoded Type III secretion system is required to protect the pathogens within the *Salmonella* containing vacuole (SCV) against the effectors functions of innate immunity. It has been reported that SPI-2 prevents localization of the phagocyte oxidase (Vazquez-Torres *et al.,* 2000) and the inducible nitric oxide synthases to the SCV (Chakravortty *et al.,* 2002).

Type III secretion systems- Central to the pathogenesis of *S. enterica* is the function of specialized protein secretion systems, known as Type III secretion system (TTSS). TTSS are specialized virulence devices that have evolved indirect translocation of bacterial virulence proteins into the host cell cytoplasm. Type III secretion systems are composed of several proteins that form a remarkable needle-like organelle in the bacterial envelope (Galan, 1998). So far the presence of two SPIs (SPI-1 and SPI-2) each encoding a TTSS, have been described for *Salmonella* species and may reflect the flexibility of this highly successful pathogen in causing different forms of diseases (Fierer and Guiney, 2001).

Regulatory proteins, toxins, plasmids and Vi antigens- Regulatory proteins that control the synthesis of multiple proteins at the level of gene transcription are also essential to *Salmonella* pathogenesis (Behlau and Miller, 1993).

Non-typhoidal *Salmonella* also carry a variety of virulence plasmids which might play a role in multiplication inside the cell, destabilizing the cytoskeleton of the eukaryotic cell and also might be involved in resistance of *Salmonella* species to the bacteriolytic activity of serum. Enterotoxin may also play a role in *Salmonella* gastroenteritis. An enterotoxin antigenically similar to Cholera toxin also has been identified (Aguero *et al.,* 1991). Flagella phase variation that is exploited by the majority of flagellated *Salmonella* might be related to escaping the host defense system (Van Asten and van Dijk, 2005). The VI antigen of *S.Typhi* prevents antibody mediated opsonization, increases resistance to peroxide, and confers resistance to complement activation by the alternative pathway and to complement mediated lysine (Looney and Steigbigel, 1986).

2.1.8. Pathogenesis

Salmonellosis in the human host is generally associated with *Salmonellaenterica* subspecies *enterica* and acute infections can present in one of four ways: enteric fever, gastro-enteritis, bacteremia, and extra intestinal (EI) focal infection. As with other infectious diseases the course and outcome of the infection are dependent upon a variety of factors including inoculating dose, immune status of the host and genetic background of both host and infecting organism (Getenet, 2008). Broadly speaking the *Salmonellaenterica* from human infections

can be subdivided in to two groups: the enteric fever (typhoidal) group and non-typhoidal *Salmonella* (NTS), which typically cause gastroenteritis but can cause invasive disease under certain conditions (Selander *et al.,* 1990).All *Salmonella* infections begin with the ingestion of organisms in contaminated food or water (Fuaci and Jameson, 2005 and Francis *et al.,* 1992). The infectious dose of *Salmonella* varies from 103 to 106 colony-forming units. This variability probably reflects the ability of *Salmonella*e to resist the low pH of the stomach a powerful component of host defense (Fuaci and Jameson, 2005). After leaving the stomach, *Salmonella* must traverse the mucosal layer overlaying the epithelium of the small intestine. After crossing the mucosal layer overlaying the intestinal epithelium, *Salmonella* interacts with both enterocytes and Micro folds cells (Mcells) (Francis *et al.,* 1992).

The organisms are rapidly internalized and transported into sub mucosal lymphoid tissue where they may enter into systemic circulation. *Salmonella* have also the ability to induce non phagocytic epithelial cells by a process known as bacterial mediated endocytosis. This process involves the formation of large membrane ruffles around the organism and cytoskeleton rearrangement (Francis *et al.,* 1992). *Salmonella* is then internalized within bound vacuoles through which organisms‟ trancytose from the apical to the basolateral surface (Rathman *et al.,* 1997). Once it crosses the intestinal epithelium, *Salmonella* serotypes that cause systemic infections enter macrophages, and migration of infected macrophages to other organs of reticulo-endothelial systems probably facilitates the dissemination of bacteria in the host (Getenet, 2008).

Gastroenteritis due to NTS may persist with fever, nausea, vomiting, abdominal pain and symptoms may continue for over a week. In contrast, the early symptoms of enteric fever are often vague, and may include a dry cough, severe headache, anorexia, fever and a tendency to constipation rather than diarrhea (Parry *et al.,* 2002). If enteric fever is not treated on time, serious complication like hemorrhage from ulcers can occur during the third week of illness or perforation of the peyer‟s patches (PP) can cause generalized peritonitis and septicemias; these are the commonest cause of death in typhoid fever. With the introduction of early and appropriate antibiotic therapy, the average case fatality rates for typhoid are less than 1% (Everest *et al.,* 2001).

2.1.9. Clinical futures

Both human and animals are susceptible to *Salmonella* infection. While some of these infections cause disease, the majority probably leads to subclinical cases resulting in a healthy carrier state with intermittent excretion of the *Salmonella* in faeces. Whether a human develop disease following ingestion of *Salmonella* depend on dose of organism, the species of *Salmonella* and up on the specific and non-specific immunological factors. Species such as *S. typhimurium* and *S. enteritidis* usually causes gastroenteritis (food poisoning). The majority of food poisoning outbreaks caused by *Salmonella* follow the consumption of food directly or indirectly associated with infection in animals. The chain of transmission is often from contaminated animal food staffs to animal and then from contaminated animal carcasses to man (Quinn *et al.*, 1999).

2.1.10. *Salmonella* infections in animals

Salmonella have a wide variety of domestic and wild animal hosts. The infection may or may not be clinically apparent. In the subclinical form, the animal may have a latent infection and harbor the pathogen in its lymph nodes, or it may be a carrier and eliminate the agent in its fecal material briefly, intermittently, or persistently. In domestic animals, there are several well-known clinical enteritis due to species-adapted serotypes, such as *S. pullorum* or *S.abortus equi.* Other clinically apparent or in apparent infections are caused by serotypes with multiple hosts (PAHO, 2001).

The principal causes of clinical salmonellosis in cattle are serotype *Dublin* and *S. Typhimurium*. Other serotypes can sometimes be isolated from sick animals. Salmonellosis in adult cattle occurs sporadically, but in calves it usually acquires epizootic proportions. The disease generally occurs when stress factors are involved. Serotype *dublin*, adapted to cattle, has a focal geographic distribution. In the Americas, outbreaks have been confirmed in the western United States, Venezuela, Brazil, and Argentina. It also occurs in Europe and South Africa. In adult cattle, the disease begins with high fever and the appearance of blood clots in the feces, followed by profuse diarrhea, and then a drop in body temperature to normal. Signs of abdominal pain are very pronounced. The disease may be fatal within a few days or the

animal may recover, in which case it often becomes a carrier and new cases appear. Calves are more susceptible than adults, and in them the infection gives rise to true epidemic outbreaks, often with high mortality. Septicemia and death are frequent in newborns. The carrier state is less frequent among young animals and occurs primarily in adult cattle. The infection is almost always spread by the feces of a cow that is shedding the agent, but it may also originate from milk (PAHO, 2001).

Swine are host to numerous *Salmonella* serotypes and are the principal reservoir of *S.choleraesu is*. Serotypes that attack swine include *S. enteritidis*, *S. Typhimurium*, and *S. dublin*. *S. choleraesuis* is very invasive and causes septicemia; it may be isolated from the blood or from any organ. Swine are particularly susceptible and experience epidemic outbreaks between 2 and 4 months of age, but the infection also appears in mature animals, almost always as isolated cases. The most frequent symptoms are fever and diarrhea. The infection usually originates from a carrier pig or contaminated food. Infection by other serotypes may sometimes give rise to serious outbreaks of salmonellosis with high mortality. Because of the frequency with which swine are infected with different types of *Salmonellae*, pork products have often been a source of human infection (PAHO, 2001).

Cases of clinical salmonellosis in sheep and goats are infrequent. The most common serotype found in gastroenteritis cases is *S. typhimurium*, but many other serotypes have also been isolated. Serotype *S. abortus ovis*, which causes abortions in the last two months of pregnancy and gastroenteritis in sheep and goats, seems to be restricted to Europe and the Middle East (PAHO, 2001). Horses are also susceptible to *Salmonellae*, particularly *S. typhimurium*. *Salmonella* enteritis occurs in these animals, sometimes causing high mortality. Calves suffer from acute enteritis with diarrhea and fever; dehydration may be rapid. Nosocomial transmission has been seen in hospitalized horses (Bauerfeind *et al.*, 1992).

In recent years, a high prevalence of infection caused by numerous serotypes has been confirmed in cats and dogs. These animals may be asymptomatic carriers or may suffer from gastroenteritis salmonellosis with varying degrees of severity. Dogs can contract the infection by eating the feces of other dogs, other domestic or per domestic animals, or man. Dogs and cats can also be infected by contaminated food. In addition, dogs can transmit the disease to

man. Treatment for these animals consists mainly of fluid and electrolyte replacement (PAHO, 2001).

Two serotypes, *S. pullorum* and *S. gallinarum*, are adapted to domestic fowl. They are not very pathogenic for man, although cases of salmonellosis caused by these serotypes have been described in children. Many other serotypes are frequently isolated from domestic poultry; for that reason, these animals are considered one of the principal reservoirs of *Salmonellae*. Pullorum disease, caused by serotype *S. pullorum*, and fowl typhoid, caused by *S. gallinarum*, produce serious economic losses on poultry farms if not adequately controlled. Both diseases are distributed worldwide and give rise to outbreaks with high morbidity and mortality. Pullorum disease appears during the first 2 weeks of life and causes high mortality. The agent is transmitted vertically as well as horizontally. Carrier birds lay infected eggs that contaminate incubators and hatcheries.

Fowl typhoid occurs mainly in adult birds and is transmitted by the fecal matter of carrier fowl. On an affected poultry farm, recuperating birds and apparently healthy birds are reservoirs of infection. *Salmonella* un-adapted to fowl also infect them frequently. Nearly all the serotypes that attack man infect fowl as well. Some of these serotypes are isolated from healthy birds. The infection in adult birds is generally asymptomatic, but during the first few weeks of life, its clinical picture is similar to pullorum disease (loss of appetite, nervous symptoms, and blockage of the cloaca with diarrheal fecal matter). The highest mortality occurs during the first two weeks of life. Most losses occur between six and ten days after hatching (PAHO, 2001).

Rodents become infected with the serotypes prevalent in the environment in which they live. Rodents found in and around food processing plants can be an important source of human infection. Of 974 free-living wild animals examined in Panama, 3.4% were found to be infected, principally by serotype *S. enteritidis* and, less frequently, by *S. arizonae* (*Arizona hinshawii*) and *Edwardsiella*. The highest rate of infection (11.8%) was found among the 195 marsupials examined. Outbreaks of salmonellosis among wild animals held in captivity in zoos or on pelt farms are not unusual. *Salmonella* infection in cold-blooded animals has merited special attention. An infection rate of 37% was found in 311 reptiles examined live or

necropsied at the National Zoo in Washington, D.C. The highest rate of infection was observed in snakes (55%) and the lowest in turtles (3%). The *Salmonellae* isolated were 24 different serotypes formerly classified under the common name of *S. enteritidis*, 1 strain of *S. choleraesuis*, and 39 of *S. arizonae*. No disease in their hosts was attributed to these bacteria, but they may act together with other agents to cause opportunistic infections (PAHO, 2001).

2.1.11. *Salmonella* infections in humans

Salmonella infections in humans can range from a self-limited gastroenteritis usually associated with non-typhoidal *Salmonella* (NTS) to typhoidal fever with complications such as a fatal intestinal perforation (OIÉ, 2000). Non-typhoidal *Salmonella* is one of the principal causes of food poisoning worldwide with an estimated annual incidence of 1.3 billion cases and 3 million deaths each year (Torpdahl *et al.*, 2007). Outbreaks of salmonellosis have been reported for decades, but within the past 25 years the disease has increased in incidence in many continents. The disease appears to be most prevalent in areas of intensive animal husbandry (OIÉ, 2000).

The incubation period in people is variable but is usually between 12 and 36 hours. The typical presenting symptom is diarrhea but this may be accompanied by nausea and abdominal pain, although vomiting is not usual. There may also be a headache and fever. While the infection is normally self-limiting and does not require antibiotic treatment, occasionally, with more invasive *Salmonella* such as *S. Virchow*, bacteremia can occur. The infection is rarely fatal in people (Gracey *et al.*, 1999).

Salmonellosis is most commonly caused by *S. enterica* subsp. *typhimurium* or *S. enterica* subsp. *enteritidis*. Secondly, *S. enterica* subsp. *typhi* and *S. enterica* subsp. *paratyphi* are the causes of typhoid fever or paratyphoid fever, respectively. *Salmonella* can replicate both inside the vacuoles of host cells and in the external environment. *Salmonella* are the second most common pathogens isolated from humans with gastro enteric disease in developed countries (Buncic, 2006).

SalmonellaTyphimurium and *S. enteritidis* occur in the gastro intestinal tract of animals, including livestock. The disease is self-limiting, but can be severe in young, elderly or otherwise IC (immune compromised) people. *Salmonella* invade epithelial cells in the ileum and proliferate in the lamina propria and profuse, watery diarrhea results. Some isolates produce a heat-labile enterotoxin, which initiates diarrhea. Sequelae include post- enteritis reactive arthritis and reiter‟s syndrome and systemic infection can result. Individuals can develop carrier status of up to 6 months in duration. The infectious dose varies, from only a few CFU to >105 CFU, so growth of the pathogen in foods has not been a factor in all cases of foodborne salmonellosis, but appears to have been in some.

Even though, any faecally contaminated food can implicated, foods known to have been vehicles of salmonellosis includes poultry, eggs, meat, milk, chocolate, coconut and frog legs (Buncic, 2006). *Salmonellatyphi* and *S. enterica* subsp. *paratyphi* cause the systemic diseases typhoid fever and paratyphoid fever, respectively. These pathogens occur in human faeces, and are spread via human faeces to the environment and to foods. Person-to-person transmission is common. The disease symptoms of typhoid and paratyphoid fevers are dissimilar to those of enteric salmonellosis (Buncic, 2006).

Salmonella penetrate the intestinal epithelium, possibly proliferating in macrophages and polymorphs, pass into mesenteric lymph nodes, liver or spleen then cause septicemia. Peritonitis and subsequent death can occur. Ulceration of the ileum can occur if organisms multiply in the bile of the gall bladder and cause re-infection. Any food could be a vehicle of infection if contaminated with human faeces. Foods known to have been vehicles of typhoid fever include raw milk, shellfish and meat. However, typhoid fever is predominantly spread by water contaminated with human faeces (Buncic, 2006).

2.1.12. Antimicrobial resistance profile of *Salmonella*

Salmonella species are leading causes of acute gastroenteritis in several countries and salmonellosis remains an important public health problem worldwide, particularly in the developing countries (Rotimi *et al.,* 2008). The situation is more aggravated by the ever increasing rate of antimicrobial resistance strains (Zelalem *et al.,* 2011). In recent years

problems related to *Salmonella* have increased significantly, both in terms of the incidence and severity of cases of human Salmonellosis. Since the beginning of the 1990s, strains of *Salmonella* which are resistant to a range of antimicrobials including the first choice agents for treatment of humans have emerged and are threatening to become a serious public health problem.

Drug resistant *Salmonella* emerge in response to antimicrobial usage in humans and in food animals. So, selective pressure from the use of antimicrobials is a major driving force behind the emergence of resistance. Multi-drug resistance to critically important antimicrobials is compounding the problem (WHO, 2005). There are reports of high prevalence of resistance in *Salmonella* isolates from countries such as Taiwan (Lauderdale *et al.,* 2006), India (Mandal *et al.,* 2004, 2006), The Netherlands (Duijkeren *et al.,* 2003), resistant isolates from France (Weill *et al.,* 2006), Canada (Poppe *et al.,* 2006), and Ethiopia (Molla *et al.,* 2003).

A particular concern with *S.Typhimurium* DT 104 is that it has resistance to many antibiotics and often acquires resistance to others. Most strains are resistant to ampicillin, chloramphenicol, streptomycin, the sulphonamides and tetracycline. Recent resistance additions include resistance to trimethoprim and of particular concern, to the fluoroquinolones. Resistance to this latter group of antibiotics is a major worry as they are among the drugs of choice for the treatment of invasive *Salmonella* in humans. There is considerable debate as to what factors result in the emergence of antibiotic resistant strains of bacteria and it is alleged that antibiotic use in animals is part of the problem. Equally the use or misuse of antibiotics in humans for example also leads to the development of antibiotic resistance. The continuing development of antibiotic resistance may lead to sufficient pressure ultimately to restrict the antibiotics available to the veterinary profession for animal treatment (Gracey *et al.,* 1999).

Antimicrobial resistant *Salmonella* are increasing due to the use of antimicrobial agents in food animals (Zewdu and Cornelius, 2009; Lynch *et al.,* 2006; Molla *et al.,* 2006;Molla *et al.,* 2003 and Threlfall, 2002) at sub-therapeutic level or prophylactic doses which may promote on-farm selection of antimicrobial resistant strains and markedly increase the human health risks associated with consumption of contaminated meat products (Zewdu and Cornelius,

2009;Molla *et al.,* 2006 and Molla *et al.,* 2003). Cattle have been implicated as a source of human infection with antimicrobial resistant *Salmonella* through direct contact with livestock and through the isolation of antimicrobial resistant *Salmonella* from raw milk, cheddar cheese, and hamburger meat traced to dairy farms. Antimicrobial use in animal production systems has long been suspected to be a cause of the emergence and dissemination of antimicrobial resistant *Salmonella* (Alexander *et al.,* 2009).

This spread of antimicrobial resistance through the food chain is regarded as a major public health issue (Lynch *et al.,* 2006 and Threlfall, 2002). The appearance of both plasmid mediated antibiotic resistant against conventional anti- *Salmonella* drugs and chromosomal resistance to quinolones and fluoroquinolones has reduced therapeutic options for *Salmonella* septicemia in humans (Nor Elmadiena *et al.,* 2012).

2.1.13. Economic and public health significance of *Salmonella* infections

Foodborne disease has emerged as an important and growing public health and economic problem in many countries during the last two decades. Frequent outbreaks caused by new pathogens, the use of antibiotics in animal husbandry and the transfer of antibiotic resistance to human are just a few examples (Rocourt *et al.,* 2003). The pathogen of *Salmonella*, belonging to intestinal bacteria family, is one of the main pathogens causing food poisoning (Lianhua, *et al.,* 2008). As the pathogen of foodborne infection, *Salmonella* is currently the leading pathogen of bacterial food poisoning in the world (Cheng, *et al.,* 2008).

The incidence of non-typhoidal salmonellosis has doubled in the United States over the past two decades. Currently, the CDC estimates that there are 2 million cases annually, with 500 to 2000 deaths. Although more than 200 serovarsof *Salmonella* are considered to be human pathogens, the majority of the reported cases in the United States are caused by *S. Typhimurium* or *S. enteritidis* (Fuaci and Jameson, 2005). Sheep and goats can be carriers of different *Salmonella* serovars, including *Salmonella enterica* serovar *Enteritidis* and *Salmonella enterica* serovar *Typhimurium*, the most important serovars for human infections (Schilling, 2012).

Contacts with small ruminants pose a potential health risk to occupationally exposed subpopulations as well as the general public, but the risk depends strongly on the serotype involved (Hoelzer *et al.,* 2011). The incidence of salmonellosis is highest during the rainy season in tropical climates and during the warmer months in temperate climates, coinciding with the peak in foodborne outbreaks (Fuaci and Jameson, 2005).

In most parts of the world, countries have seen dramatic and continuous increases in human outbreak of salmonellosis, caused by infections in animals. In 2004, in the European Union (EU) alone, 192,703 human cases of salmonellosis were reported. These and similar data from other countries almost certainly underestimate the magnitude of the problem, as many cases of salmonellosis are not reported. The Centers for Disease Control estimate the annual number of non-typhoidal salmonellosis cases in the United States of America (USA) to be approximately 1.4 million (Forshell and Wierup, 2006).

In addition to human health implications, *Salmonella* is a pathogen of significant importance in worldwide animal production and the emergence of antibiotic-resistant strains, due principally to the therapeutic use of antimicrobials in animals, is a further threat to human and animal health (Forshell and Wierup, 2006). It also generates negative economic impacts due to surveillance investigation, and illness treatment and prevention (Gómez-Aldapa, *et al.,* 2012). Financial costs are not only associated with investigation, treatment and prevention of human illness, fall in to the public and privet sectors and may be surprising, both in terms of the levels of costs incurred and the variety of affected. In the public sector, resources may be diverted from preventive activities in to the treatment of patients and investigation of the source of infection. In the private sector considerable financial burdens may be imposed on industry in general and on the food industry in particular, and last but not on the affected individual and his or her family (Sockett, 1991).

2.1.14. Salmonellosis in Ethiopia

Even though *Salmonella* populations in different geographical areas or different hosts and environmental niche may undergo different evolutionary change, due to centralization of food production and distribution and population movement, *Salmonella* strains found in different

countries of the world are believed to be clonally related (Winokur, 2001). *Salmonella* isolates in Ethiopia may have similar phenotypic and genotypic characteristics with isolates elsewhere in the world and non-typhoidal *Salmonella enterica* infection in children in Ethiopia is a major health problem and is caused by similar serovars to these reported from elsewhere in Africa: *S. Typhimurium* and *S. Enteritidis* (Getenet, 2008).

Salmonella infection most commonly occurs in countries with poor standards of hygiene in food preparation and handling and where sanitary disposal of sewage is lacking. It mainly occurs in the tropics and sub tropics in Africa, India, Pakistan South East Asia and South America (Senthikumar and Prabakaran, 2005; WHO; 2003b; Muleta and Ashenafi, 2001; Al-Lahham *et al.,* 1990; Lanata *et al.,* 1990 and WHO; 1989).

Studies indicated the widespread occurrence and distribution of *Salmonella* in Ethiopia. In recent years the number of out breaks of *Salmonella* in humans has increased considerably in the country. Much more is known now about the extent of foodborne illness and how severe it can be, not just in terms of acute illness, but also in terms of long term consequences. Studies indicated various percentages of *Salmonella* isolates in towns of Ethiopia. Moreover, high percentages of *S. typhi* isolates have been found to be resistant for antimicrobial agents (Abera *et al.,* 2010; Andargie *et al.,* 2008 and Yismaw *et al.,* 2007). In addition, the very young, elderly and immune-compromised individuals are particularly more susceptible to *Salmonella* infections at a lower infective dose than healthy adults. This is more important in developing countries such as Ethiopia where HIV/AIDS is highly prevalent and *Salmonella* is an important opportunistic infection in HIV/AIDS patients (Catherine *et al.,* 2001).

In Ethiopia, minced beef is usually used for the preparation of a popular traditional Ethiopian dish known as locally "Kitfo" and most of the time it is consumed raw or medium cooked. The habit of raw meat consumption and the presence of *Salmonella* in minced beef indicate, in addition to the poor hygienic standards in food handling in the country, the presence of great public health hazards of *Salmonella* (Muleta and Ashenafi, 2001).

2.1.15. Treatment, Prevention and Control of Salmonellosis

Gastroenteritis caused by *Salmonella* is usually a self-limiting disease (Fuaci and Jameson, 2005 and Richards *et al*., 1993) and diarrhea resolves within three to seven days and fever within seventy two hours (Fuaci and Jameson, 2005). Accordingly therapy should be directed primarily to the replacement of fluid and electrolyte losses. Therefore, antimicrobials should not be used routinely to treat uncomplicated non-typhoidal *Salmonella* gastroenteritis or to reduce convalescent stool excretion (Richards *et al.,* 1993*).* However, antimicrobial therapy should be considered for any systemic infection (Parry et al., 2002).

Antibiotic treatment usually is not recommended and in some studies has prolonged carriage of *Salmonella*. Neonates, the elderly, and the immunosuppressed (e.g., HIV- infected patients) with non-typhoidal *Salmonella* gastroenteritis are especially susceptible to dehydration and dissemination and may require hospitalization and antibiotic therapy (Fuaci and Jameson, 2005). Because of the increasing prevalence of antimicrobial resistance, empirical therapy for life threatening bacteremia or local infection suspected to be caused by non-typhoidal *Salmonella* should include a third generation cephalosporin and a quinolone until susceptibility patterns are known. Amoxicillin and trimethoprim- sulfamethoxazole are effective for treatment of long-term carriage. The high concentration of amoxicillin and quinolone in bile and the superior intracellular penetration of quinolone are theoretical advantages over trimethoprim-sulfamethoxazole (WHO, 2003a).

In many urban centers, eating and drinking in public establishments, such as Hotels, Restaurants, and Snack bars is a common practice in many countries. These establishments prepare, handle, and serve large quantities of food and drink to large groups of people within a short period of time implying a possible risk of infections if sanitary and hygienic norms are not strictly followed. The world health status review indicates that the health problem of developing nations is mainly linked to inadequate sanitation (Kumie *et al*., 2002).

Better education of food industry workers in basic food safety and restaurant inspection procedures may prevent cross-contamination. Food handling errors can lead to outbreaks. Improvements in farm animal hygiene, in slaughter plant practices, and in vegetable and fruit harvesting and packing operations may help prevent salmonellosis caused by contaminated

foods. Pasteurization of milk and treatment of municipal water supplies are highly effective prevention measures that have been in place for decades. Wider use of pasteurized egg in restaurants, hospitals, and nursing homes is an important prevention measure. In the future, irradiation or other treatments may greatly reduce contamination of raw meat (CDC, 2008). Strategies for reducing foodborne illness require a comprehensive farm-to-table approach, while *Salmonella* contamination from food handlers has been shown to make a significant contribution to human foodborne illness in several developing countries (Catherine *et al*., 2001).

Non-typhoidal S. enterica infections are a major public health problem world-wide and reduction of these diseases presents a serious and challenging problem. These diseases have several animal reservoirs. Large number of different S.enterica serovars cause gastroenteritis in humans probably makes vaccines very difficult to realize and/or use commercially. The incidence of non-typhoidal salmonellosis continues to rise along with rates of emergence of antibiotic resistant strains and increased centralization of food production. Thus, it is important to monitor every step of food production, from handling of raw products to preparation of finished foods. The prudent use of antimicrobial agents in both humans and animals is necessary to minimize the further emergence of antibiotic resistant strains (Getenet, 2008).

Furthermore, in order to control *Salmonella* infection, an individual should cook foods thoroughly, pasteurize milk and dairy products; avoid consumption of unpasteurized products, prevent cross-contamination of heat-treated foods, avoid undercooked or raw eggs, store heat-treated foods at less than 4°C or greater than 60°C to prevent growth, reduce carriage of livestock by vaccinating or dosing with antibiotics or probiotics, exclude infected or carrier-status individuals from handling food, control rodents and insects and dispose of sewage in a sanitary manner (Buncic, 2006).

3. MATERIALS AND METHODS

3.1. StudyPeriod and Area

The study was conducted between the periods of March and September 2019 in Bench Maji zone, at Mizan municipal abattoir and Butcher houses. Mizan is located in the Southern Nations Nationality and people's regional state. The study area is found at the distance of 563km from Addis Ababa south west direction. Geographically, it is located in b/n 7°0′N 35°35′E / 7.000°N 35.583°E latitude and longitude respectively with an elevation of 1451 meters above sea level. It has about 34,080 human populations of whom18, 138 are men and 15,942 women (CSA, 2007). The livestock population of the Town is 31423 cattle (BFEDDSP, 2007).

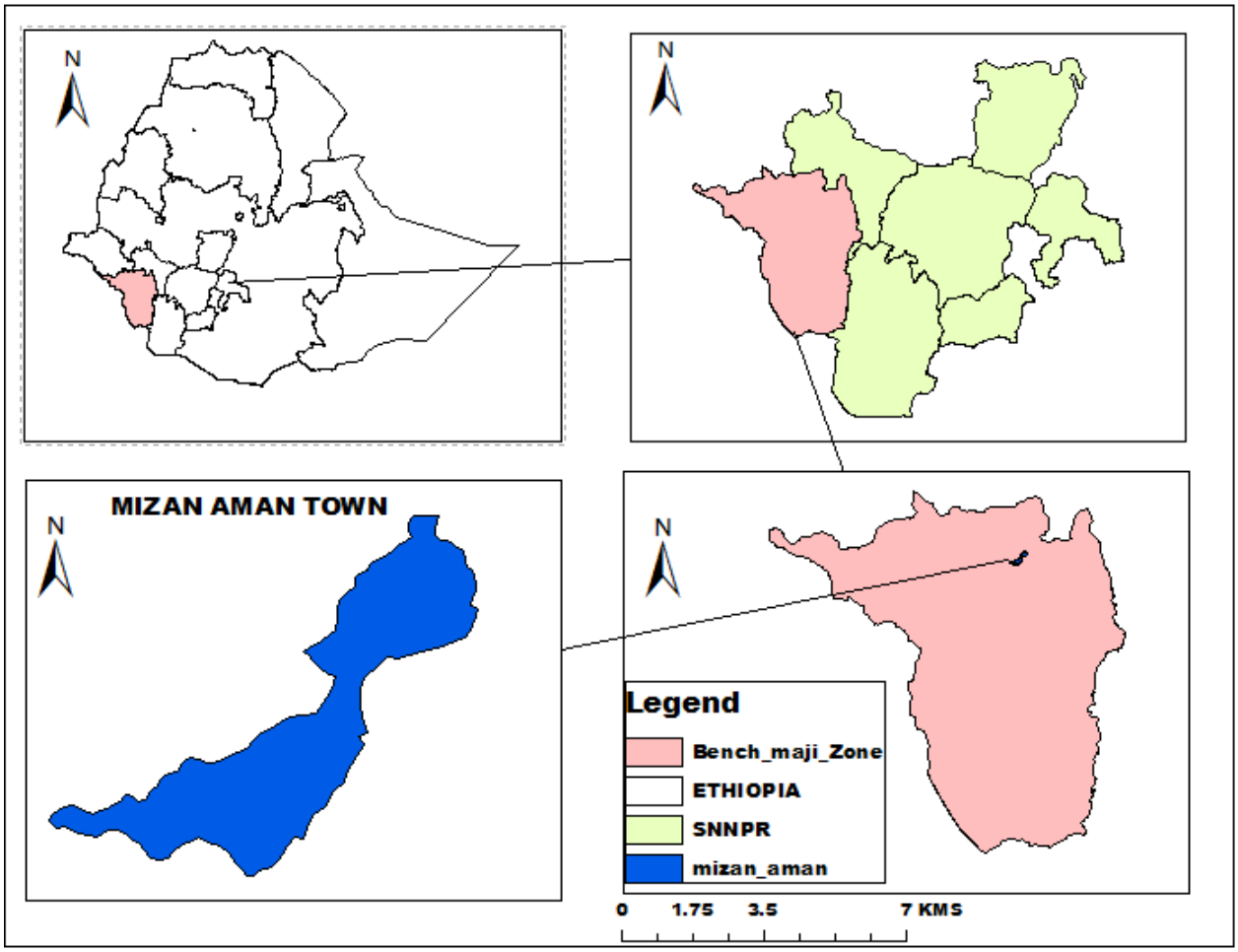

Figure 2: Map of the study area

3.2. Study Population

The study populations were all apparently healthy local indigenous zebu cattle which were brought to the abattoir for slaughtering,55 voluntarypublic members (abattoir and butcher houseworkers), and 90available materials in contact with meatand 145 Abattoir and Butcher house workers who were voluntary for interviewee.

3.2.1. Sample Size Determination

For carcasses, the sample size was calculated according to Thrusfield (2007) using 95% confidence level and 5% precision. The 12.5% expected prevalence (Wondimu *et al*, 2017 at Wolaita Sodo Abattoir) of *salmonella* from carcass in agro ecologically similar study area, in Southwest, Ethiopia was used.

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

Where n= Sample size, p= expected prevalence, d= desired level of Precision
Hence, 175 carcass samples were used.

For personnel hand swabs and material swabs, the sample size was determined purposively based on the voluntariness and availability of materials to be sampled respectively. Accordingly, 145 samples were taken. Therefore, the total sample size for carcasses, personnel hand and material swabs was 320.

For questionnaire and observational survey, a total of 145 voluntary meat handlers (60=abattoir workers and 85= butcher house workers) wereobserved and interviewed to assess their knowledge, attitude and practice on meat hygiene and safety.

3.3. Study Design

A cross-sectional study involving microbiological analysis, questionnaire andobservational survey (Annex 6) was employed.

3.4. Sampling Technique and Sample Collection

A total of 320 samples consisting of 240 from Abattoir (Carcasses swabs=175, Abattoir personnel hand swabs=25 and Abattoir material swabs=40) and 80 from Butcher houses (Butcher men hand swabs=30 and Butcher house material swabs=50) were sampled.In the municipal abattoir, 18-25 (averagely, 21) cattle were slaughtered per a day. Accordingly, a total of 14 (7 from carcass and 7 from personnel & material) samples were taken per a day and two times per a week for 3 months. Simple random sampling technique was used for carcass swabs and purposive sampling technique was used for personnel and materials swabs. Swabs from carcass were taken from the abdomen (flank), thorax (lateral), crutch, and breast (lateral) while both the right and left hands were swabbed for personnel hand swabs and all surfaces of the materials were swabbed thoroughly.

The sampling areas were delineated by using a (10 x 10 cm) aluminum foil templates. A sterile cotton tipped swab (2X3 cm) fitted with shaft, was first soaked in an approximately 10 ml of buffered peptone water (BPW) and rubbed over the delineated area horizontally and then vertically several times. Up on completion of the rubbing process, the swab was placed into the buffered peptone water used to wet the swab, breaking off the wooden shaft pressing against the inside of the universal bottle and was disposed leaving the cotton swab in the universal bottle. Other swabs of the same types was used on the other marked areas and placed into the same container. A second dry sterile cotton swab of the same type was used as before over the entire sampled area as above and this swab was placed into the same container. All samples were labeled legibly with permanent marker identifying type/source of sample and date of sampling. Finally, by using ice boxes with ice packs the samples were transported to Mizan Regional Veterinary Laboratory, South West Ethiopia.

3.4.1. Isolation and Identification of *Salmonella*

Isolation and Identification of S*almonella* organisms were carried out according to ISO 6579 (2002) standard (Annex 5) - Microbiology of Food and Animal Feeding Stuffs Horizontal method for detection of *Salmonella* spp. Accordingly, Non-selective pre-enrichment, Selective enrichment, Plating on selective media and Biochemical confirmation were used.

3.4.2. Pre-enrichment

The swabs were directly inoculated into 10 ml buffered peptone water (BPW) in screw capped bottles and incubated at 37 °C for 16-18 hrs. Each 25 ml of the swab content was inoculated into 225 ml of BPW and homogenized for two minutes with stomacher. After mixing thoroughly, the samples were incubated at 37 °C for 16-18 hours (ISO, 2002).

3.4.3. Selective enrichment

From the pre-enrichment broth after incubation and thoroughly shaking, 0.1 ml of the broth was transferred into a tube containing 10 ml of Rappaport-Vassiliadis medium (RV broth). Then 1 ml of the pre-enrichment broth was transferred into a tube containing 10 ml of Selenite cysteine broth (SC broth). The inoculated RV broth was incubated at 41.5 °C ± 1 °C for 24 ± 3 hours and the inoculated SC broth at 37 °C ± 1 °C for 24 ± 3 hours (ISO, 2002(Annex 5)).

3.4.4. Plating and identification

Xylose lysine desoxycholate (XLD) agar and Brilliant Green agar (BGA) plates were used for plating and identification purpose. A loop-full of inoculum each from the RV and SC broth was transferred and streaked separately onto the surface of XLD and BGA agar. The plates were incubated at 37°C ± 1°C for 24 ± 3 hours. After proper incubation, the plates were examined for the presence of suspected *Salmonella* colonies, which on XLD agar were pink with a darker center and a lightly transparent zone of reddish color due to the color change of the indicator whereas lactose positive *salmonellae* were yellow with or without blackening and which on BGA agar were grey-reddish/pink and slightly convex. Five *Salmonella* presumptive colonies were transferred to non-selective solid Nutrient agar medium for further confirmatory tests. Confirmation was done by using biochemical test according to ISO 6579 (ISO, 2002).

3.4.5. Biochemical Tests

3.4.5.1. Triple Sugar Iron (TSI) Agar

The Triple sugar iron agar slants were prepared with a thick butt. A loopful culture of pure growth from nutrient agar was stabbed into the butt and streaked on the slant and incubated

for 24 hours at 37°C. Typical *Salmonella* cultures showed alkaline (red) slants and acid (yellow) butts with gas production (bubbles) and formation of hydrogen sulfide (blackening of the agar). When lactose-positive *Salmonella* is isolated, the TSI slant is yellow. Thus, preliminary confirmation of *Salmonella* cultures shall not be based on the results of the TSI agar test only (ISO-6579, 2002).

3.4.5.2. Urea agar

The hydrolysis of urea releases ammonia and production of ammonia increases the pH of the medium that change color of phenol red (pH indicator) to rose pink, and later to moderate red. The basal medium was sterilized by autoclaving at 121°C for 15 minutes. When it has cooled to about 50°C, 100 ml of a 20 percent solution of pure urea previously sterilized by filtration was added and poured into test tubes. The isolates were inoculated into the urea to determine urease production. The inoculated tubes were incubated at 37°C for up to 96 hours. Then an observation was made at an interval of 4, 24, 48 and 96 hours. Urease positive cultures changed the color of the indicator to red. But no color change of the indicator if *Salmonella* is positive, since *Salmonella* is negative on urease test.

3.4.5.3. Citrate utilization test

Simmon's citrate agar was sterilized by autoclaving at 121°C for 15 minutes at 15 lb pressure and cooled for slant formation. The strains were cultured on the prepared Simmon's citrate agar medium, incubated at 37°C for 48 hours and observations were recorded. Opacity and change in color of bromothymol from green to blue indicated a positive reaction.

3.4.5.4. L-lysine decarboxylation medium

Lysine decarboxylation broth was inoculated with the loopful culture of the test organism and one was kept uninoculated control. Both tubes were incubated for 24 hours at 37°C. Turbidity and a purple color after incubation indicated a positive reaction. A yellow color indicated a negative reaction.

3.4.5.5. Indole test

Indole is a nitrogen-containing compound that can be formed from the degradation of the amino acid tryptophan by certain bacteria. Tryptone was used as a substrate because it contains much tryptophan. The indole reacts with aldehyde compound of Kovac's reagent and forms red coloured compound that is more soluble in alcohol. For indole test peptone water was prepared and the ingredients were dissolved in distilled water, dispensed in test tubes and sterilized by autoclaving at 121°C for 15 minutes. The tubes of the medium were inoculated with test isolates using sterile platinum loop and incubated at 37°C aerobically for up to 96 hours. Finally, 0.5 ml of Kovac's reagent was added to each of the inoculated and un-inoculated controls. The tubes were shaken gently and the results were recorded. Positive results were indicated by the development of red/pink ring on the top surface of the test tube for most of the family Entero-bacteriaceae other than *Salmonella* which is negative on Indole test with no colour change.

3.5. Antimicrobial Resistance Pattern Tests

The antimicrobial resistances testing of the isolates were performed by using the disc-diffusion method according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 2002) and (CLSI, 2012). Four to five well-isolated colonies from nutrient agar plates were transferred into tubes containing 5 ml of Tryptone soya broth (Oxoid, England). The broth culture was incubated at 37ºC for 4 hours until it achieved the 0.5 McFarland turbidity standards. Sterile cotton swab was dipped into the suspension, rotated several times, pressing firmly on the inside wall of the tube above the level to remove excess inoculums and swabbed uniformly over the surface of Muller Hinton agar plate (Oxiod, England). The plates were held at room temperature for 30 min to allow drying.

The resistance of the isolates were tested for the following antibiotic discs: Ampicillin (AMP) 2µg, Oxicillin (OX) 5µg, Gentamicin (HLG) 120µg, Kanamycin (K) 5µg, Ox tetracycline (O) 30µg, Erythromycin (E) 5µg, Neomycin (N) 30 µg and Penicillin G (P) 1 µg were placed at least 15 mm apart from the edge of the plates to prevent overlapping of the inhibition zones. The plates were incubated at 37ºC for 24 h. The diameter of the zones of inhibitions was compared with recorded diameters of the control organism *E. coli* ATCC 25922 and classified

as resistant, intermediate, or susceptible according to the interpretive standards of the Clinical Laboratory Standards Institute (CLSI, 2012).

3.6. Methods of Statistical Analysis

Descriptive statistics such as frequency, percentage, and/or proportion were used for prevalence, antimicrobial resistance test, questionnaire and observation survey results. Chi-squire test was used to assess significant differences of *Salmonella* status between sample source and types while Binary Logistic regression (odds ratio) was used to assess the association of possible risk factors for the occurrence of *Salmonella* using statistical package for social science (SPSS) version 20 software. The results with $P < 0.05$ were considered statistically significant.

3.7. Ethical Consideration

The study was approved by the letter of clearance obtained from Jimma University College of Agriculture and veterinary Medicine, and Bench Maji zone administration office. The data were collected after written informed consent was made with all study participants. All the rights of privacy and confidentiality of participants were protected.

4. RESULTS

4.1. Over all occurrence of *Salmonella*

The overall prevalence of *Salmonella* in this study was found to be 13.4% (43/320) with prevalence of 12.5% in abattoir and 16.2% in butcher houses. Statistical analysis of the data showed that there was no statistically significant difference (P > 0.05) on the prevalence of *Salmonella* between abattoir and butcher houses sources (Table 1).

Table 1: Proportion of Salmonella isolates from Abattoir and Butcher houses

Source of samples	Number Examined	Prevalence (%)	χ^2	P-value
Abattoir	240	30 (12.5)	0.725	0.449
Butcher house	80	13 (16.2)		
Total	320	43 (13.40		

4.2. Occurrence of *Salmonella* isolates among sample types

The specific prevalence of *salmonella* was found to be 12% in carcass swab, 16% in abattoir personnel hand swab, 12.5% in abattoir materials swab, 16.6% in butcher men hand swab and 16% in butcher materials swab.-The lowest prevalence was observed from carcasses samples among the others. The prevalence of *Salmonella* retrieval was not statistically significant (P > 0.05) among the sample types (Table 2).

Table 2: Prevalence and association of *Salmonella* recovery between sample types

Sample type	Total Observation	Frequency of Positivity	Prevalence (%)	χ^2	P-value
Carcass swab	175	21	12	1.033	0.905
Abattoir personnel hand swab	25	4	16		
Abattoir materials swab	40	5	12.5		
Butcher men hand swab	30	5	16.6		
Butcher materials swab	50	8	16		

4.3. Antimicrobial Resistance Pattern Test

Out of the total 43 isolates subjected to antimicrobial resistance test to 8 different antimicrobials, the highest level of resistance was observed for erythromycin (100%) followed by ampicillin (83.7%), oxacillin (72.09%) and neomycin (67.44%). All isolates were found to be susceptible to gentamycin (Table 3).

Table 3: Antimicrobial resistance test result of Salmonella isolates from Abattoir and Butcher houses

Antimicrobials	**Disc concentration**	**Number of isolates**		
	(µg)	**Resistant (%)**	**Intermediate (%)**	**Susceptible (%)**
Ampicillin (AMP)	2	36(83.7)	7(16.2)	-
Oxacillin (OX)	5	31(72.09)	-	12(27.9)
Gentamicin (HLG)	120	-	-	43(100)
Kanamycin (K)	5	22(51.16)	14(32.55)	7(16.27)
Oxy tetracycline (O)	30	12(27.9)	-	31(72.09)
Erythromycin (E)	5	43(100)	-	-
Neomycin (N)	30	29(67.44)	14(32.55)	-
Penicillin G (P)	1	19(44.18)	17(39.53)	7(16.27)

Out of the total isolates, 42/43 (97.67%) were resistance to at least two antimicrobial agents tested (Table 4).

Table 4: Multiple Antimicrobial Resistance Patterns of Salmonella

No. Isolates with same pattern	Antimicrobial resistance pattern	No. of antimicrobials developed resistance
12	ERY	2
10	AMP ,OX	3
8	OXY,PEN,KAN	4
6	ERY,AMP ,KAN, PEN	5
4	KAN;AMP,PEN,ERY,N	6
3	N, ERY ,PEN ,KAN, AMP,OXY	7

OXY: Ox tetracycline; ERY: erythromycin; KAN: kanamycin; AMP: ampicillin; OX: oxicillin; PEN: Penicillin N: Neomycin

4.4. Occurrence of *Salmonella* among Risk Factors

Out of 145 purposive samples expected to be potential source of contamination (abattoir worker= 25, abattoir materials = 40, butchers = 30 and butcher house materials = 50), a total of 22 (15.1%) *Salmonella* were isolated. The specific prevalence of *Salmonella* was found to be 16% (4/25), 12.5% (5/40), 16.6% (5/30) and 16% (8/50) respectively in abattoir workers, abattoir materials, butchers and butcher house materials.

The association of *Salmonella* recovery in personnel and materials with the possible risk factors by Univariable logistic regression reveled that; those personnel who were not educated (Illiterates) have 4.23 times more likely the chance of contaminating carcass than the other categories of educational status (95% CL: 0.966-18.528 with p=0.046), people who did not wash their hands during meat processing have 18.9 times more likely the chance of contaminating meat with *Salmonella* comparing with those who wash their hands at least before or after contact with meat/equipment (95% CL: 2.292-155.82 with p=0.006).

With regarding to cleaning equipment, those materials which have not been cleaned regularly have 3 times more likely the chance of contaminating meat than equipment that regularly washed (95% CL: 1.181-7.788 with p=0.021).While abattoir workers and butchers who did not knew contamination as risk have 22.7 times more likely the chance of cross contaminating carcasses in comparison to those who knew contamination as risk (95% CL: 7.367-70.180 with p=0.000) and also workers who used jewelry materials on their hands during meat processing have chance of 4.3 times more likely to contaminate meat comparing with those who did not used (95% CL1.680-11.250 with p=0.002).

Job related training and personal hygiene were not significantly associated with the occurrence of *salmonella* ($p > 0.05$; table 5). All significantly associated variables ($p < 0.25$) in univariable logistic regression analysis were taken to multivariable logistic regression analysis to control confounders.

In multivariable logistic regression analysis the occurrence of *salmonella* isolates in abattoir and butcher houses were more likely higher in materials which were not cleaned (OR=12.56; 95% CI: 0.986-160 with *P*=0.048) and people who didn't know contamination as risk

(OR=11.586; 95% CI: 1.65-80 with *P*=0.014) than other manners of cleaning equipment categories and in those who know contamination as risk respectively (table 5).

Table 5: Univariable and Multivariable Logistic Regression analysis of the association of risk factors for the occurrence of *salmonella* among Abattoir and Butcher houses

Risk factors	Categories	Frequency	Positive No. (%)	Univariable OR (95% CI)	*P-value*	Multivariable OR (95% CI)	*P-value*
ES	Illiterate	24	9(37.5)	4.2(0.9-18.5)	0.046	7.12(0.31-163)	0.219
	1-8	74	6(8.1)	0.52(0.12-2.2)	0.385	1.19(0.095-15)	0.891
	9-12	29	4(13.7)	0.17(0.01-1.8)	0.150	0.16(0.001-21)	0.468
	> grade 12	18	3(16.6)	**		**	
	before& after	28	2(7.1)	**		**	
HW	Before	70	7(10)	2.5(0.29-22.0)	0.400	0.96(0.06-15.6)	0.980
	After	13	4(30.7)	2.2(0.13-39.0)	0.578	0.63(0.05-77.0)	0.851
	not wash	34	9(26.4)	18.9(2.29-155)	0.006	3.43(0.20-57.0)	0.390
MCE	Water & DET	63	2(3.1)	**		**	
	water only	23	5(21.7)	4.09(0.99-16.8)	0.051	18.8(0.80-441)	0.068
	not wash	59	15(25)	4.16(1.27-13.6)	0.018	12.5(0.98-160)	0.048
MHW	Water & DET	78	1(1.28)	**		**	
	water only	34	7(20.5)	0.36(0.04-3.14)	0.358	0.18(0.06-6.0)	0.341
	not wash	33	14(42)	10.0(3.40-29.4)	0.000	5.4(0.73-40.78)	0.097
JRT	No	119	13(10)	0.39(0.14-1.08)	0.072	0.33(0.03-3.2)	0.346
	Yes	26	9(34.6)	**		**	
JRMT	No	128	16(12)	0.10(0.033-0.3)	0.000	0.11(0.01-1.03)	0.054
	Yes	17	6(35.2)	**		**	
UPC	No	80	15(18)	0.24(0.09-0.6)	0.007	0.74(0.09-5.8)	0.778
	Yes	65	7(10.7)	**		**	
CE	No	59	13(22)	3.03(1.18-7.7)	0.021	2.5(0.30-21.3)	0.386
	Yes	86	9(10.4)	**		**	
UD	No	101	12(11)	0.36(0.14-0.9)	0.034	0.09(0.01-1.0)	0.050
	Yes	44	10(22)	**		**	
PH	No	91	10(10)	0.83(0.33-2.1)	0.699	0.80(0.08-7.2)	0.847
	Yes	54	12(22)	**		**	
KCR	No	33	14(42)	22.7(7.36-70.1)	0.000	11.5(1.6-80.9)	0.014
	Yes	112	8(7.1)	**		**	
UJM	No	111	12(10)	**		**	
	Yes	34	10(29)	4.3(1.68-11.2)	0.002	0.26(0.04-1.7)	0.165

Keys: CE=Cleaning equipment; CI= Confidence interval; DET=Detergent; ES=Educational status; HW=Hand washing; JRMT=Job related medical test; JRT=Job related training; KCR=Know contamination as risk; MCE=Manner of cleaning equipment; MHW=Manner of hand washing; OR= odd ratio; PH=Personal hygiene; UD=Using detergent; UJM=Using jewellery materials; UPC=Using protective clothes; ** = Reference point

4.5. Questionnaireand Observational Survey Results

For questionnaire and observational survey analysis, a total of 145respondents were used, of which 60 from Abattoir workers and 85 from Butchers house workers to assess their awareness on meat hygiene and safety during meat handling.

Accordingly, table 6 shows the knowledge, attitudes and practices of 60 abattoir workers in relation to important parameters that potentially can influence the quality and safety of beef meat.Twenty two (36.66%) of the workers use unclean knives while 37 (61.66%) of them keep equipment in unhygienic places.Whilst forty three of the respondents responded that unclean hand and equipment as major causes of carcass contamination, sixteen considered falling on the ground as a major source of contamination.

Washing the hands before and after work is practiced by only four of the interviewees and thirty eight did not regularly put on clean protective clothing at work (Table 6). Only seven of them responded that the faeces, skin and dirty water could possibly cause carcass contamination. Most (65%) interviewees consider that keeping hygiene is the role of the management while some (35%) of them think the role of management is setting standards for hygiene in abattoir and workers role is maintaining standards for hygiene in the slaughterhouse.

Direct observations revealed the absence of hot water, sterilizer, carcass retention room and all processes were achieved in a single floor of the abattoir. During slaughtering equipments were placed on unclean surfaces. Knives were placed on the floor and on the skin of slaughtered animals. The protective clothes were unclean, blood tinged and frequently in contact with carcasses. There were no separate compartments for final carcasses and animals to be slaughtered (Annex 7).

Table 6: The knowledge, attitude and practice of abattoir workers

Factors	Values	Frequency	Percentage (%)
Educational status	Illiterate	14	23.23
	1-8	25	41.66
	9-12	14	23.23
	beyond grade 12	7	11.66
Placement in the abattoir	Slaughtering[a]	25	41.66
	Loading	16	26.66
	Washing stomach	11	18.33
	Washing the intestine	8	16.66
Job related training	Yes	12	20
	No	48	80
Job related medical test	Yes	6	10
	No	54	90
Know contamination as risk	Yes	39	65
	No	21	35
Clean clothing	Yes	22	36.66
	No	38	63.33
Hand washing	Before & after	4	6.66
	Before	11	18.33
	After	29	48.33
	Not wash	16	26.66
Knives are clean	Yes	38	63.33
	No	22	36.66
Unhygienic equipment placing	Yes	37	61.66
	No	23	38.33

a =Cutting the throat, flaying eviscerating, splitting the carcass and carcass washing

Table 7 shows the knowledge, attitudes and practices of 85 butchers in relation to important parameters that potentially can influence the quality and safety of beef meat. Among the eight five butchers, seventy one acquired meat selling skills from observations and fourteen of them from informal training. Forty one of the butchers did not use protective clothes and forty four of them wash their hands with only water after work. All reported that they use a single knife for cutting meat and edible offal. Twenty had worn jewelries and sixty two handled money while selling meat. Forty eight of the butchers cleaned their shop and equipment every day at end of the selling process by using water and soap.

Table 7: The knowledge, attitude and practice of Butcher house workers

Factors	Values	Frequency	Percentage (%)
Educational status	Illiterate	10	11.76
	Grade 1-8	49	57.64
	Grade 9-12	15	17.64
	Beyond grade 12	11	12.94
Received job related training	Yes	14	16.47
	No	71	83.52
Received job related medical test	Yes	11	12.94
	No	74	87.05
Apron(protective clothes)	Used	44	51.76
	Not used	41	48.23
Jewellery materials	Worn	20	23.52
	Not worn	65	76.47
Hand washing	Before and after	9	10.58
	Before	17	20
	After	41	48.23
	Not wash	18	21.17
Manner of hand washing	Using detergent and water	23	27.05
	Rinsing with water only	44	51.76
	Not wash	18	21.17
Handling money	Cashier	23	27.05
	Butcher with bare hand	62	72.94
Cleaning equipment at the end of work using water & saop	Yes	48	56.47
	No	37	43.52
Use detergents	Yes	26	30.58
	No	59	69.41
Cutting table	Single/common/	57	67.07
	Separate for d/t organs &meat	28	32.94

5. DISCUSSIONS

5.1. Over all Occurrences of *Salmonella*

In the present study, the overall proportion of *Salmonella* positive was 13.4% (43/320). Out of a total 240 samples from abattoir and 80 from butcher houses examined for Bacteriological status of *Salmonella*, 12.5% (30/240) and 16.2% (13/80) were found to be *Salmonella* positive, respectively. This finding relatively agrees with previous studies undertaken in different parts of Ethiopia which was 14.8% at Dessie (Gizachew *et al.*, 2017), 12.5% at Wolaita sodo (Wondimu *et al.*, 2017) and 13.3% at Jima (Anbessa and Ketema, 2012).

In accordance with sample types, the current finding 12%,16%&16.66% from carcass swab, personnel hand swab & pooled material swab respectively wasin consistent with the reports of 9.1%,16.7%&16.7% from carcass swab, pooled hand swab & pooled material swab respectively, in Holeta (Fufa*et al.*,2017). Also the present finding was lower than the reports of Beshatu, (2014) and Amenu, (2012) which were 17.7% from Diredawa municipal abattoir and 30% from Arbaminch municipal abattoir respectively. However, the present finding was higher than the previous studies in Ethiopia. For instance, Akafete and Haileleul, (2011) and Woldemariam *et al.*, (2005) found that the prevalence of *Salmonella* from goat carcass swab was 8.3% at Modjo and 7.5% at Bishoftu, respectively.

In the present study, even though there was no significant difference ($p > 0.05$) between the result of *Salmonella* from Abattoir (12.5%) and from Butcher houses (16.25%),but it was lower than the reports of Amenu , (2012)which were 31.5% & 28.5% from beef carcass of Abattoir and Retailer respectively, in Arbaminch. These differences could be due to differences in the hygienic and sanitary conditions practiced in the abattoirs and butcher houses. Because, the current study concerns about the municipal abattoir and butcher houses, that have poor sanitation and hygienic standard. In addition to this workers in the current abattoir and butcher were found to be with poor general and personal hygiene and lack of knowledge in hygienic processing of meat, due to lack of training regarding hygienic and sanitation of slaughtering and working environment generally and in the municipal abattoir, there is no clear division of the slaughtering process into stunning, bleeding, skinning, evisceration, chilling, cutting, or frozen delivery.

Bleeding and evisceration has been conducted on a horizontal position on the floor by incising the hide at the bottom of the abdomen without flying the skin. Workers hoisted the carcass manually using a chained pulley system after flying the skin and evisceration on the floor. There is no knife and axe sharpening machines. There is no means of sterilizing equipment. Carcasses were manually quartered using axes. There was no disinfectant, hot water and separate room for final carcass and live animals in the abattoir.

It is well recognized that, when animals are starved, *Salmonella* can survive and multiply in the rumen. Furthermore, healthy carriers intermittently excrete only a few *Salmonella*, unless they undergo some kind of stress such as transportation (Venter *et al*., 1994). Therefore, high contamination with *Salmonella* could be associated with high excretion of *Salmonella* with feces as source of contamination due to exposure to such predisposing factors as starvation, overcrowding in market and transportation.

The high level of carcass contamination with *Salmonella* is of special public health significance for a country like Ethiopia, where raw and under cooked meat is the favorite food in most areas (Akafete and Haileleul, 2011). In addition to eating raw and under cooked meat, most of the consumers does not have information about the risk of this contaminated meat, because they consider as it is safe to eat when slaughtered at abattoir therefore, consumers can also cross contaminate with other foods during processing.

5.2. Occurrence of *Salmonella* among Risk Factors

According to the present study, not receive job related training, not know contamination as risk, not cleaning equipment, people who were illiterate, not washing hands, not using detergents during cleaning equipment and washing hands; and using jewelry materials during meat processing were the major risk factors for the occurrence of *Salmonella* among carcass, abattoir personnel, butchers and materials in abattoir and butcher house since the odds value of these factors were > 1 even though there was no significant difference ($p > 0.05$) for some of the risk factors. Hence, the overall occurrence of *Salmonella* in the study area was directly or indirectly associated with the risk factors since *Salmonella* is cross contaminant of foods mainly meat. The current finding is in agreement with the studies conducted in Ethiopia,

which showed that people and equipments were found to be significantly associated with carcass contamination by *Salmonella* (Gizachew *et al.*, 2015 and Teklu *et al.*, 2011).

This may happened most probably because of more than 67.5% of slaughter house workers and butchers had only a primary school education. Similarly more than 82% of slaughter house workers and butchers did not have job related training as regards to food hygiene. These results are in agreement with reports of Endale and Hailay (2013) and Mekonnin *et al*. (2013) who reported a primary school education and lack of job relating trainings in more than half of the slaughter house workers and butchers in Mekele city, Ethiopia. Therefore, these workers could cross contaminate and not handle meat hygienically due to lack of knowledge regarding hygiene, sanitation, risk of contamination and personal hygiene. However training of food handlers regarding the basic concepts and requirements of personal hygiene plays an integral part in ensuring safe products to the consumers (Adams and Moss, 1997) and food handlers should have the necessary knowledge and skills to enable them handle food hygienically (FAO, 1990).

5.2.1. Abattoir workers

The majority 37(61.66 %) of the abattoir workers proposed unclean hand and equipment as the major causes of carcass contamination but few responded that the faces, skin and dirty water can cause carcass contamination. Besides, most consider that keeping hygiene is the role of the management while some of them think the role of management is setting standards for hygiene in abattoir and workers role is maintaining standards for hygiene in the slaughterhouse.

It is well documented that, the fecal wastes from animal and humans are important source of bacterial contamination of the environment and foods chain (Ponce *et al*., 2006), and members of *Salmonella enterica* subspecies *enterica* are widely distributed in the environment and in the intestinal tract of animals (Anjum *et al*., 2011). Thus, this research result indicates that most of the abattoir workers does not know source of meat contamination and their responsibility in hygienic management of beef meat accurately. Therefore, they can contaminate meat with such source of contamination unknowingly. The workers could not know how to minimize the risk of meat contamination if they do not know the source of meat

contamination properly and their role in hygiene of the slaughter environments.

Good health is important for workers in the meat industry. Ill persons will often be carriers of more microorganisms (pathogenic microorganisms) than is usually the case. These microorganisms may then be transmitted to the meat/food with the risk of causing disease to the consumers. Illness must always be reported to the manager and/or the meat inspector of the slaughterhouse who will decide if the worker can stay or has to leave (Skaarup, 2011). Contradictory, this study result specify that among the respondents from abattoir house workers and butchers 88.3% of them reported that they never experienced job related medical test while 11.7% of them taken job related medical test once only in their work duration.

Furthermore, every worker accountable in cattle slaughtering activity allowed slaughtering and every process from cutting the throat to final carcass preparation was covered by single person and mostly may continue to butcher shop as meat vender. This is because of most of the abattoir workers are butchers as well. As a result majority of the respondents complain that in the abattoir working quickly is preferred than slaughtering hygienically. Therefore, the workers attention is only to finish all process on time rather than slaughtering hygienically. This could result in occurrence of high cross contamination of carcass which might be a risk for the consumers. This problems could be because of the managers (concerned bodies) are not professionals, to solve such problems and they them self-do not have the knowledge of food safety and hygiene.

The slaughtering process was unhygienic and unsanitary. There was no hot water, sterilizer and retention room and equipments rest on dirty surfaces. However, Akafete and Haileleul, (2011), reported that eviscerating knife significantly associated with carcass contamination and specific attention must be given to sterilization of knifes. Motsoela *et al*. (2002) also indicated that, it is salutary to note that knives must be immersed in water for two minutes at 820C to reduce the number of contaminating microorganisms. Contradictory to these facts, in current study site the same knife was used without sterilizing (even without washing with pure water) to slaughter different cattle, cutting throat, for skin removal, for evisceration, for carcass removal, and for other process. This could cause high carcass contamination with different foodborne pathogens unless it is solved.

Correspondingly, it was found that the equipment used for slaughtering process was rested on dirty surface during working, for instance they put their knife on ground, in their own mouth, and on skin of other killed animal and then use it as it was. Similarly they use the material which put on the ground to collect water for washing carcass repeatedly; their protective clothes were full of blood, dirty and in contact with carcass while they take the finalized carcass to car (transportation access). In summery this type of area and slaughtering process can cause cross contamination of reedy to eat meat at different stage. In the same way D"Aoust, (1997) expressed that, all food that is produced or processed in a contaminated environment may become contaminated with *Salmonella* and be responsible for outbreaks or separate cases of disease as a result of faults in transport, storage, or preparation. Therefore the risk of carcass contamination might be increasing until it reaches the consumers at different stage due to above listed predisposing factors such as in contact with dirt clothes wile loading, transportation, use of contaminated water, use of contaminated materials and moving from one rail to another rail.

Removal of hides should be carried out in a manner that avoids a contact between the skin and the carcass and contact between the carcass and workers" hands, tools or equipment, which had previously contacted the hide. Knives and steels used in the de-hiding operation should be sterilized in water at 82°C (McEvoy *et al*., 200). In contrast to this information this research result indicates that, there was no separation between final carcasses and live cattle going to be killed. Consequently, there was high contact between skin of live cattle with final carcass, since there was no separate room for final carcass and live animals. McEvoy *et al*., (200) expressed that, contamination can occur by direct contact between the hide and the carcass or by indirect transfer, i.e. from workers" hands, clothes, tools or factory equipment which have had previous contact with the hide.

During the life of the animal, the hide becomes contaminated with large numbers of microorganisms derived from a wide range of sources such as faeces, soil, water and vegetation, including pathogens such as *E. coli* O157:H7 and *Salmonella*. Many of these organisms are present on the hide of animals presented for slaughter. There is a positive relationship between the level of dirt on the hide and bacterial numbers on the carcass. The

relationship is evident at sites on the carcass that are subjected to manual skinning during hide removal. Reduction of the bacterial loading on the hide of animals entering the slaughter process would limit the impact and scale of pathogen transfer from the hides to the carcass (McEvoy *et al*., 200).

5.2.2. Butchers

The hygienic practices at the butcheries were unhygienic. Most of the butchers (72.9%) handle money with bare hands while processing meat and do not put appropriate protective clothes. Similarly, Endale and Hailay (2013) reported that 91.7% of the butchers in Mekelle city handle money while processing meat. In addition, other study indicates that, handling of foods with bare hands may also result in cross contamination, hence introduction of microbes on safe food. Because meat handlers are probable sources of contamination for microorganisms, it is important that all possible measures should be taken to reduce or eliminate such contamination (Muinde and Kuria, 2005).

As the paper money circulates among different individuals it could be contaminated with several pathogens including *Salmonella* and handling carcasses with bare hands that also handle such items may result in cross contamination. In addition most butchers wash their hands after the selling process and use only water with no detergents and use single knife for edible offals and meat types and a single cutting board for all products without cleaning and sterilizing. The overall butchery practices are favorable for the contamination of beef meat.

Besides, most (56.47%) of the butchers responded that they clean their shop, clothes and equipment every day at the end of selling process using water, and few (43.5%) uses soap in addition to water. Contradictory, documented data indicates problems in cleaning with water alone as follows; blood proteins can create particular problems on porous surfaces, often giving rise to green/brown, and very resistant staining. Aged protein deposits can be quite hard, normally not scraping off easily with a fingernail.

In addition to this, soil deposits in a food plant would be bad enough if problem was simply their rather unsightly appearance. But the fact that they harbor, nourish and protect spoilage or pathogenic microorganisms that are invisible to the naked eye makes the job somewhat

harder. The soil must, of course, be removed as completely as possible by effective cleaning using the detergent. Water alone does not sufficiently wet to displace many types of soils or even to displace air from water- repellent or hydrophobic surfaces. In this case the water curls up under its own surfaces tension into droplets. Lack of wetting will prevent cleaning taking place. To achieve wetting of such surfaces, chemical agents which have particular surface properties should be employed: „surfactants" or „wetting agents" (Gracey *et al*., 1999).

5.3. Antimicrobial Resistance

Resistance to multiple antimicrobials (97.67%) which was observed in current study was in line with the reports of Olana , 2018; Abunna *et al.*, 2017 and Beshatu , 2014 which are 95.45%, 96.4% and 97.7% respectively, but higher than other studies conducted in Ethiopia. For instance, Takele *et al.*,2018; Guesh,2017; Zelalem *et al*., 2011; Endrias, 2004; Molla *et al*., 2004 and Alemayehu *et al*., 2002 reported 40.5%,72.22% , 83.3%, %,23.5%,44.8 and 52% respectively the multidrug resistance of *Salmonella* isolated from food of animal sources, animals and humans, as well higher than reports from elsewhere in the world (Fadlalla *et al*., 2012; Elgroud *et al*., 2009; Khaitsa *et al*., 2007; Al-Bahry *et al*., 2007 and Stevens *et al*., 2006), reported multidrug resistance of *Salmonella* isolates respectively as follow:16%,50% (from raw meat),(1.2%,14.1% and 23.7%) *salmonella* isolated from different type of samples,51.7% and 37.82%. This difference could be because of that, antimicrobial-resistant *Salmonella* are increasing due to the use of antimicrobial agents in food animals at sub-therapeutic level or prophylactic doses which may promote on-farm selection of antimicrobial resistant strains and markedly increase the human health risks associated with consumption of contaminated meat products (Zewdu and Cornelius, 2009; Molla *et al*., 2006 and Molla *et al*., 2003).

Zewdu and Cornelius, (2009) reported that the isolates of *Salmonella* from food items and workers from Addis Ababa were resistant to the commonly used antibiotics including streptomycin, ampicillin, and tetracycline. Furthermore, Zelalem *et al*., (2011) also indicated resistance of *Salmonella* isolates to commonly used antimicrobials including ampicillin, streptomycin, nitrofurantoine, kanamycin and tetracycline, with resistance rate of 100%, 66.7%, 58.3% and 33.3%, respectively. Similarly previous reports from Cameroon

(Akoachere *et al*., 2009, from South India (Suresh *et al*., 2006), and from Nigeria (Akinyemia *et al*., 2005)) indicated a similar 100%, 100% and 90% respectively resistance to ampicillin. The result of the current research also indicated resistance of *Salmonella* isolates to commonly used antimicrobials including erythromycin, ampicillin, oxacillin, and neomycin with resistance rate of 100%, 83%, 72%, and 67.44% respectively. However, higher resistance rate than previous reports with the exception of ampicillin and resistance to further drug as well as to penicillin (44.8%) was observed in this result. This difference could be due to the increasing rate of inappropriate utilization of antibiotics which favors selection pressure that increased the advantage of maintaining resistance genes in bacteria (Mathew *et al*., 2007 and McGeer, 1998).

It is as well recognized that recent resistance additions include resistance to penicillin. The continuing development of antibiotic resistance may lead to sufficient pressure ultimately to restrict the antibiotics available to the veterinary profession for animal treatment (Gracey *et al*., 1999). Moreover, this increase antibiotic resistance, in addition to public health problems, may lead to economic loss in the countries due to loss of exporting meat and animal products and cost of drug of choice to treat human and animals due to resistance development.

Gentamycin showed a good antimicrobial activity against these *Salmonella* isolates. It was found that all of 43(100%) isolates were susceptible to gentamycin. This result was similar with previous reports by Olana, 2018 and Abunna *et al.,* 2017 from central parts of Ethiopia among isolates of beef meat and humans from Bishoftu and dairy farm from Modjo respectively. The effectiveness of such drugs like Gentamycin could be because of that they are not widely used in countries like Ethiopia and other African countries (Zelalem *et al*., 2011). In addition to this, effectiveness of this drug could be because of this drug is not well distributed to all societies and not simply prescribed rather than it is used as drug of choice in antibiotic resistant person. In addition to this, Gentamycin is not commonly used to treat animals in Ethiopia, particularly in the current study area.

6. CONCLUSION AND RECOMMENDATIONS

The present study results revealed that high prevalence of *Salmonella*, presence of poor personal hygiene and sanitation, resistance of *Salmonella* to most antimicrobials except Gentamycin, low level of community awareness about contamination of beef meat with *Salmonella* and the associated probable risk factors for the occurrence of *Salmonella* in the study area. Consequently, beef meat provided to the consumers in the town was found to be poor quality and risk full for human health calling for urgent intervention. Based on the above conclusion the following recommendations were forwarded:

- Training programs must be provided on best practice of handling of meat for handlers and raising the level of awareness of people.
- Further studies should be conducted regarding molecular characterization and serotyping of *Salmonella* species.
- The number of abattoir works should be proportional with number of cattle slaughtered per day
- Since *Salmonella* is resistant to most common drugs, attention should be taken in selecting antimicrobials in treating *Salmonella* infection both in animals and human being based on antimicrobial susceptibility test.
- The degree of the risk of consumption of beef meat contaminated with *Salmonella* should be assessed.
- The use of standardized procedures and building of better abattoir which have partitions for all process should be applied.

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8. ANNEXES

Annexes 1: Media used and preparations for the isolation and identification of Salmonella

1. Nutrient Broth (OXOID)

Bacto-beef extract	3 g
Bacto-peptone	5 g
Distilled water	1000 ml
pH adjusted to	7.2-7.4

It was sterilized by autoclaving at 121 ^{o}C(15 lb pressure) for 15 minutes

2. Nutrient agar (OXOID)

Peptone	5 g
Beef extract	3 g
Agar	15 g
Distilled water up to	1 litre

The medium was sterilized in the autoclave at 121 ^{o}Cfor 15 minutes and poured into sterile Petri dishes in 15 ml quantities

3. Buffered peptone water (LAB M, UK)

Proteose	10.0 g
Sodium chloride	5.0 g
Disodium phosphate	3.5 g
Monopotassium phosphate	1.5 g
Final pH	7.2 ± 0.2

Preparation: Prepare by autoclaving 20 gms of media in 1 litre distilled water. Then heat to dissolve the medium and dispense in required amount. Sterilize by autoclaving at 121 ^{o}C /15 Ibs pressure for 15 minutes.

4. Rappaport vassiliadis medium (LAB M, UK, OXOID)

Soy peptone	4.5 g
Sodium chloride	7.2 g
Potassium dihydrogen phosphate	1.26 g
Dipotassium hydrogen phosphate	0.18 g
Magnesium chloride anhydrous	12.4 g
Malachite green	0.036 g
pH	5.2 ± 0.2

Preparation: Weigh 26 grams of powder and dispense into 1 litre of deionized water. Allow to soak for 10 minutes. Swirl to mix, when dissolved dispense in to 10 ml volume screw capped bottles. Sterilize by autoclaving at 115 ^{o}Cfor 15 minutes

5. Muller-Kauffmann tetrathionate broth (LAB M, UK, OXOID)

1. Thiosulphate solution:

Sodium thiosulphate	24.8 g
Sterile water	100 ml

2. Iodine solution:

Potassium iodide	20 g
Iodine	12.7 g
Sterile water	100 ml

Complete medium

Calcium carbonates	2.5 g
Nutrient broth	78 ml
Thiosulphate solution	15 ml
Iodine solution	4 ml

Phenol red	0.02 %

Preparation: Add calcium carbonate in nutrient broth and sterilize by autoclaving them. Then add iodine, thiosulphate and then phenol red under aseptic conditions. Distribute in the screw-capped test tubes

6. XLD agar (OXOID)

Yeast extract	3.0 g
Sodium chloride	5.0 g
D (+) xylose	3.5 g
Lactose	7.5 g
Sucrose	7.5 g
L (+) lysine	5.0 g
Sodium deoxcycholate	2.5 g
Sodium thiosulfate	6.8 g
Ammonium iron (III) citrate	0.8 g
Phenol red	2 ml
Agar-agar	13.5
Distilled water	1 litre

The ingredients were steamed to dissolve with final pH 7.0-7.2.

7. TSI Agar (OXOID)

Polypeptone peptone	20 g/L
Sodium chloride	5 g/L
Lactose	10 g
Sucrose	10 g
Glucose	1 g

Ferrous ammonium sulphate	0.2 g/L
Sodium thiosulphate	0.2 g/L
Phenol red	0.025 g/L
Agar-Agar	13 g
PH	7.3

Preparation: Suspend 64.50 gms in 1000 ml distilled water and heat to dissolve the medium completely. Mix well and distribute into test tubes. Sterilize by autoclaving at 15 Ibs pressure (121 ^{o}C) for 15 minutes. Allow the medium to set in slopped form with a butt about 1 inch long.

8. Simmon's Citrate Medium (OXOID)

Sodium chloride	5.0 g
Magnesium sulphate	0.2 g
Ammonium dihydrogen phosphate	1.0 g
Potassium dihydrogen phosphate	1.0 g
Sodium citrate	1.0 g
Bacto agar	20 g
Water	1000 ml
Bromothymol blue	(0.2 %) 40 ml
PH adjusted to 6.8	

Preparation: Sterilized the media by autoclaving at 121 ^{o}Cfor 15 minutes at 15 lb pressure and cooled for slope formation.

9. Urea extra pure

Melting point	132-133 o C

Insoluble matter	0.003 %
Acidity	0.05 % N
Alkality	0.05 % N
Sulphated ash	0.05 %
Chloride	0.0005 %
Sulphate	0.001 %
Copper	0.0001 %
Iron	0.0001 %
Lead	0.002 %
Biuret	0.05 %

10. Urea agar base (OXOID)

Peptone	1.0 g
Glucose	1.0 g
Sodium chloride	5.0 g
Disodium phosphate	1.2 g
Potassium dihydrogen phosphate	0.8 g
Phenol red	0.012 g

Preparation: Suspend 2.4 g in 95 ml of distilled water. Bring to the boil to dissolve completely, sterilize by autoclaving 115 ^{o}Cfor 20 minutes. Cool to 50 ^{o}Cand aseptically add one ampoule of sterile Urea solution (SR20). Mix well, distribute 10 ml amounts into sterile containers and allow setting in the slop position

11. Peptone Water for Indole Reaction (OXOID)

Peptone	20 g
Sodium chloride	5 g

Distilled water	1000 ml

Preparation: The solids were dissolved by steaming. The reaction at room temperature was adjusted to pH 7.5. The medium was dispensed in 5 ml quantities in test tubes and autoclaved at 121 ^{o}C for 15 minutes.

11. Lysine iron agar (OXOID)

Peptone	5.0 g
Yeast extract	3.0 g
Glucose	1.0 g
L-lysine	10.0 g
Ferric ammonium citrate	0.8 g
Sodium thiosulphste	0.04 g
Bromocresol purple	0.02 g

12. Mueller Hinton Agar (OXOID)

Beef	300.0 g
Casein hydrolysate	175.0 g
Starch	1.5 g
Agar	1.7 g
pH	7.3 ± 0.1 at 25 ^{o}C

Preparation: Suspend 38 g in 1 liter of distilled water. Bring to the boil to dissolve the medium completely. Then sterile by autoclaving at 121 ^{o}Cfor 15 minutes.

Annexes 2: Biochemical test result interpretation

Tests	Positive				Negative			
KIA	Slant	Butt	Gas	H_2S	Slant	Butt	Gas	H_2S
	red	yellow	crack	black	yellow	red	No crack	No black
Urease	red/purple				yellow/orange			
LDC	Blue				Yellow			
Indole	red ring on surface				yellow ring			
Citrate	Blue				Green			

Annexes 3: Sample collecting format

No	Sample collection date	Site	Types of sample	Remark
1				
2				

Annex 4: Laboratory analysis data recording format

No	Sample Code	XLD agar	BG agar	Urea test	Citrate test	TSI agar				Indole test	L-lysine Decarbox Ylation medium
						Slant	Butt	H2S	Gas production		
1											
2											
3											

Annexes 4: Standards for antimicrobial susceptibility testing of Salmonella

No	Antimicrobial Agent	Disc Code	Potency	Resistant	Intermediate	Susceptible
1	Erythromycin	ERY	15 µg	≤13	14-22	≥23
2	Amoxicillin	AMX	30 µg	≤13	14-17	≥18
3	Chloramphenicol	CHL	30 µg	≤12	13-17	≥18
4	Ciprofloxacin	CIP	5 µg	≤20	21-30	≥31
5	Gentamicin	GEN	10 µg	≤12	13-14	≥15
6	Streptomycin	STR	10µg	≤11	12-14	≥15
7	Tetracycline	TET	30 µg	≤11	12-14	≥15
8	Clindamycin	CLN	2µg	≤14	15-20	≥21
9	Spectinomycin	SPS	100 µg	≤11	12-14	≥15
10	Kanamycin	KAN	30 µg	≤13	14-17	≥18
11	Penicillin	PEN	10unit	≤26	27-46	≥47
12	Ampicillin	AMP	10 µg	≤13	14-16	≥17

Source: CLSI, (2012).

Annexes 5: Flow diagram showing ISO methods for isolation and antimicrobial sensitivity test of *Salmonella*

PRE-ENRICHMENT

Test portion, 25g + buffered Peptone water, 225ml*

16-20 h, 37 °C

SELECTIVE ENRICHMENT

Culture, 0.1ml + Rappaport (RV) broth 10ml

18-24 h, 42 °C (2 periods)

Culture, 10ml + Selenite Broth (SB) 10 ml

18-24 h, 42 °C (2 periods)

SELECTIVE DIAGNOSTIC ISOLATION

Plate onXylose lysine desoxycholate (XLD) agar and Brilliant Green Agar ((BGA) or any other solid selective medium

24 h, 35 °C or 37 °C (48 h, if necessary)

Pick five presumptive *Salmonella* colonies from each agar plate and inoculate on nutrient agar

18-24 h, 35 °C or 37 °C

BIOCHEMICAL CONFIRMATION

(TSIA, Urea broth, Simmon's citrate, Indole, LDC)

24 h, 37 °C

ANTIMICROBIAL RESISTANCE TEST

5 ml of Tryptonesoya broth

4 h, 37 °C

Muller hinton + plates + Antibiotic disc

24 h,37 °C

Annexes 6: Questioner survey format and observed hygiene practice record for knowledge, attitude and practice analysis of different key informants along the meat pathway.

1.Abattoirworkers

Date----------Questionnaire number----------

1. Nameofslaughterhouse----------

2. Nameofrespondent----------

3. Educationalstatus:a) Illiterate b)Grade1-5 c) Grade6-8 d)Grade9-12 e)Grade>12

4. Placement in slaughterhousesprocess:a)Stunner b)cuttingthe throat c) flayer

d)Evisceratore e)Splitter f)Carcasswasher g) All h)other(specify)

A.Knowledge

A1. Doyouplay any other rolein the slaughter processapart from the one mentioned above?
a)Yes b) No

A2. If yes,whichone(s)? ---------- ----------

A3. IfNo,whynot? ----------

A4. If your answerforno.4is d,howfrequentlydo youcomeacrossfaultyeviscerations? -

A5.What do youdo after faultyevisceration? ----------

A6.Howdo youhandle cattlepresentedforslaughter?

A7. Did you receiveanyjobrelated training?a)Yes b)No

A8. Ifyes for A7; where were you trained? ----------

A8.1. If there was no formaltraininghaveyoureceivedinformaltraining? ----------

A9.Have you undergoneanyjobrelated medicalteststo workin theabattoir?

a)Yes b)No

A10. When was your last medicaltest done?

a) one month b) two month c) three month d)six month e)one year

A11.What wouldcausecarcasscontamination?

a) Faeces b) Dirtywater

c) Handlingwith dirtyequipmentandhands d) Other(specify)……

A12.Ifcarcasswascontaminatedbyfaeces,whatwould youdo? (Openquestion)

a) Nothing b) Washthecarcass

c) Call themeat inspectorforadvice d) Other(specify)……

A13. Inyouropinion,doescontaminationposeanyhealthriskto meat consumers?

a)Yes b)No

A14. IfNo, why? ----------

A15.Proposewayto end carcasscontamination? ----------

B.Attitude

I will read some statements about hygiene in the slaughter process. Please indicate whether youagree or disagree. Key: SA= strongly agree,A=agree, D=Disagree, SD=stronglydisagree, andDK=don'tknow

No.	Question	SA	A	D	SD	DK
1	In this job, it is important to work quicklythan keep the carcassesclean.					
2	Peopledoingthis job aremorelikelytoget sick					
3	In this type of working environment keeping clean is Easy					
4	Asmall amount of dirt on clothing or utensils willnot causeanyharm					
5	Healthis moreimportant thanwealth					
6	Ensuringhygieneis mainlytheroleofmanagement					
7	If meat is well-cookedthenit is always safe to eat					

C. Practice (Worker observation checklist)

Cuts/wounds covered with an appropriate Water proof dressing.	Yes----------No----------
Smoking or eating or chewing while working	Smoking-------chewing----none of the two
Clothes clean and completely free from any dirty or blood	Yes----------No----------
Hand washing before, after and during cutting Meat	Before --------After -------During ---
How washed? Running water or bucket? Hot or cold? Brush or cloth? Soap?	Running water-------- bucket--------- Hot ------ cold---------Brush ------ cloth-----Soap----------
All knifes are completely clean and free from dirt and cracks and damages	Clean ----------undamaged -----
Knifes are cleaned before, after and during Use	before -------after------ during use----
How oftenandwhendo you washthe equipment?	Every day at end of the process------- Once per weak--------once per month------- other(specify)-------
Is any disinfectant used? Write name of Disinfectant	Yes----------No----------
The source of water used in abattoir	Tap----Well-----Water vendor------ other-------
Latrine available nearby	Yes----------No----------
Latrine has water, soap, paper& towels for hand Washing	Water-----soap--------paper--------towel------- tissue paper----------
Equipments rested in dirty surface duringWorking	Yes----------No----------
Strict separation between clean and dirty Areas	Yes----------No----------
Veterinary inspectors present to examine the meat to be sold.	Yes----------No----------

D. perception

D1. What constraints do youexperienceinyour work? ----------
D2. Dothey affect your abilitytoachievehighlevels of hygiene? ----------

D3. IfYes,in what way? ----------

D4. Inyour opinion, what role do you think the management should playin: ----------

a)Setting standards for hygiene in the slaughterhouse? ----------

b) Maintainingthosestandards? ----------

D5. Inyouropinion,what roledo youthinkthe workersshouldplayin? ----------

a) Maintainingstandardsforhygienein theslaughterhouse?----------

b) Doingtheir workas much as possiblequickly? ----------

2.Butchershopworkers(meatvendors).

Datecompleted: ----------Questionnairenumber----------

1. RespondentName: ----------Address: ----------Occupation: ------

2. Nameofbutchershops----------

3. Educationalstatus:a) Illiterateb) Grade1-5 c)Grade6-8 d) Grade9-12e)Grade>12

4. Did you receiveanyjob related training? a)Yes b)No

5. If yesfor4; wherewere youtrained? ----------

6. If there wasno formal traininghaveyou receivedinformaltraining? ----------

7. Howmanycarcass you receiveperday? ----------

8. What is yourselling capacityperday? ----------

9. If the meat is not sold inagiven day what willyou do/ how do youhandle?------

---

10. How many knifeyouhave and you use per day a) one b) two c) three

d) four e)more(Specify)--------

11. Howoftenand whendo youwashtheequipment? a)Everydayat endoftheselling

b)Onceperweak c)Oncepermonth d)other(specify)-------

12. Who aremostofyour customers? ----------

13.Whatwouldcausemeat contamination? (Openquestion)

a) Faeces b) Dirty water

c) Handling with dirty equipment and hands d) other (specify)……

14. In your opinion, does contamination pose any health risk to meat consumers?

a) Yes b) No

15. If No, why? ----------

16. Propose way to meat contamination? ----------

Butcher shop worker (meat vendor) observation checklist

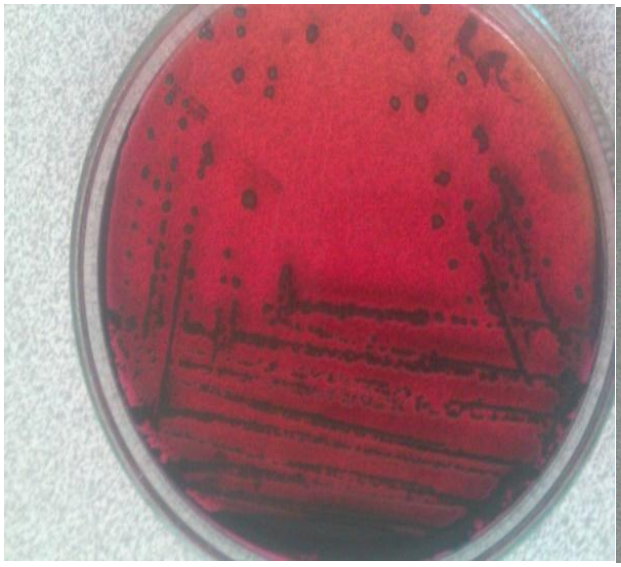
Cuts/wounds covered with anappropriate Water proofdressing.	Yes----------No----------
Smoking or eating or chewingwhileWorking	Smoking----------chewing----
Apron(anyprotectiveclothes)	Yes----------No----------
Hand washing before after and during cuttingmeat	Before---------After-------During--- Notwash----------other-------
Howwashed? Running waterorbucket? Hotorcold? Brushorcloth? Soap?	Running water----bucket------Hot -cold----- Brush ------ cloth-----Soap----------
All knifesare completely clean and free fromdirtandcracksand damages	Clean ----------undamaged-----
Knifes arecleaned before after and during Use	before ------after------during use------
Is anydisinfectant used? Write name of Disinfectant	Yes----------No----------
WearJewellery	Yes----------No----------
Handlingmoney	Cashier----------Butcherwith barehand
Cuttingtable	Single-------separatefor differentmeats ----

Annexes7: Pictures taken from the Abattoir to demonstrate observational survey

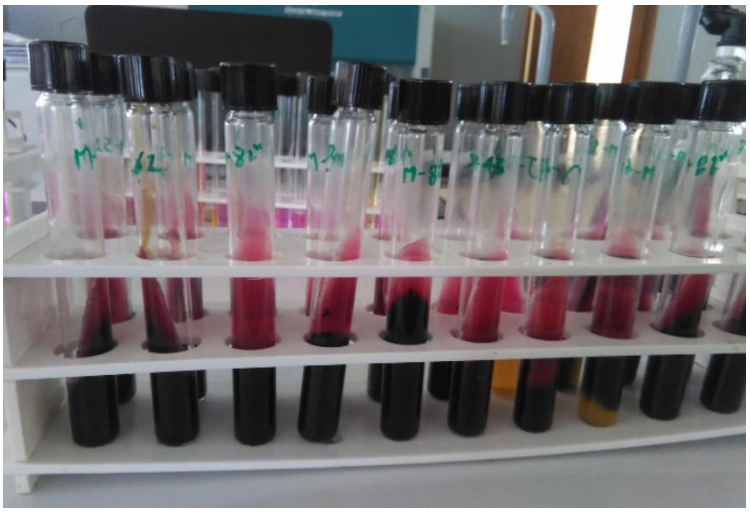



Pictures showing that every process (slaughtering, dressing, evisceration, bleeding, carcass removal and others) were processed in the same floor, thus exposed for cross contamination**.**

Annexes 8: Plating and identification of salmonella on XLD and BGA

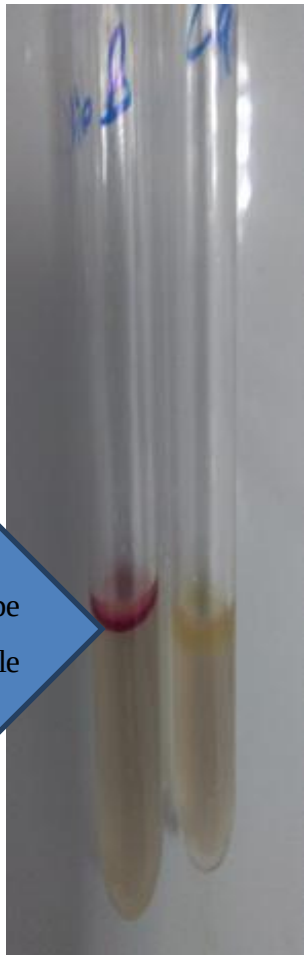


Annexes 9: Pictures showing Biochemical and Drug sensitivity test results



TSIA test

Red ring on the top surface of the test tube showing negativity of*Salmonella*on Indole



Citrate utilization test

Indole test

Antibiotic resistance testing procedure and disc diffusion appearance