JIMMA UNIVERSITY

COLLEGE OF AGRICULITURE AND VETERINARY MEDICINE

SCHOOL OF VETERINARY MEDICINE

ISOLATION, ANTIMICROBIAL SUSCEPTIBILITY PROFILE AND ASSOCIATED RISK FACTORS OF *SALMONELLA* IN RAW BEEF FROM MUNICIPAL ABATTOIR AND BUTCHER SHOPS IN JIMMA, SOUTH WESTERN ETHIOPIA

MSc THESIS

By

WONDIMU ZELEKE DESTA

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ISOLATION, ANTIMICROBIAL SUSCEPTIBILITY PROFILE AND ASSOCIATED RISK FACTORS OF *SALMONELLA* IN RAW BEEF FROM MUNICIPAL ABATTOIR AND BUTCHER SHOPS IN JIMMA, SOUTH WESTERN ETHIOPI

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A Thesis Submitted to the School of Graduate studies, Jimma University College of Agriculture and School of Veterinary Medicine, in partial Fulfillment of the Requirements for the Degree of Master of Science in Veterinary Microbiology

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I have completed my thesis research work as per the approved proposal and it has been evaluated and accepted by my advisers. Hence, I hereby kindly request the Department to allow me to present the finding of my work and submit the thesis.

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We, the thesis of advisers have evaluated the content of this thesis and found to be satisfactory, executed according to the approved proposal, written according to the standards and format of the university and is ready to be submitted. Hence, we recommend the thesis to be submitted.

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DECLARATION

First, I declare that this thesis is my own original work and that all sources of material used to this thesis have been properly acknowledged. This thesis has been submitted in partial fulfill ment of the requirements of degree of MSc in veterinary microbiology at Jimma University C ollege of Agriculture and School of Veterinary Medicine and deposited at college library. Th is thesis has not been submitted to any other institution anywhere for the award of any academic purpose.

Name: Wondimu Zeleke Desta Signature_____

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Date of submission: _____

DEDICATION

I dedicate this thesis to my My Father Zeleke Desta and Mother W/ro Woletu Mengesha who have been supporting me by praying, financially and morally, without them this would have not been possible; and to my uncle Ato Tsehay Abebe and His wife W/ro Mulualeme Bekele who made a turning point to my way of life from avery beginning upto know.

BIOGRAPHICAL SKETCH

Wondimu Zeleke Desta was born in 1980 at Dedo woreda, Jimma Zone. He attended primary education from 1986-1993 at Dedo primary and Junier secondary school. And his secondary school attended at Jimma comprehensive secondary school from 1994-1998. He gradutuated Diploma with General Agriculture in 2001 and in 2011 Bachuler of Veterinary Science (BVsc) from Jimma University College of Agriculture and School veterinary Medicine. After his graduation Wondimu worked at Meanite Shasha Woreda, Benchi Maji zone from 2004 to 2014 in Agriculture office. In 2015 He joined Jimma University School of Veterinary Medicine to pursue Master`s degree in veterinary Microbiology.

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LIST OF ABBREVIATIONS

AMR	Antimicrobial resistant
ATCC	America Type Culture Collection
ATCC	American Type Culture Collection
BGA	Brilliant Green agar
BPW	Buffered peptone water
CDC	Center for disease control and prevention
CLISI	Clinical and Laboratory Standards Institute
CSA	Central statistics agency
EFSA	European Food Safety Authority
ERS	Economic Research Service
EU	European Union
GSS	Global *Salmonella* Surveillance
ICMSF	International Commission on Microbiological Specification for Foods
ISO	International Organizations for Standardization
JZMSR	Jimma Zone Meteorology Station Report
MARI	Multidrug index
MDR	Multi-drug resistance
MR-VP	Methyl Red -Voges Proskauer
NTS	Non typhoidal *salmonella*
RVS	Rappaport Vassiliadis soy peptone broth
SCV	*Salmonella* containing vacuole
SPI-1	*Salmonella* Pathogenicity Island 1
SPSS	Statistical Package for Social Sciences
TSI	Triple sugar iron
µg	Microgram

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ABSTRACT

Salmonellosis is the leading causes of food borne disease in developing countries. It is classified into two forms namely: typhoidal and non typhoidal Salmonellosis where it is caused by two species of *Salmonella* called *Salmonella enterica* and *Salmonella bongori*. The wide spread habit of raw meat consumption of Ethiopian`s would contribute in the causes of food borne diseases in Ethiopia. Across sectional study was conducted between December 2016 and January 2018 on meat sample from abattoir and butcher to isolate *Salmonella*, antibiogram and associated contamination risk factors in Jimma town. A simple random sampling technique was used to select carcass at abattoir and for butcher shops selection. The data was analyzed by Fisher exact test using software SPSS ver 20. The overall positive samples from abattoir were 24(11.43%, 95% CI: 7.46-16.53%) out of 210 swab samples collected. From 70 apparently healthy slaughtered cattle in abattoir, 14.2% (10/70) isolates from carcasses, 8.5% (6/70) from hand and 11.4% (8/70) from knives were isolated. The overall positive samples from butcher shops were 9 (6.52 %, 95% CI: 3.03-12.02%) out of 138 swab samples collected. Among positive samples from butcher shops, 8.7% (4/46) from carcasses, 4.4 % (2/46) from hand and 6.5 % (3/46) from chopping board. The following contamination risk factors were identified had association for *Salmonella* Contamination of carcasses at abattoir and a butcher shops level. These were educational level (OR=8.40, 95% CI=1.19-59.493%, P<0.05) and (OR=9.17, 95%CI=1.15-73.24, P<0.05), respectively. Lack of job related training (OR=5.5, 95% CI: 1.065-28.42%, P<0.05); Lack perception on Contamin ation risk (OR=5.31, 95%CI=1.26-22.49%, P<0.05)); Use of unclean Knife (OR=7.6, 95% CI =0.89-65.38%, P<0.05); fecal contamination (OR=8.44, 95% CI=1.682-42.39%, P<0.05) in Jimma municipal abattoir. At Butcher shops level, unworn of protective cloth (OR=11.42, 95%CI=1.83-71.42, P<0.05); hand wash with water only (OR=7.25, 95%CI=1.21-43.44%, P<0.05); and money collecting with hand (OR=9.69, 95% CI=1.58-59.47%, P<0.05) were identified as risk factors for *Salmonella* contamination of meat. The total 33 *Salmonella* isolates (24 from abattoir and 9 from butcher shops) were subjected to 12 antimicrobial susce ptibility testing. The isolates were found highly susceptible to Ciprofloxacillin, Gentamycin and Norfloxaciline. Generally, isolates where found resistant to 9 of the 12 different types of antimicrobials tested. Most of the isolates were resistant to Tetracycline (58.3%), Ampicillin (55.6%) and Streptomycin (66.7%). Multidrug resistant isolates were observed in both abattoir (58.3%) and butcher shops (66.7%). This study shows that significant prevalence of *Salmonella* in human food (beef) in both Jimma municipal abattoir and butcher shops of Jimma town. Therefore, training about meat hygiene and ways of good hygienic handling practice is better be given for both abattoir and butcher shop workers to increase their awareness on meat hygienic handling.

Keywords: *Antimicrobial Resistance, Beef, Ethiopia, Isolation, Salmonella.*

1. INTRODUCTION

1.1. Background of salmonellosis

Salmonellosisis an infection of the bowel caused by *Salmonell*a bacteria.There are two species of *Salmonella*: *Salmonella enterica* which is further classified into six subspecies (*S.enterica, S.salmae, S.arizonae, S.diarizonae, S.hautenae and S.indica*) and *Salmonella bongori*. Accord ing to Kauffmann (1952) scheme based on antibody interactions with bacterial surface antigens, *Salmonella* has more than 2, 600 serotype. Most of the *Salmonella* serotypes are part of subspecies *S. enterica* and over 99% of human and animal infections are caused by serotypes under this subspecies. Serotypes belonging to *S. bongori* found to be predominantly associated with cold-blooded animals (Ryan *et al*., 2017).

Salmonellosis has two forms (typhodial and non-typhoidal). Typhoidal Salmonellosis caused by serotype *S. enterica* serovar typhi and *S. enterica* serovar Paratyphi A, B and C are highly adapted to humans and do not cause disease in non-human hosts. Non-typhoidal Salmonellosis are caused by all serotypes of *Salmonella* except the typhi, paratyphi A, paratyphi B and paratyphi C groups. Non-typhoidal *Salmonella* causes common food borne diseases causes gastroenteritis and bacteremia infections in humans (Getnet *et al*., 2014). This group feature *Salmonella enteritidis* and *Salmonella typhimurium* which are the two most important serotypes of Salmonellosis transmitted from animals to humans in most parts of the world (CDC, 2014).

Food animals could be infected with *Salmonella* from intensive rearing practices and use of contaminated feed (Ejeta *et al*., 2004). Cross contamination can occur during production, tran sportation preparation, storage and giving services using contaminated utensils (knives, hand and chopping board). In addition, a food handler, lack of awareness in basic personal cleanliness and safe food handling of butchers and abattoir workers enhances contamination o f meat by *Salmonella* (Garedew *et al*., 2015).

Salmonellosis induces significant morbidity and mortality effects both in human and animal and has also a substantial global socio economic impact. Globally, non-typhoidal *Salmonella* causes diarrheal disease which is estimated to be 93 million enteric infections and 155,000 diarrheal deaths each year (Majowicz *et al*., 2010).

Salmonella is the most common foodborne pathogen in the United States, causing approximat ely 17.6 illnesses per 100,000 persons, 2,290 hospitalizations, and 29 deaths in 2010 (Scallan *et al*., 2011). In Africa, it causes an estimated 32,000 deaths per year (WHO, 2015). In European Union (EU), over 100,000 human cases are reported each year. European Food Safety Authority (EFSA) has estimated that the overall economic burden of human Salmonellosis could be as high as EUR 3 billion a year (EFSA, 2014). In addition, there are economic burden of Salmonellosis in Sub-Saharan Africa which is estimated to be $210 per outpatient, $5,797 per inpatient with gastrointestinal infection, $16,441 per inpatient with invasive infection and $4.63 million per premature death (Olobatoke, 2017).

In Ethiopia, the rate of infection with *Salmonella* among food handlers ranged from 1% upto 21%. The overall pooled prevalence of *Salmonella* in raw meat samples were 5.57%; (4.53%) beef, (3.86%) goat meat, (8.02%) mutton (3.52%) pork and (21.01%) camel collected from abattoirs and from markets pooled prevalence of *Salmonella* is 11.7%; (8.34%) minced beef, (11.86%) mutton,(12.59%) pork, (13.53%) chicken and 10.8% of milk samples from farms were positive. Similarly, the pooled prevalence estimates of *Salmonella* in humans are 8.7% in diarrheic children, 5.7% in diarrheic adults and 1.1% in carriers and 57.9% of the isolates were non-typhoidal *Salmonella* (Tadesse and Gebremedhin, 2015).

Antimicrobial agents used in food animal at varied concentration for growth promotion or prophylactic aids allow resistant bacteria genes to pass through the food chain from animal to human. Globally, the incidence of *Salmonella* infection associated with multi-drug resistance has increased in the last few decades (Eguale *et al*., 2015). Different studies conducted in Ethiopia revealed fragmented substantial prevalence as well as multidrug resistance of *Salmonella* isolated from food of animal sources; 53.2% in Mojo (Abunna *et al*., 2017), 71.43 % in Bahir Dar (Melaknesh and Mulugeta, 2017), 50% in Assela (Beyene *et al*., 2016), 81.25 % in Wolayita Sodo (Mekuriaw *et al.,* 2016), 69%in Addis Ababa (kebede *et al*., 2016), 75.5% in Gonder (Garedew *et al*., 2015

Food borne pathogens are the leading causes of illness and death in developing countries resulting in the loss of labor force which could have contributed in the economic growth (Fratamico *et al*., 2005; Tasse *et al*., 2010). In Ethiopia, it is difficult to evaluate the burden of food borne diseases, because of the limited scope of studies and lack of coordinated epidemiol

ogical surveillance systems (Kebede *et al*., 2016). The incidence of food borne *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 (Garedew *et al*., 2014; Tadesse, 2014) and in different food animals and food products (Ejeta *et al*., 2004; Garedew *et al*., 2012).

1.2. Statement of problem

Contamination of carcasses at abattoir might be raised from slaughtering lairage which contaminated from shedding of carrier animals and/or from stomach or intestinal contents. The poor hygienic standards in food handling practices, the habit of raw meat consumption with the presence of *Salmonella* indicates a great public health hazards due to Salmonellosis. Jimma municipal abattoir serves the people of Jimma town by slaughtering the animals derived from the different area. On average 60 cattle have been slaughtered per day. In Jimma town butcher shops take the meat from the abattoir and sells to consumers. Even though all the above studies were reported as *Salmonella* spp. are the leading food borne pathogens in different part of the country, there is no report on the microbial quality, hygienic status and practices in abattoir and butcher shops of beef meat in Jimma town. Therefore, isolation, identification, antimicrobial susceptibility test of *Salmonella* from raw beef cattle slaughtered at Jimma municipal abattoir and meat retailers (butcher shops) multifaceted with increased consumption of raw/minced meat (locally known as '*kitfo*', '*Kurt*', '*dullete'*) and assessment of associated risks factors of *Salmonella* contamination in beef are the setting of this study.

Objectives

- To isolate, identify and determine isolation frequency of *Salmonella* from beef meat slaughtered at Jimma town municipal abattoir and butcher shops.
- To determine the antimicrobial sensitivity profile of the isolated *Salmonella* species.
- To assess knowledge gap, attitude and hygiene practices of abattoir and butcher shops workers, and beef meat consumers on zoonotic implication of Salmonellosis in Jimma town.
- To assess the risk factors of beef contamination by *Salmonella* in both abattoir and butcher shops of Jimma town.

2. LITERATURE REVIEW

2.1. Overview of Food Hygiene and Food Safety

Foodborne diseases can be defined as diseases commonly transmitted through food. It compri ses a broad group of illnesses caused by microbial pathogens, parasites, chemical contaminant s and biotoxins. The burden of disease can be defined as the incidence and prevalence of morb idity, disability, and mortality associated with acute and chronic manifestations of diseases (Assefa *et al*., 2015).

The consumption of foods contaminated by foodborne pathogenic microorganisms and toxins produced by them cause deaths, illnesses, hospitalization and economic losses. Due to the wid e spread nature of food-borne diseases particularly gastrointestinal infections represent a very large group of pathogens with a strong negative impact on public health (WHO, 2007).

According to WHO global estimate report, 31 foodborne hazards causing 32 diseases are: 11 diarrhoeal disease agents (1virus, 7 bacteria, and 3 protozoa), 7 invasive infectious disease ag ents (1virus, 5 bacteria, and 1 protozoan), 10 helminths and 3 chemicals (WHO, 2015). From diarrhoeal diseases causing 7 are bacterias (Campylobacter spp., Entero pathogenic E. coli (EPEC), Enterotoxigenic E. coli (ETEC), Shiga toxin producing E. coli, nontyphoidal *Salmone lla enterica*, Shigella sp., Vibrio cholera (V. cholerae). From Invasive infection causing 5 bact erias are Brucellas spp, Listeria monocytogenes, Mycobacterium bovis (M. bovis), *Salmonell a* paratyphi A (*S. paratyphi A), Salmonella typhi(S. typhi)* (WHO,2016).The main causes of fo od borne illness are bacteria (66%), chemicals (26%), virus (4%) and parasites (4%).The two most common types of food borne illness are intoxication and infection.Intoxication occurs when toxin produced by the pathogens cause food poisoning, while infection is caused by the ingestion of food containing pathogens (Addis and Sisay, 2015).

Each year as many as 600 million or almost one in 10 people in the world fall ill after consum ing contaminated food. Among these people, 420, 000 people die, including 125,000(1/3) chil dren under five years (WHO, 2015).

2.2. Food hygiene and food safety practices in Ethiopia

Food borne diseases are common in developing countries including Ethiopia due to the prevai ling 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 foo d handlers. These diseases are mainly caused by *Salmonella spp*, Campylobacter, Listeria, *E.c oli* and Mycobacterium. Salmonellosis cause siginificant morbidity and mortality in both hum an and animal and has a subtantial global socio-economic impact (Zelalem *et al*, 2015).

According to the National Hygiene and Sanitation Strategy program Minister of Health (MoH 2005) reported, in Ethiopia more than 250,000 children die every year from sanitation and hygiene related diseases. 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 me ntioned as contributing factors for outbreak of food borne diseases (Du and Venter, 2005)

2.3. History of *Salmonella* and Its Characteristics

The *Salmonella* bacterium was first described in 1886 by Theobald Smith. Two American vet erinarians, Salmon and Smith who isolated the bacterium causing hog cholera from infected p igs (Salmon and Smith, 1886).The name *Salmonella* was subsequently adopted in honor of Dr . Salmon (Schultz, 2008). Salmonellosis is an infection disease of the bowel caused by the *Sal monella* bacteria.The Genus *Salmonella* belongs to the Domain Bacteria, Phylum Proteobacte ria, Class Gamma Proteobacteria, Order Enterobacteriales, and Family, are facultative anaero bic, gram negative and straight rod shaped usually motile with peritrichous flagella (except *S. pullorum* and *S. gallinarum)* (Lopes *et al*., 2016). The cells are typically 0.7-1.5 μm by 2-5 μm. *Salmonella* multiply optimally at a temperature of 35^0C to 37^0C (mesophile), pH about 6. 5-7.5 (neutrophile) and water activity between 0.94-0.84. They are facultative anaeroe, ferme nt glucose usually with production of gas (except *S. typhi and S. Dublin*), but failed to ferment lactose, sucrose, salicin and urea, reduce nitrate to nitrite and most are phototropic bacteria. Given the close relationship with E. coli and the many years devoted to its study, the overall metabolism of *Salmonella* is well understood. They are also able to multiply in the environme nt with low level or no oxygen. The bacteria are sensitive to heat and will not survive a

temperature above 70^0C; so it is sensitive to pasteurization, but resist to drying even for years. Especially in dried feces, dust and other dry materials such as feed and certain food. Biochemical features used to identify *Salmonella* include hydrogen sulphide production, lysine and ornithine decarboxylation, and non hydrolysation of urea (Kemal, 2014).

2.4. Classification of *Salmonella*

Genus *Salmonella* is split into just two species: *Salmonella enterica* and *Salmonella bongori*, with *S. enterica* being split into six additional subspecies (*Salmonella enterica subsp. enterica or Subspecies I, Salmonella enterica subsp. salamae (II), Salmonella enterica subsp. arizonae (IIIa), Salmonella enterica subsp. diarizonae (IIIb), Salmonella enterica subsp. houtenae (IV) and Salmonella enterica subsp. indica (VI)*(Ryan *et al*., 2017). Among all the subspecies of *Salmonella*, *S.enterica* subsp. *enterica* (I) is found predominantly in mammals and contributes approximately 99% of *Salmonella* infections in humans and warm-blooded animals. In contra st, the other five *Salmonella* subspecies and *S. bongori* are found mainly in the environment and also in cold-blooded animals, and hence are rare in humans (Brenner *et al*. 2000 ; Eng *et al*., 2015).

According to The Kauffman–White (Kauffmann and Edwards. 1952) classification scheme is a system employed in the classification of *Salmonella* into serotypes taking cognizance of their surface antigens, the O (somatic) and H (flagella) antigens (Smith *et al*., 2016). A serotype (as well as a serovar) is determined by the somatic (O), flagella (H) and the Vi or capsular antigen factor antigens present in the cell wall of *Salmonella* organisms. The total number of O or H factors present in each serotype varies from one to four different factors. The O factors determine the grouping, while the H factors completely define the serotype identity of a *Salmonella* strain. With several monophasic exceptions, the H antigens for each s erotype are usually diphasic (Agasan *et al*., 2002). The subspecies are further divided into serotypes.There were 2610 serovars in the genus *Salmonella* 2587 belongs to *Salmonella ente rica* and 23 to *Salmonella bongori*) (Achtman *et al*., 2012). A full breakdown of the numbers belonging to each subspecies can be seen in (Figure 1and Table.1).

Table 1: *Salmonella* serotype classification

Salmonella species and subspecies	No. of serotype within subspecies	Usual habitat
S. enterica subsp. enterica(I)	1547	warm- blooded animals
S. enterica subsp. salamae (II)	513	Cold- blooded animals and environment
S. enterica subsp. arizonae (IIIa)	100	Cold- blooded animals and environment
S. enterica subsp. diarizonae(IIIb)	341	Cold- blooded animals and environment
S. enterica subsp. houtenae(IV)	73	Cold- blooded animals and environment
S. enterica subsp. indica(VI)	13	Cold- blooded animals and environment
S. bongari (V)	23	Cold- blooded animals and environment
Total	**2610**	

Source: Achtman *et al*. (2012).

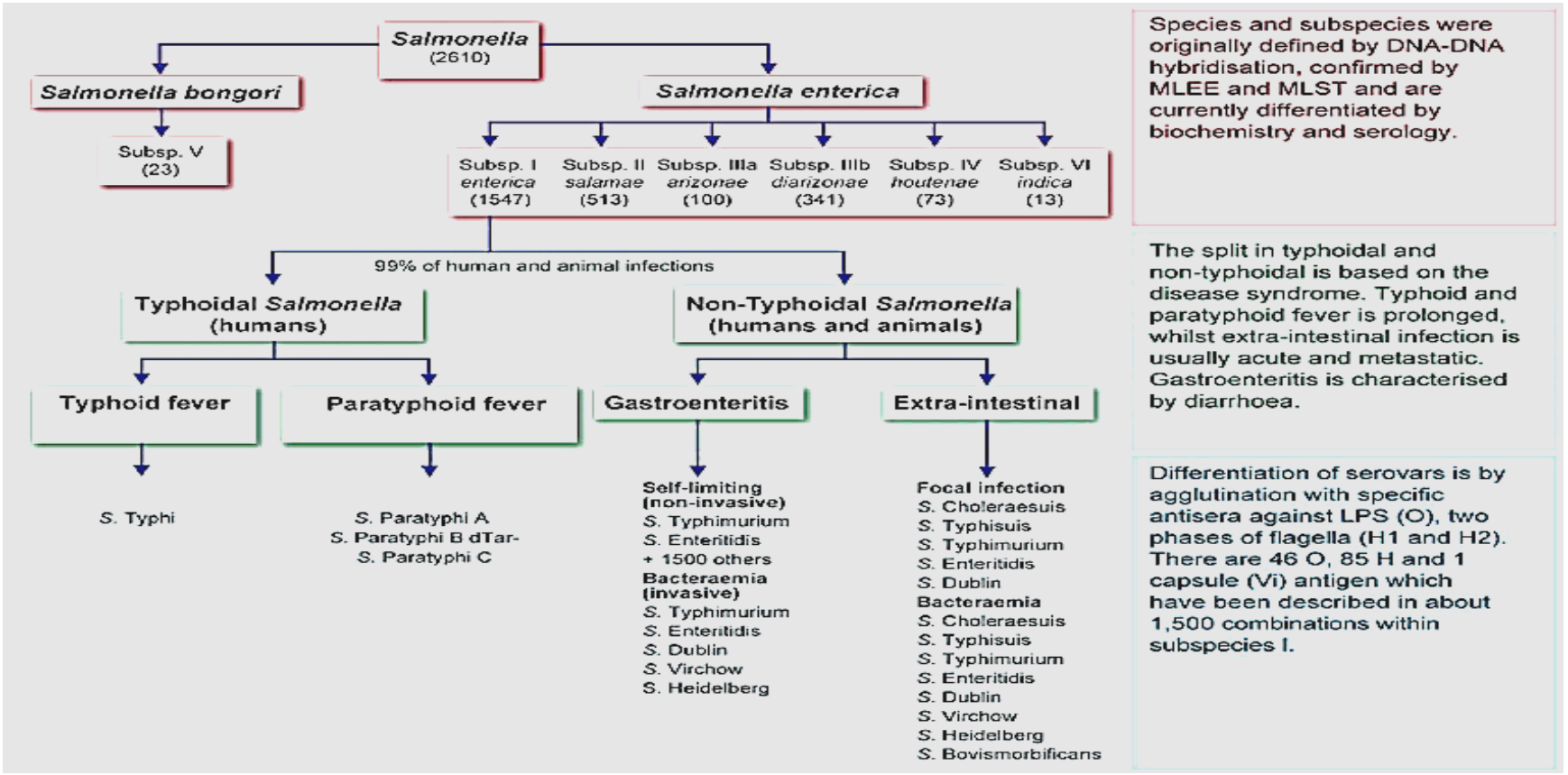

Figure 1: General overview of the current classification of *Salmonella* enterica (Achtman *et al*., 2011).

2.5. Epidemiology of non-typhoidal *Salmonella* species

Epidemiological patterns of *Salmonella* differ greatly between geographical areas depending on climate, population density, land use, farming practice, food harvesting and processing technologies and consumer habits (Beyene *et al*., 2016). For epidemiological purposes, the *Salmonella* can be placed into three groups; the first are those that infect humans only. This includes, *S. Typhi, S. Paratyphi A, S. Paratyphi C* (Gunn *et al*., 2014). This group includes the agents of typhoid and paratyphoid fevers, which are the most severe of disease caused by *Salmonella*. The second was the host-adapted serovars (some of which are human pathogens and may be contracted from food), included are *S. gallirinum* (poultry), *S. Dublin* (cattle), *S. abortus-equi* (equine), *S. abortus-ovis* (sheep) and *S. cholera¬suis* (swine) (Uzzau *et al*., 2000). The third is unadapted serovars (no host preference). There are pathogenic for humans and other animals. The epidemiology of the *Salmonell*a is complex, which often make animals control of the disease is difficult. Animals are the reservoir of food born disease of *salmonella* (Addis and Sisay, 2015).

Non-typhoidal *Salmonella* (NTS) is food born disease which known by causing of diarrhea globally is estimated to cause 93 million enteric infections and 155,000 diarrheal deaths each year (Majowicz *et al*., 2010). Approximately 38% of identified food-borne infections were caused by *Salmonella* contamination in United States (Crim *et al*., 2014;Yang *et al*. 2015).The incidence of NTS associated disease is estimated to cause 690 cases per 100,000 populations in Europe while the incidence of NTS infection in Israel is around 100 cases per 100,000 annually (Weinberger and Keller 2005). The World Health Organization (WHO) European region reported non-typhoid salmonellosis causes almost 2000 deaths annually. European Food Safety Authority (EFSA) has estimated that the overall economic annual burden of human Salmonellosis could be as high as €3 billion (ESFA, 2014).The WHO African region was estimated to have the highest burden of food-borne diseases per population. More than 91 million people fall ill and 137,000 die each year due to food-borne disease (WHO, 2015). *Salmonella* the United States, causing approximately 17.6 illnesses per in 100,000 persons in food-borne pathogen, 2,290 hospitalizations and 29 deaths in 2010 (Scallan *et al*., 2011).

Globally, typhoid fever is estimated to cause 21.7 million illnesses and 217 000 fatalities annually (Marks *et al*., 2017). In 2002, it was estimated that a total of 408,837 cases occurred in Africa (Crump *et al*., 2004). However, the exact distribution of typhoid fever in Africa is not well documented due to the non-availability of facilities capable of performing the blood culture tests essential for the diagnosis of typhoid fever and it is commonly attributed to malaria (Olobatoke, 2017). Enteric fever (typhoid and paratyphoid fevers) is a febrile illness caused by *S. typhi* and *S. paratyphi A, B and C* (Smith *et al.,* 2016). Typhoid cases are stable with low numbers in developed countries, usually causes mortality in 5 to 30% of typhoid-infected individual in the developing world (Pui *et al*, 2011).

2.6. Risk factors of *Salmonella* infection

Salmonella infection in meat animals a rises from intensive rearing practices and the use of c ntaminated feeds (Ejeta *et al*., 2004). 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 other animals in the slaughter plant. The meat from affected animals may con tain *Salmonella* and so cause food contamination in humans. *Salmonella* also inhabit the intes tinal tract of a wide range of the common meat animal species. Physical contamination of the carcass or organs by stomach or intestinal contents is a significant route of transmission for *Salmonella*. Also Contamination with *Salmonella* can occur during production, transportation , preparation, storage and service. At butchers' house, meat contamination could occur due to different possible reasons; storing food in unclean utensils, holding food at a temperature that would permit microbial growth, utilization of water of questionable hygienic quality, using packaging materials that is not of food-grade quality, vending site that had no facilities for waste disposal and utilization of unclean utensils. In addition, lack of awareness in basic personal cleanliness and safe food handling of butchers enhances contamination of meat by microbes (Garedew *et al*., 2015). Consumers should be able to assume that all food offered for sale is safe for its intended use. Primary responsibility for food safety lies with those who produce, process and trade including farmers, slaughterhouse operators, food processors, wholesale and retail traders, caterers, etc. (Tesfahun *et al*.,2016).

Although the risk factors for NTS infections in Africa have not been properly characterized, they may broadly be categorized into environmental and host factors. The environmental fact

ors include food/water contamination, nosocomial infections, direct/indirect contact with animals, and transmission between humans. It is thought that poor socioeconomic conditions in rural African settings, coupled with lack of access to clean water and proper waste disposal may also be responsible for the high burden of the disease (Majowicz *et al*., 2010). Host factors however, include age, malnutrition, disease conditions (e.g. sickle cell, malaria, schistosomiasis and HIV infections) and recent antibiotic. The relationship between malaria and NTS bacteremia has well been established in Africa, particularly in children (Park *et al*., 2016) in their multicenter study in 13 sites across sub-Saharan Africa, found a positive correlation between invasive NTS disease and malaria endemicity (MacLennan, 2012).

NTS infections appear to be age specific with higher prevalence in children less than 3 years old and adults above 50 years of age (Sigauque *et al*., 2009). In addition, recent antibiotic use coupled with malnutrition can destabilize normal intestinal flora and compromise mucosal integrity thereby increasing the risk of NTS gastroenteritis (Morpeth *et al*., 2009). Individuals with severe clinical immunosuppression are more prone to NTS infections and usually present with primary bacteraemia, leukopenia and opportunistic infections without gastroenteritis. For instance, HIV infected persons have higher risk of NTS infections compared with the general population. Among these individuals, non-typhoidal *Salmonella* spp is one of the most common causes of bacteraemia, often multidrug resistant and associated with high mortality(24%-80%) and recurrence rates (43%)). Surveillance studies showed the association of NTS infections with yearly seasonality, with highest incidence recorded during the summer months (Kingsley *et al*., 2009).

2.7. Economic and Public Health Significance of *Salmonella* Infections

In Sub-Saharan Africa, the socio-economic burden of the disease is difficult to quantify due to lack of a standard method of assessment compounded by under-reporting in many cases. In addition, there is usually no reliable data in many of the countries from which information's can be obtained for proper analysis of the disease cost per case. In the U.S however, direct cost of human Salmonellosis is usually measured in terms of medical costs and loss in productivity. The average economic burden of Salmonellosis was estimated to be $210 per outpatient, $5,797 per inpatient with gastrointestinal infection, $16,441 per inpatient with invasive infection and $4.63 million per premature death (Olobatoke, 2017). In Ethiopia, the rate of infection

with *Salmonella* and Shigella among food handlers ranged from 1 to 7.5% (Mama and Alemu, 2016).

The USDA's Economic Research Service (ERS) estimated that *Salmonella* infections from all sources cost about $2.65 billion per year. That is based on an estimate by the Centers for Disease Control and Prevention (CDC) of almost 1.4 million *Salmonella* cases annually from all sources, with 415 deaths. The estimated average cost per case is $1,896 .The European Uni on (EU**),** over 100,000 human cases are reported each year. EFSA has estimated that the overa ll economic burden of human Salmonellosis could be as high as EUR 3 billion a year (EFSA, 2014).

2.8. *Salmonella* Sources of Infections and Mode of Transmission

Salmonella is ubiquitous in nature and can be found in soil and water environments. However, intestinal tracts of both domestic and wild animals remain the primary reservoirs of NTS (Hendriksen *et al*., 2011). Food animals could be infected with *Salmonella* from intensive rear ing practices and use of contaminated feed (Ejeta *et al.,* 2004). During slaughter, *Salmonella* is passed from the intestinal tract of the host through fecal contamination to meat products. Similarly, some *Salmonella* serovars particularly *S. enteritidis* may colonize chicken ovaries and can be transmitted through eggs. Thus infection in humans may be acquired from contaminated meat or eggs, particularly when under-cooked. Chicken meat in particular has been incriminated as the most probable route of NTS transmission. Fruits and vegetables may also be contaminated with waste water from animal reservoirs, resulting in human infections (Hanning *et al.,* 2009). Other additional routes of human infection by NTS have been postulated in developing countries and these include hospital acquired infection, direct and indirect animal contact with pet and food animals, and human-to-human transmission (Hale *et al*., 2012).

The contamination of meat by bacterial pathogens such as *Salmonella* can occur at any stage of the meat production chain, including slaughtering, processing, and distribution (Doulgeraki *et al*., 2012). Furthermore, even though the cooking process significantly reduces the load of microorganisms in foods, cooked meat may become re-contaminated by food handlers and the processing environment (Carrasco *et al*., 2012). The occurrence of pathogenic bacteria such as

Salmonella in raw and cooked meat has been found to be related to poor hygienic practices at different stages of the meat chain (Cardinale *et al*., 2005).

2.9. Clinical future of Salmonellosis

Based on the clinical patterns in human Salmonellosis, *Salmonella* strains can be grouped into typhoid *Salmonella* and non-typhoid *Salmonella* (NTS). In human infections, the four differen t clinical manifestations are enteric fever, gastroenteritis, bacteraemia and other extraintestinal complications, and chronic carrier state (Sheorey and Darby, 2008).

2.9.1. Enteric fever

Enteric fever is an infection caused by *Salmonella enterica* serotype Typhi and Paratyphi. *Salmonella Typhi* is the aetiological agent of typhoid fever, while paratyphoid fever is caused by *S. Paratyphi A, B and C*. Since the clinical symptoms of paratyphoid fever are indistinguis hable from typhoid fever, the term enteric fever is used collectively for both fevers, and both *S. Typhi* and *S. Paratyphi* are referred as *typhoid Salmonella.* Humans are the sole reservoir for the two strains of typhoid *Salmonella* .Enteric fever is characterized by an incubation period of one week or more, with prodromal symptoms such as headache, abdominal pain and diarrhea (or constipation), followed by the onset of. Diarrhea is more commonly observed in children, whereas patients with immunosuppression are more likely to develop constipation. T he symptoms of enteric fever are nonspecific and may include fever, anorexia, headache, letha rgy, myalgias and constipation. This disease can be fatal, due to meningitis or septicemia, if not treated quickly (Parry *et al*., 2010).

2.9.2. Gastroenteritis

Salmonella strains other than *S. Typhi* and *S. Paratyphi* are referred to as NTS, and are predo minantly found in animal reservoirs, abdominal cramps and myalgias. Symptoms such as hepatomegaly and splenomegaly are less commonly observed in patients infected with NTS infections are characterized by gastroenteritis or stomach flu‘, an inflammatory condition of the gastrointestinal tract which is accompanied by symptoms such as non-bloody diarrhoea, vomiting, nausea, headache NTS (Hohmann, 2001). Compared to typhoid infections, NTS infections have a shorter incubation period (6–12h) and the symptoms are usually self-

limiting and last only for 10 days or less (Crump *et al*. 2008). Gastrointestinal complications of NTS infections include cholecystitis, pancreatitis and appendicitis, while the perforation of the terminal ileum has no association with NTS infections (Hohmann, 2001). Infants, young children, elderly people and immune compromised patients are highly susceptible to NTS infections and develop more severe symptoms than normal individuals (Scallan *et al*., 2011).

2.9.3. Bacteraemia and other extraintestinal complications

Salmonella bacteraemia is a condition whereby the bacteria enter the bloodstream after invading the intestinal barrier. Almost all the serotypes of *Salmonella* can cause bacteraemia, while *S. Du blin* and *S.cholearaesuis* are two invasive strains that are highly associated with the manifestatio ns of bacteraemia. Similar to enteric fever, high fever is the characteristic symptm of bacteraemi a, but without the formation of rose spots as observed in patients with enteric fever. In severe conditions, the immune response triggered by bacteraemia can lead to septic shock, with a high mortality rate. The clinical manifestation of bacteraemia is more commonly seen in NTS infecti ons than in typhoid *Salmonella* infections. The difference in the clinical manifestation is believe d to be associated with the presence of the *spv* (*Salmonella* plasmid virulence) gene in NTS whi ch causes non typhoidal bacteraemia, based on genetic analysis (Guiney and Fierer, 2011). Although the mechanism of the gene to enhance the virulence traits of NTS remains unclear, expression of the gene is required to prolong apoptotic cell death and this may allow the bacteria to persist in the host cells for a longer period. Approximately 5% of patients infected with NTS develop bacteraemia and, in some cases, extraintestinal complications occur, with the lung being the most commonly compromised organ. Other extraintestinal complications include cellulitis, urinary tract infections, pneumonia, endocarditis and meningitis (Arii *et al*. 2002).

2.9.4. Chronic carrier state

The status of chronic carrier is defined as the shedding of bacteria in stools for more than a year after the acute stage of *Salmonella* infection. Since humans are the only reservoir of typhoid *Salmonella*, carriers of *S. Typhi* and *S. Paratyphi* are responsible for the spreading of enteric fever in endemic regions, as the common transmission route is the ingestion of water or food contaminated with the faeces of chronic carriers. About 4% of patients with enteric fever, predominantly infants, elderly people and women, may become chronic carriers

(Gonzalez-Escobedo *et al*., 2011). In contrast, the carrier state of NTS is less frequent, with an occurrence rate of 0.1% in patients with non-typhoidal Salmonellosis. This is because the primary reservoir of NTS is animals, instead of humans (Hohmann, 2001).

2.10. Diagnosis of Salmonellosis

Diagnosis is based on the isolation of the organism either from tissues collected aseptically from necropsy or feces, milk, blood, rectal swabs or environmental samples. When infection of the reproductive organs or conceptus occurs, it is necessary to culture fetal stomach contents, placenta and vaginal swabs and, in the case of poultry, egg contents. However, Salmonellosis is particularly difficult to determine in clinically normal carrier animals. Because of the multitude of *Salmonella* serovars serotyping is of great importance. It represents an important prerequisite for the detection of the source of infection and the route of transmission (OIÉ, 2000).

2.11. Isolation and Identification of *Salmonella*

The isolation and identification of *Salmonella* can be performed using techniques recommend ed by International Organizations for Standardization (ISO-6579, 2002). ISO-6579:2002 is sensitive, but complex and expensive. In food, *Salmonella* may also be present in low numbers in addition to a lot of other micro-organisms, and they may be injured. To diminish the risk of obtaining false negative results, a non-selective pre-enrichment of large faeces or food sample, a combination of two selective enrichments and plating on two selective media are performed; Pre-enrichment in non-selective medium (buffered peptone water); Selective enrichment in Tetrathionate broth (Müller-Kauffmann), Rappaport Vassiliadis soy peptone (RVS) broth and selenite F broth; Subcultivation on Xylose Lysine Desoxycholate (XLD) agar and on Brilliant Green agar (BGA) (or another selective agar media).Is carried out after 24 h and 48 h of incubation. Up to five colonies per plate have to be confirmed, which may potentially involve the confirmation of up to 40 presumptive colonies (ISO-6579, 2002). Conventional cultural methods for the detection of foodborne *Salmonella* species generally consist of five distinct and successive steps. These are pre enrichment in nonselective media

and selective enrichment in broth media, plating on differential agar, biochemical screening a nd serological conformation (ISO 6579:2002).

2.12. Salmonellosis in Ethiopia

Salmonella species are leading causes of acute gastroenteritis in several countries and remains an important public health problem worldwide, particularly in the developing countries. It is the most common food borne disease in developing countries, although incidence rates vary according to the country. The fecal wastes from infected animals and humans are important sources of bacterial contamination of the environment and the food chain. In Ethiopia, as in other developing countries, it is difficult to evaluate the burden of Salmonellosis because of the limited scope of studies and lack of coordinated epidemiological surveillance systems. In addition, under-reporting of cases and the presence of other diseases considered to be of high priority may have overshadowed the problem of Salmonellosis (Getnet *et al*., 2014).To date, published or unpublished research reports from different health institutions in Ethiopia show that Salmonellosis is a problem and that *Salmonella* exist in humans, animals, animal food products and other foods in the country (Heko, 2017).Some studies conducted from different source show as meat, super market, and from hospitals.At abattoir; Sibhat *et al*.(2011), Nyeleti *et al*.(2000), Alemayehu *et al.* (2003), Teklu and Nigussie (2011), Molla *et al.* (2004); mince meat Ejeta *et al.* (2004), Molla and Mesfin (2003), Tibaijuka *et al*.(2003), Nyeleti *et al* .(2000), Edris *et al*. (2011), Zewdu and Cornelius (2009);dairy farm Addis *et al*. (2011), Tesfa w *et al*.(2013); hospital cases Beyene *et al*.(2011), Reda *et al*. (2011),Adabara *et al*.(2012).

2.13. Antimicrobial Susceptibility Tests and Resistance Profile of *Salmonella*.

Generally, the increased application of antimicrobials in veterinary and human medicine has b e en implicated as a contributing factor in the emergence of antimicrobial resistan pathogens a nd the evolution of multiple drug resistant strains.The increased level of drug resistant *Salmon ella* has become a problem in all countries, though the extent varies. Developing countries tend to have a highlevel of resistant *Salmonella*. Antimicrobial use in animal production systems has long been suspected to be a cause of the emergence and dissemination of antimicrobial resistant (AMR) *Salmonella*. Using antimicrobial agents for cattle have been implicated as a source of human infection with AMR *Salmonella* through direct contact with

livestock and consumption of raw milk, meat and contaminated material. AMR *Salmonella* are increasing due to the use of antimicrobial agents in food animals at sub therapeutic level or prophylactic doses that may promote growth and markedly increase the human health risks associated with consumption of contaminated milk and meat products (Zewdu and Cornelius, 2009) through mutation, acquisition of resistance encoding genes and irrational use of antimicrobials in food animals (Beyene *et al*., 2011).

Antimicrobial resistant bacteria pose a growing problem of concern, worldwide since the bacteria can be easily circulated in the environment. Effectiveness of current treatments and ability to control infectious diseases in both animals and humans may become hazardous. A relatively high number of strains are resistant to the antimicrobial commonly used in the therapeutic protocol of many humans and animal infections (Roca *et al*., 2015).

Globally, the incidence of *Salmonella* infection associated with multi-drug resistance (MDR) has increased in the last few decades. In Ethiopia, although there are a few studies on prevalence of *Salmonella* and antimicrobial susceptibility in humans, animals, and food of animal origin, there is no integrated surveillance and monitoring to establish the major serotypes responsible for non typhoidal Salmonellosis in humans and the associated risk factors. Moreover, most of the studies conducted in humans involved pediatric diarrheic patients and the isolates recovered from these patients were not serotyped (Eguale *et al*., 2015).

Different classes of antibiotics are used for the treatment of Nontyphoidal Salmonellosis. There has been increasing number of reports of drug resistance in *Salmonella* and it is quite worri some because *Salmonella* is one of the most common causes for food-borne gastroenteritis worldwide. There are several studies confirming the use of antibiotics in the food of animals as the source for multidrug resistant *Salmonella* serovars. Multidrug resistance is often associated with mobile genetic elements like plasmids and transposons that encode multiple resistance genes. With globalization and increasing global travel, worldwide spread of these multidrug resistant strains is of particular concern (Maanasa and Harish, 2017).

There are three test methods (disk diffusion, broth dilution and agar dilution). Antimicrobial susceptibility testing methods that consistently provide reproducible and repeatable results is when followed correctly (CLSI, 2015). *Salmonella* isolates were subjected to in-vitro susceptibility test against commonly used antimicrobial agents using disk diffusion method following guidelines established by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2015). Antimicrobials used for susceptibility testing of *Salmonella* species were Amoxicillin (AML25 µg), Ampicillin (AMP10.µg), Tetracycline (TE30.µg), Gentamicin (CN10.µg), Sulfamethoxazole-Trimethoprim (SXT25.µg), Ceftriaxone (CRO30.µg), Nitrofuranthoin (F10.µg), and Nalidixic acid (NA-30 .µg)(CLSI, 2015).

2.14. *Salmonella* Antimicrobial Resistance patterns in Ethiopia

In recent years, *Salmonella* related diseases have been documented by several food related studies conducted in different parts of Ethiopia. An increased in the resistance of *Salmonella* to commonly used antimicrobials has been also noted in both public health and veterinary sectors in Ethiopia. Antimicrobial resistance is a natural consequence of infectious agents' adaptation to exposure to antimicrobials used in medicine, food animals, crop production and use of disinfectants in farms and households. However, scarcity of surveillance data on the incidences of *Salmonella* species associated with eggs and its antimicrobial resistance pattern in the poultry farm is a major epidemiological issue. Despite some attempts to study prevalence of *Salmonella* in Ethiopia, mainly in meat and meat products (Tessema *et al*., 2017) *Salmonella* isolated from different sources in Ethiopia reflect variable resistance profiles to different antimicrobial agents used in medical and veterinary medicine.The frequency of studied bacteria corresponds with the frequency of their implication in infectious diseases in the region, their potential for transmission to humans and their high rates of resistance to available treatment regimens (Ampicillin, Chloramphenicol, Ciprofloxacillin, Cotrimoxazole, Gentanycin, and Tetracyclin) (Melaku and Minyahil, 2017). In studies conducted in Ethiopia, Addis *et al.* (2011) reported low resistance (0-16.7%) to Chloramphenicol in *Salmonella* isolates from human cases, but Reda *et al.* (2011), Beyene *et al*. (2011) and Asrat (2008), respectively, reported 62.3%, 81.4% and 83.7% resistance to the same drug. In non-human cases, however, Wandili *et al.* (2013), and Li *et al*. (2012) reported lower resistance rates to most of the drugs they used for investigation.

Frequent resistance to tetracycline by S*almonella* from animal and human cases was reported from Ethiopia (Asrat, 2008; Beyene *et al.*, 2011; Addis *et al*., 2011).

2.15. Treatment

Treatment of non-typhoidal *Salmonella* infection is different from typhoidal infection. In treatment of non-typhoidal *Salmonella* infection antibiotics should not be used routinely, as used in typhoid. Antibiotic should be only used if required as most infection with non-typhoidal *Salmonella* is self-limiting type and duration of diarrhea and fever are not much affected by use of antibiotics. Additionally, antibiotic therapy can increase relapse of infection and prolong the duration of gastrointestinal carrier states. The main treatment should be aimed at correcting dehydration that may arise due to prolonged diarrhea by fluid and electrolyte replacement (Kemal, 2014). 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. The high concentration of amoxicillin and quinolone in bile and the superior intracellular penetration of quinolone are theoretical advantages over trimethoprim sulfamethoxazole (WHO, 2003).

2.16. Prevention and Control

Control and prevention of Salmonellosis is difficult because of its distribution in nature in all types of climate and harbored in human and many various animal species. Furthermore, compared with other Gram-negative organisms, *Salmonella* are relatively resistant to different environmental factors therefore, a periodic surveillance of the level of *Salmonella* contaminati on in the different food animals, food products and environments is necessary to control the spread of the pathogen and infection in human(Abunna *et al*., 2017).

There is no vaccine to prevent Salmonellosis in human whereas, vaccine against *Salmonella typhi* has been developed, especially in children, but is only 60% effective. Currently, only one vaccine exists against typhoid, an illness caused by *Salmonella* bacteria. It is particularly recommended for travellers (from the age of 2) to countries where hygiene is left wanting. It is administered in only one injection given at least two weeks before departure and provides

partial protection (from 50 to 80 per cent) for three years (Tennant *et al*., 2016; UTMBG, 2017).

People should not eat raw or uncooked meat, they should not drink raw milk or unpasteurized dairy product, cross contamination of food should be avoided. Uncooked meat should be kept separate from cooked food ready to eat. Hands, cutting boards or knifes and other utensils should be washed thoroughly after handling uncooked food. Hands should be washed before handling any food and in between handling different food items. People should have to wash their hands after contact with animal‘s feces (Bhunia, 2008).

Treating municipals water supply for reducing risk of *Salmonella* infection, improvement in farm animal hygiene in slaughter process in food harvesting and in packaging operation have helped to prevent Salmonellosis. A periodic surveillance of the level of *Salmonella* contamination in different food product and environment is necessary to control spread of the pathogen. Reducing *Salmonella* prevalence requires comprehensive control strategy in animal and animal food stuff with restriction in the infected flock until they have been cleaned from infection. Ensuring safe food production requires knowledge on the nature and origin of animal, animal feed, the health status of animals at the farm, the use of veterinary medicinal data regarding anti-mortem and postmortem findings and the risk association with post harvests production strategies (Kemal, 2014).

Prevention and control in animals Condition that contribute to an increasing incidence of epidemic Salmonellosis include large herd size, more intensive and crowded husbandry and the trend of free-stall barn with loose housing, which contribute to the feacal contamination of the entire premise. When Salmonellosis has been confirmed in a herd, the following control measure should be considered; isolate obviously affected animals to one group if possible, treat severely affected animals, affected animals institute measure to minimize public health concern like (no raw milk should be consumed) physically clean the environment and disinfect the premise following resolution of the outbreak or crises period (Huston *et al.,* 2000).

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted between December 2016 and January 2018 in Jimma town which is located in Jimma Zone Southwestern Ethiopia at distance of 352Km from Addis Ababa. The town is bordered by Kersa Wereda in the east, with Manna Wereda in the north, Manna and Seka Chekorsa werda in the West and Dedo wereda in the South. Geographically, the area lies within a latitude of 7°40′N and longitude 36°50′E. And it has an average altitude of 1780 meter above sea level. The climatic condition of the town is in *Weina Dega* with moderate heavy rainfall from June to September with mean annual rainfall of 1500 mm. The town is generally characterized by warm climate with mean annual minimum and maximum temperature of 14°C and 30°C respectively. This is considered ideal for agriculture as well as human settleme nt (JZMSR, 2004).

According to CSA (2007) report population and housing census of Ethiopia, the total population of the Jimma town is 177,900, with 49.7% and 49.3% female and male, respectively (CSA, 2015). Jimma town has the total area of 4,623 hectares and divided into 13 urban Kebele. According to CSA (2016/17) agricultural survey report, the livestock population of the Jimma zone has estimated to 2,324,110 cattle (CSA, 2016). The present study was conducted on abattoir and butcher shops found in Jimma town. There is one municipal abattoir and 74 meat butchers who directly get slaughter service from the abattoir. Many people consume raw meat products like minced raw meat locally called '*kitfo'* raw chopped meat locally called '*kurt*' and '*dullet*'.

3.2. Study Population

The study population was comprised carrcass of cattle slaughtered at Jimma municipal abattoir and meat from butchershops as well as their workers.

3.3. Inclusion and Exclusion Criteria

3.3.1. Inclusion Criteria

All cattle of both sex without distinction to breed and age and slaughtered in Jimma municipal; All abattoir workers who were direct contact with carcasses during carcasses production; All beef butcher shops; All meat cutters at butcher shop;Knife which used for

evisceration, dressing and splitting of carcasses

3.3.2. Exclusion criteria

Other livestock slaughtered in Jimma town abattoir other than cattle and fetuses were not included in to the study; Cattle not slaughtered within the abattoir; Abattoir worker who were not directly employed on carcass production and Other types of meat seller not included, other than beef.

3.4. Study Design

A cross sectional study design was used with simple random sampling technique to select samples of carcass from both abattoir and selected 46 butcher shops.

3.5. Sample Size Determination

3.5.1. Sample Size

Sample size was determined according to formula developed by (Thrusfield, 2007).

$$n = \frac{1.96^2 \times P_{exp}(1 - P_{exp})}{d^2}$$

Where: n= required sample size; P =expected prevalence; d=absolute precision.

For this study purpose the sample size was calculated based on pooled expected prevalence of 4.53%, 95% confidence interval and 5% error margin from municipal abattoir (Tadesse and Gebremedhin, 2015). Accordingly, the minimum sample size was 67 and the sample size was inflated by 5% to increase the precision of the estimate resulted and 70 samples were collected from abattoir.

For butcher shops the sample size was calculated based on pooled expected prevalence of 4.53%, 95% confidence interval and 5% error margin (Tadesse and Gebremedhin, 2015). Accordingly, the minimum sample size was 118 and the sample size was inflated by 5% to increase the precision of the estimate resulted 124 samples. But, there is only 74 butcher house in the town and sample size adjustment was made by using the finite population correction factor formula developed by Rose *et al*. (2014).

$$n_a = \frac{n^r}{1+\frac{n^{r-1}}{N}}$$

Where: n_a= the adjusted sample size, n^r = the original required sample size and N = population

Hence there were n^r= 124 and N= 74.

$$n_a = \frac{124}{1+\frac{124-1}{74}} = \frac{124}{1+\frac{123}{74}} = \frac{124}{1+1.7} = \frac{124}{2.7} = 46$$

Finally, adjusted sample size for butcher shops was 46.

3.6. Sampling Procedures and Sample collection

The standard procedure of Meat Hazard Analysis and Critical Control Point (2002) was followed for sample collection from carcasses (Annex 11). Briefly, after random selection of the carcass, the pooled carcass swab was taken from neck, brisket, flank and rump of slaughtered cattle by swabbing vertically, horizontally, and diagonally for not less than 20 seconds using a sterile 100cm^2 template and putting as much pressure as possible using the sterile cotton tipped swab (2X3 cm) fitted with shaft (stick) which is inserted into buffered peptone water (Annex 6) and the stick was broken (Figure 2). In order to prevent duplication of the isolation the swabs was taken from the knife and hands of the workers before touching the carcasses. Then the swabs were taken immediately following carcass splitting (dressing) from the carcass also. The swab samples in BWP were stored in an ice-box at 4^{0}C and then transported to the Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) Microbiology laboratory within an hour of sample collection.

Lists of butcher shops were obtained from Urban Agricultural, Environmental and Health Office of Jimma town, then using simple random techniques from 74 butcher shops 46 were selected for sample collection and from selected butcher shop the source of samples (Out of 210 swab samples collected from abattoir, 70 were from carcass, 70 from slaughter hand and 70 from working knifes collected. Among 138 swab samples collected from butcher shops, 46 collected from carcass, 46 from hands and 46 from chopping boards).

Questionnaire and observation was used to collect data on risk of *Salmonella* contamination to meat, hand and knives. These are educational level, personnel hygiene, and knowledge of

source of contamination, meat storage facility, and way of beef consumption, hygienic status of abattoir and butcher shops, availability and accessibility to clean water (Annex 1, 2 and 3). The knowledge and practice of consumers of meat was also assessed using questionnaire tool on risk of *Salmonella* as zoonosis and transmission from meat to human.

3.7. Study Methodology

3.7.1. Isolation and Identification

Isolation and identification of the *Salmonella* was done using techniques recommended by International Organizations for Standardization (ISO-6579, 2002) (Annex-4). Briefly the following isolation activities were done.

3.7.1.1. Pre-enrichment and selective enrichment

The swabs in Buffered peptone water were incubated at 37°C for 18-21 hrs. One ml of the pre-enriched sample was pipetted into 0.1ml of pre-enriched broth was transferred into a tube containing 10 ml of Rappaport-Vassiliadis medium (RV broth) and incubated at 41.5°C for 24 hours (Figure 3) Another 9ml Selenite F broth and incubated at 37°C for 24 hours (Figure 4) (Annex 4).

3.7.1.2. Plating out and identification

A loop full of inoculums from each RV and Selenite F broth cultures were plated onto XLD and BGA plates and incubated at 37°C for 24 hours. After incubation, the plates were examined for the presence of typical and suspect colonies (Figure 5 and 6). The suspected colonies were stored on nutrient broth at +4^{o}C (Annex 4).

3.8. Biochemical tests for Identifications of *Salmonella* Isolates

Young colony were cultivated on *Salmonella* selective media (XLD or BGA) then inoculated for biochemical tests by using; Triple Sugar Iron (TSI) agar, Lysine Iron agar, Voges-Proskauer (VP) broth, Urea broth, Indole test, and Citrate utilization tests. The detail procedures used for identification of *Salmonella* was shown in (Annex 5).

I. Triple Sugar Iron Agar

Salmonella isolates were identified by confirmatory biochemical tests, where one is, its sugar fermentation ability. The presumptive *Salmonella* colonies were directly butt stabbed and streaked onto the TSI agar slant and incubated with loosened caps for 24 h at 37^0C. *Salmonella* can ferment glucose, sucrose and lactose and produce H_2S which is indicated as blackish discoloration of the inoculated media. The presence of alkaline (red) slant and acid (yellow) butt, with or without production of H_2S was considered as presumptive for *Salmonella* (Andrews and Hammack, 2003) (Figure 7).

II. Lysine Iron Agar

An inoculum from a pure culture was transferred aseptically to a sterile tube of lysine decarboxylase agar. The butt was stabbed and the slant was streaked and incubated at 37^0C for 24 hour (Figure 8). The final results are then obtained by observing the tube at 48 hours. Change back to purple from yellow indicates a positive test for lysine decarboxylase. Failure to turn yellow at 24 hours or to revert back to purple at 48 hours indicates a negative result (ISO 6579, 2002).

III. Simmons Citrate Agar

To check for citrate utilization as a sole source of carbon the slant was streaked and the tube was incubated at 37^0C for 24 hrs. The presence of growth and color change from green to blue is considered as presumptive for *Salmonella (*Figure 2) (ISO-6579; 2002).

IV. Urease test

Suspected isolates were inoculated on urea broth and was incubated at 37^0C for 24 hrs (Figure 3). The absence of color change is negative for *Salmonella* (ISO-6579; 2002)(Annex-5)

V. Voges-Proskauer test (VP)

An amount of 2 ml of sterile glucose phosphate peptone water were inoculated with 5 ml of test organisms. It was incubated at 37^0C aerobically for 48 hours. A very small amount (knifepoint) of creatine was added and mixed. 3 ml of sodium hydroxide were added and shacked well. The bottle cap was removed and left for an hour at room temperature. It was observed closely for the slow development of pink color for positive cases. In negative isolate for *Salmonella* there is no development of pink color (Figure 11) (Annex 5).

VI. Indole test

Two ml of peptone water was inoculated with 5 ml of bacterial culture and incubated for 24hours. Kovac‘s reagent (0.5 ml) was added and mixed thoroughly. The tube was then allowed to stand for a while. The appearance of red color on the whole medium is considered as a positive test for the production of indole by the organisms (Figure 12) (Bachoon, 2008) (Annex 5).

3.9. Antimicrobial susceptibility testing

Susceptibility of the *Salmonella* isolates to a panel of locally available new generation and com monly used 12 antimicrobials in both human and animal health sectors were determined using the Kirby-Bauer disk diffusion method according to the guidelines of the Clinical and Laborato ry Standards Institute (CLSI, 2015). The following antimicrobials (Sensi-Discs, Becton, Dickin son and Company, Loveton, USA) and disc potencies (µg) were used: namely Ampicillin (10), Cefoxitin (30), Chloramphenicol (30), Ciprofloxacin (5), Gentamicin (10), Kanamycin (30), an d Nalidixic acid (30), Neomycin (30), Norfloxacillin (10), Streptomycin (10), Trimethoprim (5) and Tetracycline (30). Briefly, the *Salmonella* spp were suspended in physiological saline unt il the turbidity was equivalent to 0.5 McFarland standards. The bacterial suspensions were inoc ulated on to Mueller Hinton agar, and disks were placed on the inoculated agar. The inoculated plates were incubated at 37ºC for 24 h). The interpretation of the categories of susceptible, intermediate or resistant was based on the CLSI guidelines (Annex-7) (Figure 13). For the purpose of analysis; all readings classified as intermediate were considered as resistant unless indicated. E. coli ATCC 25922 was used as a control organism (CLSI, 2015).

3.10. Data Management and Analysis

Data generated from laboratory investigations and questionnaire surveys were recorded and coded using Microsoft Excel spread sheet (Microsoft Corporation). The statistical analysis (both descriptive and inferential analysis) was done using Statistical Package for Social Sciences (SPSS) IBM version 20 software). Fisher exact test statistical tool was used to analysis the association between the *Salmonella* isolates (as response variable) and hypothesized contamination risk factors of beef meat (independent variables). P-value <0.05 was considered statistically significant.

3.11. Quality Control

Study populations were selected before data collection. Questionnaire was administered and adjusted for its appropriateness prior to the actual data collection. Samples were identified and coded clearly and appropriately. The collected samples were transported under cold ice box and stored at 4^{o}C until tests performed. Standard Operational Procedures (SOPs) were followe d at each laboratory activities.

4. RESULTS

4.1. Identification of *Salmonella* by Conventional Culture

Out of 210 swab samples collected from abattoir 24 of them were found positive for *Salmonella*.Where 10 from carcasses, 6 from hand and 10 from knife. On other hand out of 138 swabs collected from butcher shops 9; 4, 2, and 3 samples were found positive for *Salmonella* from meat, hands of meat cutter and chopping board respectivel. The overall prevalence of *Salmonella* infection for sample collected from abattoir was found to be 11.4 % (24/210) while those from butcher shops was 6.52 %(9/138)(Table 2).

Table 2: Frequency of *Salmonella* isolates from abattoir and butcher shops of Jimma, Ethiopia

Sample sours	Sample location	Sample tested	No of Positive (Prevalence %)	95% CI
Abattoir	Carcasses	70	10 (14.29)	7.07 - 24.71
	Hand	70	6 (8.57)	3.21 - 17.73
	Knives	70	8 (11.43)	5.02 - 21.28
	Total	**210**	**24 (11.43)**	**7.46 - 16.53**
Butcher shop	Carcasses	46	4 (8.70)	2.42 – 20.79
	Hand	46	2 (4.35)	0.53 – 14.84
	Chopping board	46	3 (6.52)	1.37 – 17.90
	Total	**138**	**9 (6.52)**	**3.03 -12.02**

4.1.1. Risk factors for beef contamination at abattoir

AChi-square analysis revealed that on (Table 3) the following contamination risk factors have an association with *Salmonella* isolates from abattoir (educational status, job relate training, co ntamination risk perception, neatness of knives, source of contamination). There was differenc e in proportion of *Salmonella* positive result among different educational status of Jimma abatt oir workers. From the total 8 uneducated personals, 5(62.5%) swab sample obtained were *Salmonella* positive, out of 28 swabs from workers who attended primary school, 6(21.43%)

swabs were *Salmonella* positive. Educational status was statistically associated with *Salmonell a* isolation (OR=8.40, 95%CI=1.186-59.493%, P=0.049) with higher contamination rate in respondents having low level of education than those attended primary and secondary educational levels. The probability of carcasses contamination have 8 times more chance than abattoir worker those attended secondary school (Table 3).

The higher prevalence was observed in respondents unattended job related training. Out the 29 untrained butcher men interviewed, 11(37.93%) of them were found positive for contamination of carcasses by *Salmonella* whereas out of 20 samples collected from 20 trained personnel on food handling practice, only 2 (10.00%) samples were found positive for *Salmonella* isolates. The probability of *Salmonella* isolation was 6 times more likely on those did not taken job related training abattoir workers than who attended job related training (OR=5.5, 95% CI: 1.065-28.416%, P= 0.047) (Table 3).

Higher frequency of *Salmonella* positive results 6(54.55%) was revealed among sample obtain ed from 11 abattoir workers those have lack of perception on contamination of carcasses in sla ughtering process as a risk whereas out of 38 workers had perception on contamination of carc asses in slaughtering process as a risk, 7(18.42%) personnel were isolated positive outcome. This result shown us personnel who had lack of knowledge on contamination in slaughtering process were significantly associated for positive result of *Salmonella* (OR=5.31, 95% CI=1.26 -22.489%, P=0.047). Contamination of carcasses with *Salmonella* has 5 more likely on abattoir worker those who had lack of perception on contamination as risk than those have perception on contamination as risk (Table 3).

Out of 34 workers used unclean knifes, 12 (35.29%) of them were *Salmonella* positive resulted. However, abattoir worker who used clean knife were 15 among them 1(6.67%) explored *Salmonella* positive outcome. Dressing of carcasses at abattoir by using unclean knife was 7.6 times more probability to contaminate the carcass than by using clean knife. Therefore, use of unclean knife was significantly associated with the positive isolates of *Salmo nella* (OR=7.6, 95% CI=0.892-65.376%, P=0.043) (Table 3).

Carcass was contaminated at abattoir during slaughtering from different source of contaminants, Out of 14 fecal contaminated carcass, 8 of them were *Salmonella* positive

Similarly, out of 13 dirty equipment s and hand sampled 2 samples of were also *Salmonella* positive. Water used and floor of slaughter house were also a source of contamination, Out of 22 samples collected from both sources, 3 (13.64%) sample were *Salmonella* positive. Fecal source of contamination were significantly associated with *Salmonella* contamination of positive results (OR=8.44, 95% CI=1.682-42.39%, P=0.014) fecal source contamination of carcasses risk was 8 times more than water and floor and the risk of *Salmonella* contamination of due to dirty equipment and hand was one times more likely higher as compared to water and floor (OR=1.15, 95%CI=0.166-7.99) (Table 3).

Even though positive results observed in samples collected from dirty apron and improperly placed equipment's, both are not statistically associated the positive results (P>0.05) as shown in table.3

Table 3: Risk factors for beef contamination by *Salmonella* at Jimma abattoir workers

Factor	Category	No of sample	Positive Sample (%)	95% CI	OR (95%CI)	Fisher exact test P-value
Educational Status	Uneducated	8	5(62.5)	24.49-91.48	8.40(1.186-59.493)	0.049
	Primary School	28	6(21.43)	8.30-40.95	-	
	Secondary School	13	2(15.38)	1.92-45.45	R	
Job related Training	No	29	11(37.93)	20.69 – 57.74	5.50(1.065-28.416)	0.047
	Yes	20	2(10.00)	1.23 – 31.70	R	
Contamination risk perc eption	No	11	6(54.55)	23.38 – 83.25	5.31(1.256-22.489)	0.047
	Yes	38	7(18.42)	7.74 – 34.33	R	
Clean clothing	No	39	11(28.21)	15.00 – 44.87	1.57(0.287-8.595)	0.709
	Yes	10	2(20.00)	2.52 – 55.61	R	
Neatness of Knives	No	34	12(35.29)	19.75 - 53.51	7.6(0.892-65.376)	0.043
	Yes	15	1(6.67)	0.17 – 31.95	R	
Source of contamination	Fecal	14	8(57.14)	28.86 – 82.34	8.44(1.682-42.39)	0.014
	Handling with dirty equ ipment and hand	13	2(15.38)	1.92 – 45.45	1.15(0.166-7.990)	
	Water and floor	22	3(13.64)	2.91 – 34.91	R	
Proper placing of equipment	No	8	1(12.50)	0.32 – 52.65	2.90(0.321-26.158	0.663
	Yes	41	12(29.27)	16.13 – 45.54	R	
Total		**49**	**13 (26.53)**	**0.1495-0.4108**		

Key: R= reference

4.1.2. Butcher shop questionnaire survey of carcasses contamination associated risk factors and *Salmonella* occurrence

As a chi-square result shown in table 4 among the butcher men uneducated have a statistically significant associated contamination of carcasses for *Salmonella* isolates (OR=9.17,95%CI 1.15-73.239%), P=0.037). Out of 9 uneducated butcher men, 4(44.4%) were positive swab sampled, but the remaining 14 personnel who attended primary level and 23 secondary school among that 1(7.14%) and 2(8.70%) frequency of *Salmonella* isolate respectively. Butcher worker had 9 times more to probability of contamination of carcasse by *Salmonella* than those attended secondary school (Table 4).

With regard to the manner hand washing before handling carcasses during selling, 15 butcher men washing their hands with water only. Among these, 5 (33.3%) of them were *Salmonella* positive. However, the remaining 31 butcher men were Using detergents and water to wash their hands, of which 2(6.45%) were *Salmonella* positive. Manner of hand had a Statistically Significant association with *Salmonella* isolate isolates from butcher house (OR=7.25, 95%CI=1.210-43.442%, P=0.029). The risk of Carcasses contamination with *Salmonella was* 7 times more likely higher in meat cutters wash their hands with water only than those wash their hands with water and detergent (Table 4).

In this study from 46 shops, 12 butcher men without worn garment (gown) while selling meat, 5 (41.67%) of *Salmonella* was isolated from the butcher shops by men without wearing garment; the remaining 34 worn garment and 2 (5.88%) of *Salmonella* were isolated from butcher those worn protective garment. There was statistically significant association of wearing garment during selling meat and the positive isolate of *Salmonella* in butcher shops (OR=11.42, 95% CI=1.829-71.419%, P=0.009). A carcasses swab sample from butcher men selling meat without wearing of garment had 11 times more chance of contamination of carcasses (Table 4).

In current study Meat cutter in butcher shops collecting money with hand during selling meat was strongly associated for *Salmonella* positive (OR=9.69, 95% CI=1.578-59.47%, P=0.014) Thirty two butcher shops collecting money by cashier, of which 1(3.12%) were *Salmonella* positive. On other hand, 14 butcher men collected money with hand while selling meat; *Salmonella* was isolated from 6(42.86%) personnel. The risk of carcass to be contaminated by

Salmonella was 10 times more likely higher in butcher men collecting money by themselves during selling of meat than those butcher men collecting money by cashier (Table 4).

Table 4: Risk factors for beef contamination by *Salmonella* at Jimma butcher shops

Factor	Category	No of Sample	Positive Sample (%)	95 % CI	OR (95%CI	Fisher exact test P-value
Educational Status	Uneducated	9	4(44.44)	13.70-78.8.	9.17(1.15-73.239)	0.037
	Primary	14	1(7.14)	1.27-31.47	-	
	Secondary	23	2(8.70)	1.07-28.04	R	
Wearing protective Cloth	Not used	12	5(41.67)	15.17 -72.33	11.42(1.829-71.419)	0.009
	Used	34	2(5.88)	0.72- 19.68	R	-
Manner of hand washing	Rinsing with water only	15	5(33.3)	11.82 -61.62	7.25(1.210-43.442)	0.029
	Using detergents and water	31	2(6.45)	0.79 -21.42	R	-
Handling of money	Butcher with hand	14	5(35.71)	12.76 -64.86	9.69(1.578-59.474)	0.014
	Cashier	32	2(6.25)	0.77 -20.81	R	-
	TOTAL	**46**	**7(6.34-28.87)**			

Key: R= reference

4.2. Consumer Knowledge, Attitude and Practice (KAP) towards Risk of Consumption of Raw Beef in Jimma, Ethiopia

One hundred thirty eight interviewed based on the questionnaire comprised the area of interest such as demography, habites of raw meat consumption, preference of meat and knowledge on S*almonella* was administered. From interviewed consumers 56 (40.6%) prefer to buy fresh beef; 89(64.5%) consuming raw beef and 61(44.2%) have the history of food poisoning. The majority 126(91.3 %) believe that Meat slaughtered in abattoir is always safe to eating and 121(87.7%) cooked meat is always to consume. 102(73.9%) of the participant did not heard about *Salmonella* bacteria and 110(79.7%) did not know the consumption of raw beef the source of *Salmonella* (Table 5).

Table 5: Knowledge, Attitude and Practice of the Beef Consumers

Factor	Category	Frequency	Percentage (%)
Sex of respondent	Male	98	71.0
	Female	40	29.0
Age	15-30 yrs.	40	29.0
	31-45 yrs.	54	39.1
	46-60 yrs.	27	19.6
	61 yrs. and above	17	12.3
Educational status	Illiterate	0	0
	Primary school	56	40.6
	Secondary school	62	44.9
	University	20	14.5
Priority criterion to	Freshness	56	40.6
Purchase beef meat	Low cost	45	32.6
	Low fat content	37	26.8
Consume raw beef	Yes	89	64.5
	No	49	35.5
Do you think cooked	Yes	121	87.7
Meat is safe to eat?	No	17	12.3
Historyof foodpoisoning	Yes	61	44.2
	No	77	55.8
Is Meat slaughtered in abattoir	Yes	126	91.3
Always safe to eating?	No	12	8.7
Haverefrigerator?	Yes	62	44.9
	No	76	55.1
Have you Heardavbout	Yes	36	26.1
Salmonella?	No	102	73.9
Do you Know meat can act	Yes	28	20.3
as Source of *Salmonella?*	No	110	79.7

4.3. Antmicrobial Susceptibility Test on *Salmonella* Isolates both from Abattoir and Butcher Shops

4.3.1. Frequency of Mono Antimicrobial Resistance Distribution

Antmicrobial susceptibility features of 33 *Salmonella* isolates which were isolated from abatto ir and butchers shop were tested to 12 antimicrobials to evaluate their resistant level. Among these 24 isolates from abattoir, 16(66.7%), 14(58.3%) and (14)58.3% isolates were highly resistant to Streptomycin, Tetracycline and Ampicillin, respectively. However no isolate was resistant to Norfloxaciline, Gentamicin and Ciprofloxaciline drugs. Pertaining to intermediate resistance of an isolate to a panel of antimicrobials, a relatively higher intermediate resistance was observed to Chloramphenicol (29.2%), Neomycin (29.2%), Kanamycin (25%) and Nalidixic acid (20.8%). In addition to this, all (100%) isolates from abattoir were susceptible to Norfloxaciline, Gentamicin and Ciprofloxacillin (Table 6) ((Annex 8).

Of the nine isolates of *Salmonella* from butcher shops, 6(66.7%), 5(55.6%) and 5(55.6%) isolates were resistant to Streptomycin, Tetracycline and Ampicillin,respectively. while no isolate of *Salmonella* from butchershops was resistant to Norfloxaciline, Gentamicin and Cipr ofloxacillin drugs . A relatively higher intermediate resistance of an isolate to Chloramphenic ol (22.2%) was observed compared to the rest antimicrobials. However, none of an isolate showed an intermediate resistance to Trimethoprim, Norfloxaciline, Gentamicin and Ciprofloxacillin (Table 6) (Annex 9).

Table 6: Mono Antimicrobials Profile (Resistant/Susceptible) Isolates of *Salmonella* from Abattoir and Butcher Shops in Jimma

Antibiotics	Resistant (%)		Intermediate (%)		Susceptible (%)	
	Abattoir	Butcher	Abattoir	Butcher	Abattoir	Butcher
Tetracycline	**14(58.3)**	**5(55.6)**	3(12.5)	1(11.1)	7(29.2)	3(33.3)
Streptomycin	**16(66.7)**	**6(66.7)**	3(12.5)	1(11.1)	5(20.8)	2(22.3)
Trimethoprim	2(8.33)	2(22.2)	0	0	**22(91.67)**	**7(77.8)**
Chloramphenicilole	4(16.7)	2(22.2)	7(29.2)	2(22.2)	13(54.2)	5(55.6)
Kanamycin	4(16.67)	3(33.3)	7(29.17)	0	13(54.6)	6(66.7)
Norfloxaciline	0	0	0	0	**24(100)**	**9(100)**
Gentamicin	0	0	0	0	**24(100)**	**9(100)**
Ampicillin	**14(58**.3)	5(55.6)	4(16.67)	1(11.1)	6(25)	3(33.3)
Ciprofloxacin	0	0	0	0	**24(100)**	**9(100)**
Cefoxitin	2(8.33)	1(11.1)	3(12.5)	1(11.1)	19(79.2)	7(77.8)
Neomycin	5(20.8)	3(33.3)	7(29.2)	1(11.1)	12(50)	5(55.6)
Nalidixic acid	2(8.33)	2(22.2)	7(29.2)	2(22.2)	15(62.5)	5(55.6)

4.3.2. Multi-drug resistance isolates of *Salmonella*

Out of 24 isolates from abattoir, 14(58.3%) were resistant to two or more panel of antibiotic discs. Among 14 isolates,5(35.7%) were resistant to three antibiotics (Tetracycline, Ampicilli n and Streptomycin) followed by 2 isolates which was resistant to six antibiotics (Tetracycline ,Streptomycin, Chloramphenicol ,Kanamycin, Ampicillin and Neomycin) whereas only two is olates from butcher shops were resistant to five different antibiotics as demonstrated in (Table.7).

Table 7: Multiple Antimicrobials Resistance Profile of *Salmonella* Isolates from Abattoir and Butcher Shops in Jimma Town, Ethiopia

No Of Antibiotic	**Antimicrobials shown resistance(No of isolates)**	**No of Isolates (%)**
	Resistance antimicrobials to abattoir isolates	**14(58.3%)**
Two	TE,AMP	1
Three	TE,S,AMP	5
Four	TE,S,AMP,CXT	1
Five	TE,S,AMP,CXT,NA	1
	TE,S,C,K,AMP	1
Six	TE, S,C ,K, AMP ,Neo	2
	TE, S,,AMP , Neo ,NA	1
	TE ,S ,AMP ,CXT ,Neo ,NA	1
Seven	TE,S ,W ,C, K, AMP, Neo	1
	Resistance antimicrobials to butcher shop isolates	**6(66.7%)**
Two	TE ,S(1)	1
Four	TE, K ,AMP ,Neo (1)	1
Five	C ,AMP ,CXT ,Neo ,NA(1	1
	TE ,S,W,K ,AMP(2);	2
Six	TE ,S ,C ,AMP ,Neo ,NA(1)	1
Total		**20(60.61%)**

Keys: TE-Tetracycline, AMP- Ampicillin, S-streptomycin, CXT-cefoxitin, NA- Nalidixic acid, K-Kanamycin, Neo- Neomycin, W-Trimethoprim, C-Chloramphenicol

4.3.3. Multi drug resistance index (MDRI)

Multiple antibiotic resistances (MAR) index is a measure of the extent of the isolates' resistance to antimicrobial agents within the group of antibiotics studied. Multi drug resistance index (MDRI) of individual isolate is computed by dividing the number of antibiotics to which the isolates was resistance by the total number of antibiotics to the

isoltates was exposed. There fore MDRI of *Salmonella* isolates from both abattoir and butcher shops were calculated according to Olaobja and Onitade (2015). The Value lowerthan 0.2 wil l be considerd as lower risk, as well as value higher than 0.2 will have high risk. The result fro m the MARI studies showed that all the organisms had MARI values >0.20, with the highest value exhibited by *Salmonella* isolates from abattoir was (0.25), and the least MARI value was seen in (0.58) Isolates from butchershops revealed MARI values >0.20 with the highest value was 0.42 and the list value was 0.33 (Table 9).

Table 8: Distribution of MAR index among *Salmonella* isolates from abattoir and butcher shops.

Source of sample	Total No of resistanct	MAR index							
		0.58	0.50	0.42	0.33	0.25	0.17	0.08	00
Abattoir	24	1	4	2	1	5	1	3	7
Butchershop	9	Nil	Nil	4	1	Nil	1	2	1

Table 9: Percentage of *Salmonella* isolates with MAR index >0.2

Source of sample	MAR index (%)					Total (%)
	0.58	0.50	0.42	0.33	0.25	
Abattoir	1(7.7)	4(30.8)	2(15.4)	1(7.7)	5(38.5)	13(54.1)
Butchershop	Nil	Nil	4(80.0)	1(20.0)	Nil	5(55.5)

5. DISCUSIONS

In this study, the prevalence and antimicrobial resistance patterns of *Salmonella spp*. isolated from meat sample collected from abattoir and butcher shops were assessed. This study showed 11.43% and 6.52% prevalence of *Salmonella* isolated from abattoir and butcher shops, respectively. The overall frequency of *Salmonella* isolates was (11.43%) in samples collected from abattoir. This finding was agreed with 10.9% previously reported by Sibhat *et al.* (2011) in commercial slaughterhouse in Debre Zeit, Ethiopia. However, this result was relatively higher than 8.5% previously reported by Beyene *et al*. (2016) at Asella municipal abattoir. The result of this study was higher than 0.85% previously reported by Shilngale *et al*. (2015) in three beef abattoirs in Namibia as well as 1.5% reported by Ahmed, (2014) in Kano abattoir, Nigeria. However, the current result was lower than 20.8% previously reported by Wassie *et al*. (2017) from Gonder and 26.6% reported by Heko *et al*. (2016) from Addis Abeba. The difference in frequency of *Salmonella* isolates in different reports from different parts of the country could be due to difference in sampling and isolation procedures, variability in sampled populations, diverse geographical origins of cattle, numbers of cattle, study design, season, abattoir conditions and treatment with antimicrobial substances during the process. In my observation at abattoir there was poor in sanitation keeping because there was no access of water, problem of blood drainage and waste material are disposed near to slaughtering house. This problem might be factor for poor hygiene to the abattoir. Kusumaningrum *et al*. (2004) also stated that poor hygienic are the source of carcasses contamination in abattoirs.

This finding showed 6.52% *Salmonella* isolation frequency from butcher shops. This result was similar with 6.7% of *Salmonella* isolated in beef in retail shop of Harames University Mengistu *et al.* (2017). The current result was higher than the result from Dire Dawa retail shops (1%) as reported by Mengestu *et al*. (2017). However, this result was lower than some reports from other part of Ethiopia like, 12 % from raw meat in Gondar as reported by Mebrat *et al.* (2016), and 17.3% by Garedewe *et al*. (2015) in Gondar town. During the study period, some butcher houses there were poor personnel hygiene and equipment (knifes, table and balance) (personal observation). This could be contributed for carcasses contamination. The difference in prevalence could also be due to hygienic, condition sample size, geography,

sample type and source, sample processing unit, sampling techniques and distribution of *Salmonellae* in the lot examined.

The proportion of *Salmonella* isolates from carcass was 14.23% abattoir The present finding of *Salmonella* from beef carcasses in abattoir was similar with the finding (13.3%) of Dabassa and Bacha, (2012) in Jimma abattoir. The current result is also in line with the report of Heko *et al*. (2016) who reported 11.8% from beef carcasses in Addis Ababa abattoir. The higher prevalence of *Salmonella* was found in Senegal on beef meat from abattoir by Stevens *et al.* (2006) who reported 42.8% prevalence. Also 8.7% of *Salmonella* was detected from samples of butcher shop. This was consistent with the work done by Endrias, (2004) on minced beef in Addis Ababa. However, this result is far away from 70% report by Melaknesh and Mulugeta, (2017) from fresh meat in retail shops of Bahir Dar town and 35.5% report by Garedewe *et al*. (2015) from butcher shop in Gondar town. Higher percentage of contamination of *Salmonella* could be reported in carcasses that have undressed from lack of clean water, poor personnel hygienic condition and poor cutting material hygiene. Contamination of beef at abattoir could be from intestinal tract breakage and fecal leakage during evisceration, from lairage due to lack of care. And at butcher shops might be contaminated at abattoir during slaughtering processor recontamination of carcasses during handling, storage, transportation and processin g at butcher shops. The result difference might be due to poor sanitation and sample size used.

Isolation of *Salmonella* was also performed from personnel‘s hands, at both abattoir (8.5%) and meat cutter at the butcher shop (4.4%). The prevalence of *Salmonella* (8.5%) on hand swab from abattoir workers result was in line with 9.9% previously reported by Sibhate *et al*. (2011) from hand swabs of abattoir workers in Debre Zeit and 7.5% reported by Wassie *et al*. (2017) in butcher shops and abattoir from Gonder. Higher results reported by Garedewe *et al.* (2015) who found 24.1% from swabs of hands of butcher shops in Gonder. The hand of abattoir workers can be contaminated during or after they contact with gastrointestinal tract, also the hands of butcher shop workers can get the *Salmonella* after they handling money, contact with their hair, shake with their friends or customers and absence of adequately washing of their hands before and after processing. In my observation abattoir workers who handled rumen content, gastro intestinal tract were handling the carcasses without washing their hands. Hands of abattoir workers and beef retailers were a source of carcasses contamina tion acquire from direct contact with contaminated carcasses or equipment (Ntanga *et al.*,

2014).

The isolation frequency of *Salmonella* at abattoir on working knife was 11.4% which was comparable with the finding of Gurmu and Gebretinsae (2013) who reported 14.29% from knives used by butchers. On other hand, this result was slightly higher than 7.4% report by Teklu and Nigussie (2011) in Modjo an export abattoir. However, this result was lower than the 30.7% finding of Heko (2017) in Addis Ababa. The instruments used in dressing and killing like knives, saws, cleavers and direct contact with hair, the vessels, receptacles and the personnel may all act as sources of contamination during slaughter (Biswas *et al*., 2011). In my observation at abattoir workers were put Knifes used not cleaned or disinfected throughout the slaughtering and often put on floor, on stomach content and unclear area in addition to absence of separation of cleaned and dirty knife.

The frequency of *Salmonella* isolation found from butcher shops cutting (chopping) boards was 6.5%. This result was almost comparable to 5.6% reported by Garedew *et al*. (2015) from chopping board of butcher shops in Gonder and 5.7% reported by Wassie *et al*. (2017) in Gonder at butcher shops and abattoir. However, this finding was lower than 17.7% previously reported by Heko, (2017) in Addis Ababa. The sampled butcher shops in this study were used wooden boards for meat rail which could be the risk for meat contamination when the manner of its cleaning was inadequate and poor. It was observed that at study area butcher shops use offal '*Melas-Senber*' and meat were hanged in the same room at butcher shops and sometime at closed proximity, during eviscerations settled with intestinal organ in the floor which provide high opportunity for contamination. In butcher shops equipment were used shared (the knives, the cutting board, and the weighing balance) between offal and meat. Washing with water and detergent was done once daily. Frequent and proper cleaning of equipment was not observed. In some butcher shops the surface of equipment was cleaned during working hours through wiping with a cloth which could be a source of contamination.

In this study the Educational level of both abattoir and butcher workers were statistically significant association with the prevalence of *Salmonella isolates* (P= 0.049 and 0.037) respectively (Table 3 and 4*)*. The carcasses contamination rate of Salmonellosis reduced from people with low education level to those with high levels of education. The probability of carcasses contamination has 8 and 9 times more among abattoir and butchershop workers

respectively those uneducated one when compared with attended secondary school. This finding is in comparable with the study previously reported by Ntanga *et al*. (2014) in Tanzania that found educational level of workers was the risk factors of positive results. Educational level and training of food handler are important for basic concept and requirements of personal hygiene and its environment plays an important part in safeguarding the quality of food products to consumers (Nel *et al*., 2004). Best conception of hygiene practices has been attributed to those employees with basic level (least a primary) of education, while bad practices to those who were illiterate (Afnabi *et al*. 2014**)**.

The lack of knowledge on hygeinic meat handling practice of untrained abattoir workers, was found to be relatively high as 29 (59.1%) as compared with trained workers. 11(37.93%) *Salmonella* was isolated from personnel those were untrained on job related activities. This study shown significant statistical association with *Salmonella* isolats (P=0.047). The untrained personnel were 6 times more chance of contaminating the carcasses with *Salmonella* than the trained personnel. This finding was line with Niyonzima *et al*. (2017) who reported in Rwanda that job related training is found to be significantly associated with a decreased risk of *Salmonella* occurrence in retailed meat. Job related training of food handlers regarding basic concepts and requirements of personal hygiene play an integral part in ensuring safe products to the consumers (Haileselassie *et al*., 2013). The results indicated that there was a poor knowledge on meat hygiene practice in both abattoir and butcher shops workers of Jimma town. Hence, there is a need for more effective training in both personal and general hygiene practices for these workers.

In study Higher frequency of *Salmonella* isolates 6(54.55%) was revealed among sample obtained from 11 abattoir workers those have lack of perception on contamination of carcasses in slaughtering process as a risk, This result shown us personnel who had lack of knowledge on contamination in slaughtering process were significantly associated for positive result of *Salmonella* (OR=5.31, 95%CI=1.26-22.489%, P=0.047). Contamination of carcasses with *Salmonella* has 5 more likely on abattoir worker those who had lack of perception on contamination as risk than those have perception on contamination as risk. This result was comparable with the previously reported by Alhaji and Baiwa. (2015) from Nigeria, Aware of the effects of improper operations on public and environmental health had associated with the

knowledge of good hygienic and sanitary operations in slaughterhouses. In abattoir during slaughtering process (stunning, skinning, evisceration, carcass splitting, refrigeration and eventually cutting and deboning) carry out there were contribute of contamination of carcasses by *Salmonella* throughout the cattle slaughtering process (Niyonzima *et al*., 2017).

The result of this study showed that, lack of wearing personal protective cloth during handling of meat were statistically significant association with *Salmonella* positive results in butcher workers (P=0.09). The probability of carcasses contamination with *Salmonella* was 11 times more likely in butcher men not used protective clothes during meat selling than personnel used protective cloth. This study was comparable with the Chepkemoi *et al.* (2015) reported from, small and medium enterprise butcheries in Nairobi and Isiolo Counties, that di d not wear protective clothing had risk factor for carcasses contamination. The purpose of wea ring personal protective equipment is to protect both the food products and the meat handler from cross contamination (Nel *et al*., 2004). This was due to the butcher men used in this study were had no awareness about the importance of wearing of protective cloths such as white gown, aprons, hairnets and gloves, are barriers against microorganisms that may be transferred from handlers to meat. Many studies were also identified as bare hands, dirty clothes and workers hairs were could be the sources of microbial contaminating meat (Cardinale *et al*., 2005; Lues *et al.*, 2006; Heinz and Hautzinger, 2007).

Meat cutter in butcher shops handling money with hand while selling meat have statically significantly contributing to contamination of *Salmonella* (OR=9.7, P=0.002).The isolation frequency of *Salmonella* was 10 more times contamination of carcasses butcher men collecting money with hand during selling than those collecting money by cashier. The Result of this study was disagreed with previously studied by wassie *et al.* (2013) in Gonder, handling of money while selling meat was no stastically association with contamination of carcasses by micro organisms. In this study high proportion of *Salmonella* was isolated from butcher men collecting money by bar hand during selling meat. The person handling money should not be allowed to handle food during retailing or serving. This is because money is dirty and can contaminate food. The unhygienic conditions and habits of handling money in circulation usually subject the money to contamination with a variety of microorganisms. The money can thereafter act as a vehicle for contaminating the hands of the food seller/handler and thus cross contamination of food (Alemu, 2014).

Fecal as source of contamination was statistically significantly association with the *Salmonell a* positive result (P=0.014). Fecal contamination shows 8 times more likely contributing to meat *Salmonella* contamination than floor and water contamination to the carcass. And Handling of carcasses with dirty equipment and hand also had 1 more the chance of contamination of carcasses than water and floor at Jimma municipal abattoir. This result is in agreement with the previously reports from Sudan by Abdalla *et al*. (2009). Fecal wastes from animal and humans are important source of bacterial contamination of the environment and foods chain (Cabral *et al*., 2010), and members of *Salmonella enterica* subspecies *enterica* are widely distributed in the environment and in the intestinal tract of animals (Anjum *et al*., 2011). Animal hides and visceral contents are recognized to be the major sources of microbial contaminations of meat carcasses and these contaminations occur mainly during the hide removal and evisceration processes (Sibhat *et al.,* 2011). The ligature of esophagus and rectum during the evisceration process was not practiced in slaughterhouses which might have also contributed to high microbial contamination of the processed carcasses (Niyonzima *et al*., 2017).

Using uncleaned knife for splitting of carcasses was also significantly associated for contamination of meat by *Salmonella* in the abattoir (*P*=0.043). The chance of contamination of carcass by *Salmonella* was 8 more likely when using dirty knives than clean knifes. The obtained result is comparable with the finding of Muluneh and Kibret.(2015) in Bahir Dar town who reported washing knife before beginning slaughtering was reducing the risk of contamination by *Salmonella* in which carcass processed with unwashed knife has 3.15 times chance of contamination than that processed with washed knife. Cutting equipment are the major contaminants of fresh meat in the slaughter plant. The higher bacterial loads recorded on meat carcasses from the slaughterhouses would be the possibility of cross contamination associated to the use of non-sanitized knives. The use of a two-knife system to prevent microbial cross-contamination that might be associated to the utilization of contaminated knives during the slaughtering operations recommends. This system consists in using one knife in slaughtering operations while the other one is being sanitized in hot water at 82°c and above or by another knife sanitation method with equivalent effect (Niyonzima *et al*., 2017).

In this study, hand washing before handling meat of the butcher men respondents were statistically associated with the *Salmonella* positive results (OR=7.25, P=0.029). Washing

hand with water only was 7 times higher than in those washing their hands with detergent and water. *Salmonella* isolate among all butcher shop, 2(6.45%) were washing their hands with detergent and water, but 5(33.3% of them washing their hand only rinsing with water before contact to meat. This result was agreed with the report of Ntanga, *et al*. (2014) from Tanzania, the habits of washing their hands with water and soap before and after sale of meat risk factors contributing to microbial contamination of beef along the production chain from abattoir to retail meat outlets. In the present study some 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. Reasons for food handling personnel not washing their hands at appropriate times are laziness, time pressure, inadequate hand washing facilities and supplies, lack of accountability, and lack of involvement by industry management and workers in supporting proper hand washing. From our observation in butcher shops were not practicing hand washing due to lack of hand-washing facilities washing basin, soap, etc. as reported by Todd *et al*. (2010).This result has shown that there are poor habits of hand washing which contributes carcasses contamination. Access to hand washing facilities problem was observed in both at abattoir and in some butcher houses, especially at abattoir no facilities at whole, where at butcher house hand washing sink are seated far from the shops mostly near to latrine, this may contribute for contamination of meat.

Of the total 138 beef consumers interviewed,57(41.3%) of them prefer beef meat consumptio n. The respondents preferred to eat 30(21.7%) raw meat, 28(20.3%) fried meat, 43(31.1%) cooked meat and 37(26.8%) all types. However, *Salmonella* infections are mainly transmitted by consuming raw and undercooked meat (Rounds *et al*., 2010). This research outcome shows that 56(40.6%) of the respondents were learned to primary school only who do not have adequate knowledge to consume safe. The respondents also reported that 61(44.2%) of them usually suffered from food poisoning, 126(91.3%) of the them consider that meat slaughtered in abattoir is always safe to eat and prefers to buy, 102(73.9%) of them did not heard about *Salmonella* bacteria but they heard typhoid disease and 110(79.7%) of then did not know meat as source of *Salmonella*, However corresponding to the finding of the present study and other findings, consumers of contaminated meat could be predisposed to Salmonellosis without taking care during preparation and processing due to lack of information.

The highest sensitivity for Norfloxaciline, Gentamicin and Ciprofloxacin were recorded in 100% of isolates from both abattoir and butcher shop. This finding was consistence with the high susceptibility finding of *Salmonella* isolate to Norfloxaciline, Gentamicin and Ciprofloxa cin (100%) as Wolde and Bacha (2017) reported from in Jimma town. Might be because they are not widely used in Ethiopia for animal's treatment, those resistance drugs can easily available for both treatment of animal. Except the three drugs Norfloxaciline, Gentamicin and Ciprofloxacin, all isolates were resistant to at least a single antimicrobial agent. Tetracycline and Ampicillin (58.2%); streptomycin (66.7%) was the antimicrobial that presented the higher frequency of resistant among *Salmonella* isolates of in both abattoir and Butcher shops, followed 33.3% kanamycin in both abattoir and butcher shops and 22.2 % Trimethoprim and chloramphenicol in butcher shops; 20.8% and 16.7% resistant to Trimethoprim and chloramp henicol in abattoir.

Resistant to tetracycline 14/24 (58.3%) isolates in abattoir was comparable with Madoroba *et al*. (2016) who reported 51.9% resistance in South Africa abattoir. 5/9(55.6%) Tetracycline resistance was recorded in butcher shops isolates this finding was agreed with the 58.3% reported in Addis Ababa by Eguale *et al*. (2015) and the higher resistance (75%) to tetracyclin e was found by Teshale *et al*.(2015) in food handler in Jimma town. This higher resistance profile of *Salmonella* isolates to tetracycline might be attributed to high level of utilization of this drug both in veterinary and human medicines due to its relatively cheaper price and readily availability to the local community in the current study area (Garedwe *et al*. 2015).

Isolates of *Salmonella* 14/24(58.3%) and 5/9(55.6%) was recorded resitance to Ampicilline from abattoir and butcher shops, respectively. The present Ampicillin resistance level was similar with the report of Beyene *et al*. (2016) in Assella Abattoir (58.3%). The current Ampicillin resistance level was lower than the finding of 88.7% recorded by Garedew *et al*. (2015) in butcher shops in Gondar town. On other hand higher than the reports of , 39.3 % by Abunna *et al*.(2017) Mojo; 16.4% by Eguale *et al*.(2015) in Gonder; 39.2 % by madaroba *et al*.(2016) in South Africa. The high rate could may due to frequent use and easily available in everywhere in the country.

The resistance level of the abattoir and butcher shop isolates to Streptomycin was in this study was higher than the resistance reports of Wolde and Bacha, (2017) 50.8% in Jimma town.

Contrary, higher resistance (100%) of *Salmonella* isolates to Streptomycin was found by kebede *et al.* (2016) in mentioned in Addis Ababa abattoir Enterprise. Detection of antimicrob ials resistant *Salmonella* might be associated with their frequent usage both in livestock and public health sectors as these antimicrobials are relatively cheaper and commonly available.

Moderate rate resistance to kanamycin, 33.3% in both abattoir and butcher shops isolates obtained in this study. This finding is agreed with the studies conducted by Eguale *et al*. (2015) in Addis Ababa found 34.3% and with the result in South Africa reported by (Madaroba *et al.*, 2016) in proportion of 29.1%. Whereas as the report of Beyene *et al*. (2016) described (16.7%) *Salmonella* isolates resistant to kanamycin from Selected Dairy Farms and Abattoir, in Asella town.

Salmonella isolates from abattoir and butcher shops also showed moderate 20.8% and 22.2 % respectively, resistance level to Trimethoprim. Lower than this finding was conducted by Melaknesh and Mulugeta. (2017) Retail Shops of Bahir Dar City reported 9.5%. and closed result was 30.2% described by Garedw *et al*.(2015) in Asella town. The reason for the high antimicrobials resistance could be the wide use of antimicrobials in therapy (Tesfay *et al*., 2014). This difference could be due to the increasing rate of inappropriate utilization of antimicrobials which favors selection pressure that increased the advantage of maintaining resistance genes in bacteria (Mekuriaw *et al*., 2016), this increase antimicrobials 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

This study showed the presence of multidrug resistant S*almonella* isolates from abattoir and butcher shops. A Considerably higher frequency of the isolates 14(58.3%) out of 24 in abattoir and 6(66.7%) out of 9 butcher shops isolates were resistant to two or more of the antimicrobials. Multidrug resistance was considered when an isolates is resistant simultaneous ly to two or more drugs (Gabrie *et al*., 2012). The number of isolates resistance to three drugs was higher in abattoir and followed by six drugs resistance isolates. Five drugs resistance isolates were higher in butcher shops. In general, as the number of drugs got higher the number of resistant isolates decreased that indication combined use of antimicrobials may be useful for effective treatment. Different multidrug resistance profile of *Salmonella* isolates

were previous reported from Ethiopia (Abunna *et al.*, 2017; Enyew *et al.*, 2016; kebede *et al*., 2016; Melaknesh and Mulugeta, 2017; Garedew *et al*., 2015; Beyene *et al.*, 2016; Eguale *e t al*., 2015) and In other parts of Africa Madoroba *et al*. (2016) from South Africa from differe nt food animals, food products and human. The increasing development of multidrug Resistant bacteria is signaling serious alarm from treatment point of view or the possible transfer of resistant genes to other related pathogens (Landecker, 2016).

The result of the multiple antimicrobials resistance index showed that *Salmonella* isolates had the least resistance index (0.58) from abattoir isolates and (0.33) butcher shop which could be the result of a low number of isolates resistant to antimicrobials or may be due to the efficacy of tested antimicrobials against them. The highest MARI of (0.25) and (0.42) was observed among *Salmonella* isolates which could be because higher number of isolates showed resistance against many of the antimicrobials used against them from abattoir and butchershop s, respectively. Antibiotic resistance poses a threat to bacterial illnesses.The presence of these multi-drug resistant isolates of *Salmonella* from Abattoir and butchershops samples could facilitate transmission of antimicrobials resistance. For the general public, antimicrobials resistance limits the number of effective drugs available leading to fewer treatment options for the sick. There is therefore this emphasizes need to control contamination of carcasses by *Sal monella* from food animal sources to avert the occurrence of Salmonellosis.

6. CONCLUSIONS AND RECOMMENDATIONS

The isolation frequency of *Salmonella* from Jimma town abattoir and butcher shops was (11.2 3%) and (8.7%) respectively. Among all hypothesized risk factors of *Salmonella* meat contam ination, education status, Job related training, perception of contamination risk, neatness of kn ife, wearing protective cloth, manner of hand washing, handling of money and fecal contamin ation, was found to be the contributor of *Salmonella* contamination. Antimicrobial susceptibili ty test showed that of *Salmonella* isolates are (100%) susceptible to Gentamycin, Norfloxacilli n, and ciprofloxacillin whereas resistance was high to Tetracycline, Ampicillin and Sterptomy cineThese drugs are popular and commonly used in the veterinary and human medicines sector in Ethiopia. The use of those drugs for future is under questioned. This might limit therapeutic choice to manage Salmonellosis and other bacterial diseases both in animal and human health care. Lack of public awareness about *Salmonella*, its contamination of beef meat and its transmission to human were found be high in the study area. Based on the above conclusion the following recommendations are forwarded:

- The awareness of both abattoir and butcher shop those have direct contact with meat, better be developed by giving job related training on keeping personal, meat, working materials and environmental hygiene.
- During evisceration, dehiding and splitting of carcasses emphasis have to be given to reduce contamination. Attention should be paid on providing clean or potable water supply, using clean equipments, to Jimma town abattoir by the conserning body to solve hygienic problem. And if money is collected by cashiers, wearing protecting cloths, developing the habits of hand washing befor and after contact with carcasses meat cutter at butcher shops.
- Proper antimicrobial prescription for Gentamycin, Norfloxacillin, ciprofloxacillin and cefoxitin in veterinary and human practices. Continuous monitoring and assessment of the resistant pattern in *Salmonella* to guide appropriate antimicrobial therapy is advisable.
- Further studies are needed to describe all the virulence gene and serotype of pathogenic *Salmonella* strain for the emergency of drug resistance isolates and developing prevention and control measure.

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8. ANNEXES

Annex 1. Questioner survey format and observed hygiene practice record for knowledge, attitude and practice analysis of different key informants along the meat pathway Abattoir workers

Date……..Questionnaire number……

1. Name of slaughterhouse……
2. Name of respondent ……
3. Educational status: a) Uneducated b) Grade 1-8 c) Grade 9-12
4. Placement in slaughterhouses process: a) Stunner b) cutting the throat c) flayer d) Eviscerator e) Splitter f) Carcass washer g) All h) other (specify) ……

A. Knowledge

1. If your answer for no.4 is d, how frequently do you come across faulty eviscerations? ……..
5. What do you do after faulty evisceration? ……
6. How do you handle beef presented for slaughter? ……
7. Did you receive any job related training? A) Yes b) No
8. If yes for 7; where were you trained? ……...

8.1. If there was no formal training have you received informal training? A) Yes B) No

9. Have you undergone any job related medical tests to work in the abattoir? a) Yes [ ] b) No [ ]
10. When was your last medical test done? A) one month b) two month c) three month d) six month e) one year
11. What would cause carcass contamination? 1. Faeces 2.Dirty water 3.Handling with dirty equi pment and hands 4. Other (specify)……
12. If carcass was contaminated by faeces, what would you do? (Open question)……

1. Nothing 2. Wash the carcass 3. Call the meat inspector for advice 4. Other (specify)……

A13. In your opinion, does contamination pose any health risk to meat consumers? a) Yes b) No

14. If No, why? ……
15. Propose way to end carcass contamination? ……...
16. Do you know that contamination of carcasses occurs during slaughtering process (stunning, evisceration, carcass splitting, storage and eventually cutting and deboning) preform? A/ Yes ( ), B/ No ( )

Section B. Attitude I will read you some statements about hygiene in the slaughter process. Please indicate whether you agree or disagree. Key: SA= strongly agree, A =agree, D=Disagree, SD=strongly disagree, and DK= don‟t know.

No	Question	SA	A	D	SD	DK
1	In this job, it is important to work quickly than keep the carcasses clean.					
2	People doing this job are more likely to get sick					
3	In this type of working environment keeping clean is easy					
4	A small amount of dirt on clothing or utensils will not cause any harm					
5	Health is more important than wealth					
6	Ensuring hygiene is mainly the role of management					
7	If meat is well-cooked then it is always safe to eat					

Section C. Practices (Butcher observation checklist)

Cuts/wounds covered with an appropriate waterproof dressing.	Yes [ ] No [ ]
Smoking or eating or chewing while working	Smoking [ ] chewing [ ]
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 often and when do you wash the equipment?	Every day at end of the process[ ] Once p er 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[ ]
Equipment rested in dirty surface during Working	Yes[ ] No[ ]
Strict separation between clean and dirty Areas	Yes[ ] No[ ]
Veterinary inspectors present to examine the meat to be sold.	Yes[ ] No[ ]

Section D. perceptions

1. What constraints do you experience in your work? ……

2. Do they affect your ability to achieve high levels of hygiene? ……

3. If Yes, in what way? ……

4. In your opinion, what role do you think the management should play in…….

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

b) Maintaining those standards? ……

5. In your opinion, what role do you think the workers should play in? ……

a) Maintaining standards for hygiene in the slaughterhouse? ……

b) Doing their work as much as possible quickly? ……

ANNEX 2: Structured and observational questionnaire for conducting survey butchers shops in Jimma town – Ethiopia

ANNEX 1: Structured and observational questionnaire for conducting survey butchers shops in Jimma town – Ethiopia

Section A. Personal Identification

1. Date completed……. Questionnaire number…….
2. RespondentName……Address……. Occupation……
3. Name of butcher shops ……
4. Section B. Demographic characteristics
5. Gender A. Male B. Femal
6. Educational status: a) uneducated b) Grade 1-8 c) Grade 9-12 d) Grade >12
7. Age A. 15- 25 year B. 26- 35 year C. 36- 45 year D.46- 55 F. ≥ 56 year

SECTION B: Training on meat handling hygiene and owning of medical certificate.

8. Have you attended any job related training on meat handling hygiene? 1) Yes [ ] 2) No [ ]

9. What was the frequency of training? 1) Annually [ ] 2) Twice a year [ ] 3) other (specify).....

10. If yes for 8; where were you trained? ……

11. Do you/ your conduct medical check-up prior to employment? 1) Yes [ ] 2) No [ ]

12. How frequently do you conduct the medical check-up after employment? 1) Once a year [ ] 2) twice a year [ ] 3. Other (specify).....

SECTION C: Knowledge of butcher man in relation to hygienic handling of meat in the butcheries

13. Are there sanitary regulation systems 1)yes [ ] 2) No[ ]

14. How often and when do you wash the equipment? A) Every day at end of the selling b) Once per weak c) Once per month d) other (specify) ……

15. Food handlers with unhygienic practice could be the source for meat contamination A. Yes [ ] B) No [ ] C) Not sure [ ]

16. Contacting offals then meat with bare hands cause meat contamination A) Yes [ ] B) No [ ] C) Not sure [ ]

17. Microbial contamination can cause severe diseases that end in hospitalization and sometimes death A) Yes [ ] B) No [ ] C) Not sure [ ]

18. Do you wash your hands before handling meat? 1) Yes[ ] 2) No[ ]

19. How many carcass you receive per day? ……

20. What is your selling capacity per day? ……

21. If the meat is not sold in a given day what will you do/ how do you handle? ……

22. How many knife you have and you use per day a) one b) two c) three d) four e) more (Specify)…

23. Who are most of your customers? ……

24. What would cause meat contamination? (Open question) a) Faeces b) Dirty water c) Handling with dirty equipment and hands d) Other (specify)……

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

26. If No, why? ……

27. Propose way to meat contamination? ……

SECTION D. Practices (Butcher observation checklist

28. Cuts/wounds covered with an appropriate waterproof dressing. Yes [ ] No [ ]

29. Smoking or eating or chewing while Working Smoking [ ] chewing [ ]

30. Apron (any protective clothes) Yes [ ] No [ ]

31. Hand washing before after and during cutting meat A.Before [ ] B. After [ ] C.During [ ] D.Not wash [ ] E. other [ ]

32. How washed? Running water or bucket? Hot or cold? Brush or cloth? Soap? A. Running water [ ] B. bucket[ ] C. Hot[ ] D. cold[ ] E. Brush[ ] F. cloth[ ] G. Soap[ ]

33. All knifes are completely clean and free from dirt and cracks and damages Yes [ ] No

34. Knifes are cleaned before after and during Use Before[ ] after[ ] during use[ ]

35. Is any disinfectant used? Write name of Disinfectant Yes[ ] No[ ]

36. Wear Jewellery Yes [ ] No [ ]

37. Who handlers the money in the butchery?1) Cashier [ ] 2) The same person cutting the meat [ ]

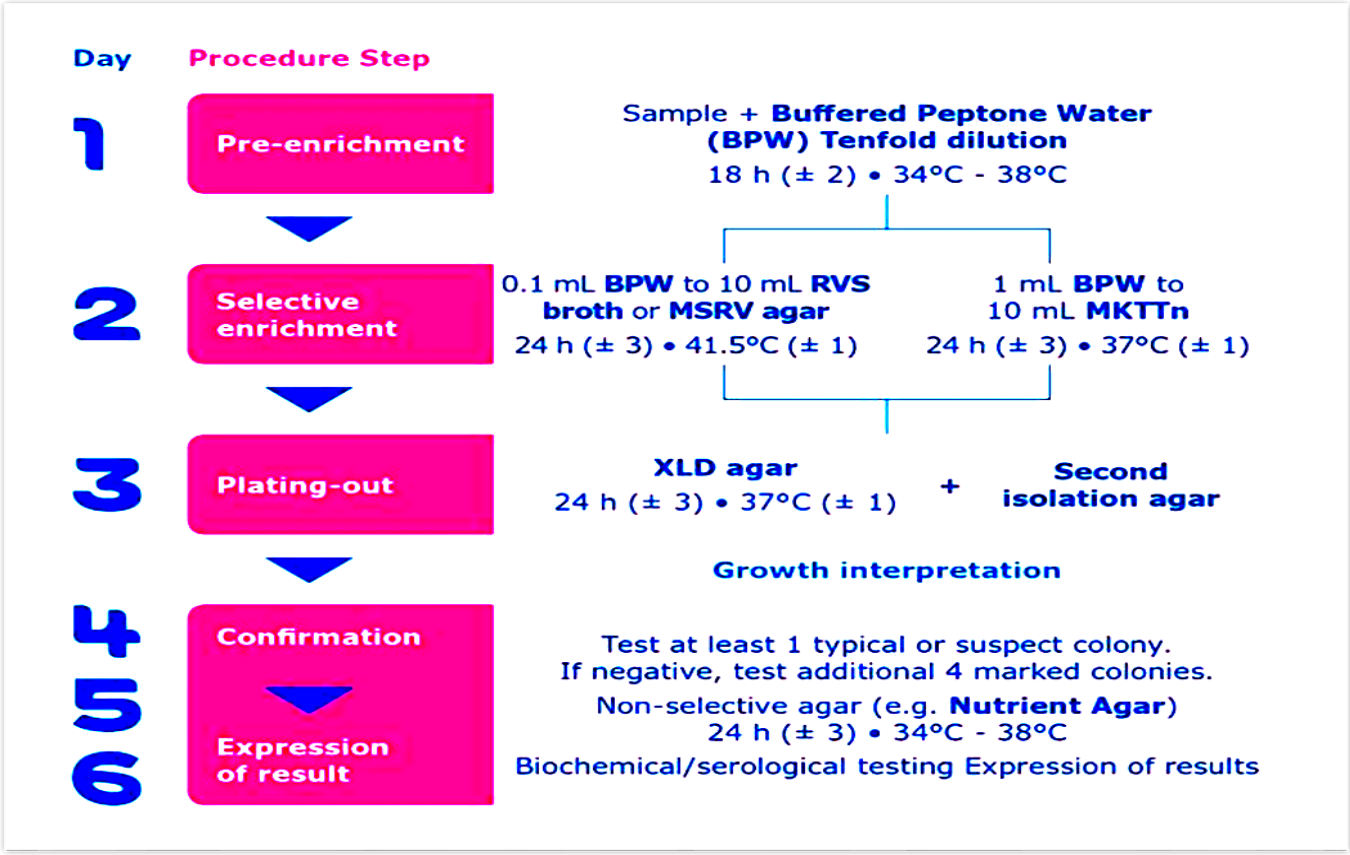
38. Cutting table Single [ ]separate for different meats [ ]

ANNEX 3: Knowledge, attitude and practice of the consumers

1. Date completed: …… Questionnaire number…….
2. Respondent Name: ……Sex: … Age … Address… Occupation: ……
3. Educational status: a) uneducated b) primary school c) high school D) University
4. From where you buy/beef meat mostly? ……..
5. What is your priority criterion to purchase meat? a) Freshness b) low cost (cheapness) c) low fat content d) healthiness e) mixed
6. Which type of red meat you prefer? A) Beef b) sheep c) goat d) all of them
7. How do you consume red meat? A) Raw b) fried c) cooked d) All type
8. Do you consume raw beef meat? A) Yes b) No
9. Do you think that cooked meat is always safe to eat? A) Yes b) No
10. 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
11. History of food poisoning? A) Yes [ ] b) No [ ]
12. If yes symptoms? ……
13. How many times? A) Once b) twice c) several times

14. If yes for no.27, what type of action taken? A) Medical examination and antibiotic treatment b) other..
15. Do you know any food poisoning/GIT disturbance associated with consuming of raw meat?
16. What are the symptoms? ……
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

ANNEX 4: ISO STANDARD 6579-2002 HORIZONTAL STANDARD FOR THE DETECTION OF *SALMONEL*



Day 1: Non-selective pre-enrichment ; Swab with a sterile wood spatula, place the sample into an universal bottle and add 10 ml buffered peptone water to obtain 1 part sample + 9 part buffer. Mix. Incubate at 36°C (+/- 1°C) overnight (18-24 hours).

Day 2: Prepare selective enrichment (I) and (II); Use a pipette to transfer 1 ml of the pre enrichment broth to 10 ml selenite F broth (Label as Tube I) Use a micro-pipette to transfer 0.1 ml (100 uL) of the pre-enrichment broth to 10 ml Rappaport-Vassiliadis soy peptone (RVS) broth. (Label as Tube II) Incubate Tube I: selenite F broth (at 36.0°C ± 1°C and Tube II: Rappaport-Vassiliadis soy peptone (RVS) at 41.5°C± 0.5°C overnight (18-24 hours).

Day 3: Spread on selective agar plates; Spread a 10 µl loop full from the inoculated and incubated selenite F broth (I) and RVS broth (II) on XLD and on BGA agar plates and incubate at 36.0°C ± 1°C overnight (18-24 hours).

Day 4: Selection and Subculture of Suspect *Salmonella* Colonies; Examine the XLD plates: A typical *Salmonella* colony has a slightly transparent red halo and a black center; a pink-red zone may be seen in the media surrounding the colonies. Note the presence of typical *Salmonella*- like colonies on XLD with a + in the record sheets.

Examine the BGA plates: Typical *Salmonella* colonies on a BGA agar plate appear red and impart a red/pink colour to the surrounding agar. Other enterics typically appear green or yellow. Note the presence of typical *Salmonella*-like colines on BGA with a + in the record sheets. Plate two suspect colonies from XLD agar and BGA onto non-selective media (e.g. nutrient agar) for biochemical confirmation and serotyping

Day 5-7: Biochemical Identification and Serotyping:

ANNEX 5: Biochemical Procedures and interpretation of the results

1. **Non-selective pre-enrichment**
   - ✓ Incubate the initial suspension buffered peptone water at 37 °C ± 1 °C for 18 h ± 2 h**.**
2. **Selective enrichment**
   - ✓ Transfer 0,1 ml of the culture obtained in to a tube containing 10 ml of the RVS broth transfer
   - ✓ 1 ml of the culture obtained in to a tube containing 10 ml of selenite F broth

✓ Incubate the inoculated RVS broth at 41.5 °C ± 1 °C for 24 h ± 3 h and the inoculated selenite F broth at 37 °C ± 1 °C for 24 h ± 3 h. Care should be taken that the maximum allowed incubation temperature (42.5°C) is not exceeded.

3. Plating out and identification

- ✓ After incubation for 24 h ± 3 h, using the culture obtained in the RVS broth inoculate by means of a loop the surface of one large-size Petri dish containing the first selective plating-out medium (XLD agar), so that well-isolated colonies will be obtained.
- ✓ In the absence of large dishes, use two small dishes one after the other, using the same loop.
- ✓ Proceed in the same way with the second selective plating-out medium Brilliant Green Agar using a sterile loop and Petri dishes as above.
- ✓ After incubation for 24 h ± 3 h, using the culture obtained in the selenite F broth repeat the procedure described in with the two selective plating-out media.
- ✓ Invert the dishes XLD and BGA so that the bottom is uppermost, and place them in the incubator set at 37 °C for the first plating-out medium . The manufacturer's instructions shall be followed for the second plating-out medium.
- ✓ After incubation for 24 h ± 3 h, examine the plates XLD and BGA for the presence of typical colonies of *Salmonella* and atypical colonies that may be *Salmonella*. Mark their position on the bottom of the dish.
- ✓ Typical colonies of *Salmonella* grown on XLD agar have a **black centre** and a lightly transparent zone of reddish colour due to the colour change of the indicator.

NOTE *Salmonella* H_2S negative variants (e.g. *S.* Paratyphi A) grown on XLD agar are pink with a darker pink centre. Lactose-positive *Salmonella* grown on XLD agar are yellow with or without blackening.

- ✓ Incubate the second selective solid medium at the appropriate temperature and examine after the appropriate time to check for the presence of colonies which, from their characteristics, are considered to be presumptive *Salmonella*.

4. **Confirmation**

A. General

✓ If shown to be reliable, commercially available identification kits for the biochemical examination of *Salmonella* may be used. The use of identification kits concerns the biochemical confirmation of colonies. These kits should be used following the manufacturer's instructions.

NOTE. The recognition of colonies of *Salmonella* is to a large extent a matter of experience, and their appearance may vary somewhat, not only from serovar to serovar, but also from batch to batch of the selective culture medium used.

B. Selection of colonies for confirmation

- ✓ For confirmation, take from each dish (two small-sized dishes or one large-sized dish) of each selective medium at least one colony considered to be typical or suspect and a further four colonies if the first is negative.
- ✓ It is recommended that at least five colonies be identified in the case of epidemiological studies. If on one dish there are fewer than five typical or suspect colonies, take for confirmation all the typical or suspect colonies.
- ✓ Streak the selected colonies onto the surface of pre-dried nutrient agar plates in a manner which will allow well-isolated colonies to develop. Incubate the inoculated plates at 37 °C $\pm$ 1 °C for 24 h $\pm$ 3 h.

C. Use pure cultures for biochemical confirmation.

a. Biochemical confirmation

General: By means of an inoculating wire, inoculate the media specified in with each of the cultures obtained from the colonies selected in:

I. TSI agar

- ✓ Streak the agar slant surface and stab the butt. Incubate at 37 °C $\pm$ 1 °C for 24 h $\pm$ 3 h.
- ✓ Interpret the changes in the medium as follows.

I. Butt

Yellow	Glucose Positive (Glucose Used)
Red or unchanged	Glucose negative (glucose not used)
Black	Formation Of Hydrogen Sulfide
Bubbles Or Cracks	Gas Formation From Glucose

b. Slant surface

Yellow- lactose and/or sucrose positive (lactose and/or sucrose used)

Red or unchanged- lactose and sucrose negative (neither lactose nor sucrose used)

- Typical *Salmonella* cultures show alkaline (red) slants and acid (yellow) butts with gas formation (bubbles) and (in about 90 % of the cases) 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

II. **Urea agar**

✓ Streak the agar slant surface. Incubate at 37 °C ± 1 °C for 24 h ± 3 h and examine at intervals.

✓ If the reaction is positive, splitting of urea liberates ammonia, which changes the colour of phenol red to rose-pink and later to deep cerise. The reaction is often apparent after 2 h to 4 h.

III. **L-Lysine decarboxylation medium**

✓ Inoculate just below the surface of the liquid medium. Incubate at 37 °C ± 1 °C for 24 h ± 3 h.

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

IV. **Detection of *β*-galactosidase**

✓ Suspend a loopful of the suspected colony in a tube containing 0,25 ml of the saline solution

✓ Add 1 drop of toluene and shake the tube. Put the tube in a water bath set at 37 °C and leave for several minutes (approximately 5 min). Add 0,25 ml of the reagent for detection of *β*-galactosidase and mix.

✓ Replace the tube in the water bath set at 37 °C and leave for 24 h ± 3 h, examining the tube at intervals.

✓ A yellow colour indicates a positive reaction. The reaction is often apparent after 20 min.

✓ If prepared paper discs are used, follow the manufacturer's instruction

V. **Medium for Voges-Proskauer (VP) reaction**

✓ Suspend a loopful of the suspected colony in a sterile tube containing 3 ml of the VP medium.

✓ Incubate at 37 °C ± 1 °C for 24 h ± 3 h.

- ✓ After incubation, add two drops of the creatine solution, three drops of the ethanolic solution of 1-naphthol and then two drops of the potassium hydroxide solution; shake after the addition of each reagent.
- ✓ The formation of a pink to bright red colour within 15 min indicates a positive reaction.

VI. **Medium for indole reaction**

- ✓ Inoculate a tube containing 5 ml of the tryptone/tryptophan medium with the suspected colony.
- ✓ Incubate at 37 °C ± 1 °C for 24 h ± 3 h. After incubation, add 1 ml of the Kovacs reagent.
- ✓ The formation of a red ring indicates a positive reaction. A yellow-brown ring indicates a negative reaction.

ANNEX 6: Composition and preparation of culture media and reagents

1. Buffered Peptone Water

Buffered Peptone Water is a pre-enrichment medium used for increasing the recovery of injured *Salmonella* species from foods prior to selective enrichment and isolation.

1.1.Composition of Ingredients Gms / Litre: Proteose peptone 10 gram, Sodium chloride 5 gram, Disodium phosphate, anhydrous 3.5gram, Monopotassium phosphate 1.5gram, Final pH (at 25°C) 7.2±0.2. Formula adjusted, standardized to suit performance parameters

1.2 Preparation Suspend 20.00 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely.Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. If desired, aseptically add rehydrated contents of 1 vial of EC O157 : H7 Selective Supplement (FD247) for isolation of *Escherichia coli* O157 from foods to previously molten and cooled to 45-50°C medium Mix well and dispense ino sterile tubes or flasks as desired

2. Rappaport-Vassiliadis medium with soya (RVS broth)

2.1 Solution A

2.1.1. Composition: Enzymatic digest of soya 5 gram, Sodium chloride 8 gram, Potassium dihydrogen phosphate (KH_2PO_4) 1,4 gam, Dipotassium hydrogen phosphate (K_2HPO_4) 0,2 gram, Water 1 000 ml

2.1.1 Preparation: Dissolve the components in the water by heating to about 70 °C if necessary. The solution shall be prepared on the day of preparation of the complete RVS medium.

2.2. Solution B

2.2.1. Composition: Malachite green oxalate 0,4 gram, Water 100 ml.

2.2.2. Preparation: Dissolve the malachite green oxalate in the water. The solution may be kept in a brown glass bottle at room temperature for at least 8 months.

2.2.3. Preparation: Add to 1 000 ml of solution A, 100 ml of solution B and 10 ml of solution C. Adjust the pH, if necessary, so that after sterilization it is 5,2 ± 0,2. Before use, dispense into test tubes (6.9) in 10 ml quantities. Sterilize for 15 min in the autoclave (6.1) set at 115 °C. Store the prepared medium at 3°C ± 2°C. Use the medium the day of its preparation. NOTE The final medium composition is: enzymatic digest of soya, 4,5 g/l; sodium chloride, 7,2 g/l; potassium dihydrogen phosphate (KH2PO4 + K2HPO 4), 1,44 g/l; anhydrous magnesium chloride (MgCl 2), 13,4 g/l or magnesium chloride hexahydrate (MgCl2·6H2O), 28,6 g/l; malachite green oxalate, 0,036 g/l.

1.3. Selenite-F-broth (Oxoid Ltd, England):

According to manufacturer, the medium was prepared by dissolving 5.0 grams peptone, 4.0 grams mannitol, 10 grams disodium hydrogen phosphate and 4.0 grams sodium hydrogen Selenite in one liter of distilled water, the pH was adjusted to 7.0 and sterilized by steaming for 20 minutes, mixed well and dispensed into sterile containers.

1.4. Xylose lysine deoxycholate agar (XLD agar)

1.4.1. Base medium

1.4.2. Composition Yeast extract powder 3 gram, Sodium chloride (NaCl) 5 gram, Xylose 3,75gram, Lactose 7,5gram, Sucrose 7,5gram, L Lysine hydrochloride 5gram, S odiumthiosulfate 6,8gram, Iron(III) ammonium citrate 0.8gram Phenol red 0.08 gram, Sodium deoxycholate 1gram, Agar 9 gram to 18 gram,) Water 1000 ml.

1.4.3. Preparation: Dissolve the dehydrated base components or the dehydrated complete base in the water by heating, with frequent agitation, until the medium starts to boil. Avoid overheating. Adjust the pH, if necessary, so that after sterilization it is 7,4 $\pm$ 0,2 at 25 °C. Pour the base to tubes or flasks (6.9) of appropriate capacity. Heat with frequent agitation until the medium boils and the agar dissolves. Do not overheat.

1.4.4. Preparation of the agar plates: Transfer immediately to a water bath (6.5) at 44 °C to 47 °C, agitate and pour into plates. Allow to solidify. Immediately before use, dry the agar plates carefully (preferably with the lids off and the agar surface downwards) in the oven (6.2) set between 37 °C and 55 °C until the surface of the agar is dry. Store the poured plates for up to 5 days at 3 °C $\pm$ 2 °C.

2.5 . Nutrient agar (Oxoid Ltd, England):

2.5.1. Composition: It consists of (grams per liter) lab-lemco powder 1.0 gram, yeast extract 2 grams, peptone 5 grams, sodium chloride 5 grams and agar 15 grams. 28 grams of medium were added to 1 liter of distilled water. and boiled to dissolve completely, the pH was adjusted to 7.4, and then the medium was sterilized by autoclaving at 121º C for 15 minutes and distributed aseptically in 15 ml amounts into sterile Petri dishes. Nutrient agar slops were also prepared and stored in refrigerator at 4º C until used.

2.6. Triple sugar Iron Agar medium (TSI) (Oxoid):

2.6.1. Compostion: It contains (grams per liter) Lab-Lemco powder (Oxiod L29) 3 grams, yeast extract (Oxoid L20) 3 grams, peptone (Oxoid L37) 20 grams, sodium chloride 5 grams, lactose 10 grams, sucrose 10 grams, dextrose 1 gram, ferric citrate 0.3, sodium thiosulfate 0.3, phenol red 0.025 gram and agar No. 3 (Oxoid L13) 12 grams.

2.6.2. Preparation: Triple sugar iron agar was prepared by adding 65 gram of powder to 1 liter of DW, the pH adjusted into 7.4, then boiled to dissolve completely, mixed well, distributed in 5 ml amount into McCarteny bottles and sterilized by autoclaving at 121º C for 15 minutes. The medium was allowed to set in a slope position about one inch butt and stored at 4º C.

2.7. Christensen's Urea Agar (Oxoid Ltd, England):

The medium was composed of (grams per liter) peptone 1.0 gram, dextrose 1.0 gram, sodium chloride 5.0 grams, disodium phosphate 1.2 grams, potassium dihydrogen phosphate 0.8 gram, phenol red 0.012 gram and agar 15 grams. According to the manufacturer instructions, 2.4 grams of dehydrated medium were dissolved in 95 ml of distilled water by boiling, pH was adjusted to 6.8, sterilized by autoclaving at 115º C for 20 minutes, then cooled to 50º C and aseptically 5 ml of sterile 40% urea solution were added. The medium was poured into sterile screw-capped bottles 10 ml each, and then allowed to set in the slope position.

2.8. L-Lysine decarboxylation medium

2.8.1. Composition: L Lysine monohydrochloride 5,0 gram, Yeast extract 3,0 gram, Glucose 1,0 gram, Bromocresol purple 0,015 gram.Water 1 000 ml.

2.8.2. Preparation: Dissolve the components in the water, by heating if necessary. Adjust the pH, if necessary, so that after sterilization it is 6,8 $\pm$ 0,2 at 25 °C. Transfer the medium in quantities of 2 ml to 5 ml to narrow culture tubes with screw caps. Sterilize for 15 min in the autoclave (6.1) set at 121 °C.

2.9. β-Galactosidase reagent

2.9.1. Buffer solution

2.9.1.1. Composition: Sodium dihydrogen phosphate (NaH_2PO_4), Sodium hydroxide, 10 mol/l solution about 3 ml and Water, to a final volume of 50 ml

2.9.1.2. Preparation: Dissolve the sodium dihydrogen phosphate in approximately 45 ml of water in a volumetric flask. Adjust the pH to 7,0 $\pm$ 0,2 at 25 °C with the sodium hydroxide solution. Add water to a final volume of 50 ml

2.10. ONPG solution

2.10.1. Composition: o-Nitrophenyl β-D-galactopyranoside (ONPG) 0. 08 g Water 15 ml.

2.10.2. Dissolve the ONPG in the water at approximately 50 °C and Cool the solution.

2.11. Complete reagent

2.11.1. Composition: Buffer solution 5 mland ONPG solution 15 ml

2.11.2. Preparation: Add the buffer solution to the ONPG solution.

2.12. Reagents for Voges-Proskauer (VP) reaction

2.12.1. VP medium

2.12.2. Composition: Peptone 7 gram, Glucose 5 gram, Dipotassium hydrogen phosphate (K_2HPO_4) 5 gram, and Water 1 000 ml.

2.12.3. Preparation: Dissolve the components in the water, by heating if necessary. Adjust the pH, if necessary, so that after sterilization it is 6,9 $\pm$ 0,2 at 25 °C. Transfer the medium to tubes (6.9) in quantities of 3 ml. Sterilize for 15 min in the autoclave (6.1) set at 121 °C.

2.13. Creatine solution (N-amidinosarcosine)

2.13.1. Composition: Creatine monohydrate 0,5 gram and Water 100 ml

2.13.2. Preparation: Dissolve the creatine monohydrate in the water

2.14. 1-Naphthol, ethanolic solution

2.14.1. Composition: 1-Naphthol 6 gram and Ethanol, 96 % (volume fraction) 100 ml

2.14.2. Preparation: Dissolve the 1-naphthol in the ethanol

2.15. Potassium hydroxide solution

2.15.1. Composition: Potassium hydroxide 40 gram and Water 100 ml

2.15.2. Preparation: Dissolve the potassium hydroxide in the water

2.16. Reagents for indole reaction

2.16.1. Tryptone/tryptophan medium

2.16.2. Composition: Tryptone 10 gram, Sodium chloride (NaCl) 5 gram, DL-Tryptophan 1 gram and Water 1 000 ml

2.16.3. Preparation: Dissolve the components in the boiling water. Adjust the pH, if necessary, so that after sterilization it is 7,5 ± 0,2 at 25 °C. Dispense 5 ml of the medium into each of several tubes. And Sterilize for 15 min in the autoclave (6.1) set at 121 °C

2.17. Kovacs reagent

2.17.1. Composition: 4-Dimethylaminobenzaldehyde 5 g. Hydrochloric acid, ρ = 1,18 g/ml to 1,19 g/ml 25 ml and 2-Methylbutan-2-ol 75 ml

2.17.2. Preparation: Mix the components

2.18. Physiological saline solution

2.18.1. Composition: Sodium chloride (NaCl) 8.5 gram and Water 1 000 ml

2.18.2. **Preparation:** Dissolve the sodium chloride in the water. Adjust the pH, if necessary, so that after sterilization it is 7,0 □ 0,2 at 25 °C. Dispense quantities of the solution into flasks or tubes. so that they will contain 90 ml to 100 ml after sterilization. Sterilize for 15 min in the autoclave .set at 121 °C.

2.19. **Simmon's Citrate Agar (Oxoid Ltd, England):**

It consist of (grams per liter) 0.2 gram of magnesium sulphate, ammonium dihydrogen phosphate 0.2 gram, sodium ammonium phosphate 1.0 gram, sodium citrate 2.0 grams, sodium chloride 5 grams, bromo-thymol blue 0.08 gram and agar 15 grams. 23 grams of dehydrated Simmon's citrate agar were suspended in one liter of distilled water, boiled to dissolved completely, the pH was adjusted to 7.0 and sterilized by autoclaving at 121ºC for 15 minutes. It was then poured into sterile screw-capped bottles and allowed to set in the slope position

2.20. Methyl Red-Voges Proskauer medium (MR-VP) (Oxoid Ltd, England):

This medium contains (grams per liter) peptone P (Oxoid L49) 5 grams, dextrose 5 grams and phosphate buffer 5 grams. It was prepared by adding 15 gram of powder to 1 liter of DW, mixed well, the pH adjusted into 7.5, distributed into test tubes in 5ml amount and sterilized by autoclaving at 121º C for 15 minutes. e. Peptone water sugars: This medium composed of peptone water and different sugars. The pH of the peptone water (900 ml) was adjusted to 7.1-7.3 before 10 ml of Andrade's indicator added, then 100 ml of 10% sugar solution (glucose or sucrose or mannitol) were added to the mixture, mixed well and distributed in 2 ml amounts into sterile test

tubes containing inverted Durham‘s tube, then sterilized by steaming for 30 minutes and stored in the refrigerator at 4º C until used

2.21. Performance standards for antimicrobial Mueller and Hinton Agar (Oxoid Ltd, England):

This medium used for cultivation of Niesseria and antimicrobial susceptibility testing. It contains of (grams per liter) beef infusion from 300 grams, casein hydrolysate 17.5 grams and agar No 1 10.0 grams, and pH adjusted into 7.4. 35 grams of powder were suspended in 1 liter of distilled water, boiled to dissolved completely, then sterilized by autoclaving at 121ºC for 15 minutes

ANNEX 7: Performance standards for antimicrobial susceptibility testing of *Salmonella*

No	Antimicrobial Agent	Disc Code	**Potency(µ)**	Resistant	Intermediate	Susceptible
1	Tetracycline		30	≤11	12-14	≥15
2	Streptomycin (S)		10	≤11	12-14	≥15
3	Trimethoprim (W)		5	≤10	11-15	≥16
4	Chloramphenicol(CHL)		30	≤12	13-17	≥18
5	Kanamycin(		30	≤13	14-17	≥18
6	Norfloxaciline (NOR)		10	≤12	13-16	≥17
7	Gentamicin (GEN)		10	≤12	13-14	≥15
8	Ampicillin		10	≤13	14-16	≥17
9	Ciprofloxacin		5	≤20	21-30	≥31
10	Cefoxitin		30	≤14	15-17	≥18
11	Neomycin (NEO)		30	≤12	13-16	≥17
12	Nalidixic acid		30	≤13	14-18	≥19

Source: CLIS, 2015

ANNEX 8: Antimicrobial susceptibility test zone of inhibition *Salmonella* isolates from butcher shops

Antimicrobial	**Zone of inhibition of Antimicrobial susceptibility test (mm) *Salmonella* isolates from butcher shops (n=9)**																															
	5	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
TE30	0	0	0	0	**2**	**1**	**2**	0	**1**	0	0	0	0	0	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S25	0	0	0	0	**2**	**1**	**3**	0	0	**1**	**1**	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
W5	0	0	0	0	**1**	**1**	0	0	0	0	0	0	**2**	0	**1**	**2**	**1**	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0
C30	0	0	0	0	0	0	0	**2**	0	0	0	**1**	**1**	**1**	**1**	0	**1**	**1**	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0
K30	0	0	0	0	0	0	**1**	**1**	**1**	0	0	0	0	**1**	**1**	**1**	**2**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NOR10	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**1**	**1**	**1**	**1**	**2**	0	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0
GEN10	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**2**	**2**	**1**	**2**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AMP10	0	0	0	**1**	0	**1**	**1**	**1**	**1**	**1**	0	0	0	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CPR15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**3**	**1**	**2**	0	**1**	**2**
CXT30	0	0	0	0	0	0	0	0	0	**1**	**1**	0	0	**1**	**2**	**1**	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0
Neo10	0	0	0	0	0	0	**2**	**1**	0	0	0	**1**	0	0	**1**	**2**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NA10	0	0	0	0	0	0	**1**	0	**1**	0	0	**1**	0	**1**	**1**	0	0	0	0	**2**	**1**	**1**	0	0	0	0	0	0	0	0	0	0

Key: TE-Tetracycline, **S**-Streptomycin, **W**-Trimethoprime, **C**-Chloramphenicol, **K**-Kanamycin, **NOR**-Norfloxacillin, **GEN**-Gentamycine, **AMP**- Ampicillin, **CPR**-Ciprofloxacillin, **CXT**-Cefoxitin, **Neo-** Neomycin, **NA**- Nalidixic acid.

Annex 9: Antimicrobial susceptibility test *Salmonella I*solates from Abattoir

Antimicrobial	***Salmonella* isolates from abattoir Antimicrobial susceptibility test Zone of inhibition (mm) (n=24)**																																	
	5	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
TE30	0	0	**1**	**1**	**4**	**4**	**4**	0	**2**	**1**	0	**1**	**1**	**1**	**1**	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S25	0	**1**	0	**1**	**3**	**6**	**5**	**1**	**1**	**1**	0	0	**1**	0	**1**	0	**1**	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0
W5	0	0	0	0	0	**2**	0	0	0	0	0	**3**	**2**	**2**	**2**	**1**	**3**	**3**	**1**	**3**	**1**	**1**	0	0	0	0	0	0	0	0	0	0
C30	0	0	0	0	0	**0**	**2**	**2**	**0**	**1**	**1**	**1**	**3**	**2**	**2**	**2**	**2**	**2**	**2**	**1**	0	**1**	0	0	0	0	0	0	0	0	0	0
K30	0	0	0	0	0	0	0	**2**	**2**	**1**	**1**	**2**	**3**	**2**	**3**	**2**	**1**	**3**	**2**	0	0	0	0	0	0	0	0	0	0	0	0	0
NOR10	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**1**	**3**	**3**	**5**	**3**	**2**	**3**	**2**	**1**	0	0	0	0	0	0	0	0	0	0
GEN10	0	0	0	0	0	0	0	0	0	0	0	**1**	**2**	**3**	**4**	**5**	**5**	0	**2**	0	0	0	0	0	0	0	0	0	0	0	0	0
AMP10	**1**	**1**	**1**	**2**	**1**	**1**	**2**	**1**	**4**	**1**	**2**	**1**	**2**	**1**	**1**	**2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CPR15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**7**	**4**	**5**	**2**	**1**	**5**
CXT30	0	0	0	0	0	0	0	0	**1**	**1**	**2**	**1**	0	**4**	**5**	**1**	**3**	**2**	**2**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0
Neo10	0	0	0	0	0	0	**2**	**3**	**1**	**2**	**1**	**3**	**1**	**2**	**3**	**2**	**2**	**2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NA10	0	0	0	0	0	0	**0**	**1**	**1**	**2**	**1**	**1**	**1**	**2**	**3**	**1**	**2**	**1**	**3**	**2**	**1**	**2**	0	0	0	0	0	0	0	0	0	0

Key: TE-Tetracycline, **S**-Streptomycin, **W**-Trimethoprime, **C**-Chloramphenicol, **K**-Kanamycin, **NOR**-Norfloxacillin, **GEN**-Gentamycine , AMP- Ampicillin, **CPR**-Ciprofloxacillin, **CXT**-Cefoxitin, **Neo-** Neomycin, **NA**- Nalidixic acid.

ANNEX 10: Plating and biochemical tests record sheet format used for *Salmonella* isolation

Sample No	Date of sample collected	Colony characteristics on		Biochemical tests									Sample (+/-)
				TSI test				Urease test	indole	methyl red	voges-proskauer	Lysine Decarboxilase (LIA)	simmons citrate	
		XLD agar	BGA agar	Butt	slant	Gas	H_2S							
1														
2														
3														
4														
5														

ANNEX 11: Carcasses sampling area

Meat (Hazard Analysis and Critical Control Point) (Scotland) Regulations 2002(2)
These Regulations extend to Scotland.

Sampling of each carcass was done using a 100 cm disposable sterile template and all samples were collected aseptically using sterile gloves. Each sterile sponge was hydrated with 10ml of sterile buffered peptone water. An additional 15ml of the remaining buffered peptone water was added to the sponge, in order to bring the total volume to 25ml. after excess air was expelled. The sponge bags were folded down, labeled and samples were packed with icepacks and shipped to the laboratory. The following sites were considered appropriate for process control:

• Cattle: *neck, brisket, flank, and rump.*

• Sheep, goat: *flank, thorax lateral, brisket, and breast.*

• Pig: *back, jowl (or cheek), hind limb medial (ham), and belly.*

• Horse: *flank, brisket, back, and rump*.

The above sampling procedure was as recommended by the Meat (Hazard Analysis and Critical Control Point) (Scotland) Regulations 2002 No. 234.

9. FIGURES

Picture taken during isolation, identification and Antimicrobial susceptibility test

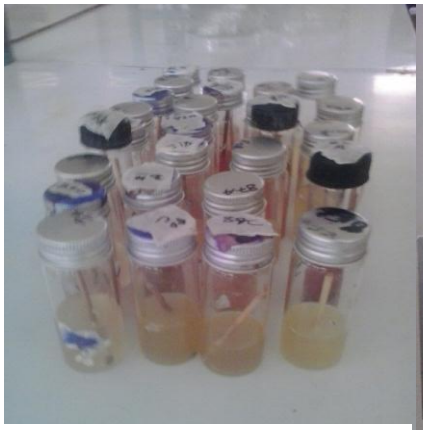


Figure 2: Swab Samples collected from abattoir in buffered peptone water**.**

Figure 5: Preenriched sample Inoculated in to enrichment Rappaport-Vassiliadis broth

Figure 4: Sample from pre-enrichment media Inoculated in enrichment Selenite F broth

Figure 4: *Salmonella* isolates from carcasses sample from abattoir grown on Xylose Lysine Desoxycholate (XLD)

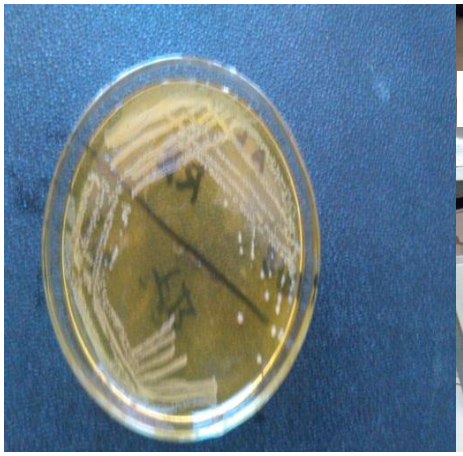


Figure 6: *Salmonella* isolates from but cher shops carcasses grown on Brilliant Green agar (BGA)

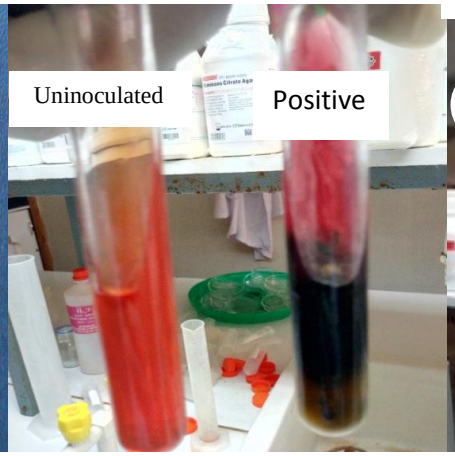


Figure 7: *Salmonella* positive results on TSI carcass sample from abattoir

Figure 6: positive result in Lysine iron agar, an isolate from knife sample of butcher shop

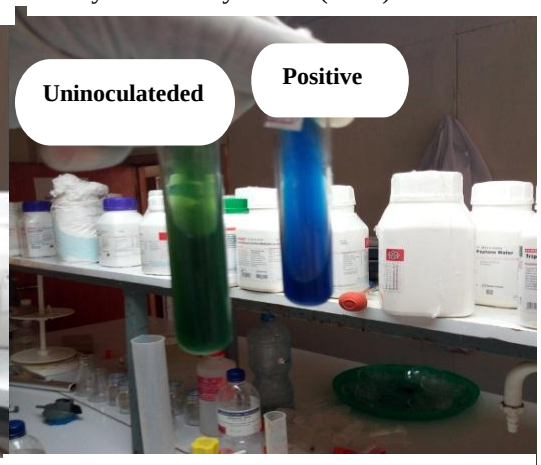


Figure 9: Isolate from sample of butcher shop chopping board tested for citrate utilization showed positive result for *Salmonella.*

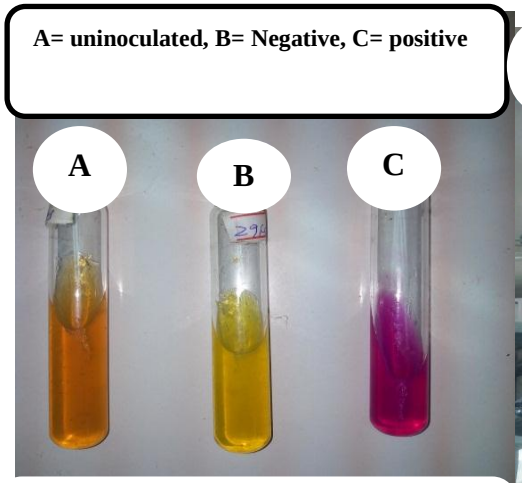


Figure 10: Isolate from sample of hand swabs from abattoir tested for Urea break down showed positive result for *Salmonella* Urea test.

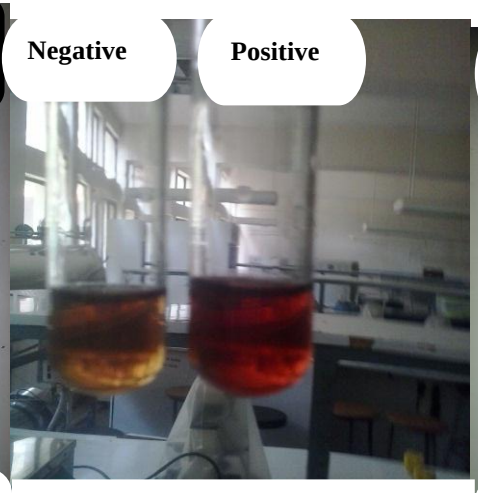
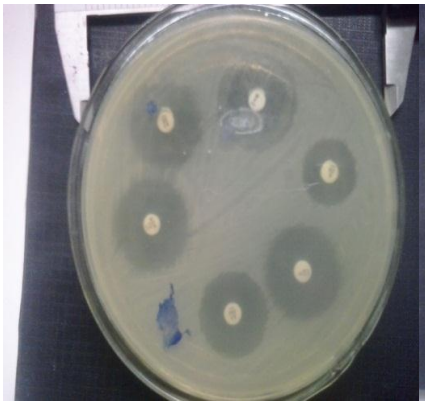
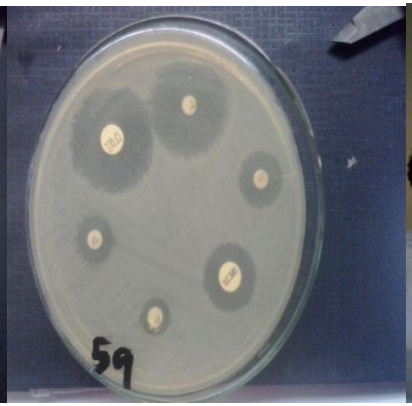


Figure 11: Isolates from butcher shop carcass *Salmonella* negative on VP test

Figure 12: Indole test for *Salmonella* isolate from Hands of butcher shops

Figure13: Antimicrobial susceptibili ty test for the *Salmonella* isolates based on the inhibition zone on the Kirby-Bauer disk diffusion, the isolate was from carcass swab slaughtered at abattoir.





Antimicrobial susceptability test Zone of inhibition

Innoculum preparation