Thesis Ref No._____

ASSESSMENT OF THE CONTAMINATION OF BEEF WITH *SALMONELLA* AND KNOWLEDGE, ATTITUDES AND BEEF HANDLING PRACTICES ALONG BEEF SUPPLY CHAIN IN DUKEM TOWN, ETHIOPIA

MSc Thesis

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MSc Program in Veterinary Public Health

June, 2017

College of Veterinary Medicine and Agriculture: Bishoftu, Ethiopia

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A Thesis submitted to School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in veterinary public health.

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

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DEDICATION

This thesis manuscript is dedicated to my families and to all my friends those who are behind my success.

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ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
BGA	Brilliant Green Agar
BPW	Buffered Peptone Water
CDC	Center for disease control and prevention
CLSI	Clinical and Laboratory Standards Institute
CSA	Central Statistical Authority
EU	European Union
FAO	Food and Agriculture Office
FBD	Food borne diseases
GALT	Gut-associated lymphoid tissue
HIV	Human Immune deficiency Virus
ISO	International Organization for Standardization
LIA	Lysine Iron agar
MHA	Muller Hinton Agar
MKTTn	Muller Kauffmann Tetrathionet with novobiocin
MoH	Ministry of Health
NTS	Nontyphoidal *Salmonella*
OIE	Office of International des Epizooties
RVS	Rappaport-Vassiliadis with Soya
Spp	Species
Subsp	Subspecies
TSI	Triple Sugar Iron agar
WHO	World Health Organization
XLD	Xylose lysine desoxycholate

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ABSTRACT

Salmonella is a major cause of food borne disease in the world, with an increasing concern for the emergence and spread of antimicrobial-resistant strains. A cross-sectional study was conducted between November, 2016 and April, 2017 to estimate the prevalence, and determine the antimicrobial susceptibility pattern of *Salmonella*, and assess the knowledge, attitudes and practices along beef supply chain in Dukem town. A total of 286 samples comprising feces, carcass swab and retail meat were collected and examined for the presence of *Salmonella* following the standard techniques and procedures. Hundred respondents consisting of 20 abattoir workers, 20 butchery workers and 60 consumers participated in the study. Systematic random sampling and purposive sampling techniques were used to generate the desired data. The overall prevalence of *Salmonella* along the beef supply chain was 6.3% (95% CI: 3.9-9.7). The specific prevalence of *Salmonella* based on sample source was 0.9%, 2.9%, and 12.7 % in feces, carcass swab, and retail meat, respectively. There was statistically significant difference along the beef supply chain(X^2 = 14.3027, P <0.05). Among the isolates, 94.4% (n=17) were resistant at least to one of the antimicrobials. All the isolates (100%) were sensitive to Kanamycin where as 94.4%, 88.9% and 83.3% of the isolates were found to be sensitive to Sufisoxazole, Tetracycline and Nalidixic acid, respectively. Multi-drug resistance was observed in 27.8% (n=5) of the isolates.

Majority (95%) of abattoir workers didn't know that contaminated carcass has public health risk. According to the respondents, falling of carcass in the dirty floor and sudden stomach cut were the major possible sources for carcass contamination. All of the abattoir and butchery workers received neither job related training nor medical check up. Most (70%) of consumers had habit of eating raw beef and 73.3% of them had no knowledge about the transmission of *Salmonella* via meat consumption. In conclusion, the study found the occurrence of *Salmonella* along beef supply chain with higher prevalence at meat retail shop and the variability in the susceptibility pattern of *Salmonella* isolates against the tested antimicrobials. It also showed that the existing beef handling practices, knowledge and attitudes about the importance of occurrence of *Salmonella* along the beef supply chain could be a potential public health risk unless the necessary intervention is in place. All stakeholders should raise awareness in minimizing the occurrence of *Salmonella* and thereby transmissions of resistant *Salmonella* to humans and risk of raw beef consumption along the beef supply chain particularly at meat retail. Moreover, identifying *Salmonella* serotypes circulating in the area and regular monitoring of the heath status of workers and hygienic condition of the slaughter house and meat retail shop is recommended.

***Key words**: Abattoir, Antimicrobial resistance, beef, contamination, Dukem, Prevalence, Salmon ella.*

1. INTRODUCTION

Food borne diseases(FBD) are diseases of infectious or toxic nature caused by the consumption of foods or water contaminated with bacteria and/or their toxins, parasites, viruses, or chemicals(WHO,2015).

They remain a real and formidable problem in both developed and developing countries, causing great human suffering and significant economic losses (WHO, 2015). Up to one third of the population of developed countries may be affected by foodborne diseases each year, and the problem is likely to be even more widespread in developing countries. For instances, in developing countries food and water-borne diarrheal diseases kill an estimated 2.2 million people each year, most of them children (FAO/WHO, 2006). One recent estimate is that the 31 major foodborne pathogens account for nearly 9.4 million people becoming sick, more than 55,961 hospitalizations, and 1351 deaths each year in the United States with an estimated economic cost of $77.7 billion annually (Scallan *et al*., 2011).

There are many and varied sources of organisms causing FBD. Most cases are caused by bacteria which arise from animal, human or environmental sources (Radostits *et al.,* 2007). *Salmonella* species, *Campylobacter* species, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 linked to illness due to consumption of meat and meat products (Mershal *et al*., 2010).

Salmonellosis is one of the major zoonotic diseases all over the world with annual estimates of 22 million cases and 200,000 deaths due to typhoid fever and 93.8 million cases of gastroenteritis and 155,000 deaths due to non-typhoidal *Salmonellae* (NTS) (Majowicz *et al*., 2010). More than 2,700 *Salmonella* serotypes are recognized to date. Many are known to cause illness in humans (Jones *etal*., 2008). Nontyphoidal *Salmonella* (NTS) spp. is one of the most important causes of foodborne disease and manifested by diarrhoea, bacteraemia and focal suppurative infections. *S.* enterica sub sp.enterica with *S.*Enteritidis and *S.*Typhimurium were responsible for most of the infections associated to humans and other mammals (Dunkley *et al*., 2009).

Of the NTS, *Salmonella* Typhimurium and *Salmonella* Enteritidis account for nearly 80% of all human isolates reported globally (Vieira *et al*., 2009).

The main sources of meat contamination include; animal/carcasses source, on farm factors, transport factors, abattoir and butchers facilities, parasites and wild animals, meat van, abattoir and retail meat outlet workers (Adetunde*et al*., 2011). Transmission is predominantly foodborne, although other modes include consumption of contaminated water, contact with infected animals and nosocomial exposure (Pegues and Miller, 2010).

Moreover, antimicrobial resistant *Salmonella* are becoming challenge. The use of antimicrobial agents in food animals at sub-therapeutic level or prophylactic doses may result in antimicrobial resistant strains and markedly increase the human health risk associated with consumption of contaminated meat products (Zelalem *et al*., 2011; Alemu and Molla, 2012). Antibiotic resistant NTS are associated with increased treatment failure and risk of invasive disease (Varma *et al*., 20 05). World wide surveillance data has demonstrated an overall increase in antibiotic resistance a mong NTS, although significant geographical and serotype variability exist (Su *et al*., 2004 ; Parr y and Threlfall, 2008).The wide spread use of antibiotics in food animals has been implicated in the increasing prevalence of antibiotic resistant NTS (Angulo,2000).

Food animals harbor a wide range of *Salmonella* serotypes and so act as source of contamination, which is of paramount epidemiological importance in non-typhoid human salmonellosis. Stress associated with transport of animals to abattoir augments shedding of *Salmonella* by carrier animals and this may contribute to the spread of the organism to carcass during slaughter process (Callaway *et al*., 2010).

The process of removing the gastrointestinal tract during slaughtering of food animals is regarde d as one of the most important sources of carcass and organ contamination with *Salmonella* at ab attoirs (Ejeta *et al*., 2004). *Salmonella* contamination in beef can occur at several stages along food supply chain includes productions, processing, distribution, retailing and also preparing and handling by consumers (El-Aziz, 2013).

Despite the presence of many studies on salmonellosis in different parts of Ethiopia (Molla *et al*., 2003a; Tadesse and Gebremedhin, 2015; Ejeta *et al*., 2004; Alemayehu *et al*., 2002; Mekuriaw *et al*., 2016), there is no citable information regarding to the status of *Salmonella* along beef supply

chain in Dukem town where raw beef consumption is prediminantly common. It is of important to assess the contamination level of *Salmonella* and its antimicrobial susceptibility pattern along the beef supply chain from public health point of view.

General objective

- To assess the public health importance of *Salmonella* along beef supply chain in Dukem town

Specific objectives:

- To estimate the prevalence of *Salmonella* along beef supply chain
- To determine the antimicrobial susceptibility pattern of *Salmonella* isolates
- To assess the knowledge, attitudes and beef handling practices along beef supply chain

2. LITERATURE REVIEW

2.1. Salmonellosis

Foodborne salmonellosis often follows consumption of contaminated animal products, which usu ally results from infected animals used in food production or from contamination of the carcasses or edible organs (Alemayehu *et al*., 2002). Salmonellosis remains among the main causes of foodborne illness in developing as well in developed countries. *Salmonella* causes 31% of food related deaths followed by *Listeria* (28%), *Campylobacter* (5%), and *Escherichia coli* O157:H7 (3%) (Michael and Samuel, 2001). *Salmonella* spp. is mainly transmitted by the fecal-oral route. They are carried asymptomatically in the intestines or gall bladder of many animals, and are continuously or intermittently shed in the feces (OIE, 2005).

2.1.1. Etiology

Salmonella is a heterogeneous bacterial genus, consisting of rod-shaped, Gram-negative, facultative anaerobe, non-spore forming bacteria which are predominantly motile by means of peritrichous flagella, except *Salmonella* Pullorum and *Salmonella* Gallinarum, which lack flagella (Yan *et al.*, 2003; Rao, 2004).

Salmonella grow optimally at a temperature of 35^0C to 37^0C, pH about 6.5 7.5 and water activity between 0.84 to 0.94 (European Commission, 2000; Food Research International, 2010). Howev er, some growth is observed in the range of 5^0C to 45^0C and within a pH range of approximately 4.0 to 9.0 (European Commission, 2000).

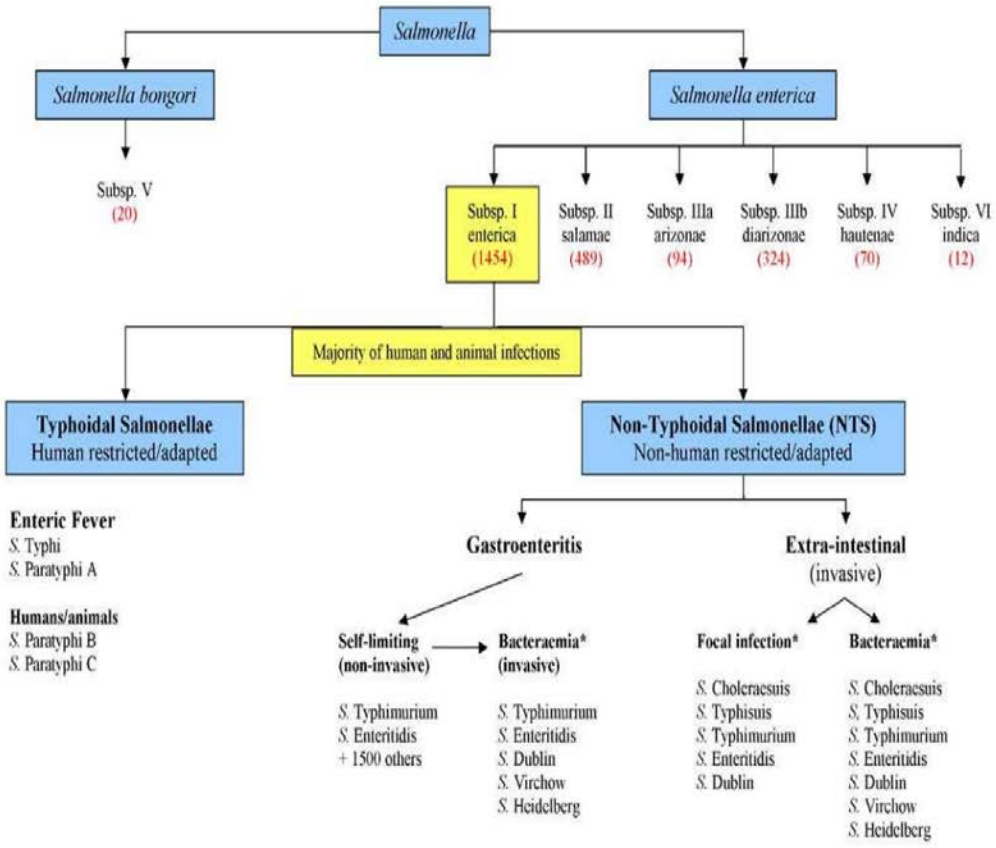
Salmonella spp. catabolize carbohydrates such as glucose, mannitol, into acid and gas the genus *Salmonella* produces usually gas from glucose except *S.* Typhi which ferments glucose and mann itol without gas production(Barbara *et al.,* 2000).Majority of *Salmonella* strains fail to catabolize lactose, sucrose, salicin and urea (Morita *et al.,* 2006).

Salmonella serotypes are generally distinguished as typhoidal and nontyphoidal *Salmonella*. NTS usually cause a self- limited acute gastroenteritis in healthy hosts. There are over 1500 NTS serotypes, the most common are *Salmonella* Enteritidis, *S.* Typhimurium and *S.* Heidelberg, and

are primarily transmitted to humans directly or indirectly from animal sources. NTS are found worldwide in domestic and wild animals and is primarily a foodborne illness. *Salmonella* Enteriti dis is the most common serotype identified in outbreaks of foodborne illness and can be isolated from a variety of hosts (Jones *et al*., 2008).

Salmonella Typhimurium is the second most prevalent serotype isolated from food, accounting for 14% of laboratory-confirmed cases of salmonellosis. *Salmonella* Typhimurium is also one of the top serotypes isolated from food-producing animals and retail meats (Acha and Szyfres, 2001). There are more than 2,700 *Salmonella* serotypes are recognized to date. Of these over 150 0 are NTS serotypes. The general classification of this genu is shown below (Figure, 1).

Figure 1: Classification of the genus *Salmonella*



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

Note: Numbers in brackets indicate the total number of serotypes included in each subspecies.

* Common serotypes are listed but other serotypes may cause bacteraemia or focal infection

2.1.2. Epidemiology

The epidemiology of salmonellosis is complex largely because there are more than 2,700 distinct serotypes (serovars) with different reservoirs and diverse geographic incidences. Changes in food consumption, production, and distribution have led to an increasing frequency of multistate outbr eaks associated with fresh produced and processed foods. All livestock species can be affected by salmonellosis with young, debilitated and parturient animals most susceptible to clinical disea se and all age group of humans also affected (Rounds *et al*., 2010). Unlike *Salmonella* Typhi and *Salmonella* Paratyphi,which have host specificity for humans, NTS can be acquired from both animal and humans (Braden,2006 ; Hohmann, 2001).

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

Globally, an estimated 93.8 million cases of gastroenteritis and155,000 deaths are caused by Non typhoidal *Salmonella* spp. annually（CDC, 2014. Under reporting is a problem worldwide. In the U.S., the incidence of salmonellosis has remained relatively stable in recent years. About 16 case s were reported per 100,000 people in 2012; however, for every reported case an estimated 29 undiagnosed cases occur. In the EU, reported cases of salmonellosis have been decreasing. In 2011, about 20 cases were reported per100, 000 people（CDC, 2014）. Surveillance data are often not available in developing countries. In Southeast Asia, it is estimated that 22.8 cases of salmonellosis occur each year. The overall mortality rate for most forms of salmonellosis is less

than1%. In hospital or nursing home outbreaks, the mortality rate can be up to 70 times higher. *S almonella* gastroenteritis is rarely fatal in healthy people（CDC, 2014).

Besides the importance of this microorganism in public health, another aspect is the cost incurred by human salmonellosis. With the increasing population in the developing world, there is an incr easing demand for meat and meat products which will force the present resource-driven system of livestock production to a demand-driven system (Zessin, 2006) which will increase the disease transmission risks. There is a multifactorial risk of foodborne hazards including salmonellosis in the developing countries due to poor sanitation and inadequate access to potable water (Henson, 2003).

2.1. 3.Mode of transmission

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

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

2.1. 4.Pathogenesis

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

The nature and severity of *Salmonella* infections in humans vary enormously and are influenced infecting *Salmonella* serovar, strain virulence, infecting dose, age, and immune status of the host. Usually, *Salmonellae* colonize the intestine by the adhesion of the bacteria to the epithelial cells using fimbrial antigens. The cells invade the intestinal mucosa and multiply in the gut-associated lymphoid tissue (GALT)(Gondwea *et al*., 2010; MacLennan *et al*., 2008).

From the infected tissues the pathogens spread to the regional lymph nodes, where macrophages form a first effective barrier to prevent a further spread (Radostits *et al.,* 2007). If the marcropha ges are unable to avoid the spread, systemic disease can occur (Liu, 2010).

During the systemic disease the bacteria spread from the GALT via the efferent lymphatics and the thoracic duct into the vena cava from where it spreads out through the body (Gondwea *et al*., 2010; MacLennan *et al*., 2008). The bacteria multiply in spleen, liver and released in large numbers to the blood stream infecting other organs. *Salmonellae* are able to survive and multiply inside host cells (Liu, 2010).

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

2.1.5. Clinical signs and Symptoms

Two to eight percent of NTS infections are associated with bacteremia, and are not always preceded by gastroenteritis. Risk factors for NTS bacteremia include immune-compromise (including HIV,malignancy,chemotherapy, steroid therapy) and extremes of age (< 3 month and greater than 50 years old).Risk factors are not apparent in up to one third of cases of NTS

bacteremia. Extraintestinal focal infections (eg. arthritis, meningitis, pneumonia) occur in 5-10% of those with bacteremia (Matheson, 2010).

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

2.1.6. Diagnosis

Diagnosis is based on isolation of *Salmonella* organisms from feces, food items or in cases of disseminated disease, from the blood by culture. Isolates of *salmonella* are needed for serotyping and antimicrobial susceptibility testing (Acha and Szyfres, 2001).

2.1.7. Treatment

Gastroenteritis caused by *Salmonella* is usually a self-limiting disease and diarrhea resolves within three to seven days and fever within seventy two hours (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. 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 immune-suppressed (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 in eradication of long-term carriage (WHO, 2012).

The high concentration of amoxicillin and quinolone in bile and the superior intracellular penetra tion of quinolone are theoretical advantages over trimethoprim sulfamethoxazole (WHO, 2012).

2.1.8. Prevention and control

A periodic surveillance of the level of *Salmonella* contamination in the different food animals, food products and environment is necessary to control the spread of the pathogen and human in fection (Norrung and Buncic, 2007).

The most serious meat safety issues affecting consumer health and triggering product recalls involve microbial and particularly bacterial pathogens (Sofos, 2008). Control of these pathogens at all stages of the farm-to-fork chain is vital to minimize the occurrence of food-borne disease in the human population (Norrung and Buncic, 2007).

Salmonellosis is the most wide spread foodborne and zoonotic problem throughout the world. Reducing *Salmonella* prevalence requires comprehensive control strategy in animals and animal food stuffs with restrictions on the infected flocks until they have been cleaned up from infections (Breytenbach, 2004). In addition, mandatory testing before slaughter should be conduc ted like the one being implemented in Sweden (Boqvist and Vagsholm, 2005).

Reservoirs for non-typhoid *Salmonella* organisms include a wide range of domestic and wild animals, such as cattle, poultry, swine, rodents, and pets like iguanas, turtles, dogs, cats, chicks, and ducklings. In humans infected with *Salmonella*, the excretion of bacteria can last throughout the course of infection and as a temporary carrier state for months. The mode of transmission may include ingestion of the organisms in food derived from infected animals or contaminated by feces of an infected animal or person. The source may be contaminated meat, poultry, eggs, milk, and their products, as well as water, fruits and vegetables. Preventive measures therefore should include the education of food handlers about hand hygiene, refrigerating foods in small portions, thoroughly cooking all foodstuffs, avoiding recontamination of cooked food, and maintaining a sanitary kitchen to prevent from contamination by rodents and insects (Varma *et al*., 2005).

Safe food production requires knowledge on the nature and origin of the animals, animal feed, the health status of animals at the farm. It also needs knowledge on the use of veterinary medicinal products, the results of any analysis of the samples taken at the farm and slaughter data regarding ante-mortem and post-mortem findings and the risks associated with post-harvest production stages (Snijders and Knapen, 2002). No part of the food chain can be regarded alone but has to be seen as part of the whole. It must also include the consumers. Additional measures to control secondary contamination could be prevention of contamination by cleaning and disinfection, hygiene of personnel and proper processing (Nowak *et al.*, 2006).

Growth of microorganisms in meat and poultry products can be controlled by maintaining a cold temperature at 10^0C, especially for *Salmonella* during transport and storage (Coleman *et al.*, 2003).

2.2. Status of *Salmonella* in Ethiopia

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

Studies conducted in different parts of the country showed the poor sanitary conditions of catering establishments and presence of pathogenic organisms like *Campylobacter, Salmonella, Staphylococcus aureus, Bacillus cereus and Escherichia coli,* (Bayleyegn *et al*., 2003; Abera *et al*., 2006; Knife and Abera, 2007; Tefera *et al*., 2009 and Mekonnen *et al*., 2013).

The incidence of foodborne *Salmonella* infections has increased dramatically in Ethiopia during the past few years. Studies conducted in different parts of the country have demonstrated the presence of *Salmonella* in human beings (Tadesse, 2014; Nyeleti *et al*., 2000) and in different food animals and food products (Nyeleti *et al*., 2000; Molla *et al*., 2003a).

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

In Ethiopia, several factors including under and mal-nutrition, HIV-AIDS, the unhygienic living circumstances and the close relations between humans and animals may substantially contribute to the occurrence of Salmonellosis. Although surveillance and monitoring systems are not in place and its epidemiology is not described, qualitative and quantitative syntheses of previous studies could shed light on the occurrence of the disease and the major serotypes that frequently cause infections (Tadesse, 2014; Nyeleti *et al*., 2000).

Even if data regarding meat borne diseases in Ethiopia are extremely scarce, a few studies conducted in different parts of the country have shown the public health importance of several bacterial pathogens associated with foods of animal origin (Bayleyegn *et al*., 2003; Ejeta *et al*., 2004: Adem *et al*., 2008; Kumar *et al*., 2009 ; Tefera *et al*., 2009). *Salmonella* remains a leading etiological agent in bacterial foodborne diseases (Jones *et al*., 2008).

2.3. Antimicrobial Resistance of *Salmonella*

Antibiotic-resistant *Salmonella* infections of both human and animal are universal concerns, particularly in developing countries where the risk of infection is high because of unhygienic living conditions, close contact and sharing of houses between animals and humans and the traditions of consumption of raw or undercooked animal-origin food items(Feasey *et al*.,2012). There is an increasing concern with this pathogen due to the emergence and spread of antibiotic-

resistant and potentially more pathogenic strains. If resistant microorganisms are common in animals, the chance that they will be transmitted to human beings is more likely (WHO, 2014).

Antimicrobial-resistant *Salmonella* spp. have been isolated from different foods of animal origin around the world, which is attributed to the inappropriate use of antimicrobials as therapeutic or prophylactic agents in human and veterinary medicine, as well as the use of growth promoters in animal production(WHO,2012). Antimicrobial resistance among non typhoid *Salmonella* serotyp es has been a serious problem worldwide. Other possibility for the emergence and spread of *Salm onella* strains resistant to antibiotics commonly used for treatment is, because this infection can be invasive and difficult to treat by the drugs of choice for invasive *Salmonella* disease (Paterson , 2006).

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

3. MATERIAL AND METHODS

3.1. Study area

Dukem town is located at 37 km South East of Addis Ababa along the main road to Adama. Geographically, the study area located by latitude $8^{o}45'25"N$-$8^{o}50'30"N$ and longitude $38^{o}51'55"E$ - $38^{o}56'5"$ E covering a total area of 35.96 km^2. It is located at an average altitude of 2100m above sea level (CSA, 2006). The mean annual rainfall of the area according to 1996 to 2003 year's meteorological data at Bishoftu station is 606.13 mm, and the mean maximum and mean minimum annual temperature of the area are $25.83^{o}C$ and $11.9^{o}C$ respectively. The maximum temperature is during February to May and the minimum temperature is from mid-October to January. According to 2007 population and housing census, Dukem town has a population of 24,222 (CSA, 2013).

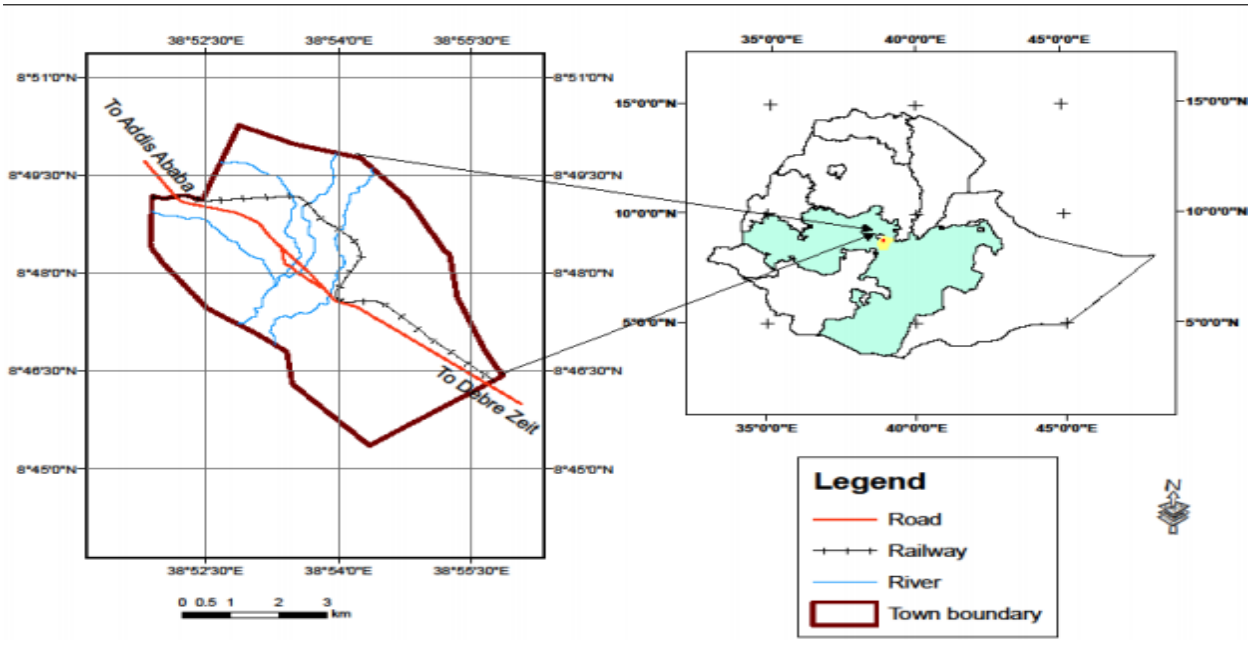


Figure 2: Map of the study area (CSA, 2006)

3.2. Study design

A cross-sectional study design was employed to generate the desired data, along the beef supply chain in the study area.

3.3. Study population

The study populations were cattle slaughtered in Dukem Municipal abattoir. Cattle presented to abattoirs originated from Dukem town and its surroundings. In addition, cattle meat handlers (abattoir workers, butchers and consumers) were also included.

3.4. Sample size determination

The sample size was determined using the formula described by Thrusfield (2005) by assuming 5% precision, 95% level of confidence interval and 7.07%, 4.53% and 8.34% expected prevalenc e of *Salmonella* in cattle feces (Tadesse and Tessema, 2014), carcass at slaughter house and meat at market (Tadesse and Gebremedhin, 2015), respectively.

$n = Z^2 p \exp (1\text{-}pexp) /d^2$,
Where n = required sample size; pexp = expected prevalence and d= desired absolute precision of 0.05, Z = 1.96

Accordingly, the minimum sample sizes required from each points of beef supply chain were 101, 67 and 118 for feces, carcass and retail meat, respectively.

All the 20 abattoir workers, 20 butchers and 60 consumer's respondents were included purposive ly based on their voluntary consent to participate in this study.

3. 5. Sampling Technique

Sample animals from abattoirs were selected by systematic random sampling technique. Identification number was given for each animal for selection during ante mortem examination and follow up during postmortem examination, depending on the number of animals slaughtered on each day.

Fecal samples were taken immediately from rectum after evisceration of the identified animals. The rump, flank, brisket and neck were sample site for beef carcasses swabbing in the abattoir. A sterile cotton tipped swab (2 × 3 cm) fitted with wooden shaft was first soaked in 10 ml of sterile buffered peptone water (BPW) (OXOID, England). Samples were taken by swabbing a 100 cm^2 area at each of these four sample sites by rubbing the applicator stick 10 times in the horizontal and 10 times in the vertical direction. Carcass swab samples were collected at the end of slaughtering process before it was prepared for loading and inserted in to the universal bottles containing10 ml BPW after cutting off the part the stick which was in contact with the hand, by binding out on the mouth of bottle (ISO, 2003). Meat cut sample (30g) from meat shops also collected and put in sterile cups.

At the end of each sample collection, every sampling bottle was labeled including date of sampling and the type of sample collected corresponding to animal identification number**.** All the samples were kept in icebox containing ice crystals and transported to Addis Ababa University, Collage of Veterinary Medicine and Agriculture, Microbiology Laboratory. The study participant s for knowledge, awareness and practices assessment were selected purposively.

3.6. Isolation and Identification of *Salmonella*

The isolation and identification of *Salmonella* was performed according to the techniques recommended by the International Organization for Standardization (ISO 6579: 2002) at the microbiology laboratory of College of Veterinary Medicine and Agriculture, Addis Ababa University. According to this ISO standard, the detection of *Salmonella* requires four successive stages namely, pre-enrichment in non-selective liquid media, enrichment in selective media, plating out and identification, and biochemical confirmation of suspected colonies.

The bacteriological media used for the study were prepared following the instructions of the manufacturer. Each 25 g of sample from meat and feces was put in a sterile Stomacher Bag and 225 ml of buffered peptone water (OXOID, England) was added and homogenized using a laboratory blender (OXOID, England) for 2 minutes and swab ample was put in 10 ml of buffered peptone water(OXOID, England). The pre-enriched samples were incubated for 18 to 24 hrs at 37°C. Following this, 1 ml and 0.1 ml of the pre-enrichment broths were transferred aseptically into 10 ml of Muller Kauffmann Tetrathionate (Merck, Germany) and 10 ml of Rappaport-Vassiliadis (RV) broth (OXOID, England), mixed, and then were incubated for 18 to 24 hrs at 37°C and 41.5°C, respectively. Following incubation, a loop-full of each culture was streaked onto the surface of xylose lysine deoxycholate (XLD) (OXOID, England) and brilliant green agar (BGA) (OXOID, England) medium and incubated at 37°C for 24 to 48 hrs.

The XLD and BGA plates were examined for the presence of *Salmonella* colonies.The formation of red colonies with black centers and of pink colonies with a red zone was inspected on XLD and BGA plates, respectively. When suspected colonies were detected, sub cultivation of 4 *Salmonella* colonies from XLD and BGA plates on to a non-selective nutrient agar media plates for biochemical tests was done.

All suspected non-lactose fermenting *Salmonella* colonies were picked up from the Nutrient Agar and inoculated into the following biochemical tubes for identification: triple sugar iron agar (TSI) (OXOID CM0277 500G), urea broth (HIMEDIA M111A-500G), lysine iron agar (LIA) (OXOID CM0381 500G), citrate broth (OXOID CM0129 500G), and then incubated for 24 to 48 hours at 37^{o}C. Colonies producing red slant (alkaline), with yellow butt (acid) on TSI with blackening (H_2S- hydrogen sulphide production) and bubbles (gas production) in butt, negative for urea hydrolysis (yellow), positive for LIA (purple slants and butt and H_2S), negative for tryptophan utilization (indole test) (yellow-brown ring), were considered to be *salmonella* positive (ISO 6579, 2002). Presumptive *salmonella* isolates for all biochemical tests were cultured on Nutrient Agar (NA) (OXOID CM0003 500G) for Antibiotic Susceptibility test. The detailed procedures of bacteriological analysis and media used in this study were done in accordance with the recommended procedures and preparations (Appendix, 8).

3.7. Antimicrobial Sensitivity Test

Phenotypic antimicrobial susceptibility testing on Mueller–Hinton agar (Oxoid) using the agar disc diffusion method (CLSI, 2015) was conducted to determine the antibiotic-resistant profiles of each isolate. The antibiotics to be used were selected among the currently available and commonly used chemotherapeutic agents for treatment of *Salmonella* infection in humans and animals. These includes ampicillin (25 mg), cefoxitin (30 mg), nalidixic acid (30 mg), nitrofurant oin (50 mg), sufisoxazole (100 mg), tetracycline (30 mg), Kanamycin(30mg), Streptomycin (10 mg) and Trimethoprim (25mg).

Four to five well isolated colonies grown on nutrient agar were transferred on to tubes containing 5ml of citrate broth. The broth culture inoculated at 37^0C for 4hrs until it achieves or exceeds the 0.5 McFarland turbidity standard. For those tubes which exceeded the turbidity standard, adjustment were made by adding the sterile saline solution to obtain turbidity usually comparable to the standard.

Sterile swab immersed in each of dilution suspension and swabbed uniformly over surface of two plates of Muller Hinton agar (Oxoid LTD,Basingstoke Hampshire, England) for each inoculums. The plates were held at room temperature for 30 minutes to allow drying. Using sterile forceps, disc impregnated with known concentration of antimicrobials were dispensed on to the surface of Muller Hinton agar plates and the Plates were incubated at 37^0C for 24hrs.

Following incubation, the diameters of the inhibition zone were measured in millimeters and interpreted in accordance with CLSI guidelines (CLSI, 2015). The zone of inhibition was measur ed and reported as susceptible (S) intermediate (I) or resistant (R) in reference to performance sta ndards for antimicrobial susceptibility testing of *Salmonella* (Appendix, 9). The development of multiple drug resistance (MDR) by the isolates considered if they were resistant to two or more antimicrobial agent tested.

3.8. Questionnaire and observational survey

The knowledge, attitudes and practices of meat handling along beef supply chain from abattoir to retail meat outlets were assessed using a semi-structured questionnaire. Questionnaires were administered to 20 retail meat outlet workers, 20 abattoir workers and 60 consumers. Moreover, direct assessment of the abattoir and meat shops were conducted using prepared observation check list. The information collected included means of transporting meat, availability of meat cold chain, frequency of check up healthy for workers, hygienic status of abattoir and retail meat outlets, availability and accessibility to clean and safe water and others (Appendices 3, 4, 5, 6 and 7).

3.9. Data Management and Analysis

Data was coded, entered and managed by Microsoft Office Excel spread sheet (2007) and analyzed by using STATA release 11 computer software. Descriptive statistics (frequency and percentage) was used to summarize the result. Chi square test with 95% confidence interval was used to assess the association of the sample sources with the *Salmonella* positivity and the difference in the proportion of *Salmonella* along the beef supply chain. P-value less than 0.05 was considered statistically significant.

4. RESULTS

4.1. Prevalence of *Salmonella*

In the present study, a total of 286 samples comprising of 118 retail meats, 67 carcass swab and 101 fecal samples were collected and analyzed. Among these samples examined, 6.3% (n= 18) were positive for *Salmonella*. The highest sample prevalence (12.71%) was found on meat at retailer which contributed 83.3% (15 of 18) of total isolates while the lowest prevalence 0.9% (1 of 101) was found on fecal sample contributed 5.5 % (1 of 18) of total isolates (Table, 1).

The result shows statistically significant variation in the prevalence of *Salmonella* along beef supply chain (X^2= 14.3027, p-value=0.001).

Table 1: The prevalence of *Salmonella* from different sample types

Sample types	Number of samples	Positive (%)	95%Conf. Interval
Feces	101	1(0.99%)	0.1-6.9
Carcass swab	67	2(2.98%)	0.8-11.5
Meat	118	15(12.71%)	7.8-20.1
Total	286	18(6.29%)	

4.2. Antimicrobial susceptibility of *Salmonella* isolates

All the 18 isolates were tested against nine commonly used antibiotics. All the isolates (100%) were sensitive to Kanamycin where as 94.4%, 88.9% and 83.3% of the isolates were found to be sensitive to Sufisoxazole, Tetracycline and Nalidixic acid, respectively (Table 1). tested. The *Salmonella* species isolated along the beef supply chain in Dukem exhibited resistance against 6 out of the 9 antibiotics used in this study. Among the isolates, 94.4% (n=17) were resistant at least to one of the antibiotics. In this respect, 72.2%, 33.3%, 22.2% and 22.2% of the isolates were resistant to Ampicillin, Nitrofurantoin, Cefoxitin, Trimethoprim, respectively (Table 1).

Table 2: Antibiotic Susceptibility Profiles of *Salmonella* isolates

Type of Antimicrobials	**Number of isolates**		
	Resistant (%)	Intermediate (%)	Susceptible (%)
Trimethoprim(TR)	4(22.2%)	2(11.1%)	12(66.7%)
Nalidixic acid(NA)	-	3(16.7%)	15(83.3%)
Kanamycin(K)	-	-	18(100%)
Cefoxitin(FOX)	4(22.2%)	4(22.2%)	10(55.6%)
Streptomycin(S)	3(16.6%)	7(39%)	8(44.4%)
Nitrofurantoin(F)	6(33.3%)	5(27.7%)	7(39%)
Sufisoxazole (RL)	-	1(5.6%)	17(94.4%)
Ampicillin(AMP)	13(72.2%)	4(22.2%)	1(5.6%)
Tetracycline(TE)	2(11.1%)	-	16(88.9%)

The majority of the isolates (83.3%, n=15) originated from retail meat and exhibited different antimicrobial and multi-drug resistant patterns. The isolate that was recovered from feces was found to be resistant to Ampicillin, Nitrofurantoin and Cefoxitin. All isolates recovered from carcass swab were resistant to Ampicillin, but 50% of these isolates were resistant to Nitrofurantoin, Trimethoprim and Cefoxitin (Table, 2).

Table 3: Resistance pattern of *Salmonella* isolates according to the sample sources

Type of Antimicrobials	**Number of resistance *Salmonella* isolates**			**Total**
	Retail meat (%) (n=15)	Carcass swab (%) (n=2)	Feces (%) (n=1)	
Ampicillin	10(66.7)	2(100)	1(100)	13(72.2%)
Nitrofurantoin	4(26.6)	1(50)	1(100)	6(33.3%)
Trimethoprim	3(20)	1(50)	-	4(22.2%)
Cefoxitin	2(13.3)	1(50)	1(100)	4(22.2%)
Streptomycin	3(20)	-	-	3(16.6%)
Tetracycline	2(13.3)	-	-	2(11.1%)
Sufisoxazole	-	-	-	-

Nalidixic acid	-	-	-	-
Kanamycin	-	-	-	-

4.3. Multiple Drug Resistance Patterns

From the 18 isolates, 9(50%) isolates were resistant to two or more drugs, and from those 5 (27.8%) isolates were showed multidrug resistant (resistance for three and more antibiotics) (Table 3). The common multiple resistance pattern was to the combination of Nitrofurantoin, Ampicillin and Trimethoprim, seen in 2 (11.1%) of the resistant isolates.

Table 4: Multiple antimicrobial resistance profile of *Salmonella* isolates

Number of antimicrobial resistance	Antimicrobial resistance patterns (number of isolates)	Number of isolates (%)
One	AMP(6),TE(1),S(1)	8(44.4)
Two	FOX+AMP(2)	4(22.2)
	F+TE(1)	
	TR+S(1)	
Three	TR+F+AMP(2)	4(22.2)
	S+F+AMP(1)	
	FOX+F+AMP(1)	
Four	TR+F+AMP+FOX(1)	1(5.6)

Key: AMP= Ampicillin; S= Streptomycin; FOX= Cefoxitin; F= Nitrofurantoin; TE= Tetracycline; TR= Trimethoprim

4.4. Questionnaire and observational survey

4.4.1. Abattoir workers

Twenty abattoir workers who had direct contact with the slaughtering process were included in this study. Out of this, 12(60%) of the workers were illiterate and two (10%) were beyond grade 12. Moreover, all of them didn't receive job related training and medical checkup (Table, 5).

Half of the respondents 10(50%) encountered faulty evisceration and all thought that washing contaminated carcass only with water can decontaminate the carcass. Half of interviewees (50%) responded that sudden stomach cut were the possible major source of carcass contamination and 19(95%) of them didn't consider carcass contamination pose health risk for consumers. None of them responded that the feces, skin and dirty water could possibly cause carcass contamination (Table, 5).

Table 2: The knowledge, attitude and practice of abattoir workers

Variables	Frequency	Percentage (%)
Educational status		
Illiterate	12	60
Grade 1-5	2	10
Grade 6-8	2	10
Grade 9-12	2	10
Beyond grade 12	2	10
Placement in the abattoir		
Slaughtering	9	45
Loading	4	20
Flaying	3	15
Washing stomach	1	5
Supervision	3	15
Job related training		
Yes	0	0
No	20	100
Job related medical test		
Yes	0	0
No	20	100
Faulty eviscerations		
Yes	10	50
No	10	50
Cause of contamination		
Dirty hand	1	5
Stomach cut	10	50
Fall floor	6	30
Dirty cloth	3	15
Ways of ending contamination		
Washing with water	20	100

Condemned	0	0
Retention	0	0
How long you stay in this work		
Six months	4	20
One year	0	0
2-4 years	9	45
5-8 years	5	25
Above 8 years	2	10
Clean clothing		
Yes	2	10
No	18	90
Hand washing		
Before work	5	25
After end of work	13	65
Before and after work	2	10
Hygienic equipments placing		
Yes	0	0
No	20	100
Know contamination as risk		
Yes	1	5
No	19	95
Raw meat consumption		
Yes	15	75
No	5	25
Food infection		
Yes	1	5
No	19	95

Direct observations revealed the absence of hot water, sterilizer and carcass retention room in the abattoir. Washing hands before and after work was practiced only by two of the interviewees (10%) and eighteen (90%) did not regularly put on clean protective clothing at work (Table, 5).The protective clothes were unclean, blood tinged and frequently in contact with carcasses and no hygienic placement of equipment. There were no separate compartments for bleeding and clean area and large sized carcasses were in direct contact with the ground and some carcasses suddenly falling on the ground (Appendix, 6). The latrine is constructed far away from the abatto ir and has no water, soap or other cleaning materials.

4.4.2. Butchery workers

Most of butchery workers 11(55%) had only primary education and 3(15%) were Illiterate. None of them took either job related training or medical checkup. Seventy five percent (n=15) of the butchers didn't wash their hands before touching meat and 13(65%) of them sold their meat using plastic. Most 19(95%) butchers cleaned their shop and equipments every day at end of the

selling process by using water and clothes but one (5%) reported that used soap in addition to water and clothes. All responded that they wash their protective clothes once per week (Table, 6).

Table 3: The knowledge, attitude and practice of butchery workers

Variable	Frequency	Percentage (%)
Level of education		
Illiterate	3	15
Primary Education	11	55
Secondary Education	6	30
Above	0	0
Means of transport meat		
Open vehicle	0	0
Closed vehicle	20	100
Animal transport(Cart horse)	0	0
Cover on display case		
Yes	0	0
No	20	100
Clean Wall and ceiling		
Yes	16	80
No	4	20
Wall painted white		
Yes	14	70
No	6	30
Ventilation status		
Good	18	90
Fair	2	10
Poor	0	0
Cooling facilities		
Yes	0	0
No	20	100
Refrigerator		
Yes	0	0
No	20	100
Wear protective cloth		
White coat only	13	65
White coat and Head cover	7	35
White coat, Head cover and gloves	0	0
Wash the protective		
Once per day in the evening	0	0
Twice per day	0	0
Once after every two days	0	0
Once per week	20	100
Have sink for washing hands		
Yes	0	0
No	20	100
Wash hand before touching meat		
Yes	5	25
No	15	75
Wash hand with soap		
Yes	0	0
No	20	100

Source of water		
Municipal	20	100
Bore hole	0	0
Rain	0	0
River	0	0
Cutting board type		
Wood	20	100
Plastic	0	0
Metal	0	0
Marble	0	0
Frequency of wash materials		
Once per day in the morning	3	15
Once per day in the evening	2	10
Twice per day	15	75
More than twice	0	0
Use detergent/disinfectant		
Yes	8	40
No	12	60
Sterilize your equipment's		
Yes	0	0
No	20	100
Hot water baths for knives		
Yes	0	0
No	20	100
Ways of cleaning equipments		
Cold water with soap	20	100
Hot water only	0	0
Hot water with soap	0	0
Wiping with pieces of fabrics	0	0
Routine control of flies		
Yes	4	20
No	16	80
How long does the meat stay		
Less than 12 hours	11	55
One day	5	25
Two days	4	20
Material to wrap meat for sale		
News paper	7	35
Plastic	13	65
Used paper	0	0
Handling money while selling		
Yes	9	45
No	11	55
Job related training		
Yes	0	0
No	20	100
Job related medical checkups		
Yes	0	0
No	20	100
Different storage and display cabinets for offal's and meat		
Yes	14	70
No	6	30
Same equipment while handling meat and the offal's		

Yes	9	45
No	11	55

Direct observations revealed, nine (45%) handled money while selling meat and also nine (45%) use the same equipment while handling meat and the offal's. 13(65%) of butchers wore only white coat, 7(35%) white coat and Head cover and none of them wore white coat, Head cover and gloves. There were no cover on display case, refrigerator, sterilizer for equipment's, sink for washing hands and cooling facilities (Table, 6).

4.4.3. Consumers

There were sixty respondents included in this study who gave their opinion and experiences related to the parameters important for the study while eating meat. Out of this 46(76.7%) and 14(23.7%) were male and female respectively and 19(31.7%) learned up to high school. Most 55(91.7%) respondents prefer beef than other type of red meat and 50(83.3%) of them select the meat based on freshness but only 3(5%) consider healthiness of the meat (Table, 7).

Forty two (70%) of them consume raw beef and 44(73%) respondents didn't know transmission of *Salmonella* through meat consumption and the same number of respondents reported that meat from abattoir was always safe. Some of the respondents 23(38.3%) reported that they suffered food poisoning of which 18(78.2%) had medical attention and received antimicrobials (Table, 7).

Table 4: The knowledge, attitudes and practices of consumers

Variables	**Frequency**	**Percentage (%)**
Sex		
Male	46	76.7
Female	14	23.3
Age		
15-30	22	36.7
31-50	32	53.3
>50	6	10
Educational status		
Illiterate	4	6.7
Primary school	14	23.3
High school	19	31.7
College or university	23	38.3
Priority criterion		
Freshness	50	83.3
Cheapness	4	6.7
low fat content	3	5

Healthiness	3	5
red meat you prefer		
Beef	55	91.7
Sheep	3	5
Goat	2	3.3
Camel	0	0
Which you prefer to eat		
Boiled	0	0
Fried	0	0
Cooked	55	91.7
Raw	5	8.3
Do you consume raw beef		
Yes	42	70
No	18	30
Cooked meat is always safe		
Yes	50	83.3
No	10	16.7
How often you consume		
Every day	0	0
Once in a week	11	18.3
1-3 times in a week	0	0
Once per month	20	33.3
Most of the time	19	31.7
History of food infection		
Yes	23	38.3
No	37	61.7
Symptoms		
Nausea	7	30.4
Abdominal cramp	2	8.7
Vomiting	3	13
Nonbloody diarrhea	11	47.8
Type of action taken		
Drug	18	78.2
Traditional	1	4.3
None	4	17.4
Meat from abattoir always safe		
Yes	44	73.3
No	16	26.7
Have refrigerator		
Yes	42	70
No	18	30
Heard about *Salmonella*		
Yes	31	51.7
No	29	48.3
***Salmonella* transmitted meat consumption**		
Yes	16	26.7
No	44	73.3
Give raw beef for children		
Yes	5	8.3
No	55	91.7

5. DISCUSSION

Food borne illnesses caused by non- typhoid *Salmonella, S. aureus* and *E. coli* represents a major public health problem worldwide. These pathogens are transmitted mainly through consumption of contaminated food and the presence of these organisms in meat animals and in raw meat products has relevant public health implications (Sousa, 2008).

In the present study, the prevalence *Salmonella* in raw retail meat samples was 12%, this was in close agreement with the report of Ejeta *et al*., (2004), who reported 12% from retail raw meat samples in Addis Ababa, Ethiopia. But the current finding was lower as compared to the studies conducted in Senegal, 87 % (Stevens *et al*., 2006) and in Iran, 47% (Dallal *et al*., 2010).This difference might be the sample type and sample procedures and the detection methods employed for different studies. As Padungtod and Kaneene(Padungtod and Kaneene 2006) described earlier, the prevalence may also vary from experiment to experiment, from country to country, or from one area to another area within a country.

There is a risk of *Salmonella* infection if the meat is improperly cooked and consumed raw, or if there is cross-contamination of *Salmonella* with other foods that are consumed raw (Gallegos *et al*., 2009). Contaminated raw or undercooked red meats can serve as main vehicles of transmissi on for *Salmonella* (Garedew *et al*., 2014; Garedew *et al*., 2015). In this study the high contaminat ion of *Salmonella* at meat retail may be due to unhygienic carcass transportation, improper loading and unloading, unhygienic meat shop equipments and personnel. When meat is cut into pieces, more microorganisms are added to the surfaces of exposed tissue (Ejeta *et al*., 2004). The contamination of equipment, material, and workers' hands can spread pathogenic bacteria to non contaminated carcasses (Nouichi and Hamdi, 2009).

In the present study prevalence of *Salmonella* in feces and carcass was found to be 0.9% and 2.9% respectively. The low prevalence in the samples might be due to low *Salmonella* carrier rate among the cattle in the study populations. Of the feces samples analysed 0.9% was positive for *Salmonella* and was in agreement with Molla *et al*., (2003a) who reported that the prevalence of 1.9% in apparently healthy slaughtered cattle. The current study revealed prevalen ce of *Salmonella,* 2.9% in carcass swab samples, this result was lower than 8% reported by

Mekuriaw *et al*., (2016) in Wolaita Sodo municipal abattoir. The difference in the prevalence reported could be due to differences in study sites (abattoirs) and animal.

The observation of this study found that the carcasses fall on dirty floor, the wall, floor and equipments used were not clean. The workers had no specific place to put equipments and their clothes were blood tinged and moreover, do not practice hand washing before and after work rather most13 (65%) of them wash after finishing the slaughtering process. They hurry to finish the work rather than following hygienic slaughtering process.

The existing unhygienic practices and facilities in slaughter houses could exacerbate the contami nation of carcasses and edible organs. Fecal shedding of *Salmonella* from cattle may be intermitt ent and difficult to detect due to healthy carriers intermittently excrete only a few *Salmonellae*, u nless they undergo some kind of stress, for example during transport or holding in the lairage prior to slaughter. However, the organism appears to be fairly spread throughout bovine population (Lawan *et al*., 2011). The presence of even small numbers of *Salmonella* on carcass meat and edible offal may lead to heavy contamination of minced meat and sausage (White *et al*., 2001).

The study also suggested that overall faulty evisceration, falling carcass on dirty slaughter house floor, unhygienic equipments and personnel, improper transportation of carcass, unhygienic preparation at meat retail might be considered as a common source of *Salmonella* along the supply chain.

In the present study, all *Salmonella* isolates tested were found to be resistant to at least one antimicrobial agent. This observed resistance profile was higher than what other studies reported in Ethiopia (23.5%) (Zewdu and Cornelius, 2009), in Senegal (17%)(Stevens *et al*., 2006) and 83% reported in Thailand (Gebre, 2012). Resistance was noted to four, five, six, seven, and more antibiotics at varying proportions (Behailu and Mogessie, 2009) and 52% of the *Salmonella* isolated at the abattoir from beef were resistant to at least three antibiotics (Alemayehu *et al,* 2003).

Even though it needs a better understanding of antibiotics use in Ethiopia, this resistance variation might be due to indiscriminate use of antimicrobials in animal production without prescription in the animal health sector, which might favor selection pressure that increased the

advantage of maintaining resistance genes in bacteria (Gillings*et al*., 2008).The emergence of antimicrobial-resistance to *Salmonella* is associated with supplement of antibiotics to animal feed and for their treatments. Resistant bacteria can be transmitted to humans through foods, particularly those of animal origin (White *et al*, 2001).

In this study, majority (55.6%) of the identified isolates were resistant to two or more antimicrobials, particularly to Ampicillin (72.2%), Trimethoprim (22.2%), Nitrofurantoin (33.3%). *Salmonella* resistance in this finding was higher than in the previous studies done in Ethiopia (Zewdu and Cornelius, 2009; Tadesse and Dabassa, 2012) and other countries (Stevens*et al*., 2006; Gebre, 2012).

The development of multiple drug resistance by the isolates is serious concerns that there may critical challenge for treatments of the salmonellosis. There were a number of studies where multiple drug resistance of *Salmonella* against antibiotics reported. The remarkable rise in the occurrence of antimicrobial resistance in *Salmonella* for the mentioned antibiotics was probably an indication of their frequent usage both in livestock and in public health sectors in Ethiopia. The systematic review and meta-analysis in Ethiopia Tadesse (2015) indicated the increase in the proportion of drug-resistant *Salmonella* isolates that could be due to the irrational use of antimicrobials and inappropriateness of the prescription and dispensing methods in both the public veterinary and private health setups of the country.

Due to the relatively limited access and high price to get the newly developed cephalosporin and quinolone drugs, the reports of prevalence of antimicrobial-resistant *Salmonella* to relatively low-priced and regularly available antibiotics are alarming for a low-income society living in most developing countries, like Ethiopia. However, it is important to note that these antibiotics are commonly used in veterinary medicine, and infections with these resistant *Salmonella* isolates could lower the efficiency of antibiotic treatment. The finding of this study shows slightly lower resistance than the study reported in Nigeria (93.1%) (Ekundayo and Ezeoke, 2011).

Resistance to multiple antimicrobials (55.6%) which was observed in current study was higher than other studies conducted in Ethiopia. For instance, Alemayehu *et al*., 2002; Endrias, 2004; Molla *et al*., 2004 and Zelalem *et al*., (2011)) reported 52%, 23.5%, and 44.8% 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 (Stevens *et al*., 2006), reported multidrug resistance of *Salmonella* isolates respectively as follow 16%, 50% (from raw meats), (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 (Molla *et al*., 2003a; Molla *et al*., 2006 and Zewdu and Cornelius, 2009).

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, kanamycine and tetracycline, with resistance rate of 100%, 66.7%, 58.3% and 33.3%, respectively. Similarly previous reports from South India (Suresh *et al*., 2006), from Nigeria (Akinyemia *et al*., 2005) and from Cameroon (Akoachere *et al*., 2009) indicated a similar 100%, over 90% and 100% respectively resistance to ampicillin.

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.

The level of education and training of food handlers about the basic concept and requirements of personal hygiene and its environment plays an important part in safeguarding the safety of products to consumers. During the study it was revealed that, the abattoir and beef retail outlet workers had low level of education and this could make difficult in acceptability of modern slaughtering practices as well as adherence to strict hygienic and standard slaughtering practices that contribute to microbial contamination. The present study found that out of 20 abattoir workers interviewed, 60% were illiterate, and all (100%) had no any training regarding meat hygiene. Bhandare *et al*. (2009) reported that workers working in the abattoir in most cases in developing countries are untrained and thus, they pay no attention to the hygienic standards and as a result contribute immensely to bacterial contamination.

In order to protect both food products and meat handlers from cross contamination the abattoir and retail meat outlet workers should wear protective clothes while working (Nel *et al*., 2004). Findings from this study revealed that most of abattoir workers were not wearing protective clothes.

Large sized carcasses were in direct contact with the dirty floor that may contribute to contamination of meat from the contact part as the floor was in poor hygienic condition. These findings are similar to those reported by Adzitey *et al.* (2011) that 65% of abattoir workers dressed carcasses on bare floor in the abattoir, 16% dressed carcasses on unclean slaughter slabs and 19% on both the slaughter slabs and bare floor in which the slaughter floor and slabs were smeared with blood, rumen contents and other wastes from previous dressed animals which increased the risk of contamination of subsequent carcasses.

In the study done by Haileselassie *et al*. (2012), 53.8% of the respondents reported that sanitary measures in the abattoir were not observed making the quality of meat produced in the study area questionable, a finding which was similar to what was observed in the present study whereby majority of respondents reported that the abattoir was in poor hygienic condition which made poor quality of meat produced.

Haileselassie *et al*. (2012) found that 71 of the butcher shop workers interviewed, 11.3% did not use protective clothes and 50.7% did not cover their hair, 47.9% of the butchers handled money while serving food and 78.9% of them had worn jewellery materials which may result into cross contamination of meat with microbes. In addition other study by Endale and Hailay (2013) reported that 91.7% of the butchers in Mekelle city handle money while processing the meat. Nervy *et al*. (2011) reported that handling of carcasses and money with the same unwashed hands could be good sources of contamination. 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 be taken to reduce or eliminate such contamination (Muinde and Kuria, 2005).

In the present study some 5(25%) of the workers used only water for hand washing but all of workers had no habits of washing their hands with water and soap before and after sale of meat which contribute to contamination of meat. Muinde and Kuria,(2005) recommends that hand-

washing alone has no effect on *S. aureus* counts on hands. The reduction of bacteria on hands depends on the mechanical action, the duration and the type of soap and sanitizers being used. Hence same procedures should be advocated as all of them, did not use soap and sanitizers.

Meat handlers might be sources of contamination of beef with microorganisms. Thus it is important that all possible measures be taken to reduce or eliminate such contamination (Muinde and Kuria, 2005). Routine medical examination is important since it helps to control and prevent zoonotic diseases such as Tuberculosis. The result revealed that all workers in retail meat outlets had no a routine medical examination. Nervy *et al.* (2011) reported that careless sneezing and coughing among butchers may lead to contamination of beef. In order to protect the health of consumers and for aesthetic reasons, meat handlers should stop habit of careless sneezing and coughing when handling it.

The majority16 (80%) of retail meat outlets had no routine control of flies in their shops, some of the workers practiced daily cleaning of the shops at the evening.

In order to keep beef safe for a long period of time the refrigerators or freezers are the most important storage facilities used. It was observed during the study that all of retail meat outlets had no refrigerators in their shops. Similar results was reported by Nonga *et al*. (2010) in which only 15% of the shops had refrigerators despite the fact that majority of owners were aware with the risk of meat being spoiled following prolonged storage at room temperature.

Most of surveyed retail meat outlets had poor hygienic condition despite of daily cleaning of their shops with water. Ali *et al*. (2010) reported that butcher men lack knowledge of disinfecting and sanitizing, they clean their shops once in 24 hours with detergent and water which is not enough to maintain the hygienic environments in the butcher. Regular cleaning and disinfecting the beef retail outlets is important since it helps to reduce microbial contamination.

Of the total 60 cattle meat consumers interviewed almost all 55(91.7%) of the consumers reported that they prefer beef to other type of meat and 42(70%) prefer eating raw to other types of preparations. But it is well recognized that, *Salmonella* infections are primarily of foodborne origin (Rounds *et al*., 2010) and can be transmitted by consuming undercooked meat (Fuaci and Jameson, 2005).

Although majority of them 83.3% of the respondent prefer to use cooked meat, 70% of them prefer to eat raw beef which can be source of *Salmonella* to the consumers. Some facts indicate that *Salmonella* infections are primarily of foodborne origin (Rounds *et al*., 2010) and can be transmitted by means of using undercooked meat (Fuaci and Jameson, 2005).

Moreover, during food handling and preparation pathogenic organisms may be transferred to food items by the handler both directly or by cross contamination through hands, surfaces, utensils and equipment that have been inadequately clean and disinfected between the preparation of different types of food (Linda du and Irma, 2005).

Respondents those reported history of food poisoning was specified that the symptom of their event were mostly nausea, nonbloody diarrhea and vomiting.

6. CONCLUSIONS AND RECOMMENDATIONS

The present study showed that the occurrence of *Salmonella* along the beef supply chain and its public health importance. More specifically, it revealed high contamination of retail meat with *Salmonella*, the variability in the susceptibility pattern of *Salmonella* isolates against the tested antimicrobials and the possibility of the existing beef handling practices, knowledge and attitudes about the importance of occurrence of *Salmonella* along the beef supply chain leading to public health risk unless the necessary intervention is in place.

The majority of the isolates originated from retail meat. The isolates were susceptible to Kanamy cine, Sufisoxazole and Nalidixic acid and resistant to Ampicillin (72.2%) and Nitrofurantoin (33%). Similarly, multiple drug resistant *Salmonella* isolates were found to occur in the study area.

Majority of abattoir workers didn't know that contaminated carcass has public health risk. Falling of carcass in the dirty floorand sudden stomach cut were the major possible sources of carcass contamination. Most of consumers had habit of eating raw beef and had no knowledge ab out the transmission of *Salmonella* via meat consumption.

In light of the above conclusion, the following recommendations were forward:

- Kanamycine, Sufisoxazole and Nalidixic acid should be rationally used for treatment of Salmonellosis both in humans and Cattle to avoid the development of resistance

- Accurate diagnosis of the diseases and conducting antimicrobial susceptibility test in handling clinical case of salmonellosis to select the best effective antimicrobials

- Policies, regulations and guidelines regarding food safety at all levels along beef supply chain should be formulated and enforced so as to ensure the safety of beef consumption

- All stakeholders should raise awareness in minimizing the occurrence of *Salmonella* and thereby transmission of resistant *Salmonella* to humans and good hygienic and handling practices along the beef supply chain particularly at meat retail

- Further study to identify the source of contaminations, identify *Salmonella* serotypes, circulating in the area, molecular characterization of the resistant *Salmonella* isolates to better understand the underlying the resistant genes and elucidate mechanisms of resistance development should be undertaken

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8. APPENDICES

Appendix 1: Sample Collection sheet for laboratory analysis

SN	Type of sample	Date of collection	Collection site	Sample code	Number of sample
1					
2					
3					
4					
.					
.					
.					
286					

Appendix 2: Record sheet format for Salmonella isolation and identification

Sample number	Date of sample collected	Colony characteristics on		Biochemical test							Sample (+/-)
		XLD Agar	BGA Agar	Urease test	TSI test				L-lysine test	Indole test	
1					But	slant	gas	H_2S			
2											
3											
.											
.											
286											

Appendix 3: Questionnaire format for Abattoir workers

Date:---------- Name: _____ Questionnaire number----------

Time:---------- sex_____ occupation----------

1. Educational status

a) Illiterate b) Grade 1-5 c) Grade 6-8 d) Grade 9-12 e) Grade >12

2. Placement in slaughterhouses process

3. Do you play any other role in the slaughter process apart from the one mentioned above?

4. If yes, which one(s)?

5. If No, why not?

6. How frequently do you come across faulty eviscerations?

7. What do you do after faulty evisceration?

8. How do you handle cattle presented for slaughter?

9. Did you receive any job related training? a) Yes b) No

10. If yes where were you trained?

11. If there was no formal training have you received informal training? a) Yes b) No

12. Have you undergone any job related medical tests to work in the abattoir? a) Yes b) No

13. When was your last medical test done and how often you check?

14. What would cause carcass contamination?

15. If carcass was contaminated by faeces, what would you do?

16. In your opinion, does contamination pose any health risk to meat consumers? a) Yes b)No

17. If No, why?

18. Propose way to end carcass contamination?

19. How long you stay in this work?

20. Do you consume raw beef? a) Yes b) No

21. History food poisoning? a) Yes b) No If yes symptoms

Appendix 4: Questionnaire format for butcher shop workers

Introduction: Hello? My name is Zelalem Sisay and I am conducting a research work entitled "*Assessment of the contamination of beef with salmonella and knowledge, attitudes and beef handling practices along beef supply chain in dukem town, Ethiopia*. More specifically, I am assessing the meat handling practices at retail markets. I'm from Addis Ababa University. I would like to ask you some questions about your retail market and the meat handling practices that will help us in this work. The results of the study and related information will only be used for the purpose of this study. Your name will not be used on the sample and/or any report that might result from the study. We will use codes specific to the study. This will take about 30 minutes. Can we go ahead?

A. Basic information
   1. Date_____
   2. Questionnaire Code_____

B. General characteristics of individuals
   1. Sex: Male [ ] Female[ ]
   2. Age…….
   3. Level of Education: Illiterate [ ] Informal Education [ ] Primary Education [ ] Secondary Education [ ] Other (Specify)…….
   4. Duration of selling meat in retail outlet? .....

C. Possible risk factors for contamination of meat at retail market

1. What is the means of transporting meat from abattoir to the retail shop? Open vehicle [ ] Closed vehicle[ ] Animal transport(Cart horse)[ ]
2. Is there any cover on display case? Yes [ ] No [ ]
3. Retail shop floor is made of concrete(observe) [ ]Tile [ ]wood earthen material[ ] others(specify)
4. Wall and ceiling are clean or free of dust(observe)Yes [ ] No [ ]
5. Wall painted with white color Yes [ ] No [ ]
6. If yes, sign of dirty on the wall. Yes [ ] No [ ]
7. What is the ventilation status of display case and butchery (observe)? Good [ ] Fair [ ] Poor[ ]
   Good-ventilation allows air flow into the butchery but sieves off dust and other particles [ ]
   Fair-ventilation allows air flow but do not sieve dust or other particles or allows very little air flow [ ]
   Poor-ventilation does not allow air flow at all [ ]
8. Is there use of bulbs at the display case (observe) yes [ ] No [ ]
9. Are there meat cooling facilities at the display cabinet? (Observe) Yes [ ] No [ ]
10. Do you have a refrigerator for storage of the meat that remains from daily sale? Yes [ ] No [ ]

11. Do you use the following protective materials while selling or handling meat?(observe)

Protective materials	Response	
	Yes	No
Apron/white coat		
Head cover		
Gloves		

12. How frequent do you wash the protective (white coat and Apron)? Once per day in the evening [ ] Twice per day, morning and evening [ ] once after every two days [ ] once per week [ ] others [ ]
13. Do you have sink for washing hands Yes [ ] No [ ]
14. Do you wash your hand before touching the meat? Yes [ ] No [ ]
15. Do you wash your hand with soap Yes [ ] No [ ]
16. What is your source of water for use in the butchery? City/Municipal council [ ] borehole [ ] rain collected water [ ] River [ ] others (specify) [ ]
17. What kind of cutting board you are using? (Observe) Wood [ ] plastic [ ] Metal [ ] concrete [ ] Marble [ ]

18. How often do you wash the following butchery surfaces and equipments?

Frequency of wash	Equipments /surfaces						
	Knife	Cutting boards	Saw/Axes	Display cabinet	Hooks	Floors	
Once per day in the morning							
Once per day in the evening							
Twice per day							
More than twice							
Once in every two days							
Others (specify)							

19. Do you use detergent/disinfectant for cleaning the butchery utensils?Yes [ ] No [ ]
20. If "Yes" what types of detergent/ disinfectant do you use……..
21. Do you sterilize your equipment's ?Yes [ ] No [ ]
22. If "Yes" what are the methods used to sterilize the equipment…….
23. Do you have any hot water baths for dipping of knives? Yes [ ] No [ ]
24. Ways of cleaning butchery equipments cold water only [ ], cold water with soap [ ] hot water only [ ] hot water with soap [ ] wiping with pieces of fabrics [ ] others (specify)…….
25. Do you have routine control of flies in your butcher? Yes [ ] No [ ]
26. If "Yes" what are the methods used to control flies?.....
27.How long does the meat stay in your butchery before it is over? Less than 12 hours [ ] one day [ ] Two days [ ]
27. Material to wrap meat for sale. Newspaper[ ] Plastic [ ] Used paper[ ] Others[ ]
28. Do you collect money while handling or selling meat Yes [ ] No [ ]
26. Have you ever received any training on hygienic handling of meat? Yes [ ] No [ ]

27. Do you ever receive complaints from the consumers on the quality of the meat you sell? Yes [ ] No [ ]
28. If yes, what kind of complaint? Abdominal upsets [ ] Tough meat [ ] Dirty meat [ ] others [ ]
29. Have you gone for medical checkups in the last 6 months? Yes [ ] No [ ]
30. How frequent you go for medical checkup? Once per year [ ] every three months [ ] every six months [ ]
31. Do you have different storage and display cabinets for offal's and meat? (Observe) Yes [ ] No [ ]
32. Do you use the same equipment while handling meat and the offal's? Yes [ ] No [ ]
33. Do you believe that the butchery where you work requires some improvement for better handling of meat? Yes [ ] No [ ]
34. If yes, what kind of improvement?.....

Appendix 5: Questionnaire format for meat consumers

Date: _____ Questionnaire number---------- Name: _____ Age:------Sex:----------

address: _____ Occupation----------

1. Educational status

a) Illiterate b) primary school c) high school d) university e) Master and PhD

2. from where you buy meat mostly?

a) beef b) sheep c) goat d) camel e) all of them

3. What is your priority criterion to purchase meat?

a) Freshness b) low cost (cheapness) c) low fat content d) healthiness

4. Which type of red meat you prefer?

a) beef b) sheep c) goat d) camel e) all of them

5. How do you consume red meat?

a) boiled b) fried c) cooked d) raw e) cooked in oven

6. Do you consume raw beef a) Yes b) No

7. Do you think that cooked meat is always safe to eat? a) Yes b) No

8. How often do you consume meat?

a) every day b) once in a week c) 1-3 times in a week d) 3-5 times in a week e) once per month f) most of the time

9. When do you consume meat most of the time? a) usualy b) occasionaly c) holidays

10. History of food infection? a) Yes b) No

11. If yes symptoms?

12. How many times?

13. If yes, what type of action taken? a) Drug b) traditional (herbal) c) none d) other

14. Do you know any food poisoning/GIT disturbance associated with consuming of raw meat?

16. What are the symptoms? a) Nausea b) abdominal cramp c) vomiting d) nonbloody diarrhea

17. Do you think that meat slaughtered in abattoir is always safe to eat? a) Yes b) No

18. How do you handle meat?

19. Do you have refrigerator? a) Yes b) No

20. Have you ever heard about Salmonella as foodborne disease? a) Yes b) No

21. Do you know that Salmonella can be transmitted through meat consumption a) Yes b) No

22. What is your opinion on consumption of raw beef by sick, elder and very young children?

23. Do you give raw beef for children? Or if you know any family and related problem happened? a) Yes b) No

Appendix 6: Abattoir practice observation checklist

Date: _____Time: _____

1. Cuts/wounds covered with an appropriate waterproof dressing.Yes----No----

2. Smoking or eating or chewing while working.Smoking----chewing-----other-----

3. Clothes clean and completely free from any dirty or blood.Yes----No----

4. Hand washing before after and during cutting Meat.Before -----After ----During----

5. How washed? Running water or bucket? Hot or cold? Brush or cloth? Soap?Running water---

bucket-----Hot ---- cold------Brush ------ cloth-----Soap-------

6. All knifes are completely clean and free from dirt and cracks and damages.

Clean---------undamaged --------

7. Knifes are cleaned before after and during Use. before -------after------ during use------

8. How often and when do you wash the equipment? Every day at end of the process-------

Once per weak--------once per month----------other (specify)-------

9. Is any disinfectant used? Write name of Disinfectant. Yes----No----

10. The source of water used in abattoir. Tap------Well Water vendor-------- other-------

11. Latrine available nearby. Yes----No----

12. Latrine has water, soap, paper towels for hand Washing. Water------soap------- paper--------

towel-------tissue paper---------

13. Equipments rested in dirty surface during Working. Yes----No----

14. Strict separation between clean and dirty Areas. Yes----No----

15. Veterinary inspectors present to examine the meat to be sold. Yes----No----

Appendix 7: Butcher practice observation checklist

Date: _____Time:_____ Butchery No_____

1. Cuts/wounds covered with an appropriate waterproof dressing. Yes----No----

2. Smoking or eating or chewing while working. Smoking-------chewing------

3. Apron (any protective clothes)? Yes----No----

4. Hand washing before after and during cutting meat? Before ---------After ------- During ---

Not wash----------other-------

5. How washed? Running water or bucket? Hot or cold? Brush or cloth? Soap?

Running water-------- bucket--------- Hot ------ cold---------Brush ------ cloth-----Soap---------

6. All knifes are completely clean and free from dirt and cracks and damages?

Clean ---------undamaged --------

7. Knifes are cleaned before after and during Use? before -------after-------during use------

8. Is any disinfectant used? Write name of Disinfectant? Yes----No----

9. Wear Jewellery? Yes----No----

10. Handling money? Cashier --------Butcher with bare hand-------with glove------

11. Cutting table? Single -------separate for different meats ----

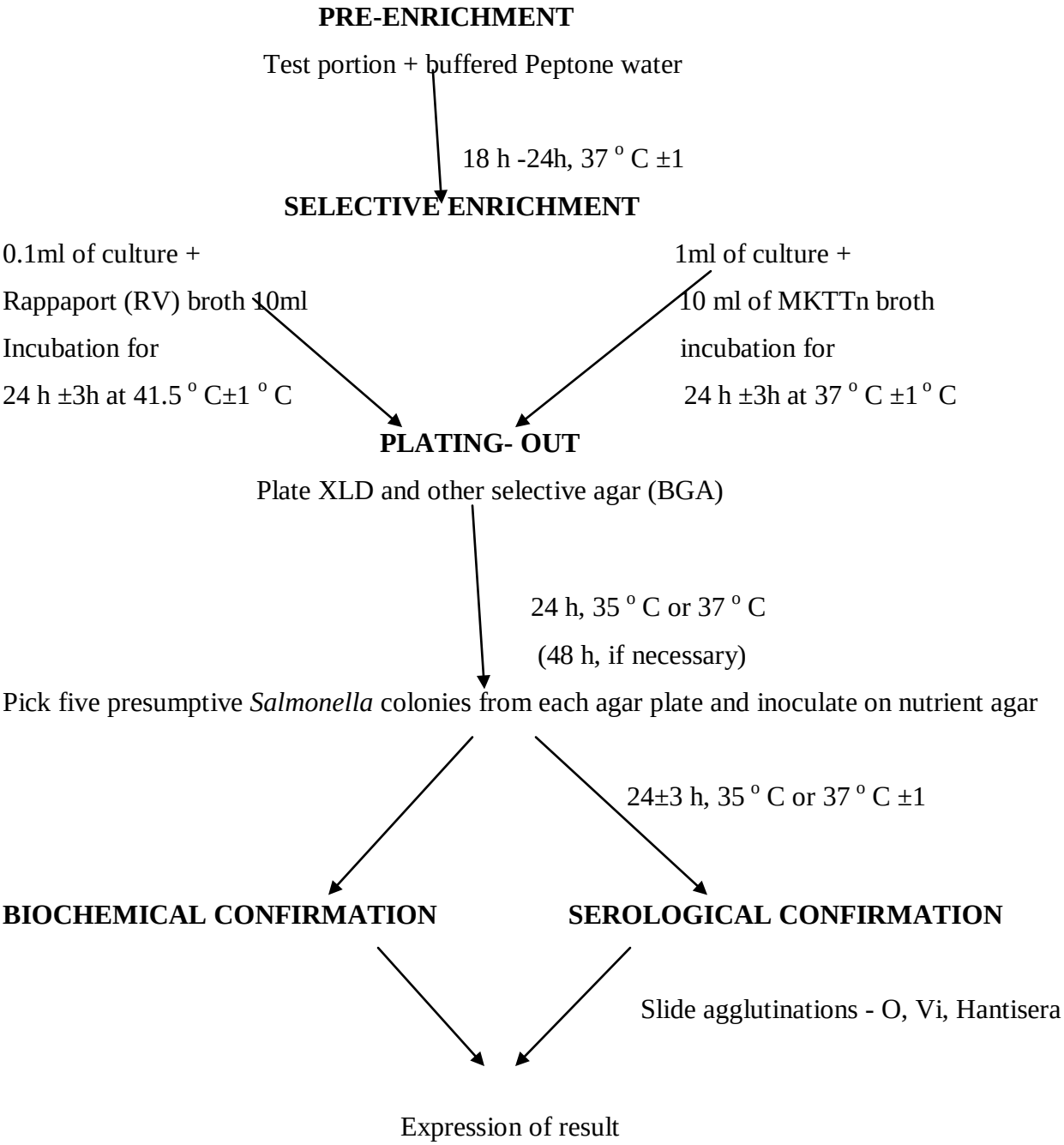
12. Cutting board type and clean? Wood-----metal-----glass------other-----clean-----dirt-----

13. Distance from main road?

14. Meat shop has open or closed? Window? open-----closed----

15. Insect presence and control method used? Present---absent----,chemical----physical---other---

Appendix 8: Flow diagram showing ISO method for detection of *Salmonella*



Sources: (ISO-6579, 2002**;** WHO, 2003).

Appendix 9: Performance standards for antimicrobial susceptibility testing of *Salmonella*

No	Antimicrobial Agent	Disc Code	Potency	Resistant	Intermediate	Susceptible
1	Trimethoprim	TR	25µg	≤14	11_15	≥16
2	Nalidixic acid	NA	30µg	≤13	14_18	≥19
3	Kanamycin	K	30µg	≤13	14_17	≥18
4	Cefoxitin	FOX	30µg	≤14	15_17	≥18
5	Streptomycin	S	10µg	≤11	12_14	≥15
6	Nitrofurantoin	F	50µg	≤14	15_16	≥17
7	Sufisoxazole	RL	100µg	≤12	13_16	≥17
8	Ampicillin	AMP	25µg	≤13	14_16	≥17
9	Tetracycline	TE	30µg	≤11	12_14	≥15

Source: CLSI, (2015).