OCCURRENCE AND ANTIMICROBIAL RESISTANCE PROFILE OF *SALMONELLA* ISOLATED FROM PIGS SLAUGHTERED IN BISHOFTU AND ADDIS ABABA, ETHIOPIA

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A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Tropical veterinary public Health

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As members of the examining board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by Musse Tadesse; Entitled as "Occurrence and Antimicrobial Resistant Profile of *Salmonella* Isolated from Pigs Slaughtered in Bishoftu and Addis Ababa, Ethiopia" and recommended that it be accepted as fulfilling the thesis requirement for the degree of Master of Science in Tropical veterinary Public Health.

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LIST OF ABBREVIATIONS

A_w	Water activity
AACA	Addis Ababa City Administration
AAMA	Addis Ababa Municipal Abattoir
AAU	Addis Ababa University
AMC	Amoxicillin/Clavulanic acid
AMP	Ampicillin
APHIS	Animal and Plant Health Inspection Service
BGA	Brilliant-Green Agar
BPW	Buffered Peptone Water
CC	Ceacal content
CCP	Critical Control Point
CI	Confidence Interval
CRO	Ceftriaxone
DNA	Deoxyribonucleic acid
DT	Definitive Type 104
FVM	Faculty of Veterinary Medicine
H_2S	Hydrogen Sulphide
HACCP	Hazard Analysis Critical Control Point
HIV/AIDS	Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome
ISO	International Organization for Standardization
K	Kanamycin
LPS	Lipopolysaccharide

m.a.s.l	meters above sea level
MDR	Multi-Drug Resistant
MH	Muller Hilton
MLN	Mesenteric lymph node
MSs	Member States
NA	Nalidixic Acid
NCCLS	National Committee for Clinical Laboratory Standards
OIÈ	Office International des Èpizootics
OT	Oxy-tetracycline
PCR	Polymerase Chain Reaction
PT	Phage type
RV	Rappaport Vassiliadis broth
S	Streptomycin
SF	Selinite F broth
SPI	Salmonella Pathogenicity Islands
Spp.	Species
Subsp.	Subspecies
SXT	Sulfamethoxazole/Trimethoprim
TSI	Triple Sugar Iron agar
USDA	United States Department of Agriculture
XLD	Xylose Lysine Desoxycholate agar
µg	Microgram

SUMMARY

Salmonellosis is considered one of the most widespread food-borne zoonoses in industrialized as well as developing countries. The presence of *Salmonella* in food animals at slaughter and the consequent cross-contamination of edible carcass tissues present a significant food safety hazard. A cross sectional study was conducted from March, 2014 to May, 2014; to determine the occurrence and antimicrobial resistance profile of *Salmonella* isolated from apparently healthy slaughtered Pigs and from the environment at slaughterhouses. A total of 583 samples (from Mesenteric Lymph nodes, Ceacal content, carcass swabs and abattoir environment) were analyzed for presence of *Salmonella.* Isolation and identification of *Salmonella* organisms was carried out by using the standard guidelines given by ISO-66579-3 (ISO, 66579-3: 2003). Of the 120 Mesenteric lymph node and Caecal content samples examined 58 (48.3%) mesenteric lymph node and 50 (41.7%) caecal content samples were detected *Salmonella* positive, respectively. Of the carcass swab samples *Salmonella* isolates were recovered from 16 (16 %) samples. Out of the 243 environmental samples (Personnel's hand, Eviscerating knife, Skin surface, De-hairing/Shaving material and Scalding water) from the slaughtering line, the highest rate of *Salmonella* was recovered from the skin surface 20 (34.5%), followed by De-hairing/Shaving materials 8(13.8%) and Eviscerating knife 7 (12.1%). Out of 167 *Salmonella* isolates tested for eight antimicrobials, all the isolates were resistant to at least one antimicrobial and 85% were resistant for more than one antimicrobial. Of which, 31.7% isolates were resistant to 2 antimicrobials; 24.5% were resistant to 3 antimicrobials; 19.2% isolates were resistant to 4 antimicrobials and 9.5% were resistant to 5 antimicrobials. The result of this study show that the wide spread of antimicrobial resistant *Salmonella* in apparently healthy slaughtered pigs and the abattoir environment which may pose a significant public health risk. Improving hygienic measures in the pork production chain is necessary in order to reduce the level of *Salmonella* infection in animals and the associated public health threats. Further large scale studies should be carried out to describe the risk factors associated with the emergence of drug resistant *Salmonella* and control of their spread across the country.

Key words: *Addis Ababa, Antimicrobial resistance, Bishoftu, Ethiopia, Salmonella, Swine*

1. INTRODUCTION

Salmonellosis is one of the major zoonotic diseases caused by organisms of the two species of *Salmonella* (S. enterica, and S. bongori) (OIE, 2010). The genus *Salmonella* is important foodborne pathogens worldwide which constitutes more than 2,500 serotypes known to cause illnesses in humans (WHO, 2005). They colonize in the gut of many species of animals, enter the human food chain, and produce gastroenteritis in people (Barrow, 2012). *Salmonella* can multiply and survive along the food chain, behaving as an infectious agent in the pre-harvest stage and as a food contaminant in the harvest and post-harvest stages. As a result *Salmonella*-reducing control measures early in the food chain may not always reduce the public health risks (EFSA, 2010).

As a food safety issue becomes a global concern and among those microorganisms which results in food borne illness *Salmonella* is a major concern for the livestock industry and public health aspect all over the world. It is estimated that 80.3 million cases of foodborne Salmonellosis occur annually in the world (Majowicz *et al.*, 2010). Several factors including under and mal-nutrition, HIV-AIDS, the unhygienic living circumstances and the close relations between humans and animals, lack of disease surveillance, monitoring systems and epidemiological data may substantially contribute to the occurrence of Salmonellosis in most developing countries like Ethiopia (Getachew, 2014).

Food-borne Salmonellosis often follows consumption of contaminated animal products, which usually results from infected animals used in food production or from contamination of the carcasses or edible organs (Alemayehu *et al*., 2003). It remains a very important zoonotic disease in humans next to Campylobacteriosis all over the world with annual estimates of 22 million cases and 200,000 deaths due to typhoid fever and 93.8 million cases of gastroenteritis and 155 000 deaths due to non-typhoidal *Salmonellae* (NTS)(WHO, 2004; Majowicz *et al*., 2010). The main *Salmonella* serovar found in pigs is S. Typhimurium, which was responsible for 13.6% of reported Salmonellosis cases in the UK (DEFRA, 2007). Among 490 verified *Salmonella* outbreaks were reported by member states (MSs) in Europe, corresponding to 26.0% of the total

reported *Salmonella* outbreaks, 7.1% of human cases caused by *Salmonella* were attributed to pig meat and products (12.2% of human cases caused by *S.* Typhimurium and 2.2% of cases caused by *S.* Enteritidis were attributed to pig meat and products) (EFSA, 2010).

In addition to its impact on the human population, Salmonellosis is also a major economic disease of Swine. *Salmonella* infections in swine are of concern for two major reasons. First, is the clinical disease in swine (Salmonellosis); and second, is that Swine's are susceptible to infections with a broad range of *Salmonella* serotypes (often occurring as subclinical infections) constituting a potential source of human exposure and illness (Garcia-Feliz *et al.,* 2007; Farzan *et al.,* 2008; Visscher *et al.,* 2011). The septicemia or enterocolitis experienced by weaned to adult-aged pigs is reported to result in millions of dollars in lost income to the pork industry (Hurd *et al.,* 2004). Although the carrier state has been defined for S. Typhimurium and S. Choleraesuis under experimental conditions little is known about the exact modes of transmission and maintenance of the disease in swine herds. Transmission is thought to occur from introduction of carrier animals into the herd, through contaminated feed, or by exposure to infected rodents or farm personnel (Rasschaert *et al.,* 2012).

An increase in human outbreaks of Salmonellosis originating from infections in animals has been seen periodically. Pork is one of the main sources for human Salmonellosis, being the source of approximately 20% (5-30%) of the human cases (Fedorka-Cray *et al*., 2000). Moreover, Multidrug resistant (MDR) *Salmonella* strains have steadily increased, probably due to continuous antibiotic pressure in human and veterinary medicine (Beyene *et al.,* 2009). The high prevalence and dissemination of multidrug resistant *Salmonella* serovars have become a growing public health concern (Winokur *et al.,* 2001; Kariuki *et al*., 2002; Gebreyes, 2003).

Therefore, reduction of *Salmonella* risks associated with pork can significantly contribute to the protection of human health. Despite some attempts to study prevalence of *Salmonella in pigs*, information on the status of *Salmonella* in pig, associated risks in slaughterhouse environment, public awareness and antimicrobial profile of the *Salmonella* isolates has not been adequately studied in Ethiopia. This indicates the need to investigate the antimicrobial resistance profiles of *Salmonella* from apparently healthy slaughtered Pigs.

Therefore the objective of this study was:

- To determine the occurrence and antimicrobial resistance profile of *Salmonella* isolated from Mesenteric lymph node, Ceacal content, Carcass of apparently healthy slaughtered Pigs and from the environment at slaughterhouses.

2. LITERATURE REVIEW

2.1. Genus *Salmonella*

2.1.1. Taxonomy and Nomenclature

Salmonellae are gram-negative, intracellular, aerobic, rod-shaped bacteria belonging to the family Enterobacteriaceae (Su and Chiu, 2007). Several nomenclature systems that divide the genus into species, subspecies, subgenera, groups, subgroups, and serotypes (serovars). Strains of *Salmonella* are classified into serovars on the basis of extensive diversity of lipopolysaccharide (LPS) (O) antigens and flagellar protein (H) antigens in accordance with the Kauffmann/White scheme (table 1). Currently the genus *Salmonella* is divided into two species: *Salmonella* enterica and *Salmonella* bongori. The species *Salmonella* enterica consist of six subspecies: S. enterica, S. salamae, S. arizonae, S. diarizonae, S. houtenae and S. indica, whereas no subspecies has been assigned to *Salmonella* bongori (Su and Chiu, 2007). Based on the combination of bacterial surface-antigens the genus *Salmonella* is subdivided into 2,541 serovars (also called serotypes) (Foley and Lynne, 2008).

According to Popoff *et al.* (2004), the majority of *Salmonella* serotypes belong to *Salmonella* enterica, subspecies enterica which consists of 1,504 serovars; Within S. enterica subsp. I (S. enterica subsp. enterica), the most common O-antigen serogroup are A, B, C1, C2, D and E Strains, these serogroup cause approximately 99% of *Salmonella* infections in humans and warm-blooded animals. Serotypes in S. enterica subsp. II (S. enterica subsp. salamae), IIIa (S. enterica subsp. arizonae), IIIb (S. enterica subsp. diarozonae), IV (S. enterica subsp. indica), and V (S. enterica subsp. bongori) are usually isolated from cold-blooded animals and the environment but rarely from humans (Le Minor and Popoff, 1997). Except for serotypes S. Typhi, S. Paratyphi A, and S. Paratyphi C, which are strictly human and whose only reservoir is man; all serotypes can be considered zoonotic or potentially zoonotic (Acha and Szyfres, 2001; Baird-Parker, 1990).

Table 1: Present number of *Salmonella* serovars in each species and subspecies (Source: Popoff *et al.* 2004)

Salmonella species and subspecies	No. of serovars
Salmonella enterica	
• Subsp.enterica (I)	1504
• Subsp.salame (II)	502
• Subsp.arizoane (IIIa)	95
• Subsp.diarizonae (IIIb)	333
• Subsp. Houtene(IV)	72
• Subsp.indica (VI)	13
Salmonella bongori	22
total	2541

The genus *Salmonella* can also be classified into three categories or groups based on their adaptation to animal host (WHO, 2005; Hafez and Jodas, 2000):

- Group 1: The highly host-adapted and invasive serovars include species of restricted and invasive *Salmonella* such as *S.* Pullorum, *S.* Gallinarum, *S.* Typhi, and *S.* Paratyphi in humans.

- Group 2: The non-host-adapted and invasive serovars consist of approximately 10 - 20 serovars that are able to cause an invasive infection in animals and humans. Currently, the most important serovars in this group are *S.* Typhimurium and *S.* Enteritidis.

- Group 3: The non-host adapted and non-invasive serovars include most serovars of the genus *Salmonella*. They may be pathogenic for animal and humans.

2.1.2. Characteristics of Salmonella

Salmonella are 0.2 - 1.5 x 2-5 um in size, Gram negative, facultative anaerobic, rod- shaped bacteria, belonging to the family Enterobacteriaceae. Members of this genus are motile by peritrichous flagella, except S. Pullorum and S. Gallinarum (D'Aoust and Maurer, 2007). Colonies are generally 2-4 mm in diameter but certain serovars, such as serovar Abortusovis, may form unusually small colonies (~1 mm diameter). *Salmonella* are chemo-organotrophic, with ability to metabolize nutrients by both respiratory and fermentative pathways. Hydrogen sulphide (H_2S) is produced by most *Salmonellae* but a few serovars like S. Paratyphi A and S. Choleraesuis do not produce H_2S. Most *Salmonellae* are aerogenic, however; S. Typhi does not produce gas (Popoff and Le Minor, 2005).

A range of environmental conditions affects the growth, death or survival of *Salmonella*. They grows between 8 ^{o}C and 45 ^{o}C (optimally at 37 ^{o}C) and at a pH of 4 to 9. A temperature higher than 70 ^{o}C rapidly kills them. Pasteurization at 71.1 ^{o}C for 15 seconds is sufficient to destroy *Salmonellae* in milk (Acha and Szyfres, 2001). Unlike the other enteric bacteria, except for *Yersinia, Salmonellae* are frequently facultative intracellular. They grows at water activity (a_w) values between 0.999 and 0.945 in laboratory media, down to 0.93 in foods, with an optimum of 0.995. While there is no growth below 0.93, the time of *Salmonella* survival increases as a_w decreases. Salt (NaCl), used as a salute to lower a_w, and as a preservative in foods, is inhibitory towards *Salmonella* at concentrations of 3-4% tolerance increases with temperature between 10 and 30 °C (Cox, 2000).

Most of the *Salmonellae* do not ferment lactose and this property has been the basis for the development of numerous selective and differential media for the culture and presumptive identification of *Salmonella* species (Andrews and Hammack, 2001; Anderson and Ziprin, 2001). The biochemical properties of *Salmonella* species show that almost all *Salmonella* serovars do not produce indole, hydrolyze urea, and de-aminate phenylalanine or tryptophan. Most of the serovars readily reduce nitrate to nitrite and most ferment a variety of carbohydrates with the production of acid, and reported to be negative for Voges-Proskauer (VP) reaction (Popoff and Le Minor, 2005). The other prominent characteristics of *Salmonella* are that most serovars

produce hydrogen sulfide (H2S) and decarboxylate lysine, arginine and orinithine with few exceptions (e. g. *S.* enterica subsp. arizonae and *S.* enterica subsp diarizonae). Most of *Salmonellae* utilize citrate with a few exceptions such as *S.* Typhi , S. Paratyphi A and a few S. Choleraesuis serovars; Whereas, lactose will not be utilized by most of the *Salmonella* serovars (Popoff and Le Minor, 2005).

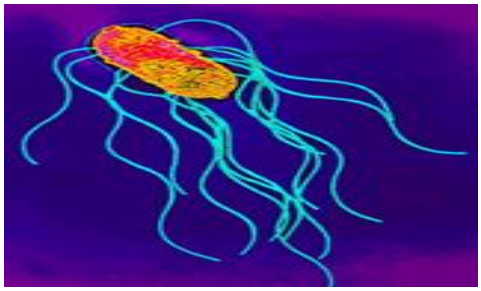


Fig.1: Morphological appearance of *Salmonella*
Source: Ana, (2005)

Variation in the amount of the LPS produced (the length of O side chains, and the degree of glycosylation); fimbrial structures; production of a protein enterotoxin, are the main virulence factors. *Salmonella* also produces a membrane-bound proteinaceous cytotoxin which may be released intracellularly as a consequence of limited bacterial lysis, inhibits protein synthesis, leading to host cell lysis and dissemination of the bacterium. Host cell lysis may also result from chelation of divalent cations by the toxin, causing disruption of host cell membranes (Cox, 2000); sequestering molecules or siderophores (enterocholin/enterobactin and aerobactin), chromosome encode factors necessary for intracellular survival, discrete chromosomal virulence gene insertions termed *Salmonella* Pathogenicity Islands (SPI). Five SPIs have been identified and are common across numerous serotypes. These determinants, along with virulence traits encoded on plasmids common to many strains, are responsible for the specific interactions that work together to allow *Salmonella* to become host adapted and a successful pathogen (Cox, 2000; Cook *et al.*, 2005).

2.2. Epidemiology of *Salmonella*

The epidemiology of foodborne diseases has changed in the last two decades because new pathogens has emerged and previously recognized pathogens have increased in occurrence or have become associated with food or new food vehicles. The ubiquity of *Salmonellae* in the natural environment contributes significantly to the continued presence of *Salmonellae* in meat animals and derived products (Zewdu, 2004). As *Salmonella* lives in the gastro-intestinal tracts of humans and other animals including birds, it is not surprising that the major food vehicles of transmission are animal-derived foods such as meat, poultry, milk, or eggs, and any food including vegetables contaminated with animal faces (Cox, 2000).

2.2.1. Global Distribution of Salmonella

Salmonellosis can be found worldwide but seems to be most common where intensive animal husbandry is practiced, especially with poor hygiene (Wray and Davies, 2003). *Salmonella* eradication programs have nearly eliminated the disease in domestic animals and humans in some countries (e.g. Sweden), but reservoirs remain in wild animals. Serovars vary in their distribution. Some, such as *Salmonella* ser. Enteritidis and *Salmonella* ser. Typhimurium, are found worldwide. Others are limited to specific geographic regions (CFSPH, 2005).

In 2002, the most common serovars from clinically ill animals reported to the CDC were, in descending order: *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Newport, *Salmonella* ser. Agona, *Salmonella* Heidelberg, *Salmonella* ser. Derby, *Salmonella* ser. Anatum, *Salmonella* ser. Choleraesuis, *Salmonella* Montevideo, *Salmonella* ser. Kentucky, *Salmonella* ser. Senftenberg and *Salmonella* ser. Dublin (CDC, 2008).

Among 1,100,000 *Salmonella* isolates from human and non-human (animal, food, feed and environment) sources, *Salmonella* Enteritidis and *Salmonella* Typhimurium were identified as the most common serotypes globally during 2000 – 2005 (Figure 2) (WHO, 2006).

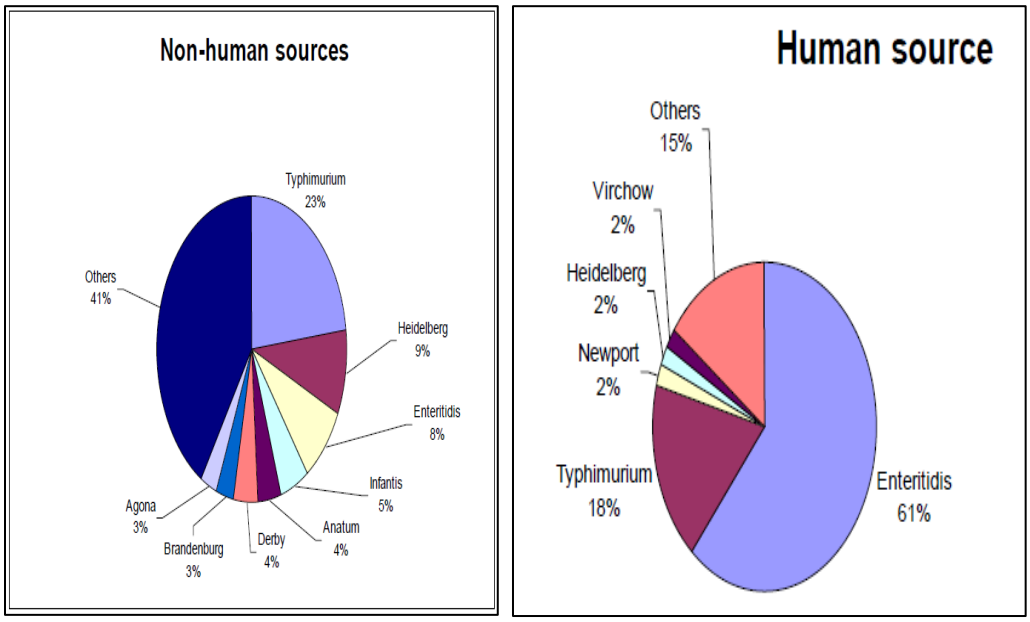


Figure 2: Distribution of Global *Salmonella* serotypes from human and non-human sources during 2000-2004 (WHO, 2006)

2.2.2. Susceptibility, Source of infections and mode of transmission

Species Affected

Salmonella species has been found in all species of mammals, birds, reptiles and amphibians that have been investigated. Fish and invertebrates can also be infected. Infections are particularly prevalent in poultry, swine and reptiles. Among reptiles, infections have been found in turtles, tortoises, snakes and lizards (including chameleons and iguanas) (OIE, 2010). Some serovars have a narrow host range; for example, *S.* choleraesuis causes disease in pigs, *Salmonella* ser. Abortusovis tends to be associated with sheep and *Salmonella* ser. Pullorum causes disease in poultry. However, most serovars can cause disease in a broad range of hosts. All species seem to be susceptible to Salmonellosis under the right conditions but clinical disease is more common in some animals than others. Clinical cases are common in cattle, pigs and horses but are relatively uncommon in cats and dogs (CFSPH, 2005).

Source attributions

Animals can become infected from contaminated feed (including pastures), drinking water or close contact with an infected animal (including humans). Birds and rodents can spread *Salmonella* to livestock. Carnivores are also infected through meat, eggs and other animal products that are not thoroughly cooked (WHO, 2006; OIE, 2010). They can be isolated from many sources including farm effluents, human sewage and water. *Salmonella* Choleraesuis has been isolated for up to 45 days from pig meat and for several months from feces or fecal slurries. *Salmonella* Typhimurium and *Salmonella* Dublin have been found for over a year in the environment (CFSPH, 2005). *Salmonella* spp. can survive for long periods in the environment, particularly where it is wet and warm. Poor sanitation, overcrowding, unfavorable weather, stress of hospitalization and surgery, parturition, parasitism, transportation, and concurrent viral infections are all factors which promote excretion of *Salmonella* by carriers and leads to activation or reactivation of disease to clinical Salmonellosis (Randell, 2001).

Mode of transmission

The feco-oral route is the most important mode of transmission of *Salmonella* in animals. Infection in animals may also occur via other routes, including the respiratory tract by inhalation of aerosol in confinement facilities, saliva and nasal secretion especially in shared waterers and also through milk and colostrum (Sheila and Simon, 2003). Insects, particularly flies, may have some role as mechanical vectors in very contaminated environments (Acha and Szyfres, 2001).

Animal feeds are frequently contaminated by a variety of serotypes, which usually enter the feed mixture in the protein supplement (Wray and Sojka, 1977). In some cases the mode of transmission is complex; for example, in poultry subsequent spread may occur via the feco-oral route or from egg to chick in the hatchery. *S.* Abortusovis may be isolated from the genitalia of both female and male sheep, but the extent to which transmission occurs by this route and is responsible for infection is disputed. In the case of *S .arizonae*, nasal carriage also appears to be an important aspect of the epidemiology. A variable percentage of animals, once infected, remain carriers and shed the organism intermittently (Gillepsie and Timoney, 1981).

Table 2: *Salmonella* serotypes in human and animals; clinical and other consequences of infection (Source: Quinn *et al*., 2003)

***Salmonella* serotype**	**Host**	**Consequences of infection**
Salmonella Typhimurium	Many animal species	Enterocolitis and septicemia
	Humans	Food poisoning
Salmonella Dublin	Cattle	Many disease conditions
		Enterocolitis and septicemia
Salmonella Choleraesuis	Pigs	Enterocolitis and septicemia
Salmonella Pullorum	Chicken	Pullorum disease
		(bacillary white diarrhoea)
Salmonella Gallinarum	Adult birds	Fowl typhoid
Salmonella Arizonae	Turkeys	Arizona or paracolon infection
Salmonella Enteritidis	Poultry	Often sub-clinical in poultry
	Many other species	Clinical disease in mammals
	Humans	Food poisoning
Salmonella Brandenburg	Sheep	Abortion

2.2.3. Pathogenesis and dynamics of Salmonella infection

Following *Salmonella* infection in hosts, pathogenesis is characterized by three phases: (1) colonization of intestines, (2) invasion of enterocytes, and (3) bacterial dissemination to lymph nodes and organs. Some *Salmonella* serovars are able to invade the tonsils 30 minutes after oral uptake/contact with the contamination source and within few hours (2h to 3h) they can colonize the mandibular lymph nodes, colon, caecum, and ileocaecal lymph nodes (Hurd *et al.*, 2001a). After experimental infection with *Salmonella* Typhimurium DT104, pigs excrete *Salmonella*

during two weeks post infection, thereafter shedding rate in faeces declined and became intermittent until the end of the five months fattening period (Scherer *et al.*, 2008). Several organs including the tonsils serve as important sites for persistence of *Salmonella* (Loynachan *et al.*, 2004).

Since *Salmonella* is able to survive and proliferate in phagocytes and leucocytes, translocation to gut associated lymphoid tissue is possible. The immune response in the intestine of the host is determined by a number of complex mechanisms including factors such as immune cell interactions with bacteria and their products (Bailey *et al.*, 2001). In several studies with pigs challenged with *Salmonella*, the development of the humoral immune response in serum was investigated. After experimental infection with *S.* Typhimurium DT 104, *Salmonella*-specific IgG antibodies are detected in the majority of pig sera between day 22 and 39 post infection (Szabo *et al.*, 2008).

2.3.4. Clinical Signs

The clinical signs vary with the infecting dose, health of the host, *Salmonella* serovar and strain, and other factors. Some serovars tend to produce a particular syndrome: for example, in pigs *Salmonella* Choleraesuis is usually associated with septicemia and *Salmonella* Typhimurium with enteric disease. Although Salmonellosis can be seen in all domestic animals, pregnant, lactating or young mammals and birds are the most susceptible (OIE, 2010; Schwartz, 1999).

The major syndromes in animals are enteritis and septicemia. Septicemia is the most common in very young calves, lambs and foals, and in pigs up to 6 months of age. The symptoms include marked depression, high fever and, often, death within 1 to 2 days. Diarrhea can occur in some animals. Central nervous system (CNS) signs or pneumonia may be seen in calves and pigs. Pigs may also develop a dark reddish or purple discoloration of the skin, particularly on the ears and ventral abdomen (Schwartz, 1999). Whereas, in adult animals and in calves over a week old chronic and acute enteritis is the most common form which is characterized by profuse diarrhea, dehydration, depression abdominal pain and anorexia (Gay, 2003). The feces are watery to pasty, often foul smelling, and may contain mucus, pieces of mucous membrane, or blood. A fever

occurs early in the infection, but can disappear by the time diarrhea develops. In dairy cows, milk production drops acutely. Intestinal salmonellosis usually lasts for 2 to 7 days. Death can occur as the result of dehydration and toxemia. Horses, in particular, often have severe enteritis and may die within 24 to 48 hours. Loss of condition, emaciation and thriftiness may be seen in surviving livestock and recovery can be slow (Wray and Davies, 2003; Barrow, 2012).

Results of clinical studies in pigs demonstrate that *S.* Choleraesuis infection can result in septicaemia, enterocolitis, pneumonia and/or hepatitis as a consequence of bacteremia, Whereas *S.* Typhimurium infection may sometimes cause enterocolitis and diarrhoea (Schwartz, 1999). Other experimental investigations in weaning piglets using an oral dose of 109 colony-forming units (cfu) of *S.* Typhimurium led to clinical signs such as fever and vomiting at the early stage of infection but also the absence of clinical signs (Scherer *et al.*, 2008; Szabo *et al.*, 2009). Differences in the clinical outcome after exposure may be due to serovar or strain specific differences in virulence and/or constitution of pigs such as susceptibility and predisposition. Both serovar and number of *Salmonella* inoculated are considered to be important factors (Osterberg *et al.*, 2009; Osterberg and Wallgren, 2008).

2.2.5. Diagnosis

Diagnosis is based on the isolation of the organism either from tissues collected aseptically at necropsy or from feces, milk, blood, tissue swabs, rectal swabs or environmental samples (Wray and Davies, 2003). *Salmonella*e may be isolated by a variety of techniques, which may include pre-enrichment in non-selective medium, enrichment media that contain inhibitory substances to suppress non-*Salmonella* organisms, and selective plating agars to differentiate *salmonella*e from other Enterobacteriaceae. Various biochemical and serological tests can be applied to the pure culture to provide a definitive confirmation of an isolated strain (OIE, 2010).

Serological tests, such as the whole blood test and serum agglutination test have been developed for the identification of infected herds but are inadequate for the identification of persistently infected animals and their sensitivity is low (Thomson *et al*., 2008; OIÉ, 2010). In recent years, molecular methods are used for diagnosis of *Salmonella* serovars (Gebreyes, 2003). PCR has

been one of the most promising new methods shown to be well suited for both rapid and sensitive detection of *Salmonella* contamination in various foods. Many of these methods have not been fully validated for faecal and environmental samples, although progress has been made (Malorny and Hoorfar, 2005), and are more suited to analysis of human foodstuffs where inhibitors of the PCR reactions are not so problematic (Kanki *et al*., 2009).

2.2.6. Treatment

Septicemic Salmonellosis can be treated with a number of antibiotics including Ampicillin, Amoxicillin, Gentamicin, Trimethoprim/Sulfamethoxazole, third generation Cephalosporins, Chloramphenicol and Fluoroquinolones. Many isolates are resistant to one or more antibiotics, and the choice of drugs should, if possible, be based on susceptibility testing. Antibiotics can favor the persistence of *Salmonella* species in the intestines after recovery, affect the intestinal flora and increase the emergence of antibiotic-resistant strains. For these reasons, antibiotics might not be used for enteric disease (Hohman, 2001; D'Aoust, 1991a; Baird-Parker, 1990)

Supportive care such as Fluid replacement, correction of electrolyte imbalances and other nursing care and appropriate antimicrobial therapy based on susceptibility test is the principal treatment for the enteric form of Salmonellosis (Hirsh, 1999; D'Aoust 1991). In man, antibiotics are not indicated in *Salmonella* gastroenteritis, except in very young and those over 65 and to individuals with severe invasive infection. Non-steroidal anti-inflammatory drugs may be given to decrease the effects of endotoxemia. Antibodies to *Salmonella* lipopolysaccharide may also be used in some cases (CDC, 2008).

2.2.7. Prevention and control

In many cases, elimination of *Salmonella* infections is a most challenging task because of the complexity and interdependence of various aspects of animal husbandry, slaughtering, and food processing (EU, 2009). The control of *Salmonella* is limited to preventing clinical disease and/or the transmission of bacteria to humans. The principles of control include prevention of

introduction and limitation of spread within a herd. During a herd outbreak, carrier animals should be identified and either isolated and treated, or culled. Treated animals must be re-tested several times to ensure that they no longer carry Salmonella. Fecal contamination of feed and water supplies should be prevented. Contaminated buildings and equipment should be cleaned and disinfected, and contaminated material should be disposed of (OIE, 2010; WHO, 2005).

Clinical Salmonellosis can be decreased by good hygiene and minimizing stressful events. Ensuring that feed supplies are free of *salmonella*e depends on the integrity of the source. Colostrum is important in preventing disease in young animals and stresses should be minimized. Adequate nutrition and hygiene prior to and during transport and within farm and abattoir could be important interventions to prevent spread of Salmonellosis (Wray and Davies, 2003). Implementing appropriate hygienic practices and coordinated control systems in the food value chain remain the best preventive measures for most *Salmonella* infection (Zewdu, 2004). Vaccines can reduce the level of colonization and shedding of *Salmonella* species into the environment, as well as clinical disease. Vaccines are available for some serovars such as S. Dublin, S. Typhimurium, S. Abortusequi and S. Choleraesuis in some countries (Wallis, 2004; Mastroeni *et al.,* 2001).

Additionally, comprehensive educational programs for the consumer and food handler, both in commercial establishments and in the home, about correct cooking and refrigeration practices for foods of animal origin, and about personal and environmental hygiene beside veterinary meat inspection and supervision is of paramount importance (IFT, 2003). The detection of personnel who are carriers of *Salmonella*, through regular stool culture is important to the food industry because it may prevent future contamination of products (Hohmman, 2001).

2.3. *Salmonella* in the pig production chain

Swine production is increasing from time to time in many parts of tropical countries. An increased demand on international market, due to increased number of pork consumer and the profit obtained from the sector make the production to increase rapidly (Serres, 2001). Swine production in Ethiopia is in its infant stage. The pig population in Ethiopia was estimated to be

29,000 (FAO, 2005). For the last number of years adequate emphasis was not given for the sector. Unlike other livestock distribution, swine farms are restricted to central part of the country near, Addis Ababa (ILCA, 1994). Currently large numbers of swine are widespread in these areas and some are kept mixed with other livestock's (Seid and Abebaw, 2010).

2.3.1. Risks at pre-harvest level

The possible sources of *Salmonella* input to pig producing and fattening farms are wide as well as the spread within a farm is affected by specific farm characteristics. In order to control *Salmonella* in pig farm, the initial step is to find out the risk factors which can be introduced in pork (Davies *et al.*, 1999; Zheng *et al*., 2007). The important risk factors that can be identified by different studies includes: feeds of animal and plant origin often contaminated with *Salmonella*, contaminated Water supply, Floor and bedding materials (Cook *et al*., 2005, Feder *et al.,* 2001; Cédric *et al.,* 2006), introduction of infected pigs mixing with the herd, transport stress e.g. noise, smells, animal mixing, and close contacts of domestic and wild animals and equipment may cause pigs to shed *Salmonella* more frequently and results in introduction and dissemination of *Salmonella* in the farm (Heijden *et al.,* 2005; Leirs *et al.*, 2004; Funk and Gebreyes, 2004; Barber *et al.,* 2002).

Salmonella infections in swine are much more common than the clinical disease. In fact, asymptomatic intestinal carriage and fecal shedding of *Salmonella* represents a very common scenario in swine herds around the world. Although only about 5-10% of pigs will be shedding *Salmonella* at any given time, virtually every farm has or will soon have infected pigs carrying the bacteria in their gastrointestinal tract (Hurd *et al*., 2004; Garcia-Feliz *et al*., 2007; Farzan *et al.,* 2008; Visscher *et al*., 2011; Rasschaert *et al*., 2012).

Several studies reported a *Salmonella*-preventive effect of a fully slatted floor; contaminated faeces flow away much faster and have a minor chance of infecting susceptible pigs in the pen. Regular cleaning of the feed tube prevents the settlement and growth of *Salmonella* and decreases the risk of seropositivity as well or a prevalence increasing impact of dry feeding systems respectively. More contacts increase the chance of an infection; however, it has to be

considered that nose-nose transmission is less frequently the reason for infection than faecal-oral transmission (Schwartz, 1999). The analysis of the blood and meat juice samples in some studies demonstrated especially the importance of hygienic principles and feeding aspects, respectively (Van der Wolf *et al*., 2001; Vorgelegt *et al*., 2011).

2.3.2. Risks at harvest and post-harvest level

After a pig has entered the slaughter process, the final *Salmonella* contamination of the dressed carcass originates from the combined impact of several sources which can be summarized as: 1) the animal itself; 2) previously slaughtered animals via the processing machinery; 3) slaughterhouse personnel; and 4) persistently contaminated equipment (Lo Fo Wong *et al.*, 2002). At slaughter the highest level of *Salmonella* colonization is detected in tonsils, jejunal and ileocaecal lymph nodes (Scherer *et al.*, 2008). The presence of *Salmonella* in those organs may pose a risk of cross contamination due to incision during sanitary inspection and processing. Once a processing line is contaminated, *Salmonella* can be isolated from the machinery, hands of workers, knives and carcasses (Small *et al.*, 2006).

Finisher pigs may harbor *Salmonella* in several tissues, especially the digestive tract including associated lymph nodes and also on the contaminated skin. Sub-clinically infected carriers may be a risk factor for horizontal transmission via contaminated faeces, during transportation to the Abattoir or while waiting in the lairage before slaughter (Vieira-Pinto *et al.*, 2005). A translocation of *Salmonella* to muscular tissue was observed in slaughtered pigs after exposure to severe stress (Fehlhaber, 2003).

According to some studies there seems to be a general positive relationship between skin contamination before slaughter (after lairage) and external carcass contamination at the end of slaughter line (Rossel, 2009). Therefore cleanness of the pigs at the early step of the slaughter process is worth considering. Another group of factors that can interfere with carcass contamination relates to the equipment of the slaughterhouse and the aerosols which are produced when the slaughter line is running. Cross-contamination between adjacent carcasses is

possible through direct contact or airborne contamination. Moreover, equipment-mediated contamination (direct contact) was considered important (Prendergast *et al*., 2008).

2.4. Public health significance

2.4.1. Salmonella infections in humans

The majority of human Salmonellosis cases are associated with the consumption of contaminated products such as meat, pork, poultry, eggs, milk, seafood, and fresh produce (Foley and Lynne, 2008). *Salmonella* infections in humans may result in distinct clinical syndromes which are characterized by a 6 to 72 hour incubation period after ingestion, including acute gastroenteritis, fever, and bacteraemia with or without focal extra-intestinal infections and reactive arthritis (Haagsma *et al.,* 2008). Most strains of *Salmonella* are known to infect man and many other animal species. Approximately 95% of cases of human Salmonellosis are associated with the consumption of contaminated products. Workers in food processing or production plants once infected with *Salmonella* are also sources of infection. Human patients, both sick and convalescent, and subclinical carriers may also shed organisms (Foley and Lynne, 2008).

Although, Salmonellosis may occur in all persons the incidence is much higher among children, the elderly and immune-compromised individuals (DEFRA, 2007; CDC, 2008). Risk factors for Salmonellosis include gastric hypoacidity, recent use of antibiotics, extremes of age, and immunosuppressive conditions. In the developing world, HIV infection is a prominent risk factor for non-typhoidal Salmonellosis and bacteremia (Crum-Cianflone, 2008). The main symptoms consist of diarrhea, vomiting, abdominal pain, and nausea (Acha and Szyfres, 2001). Typhoid fever is difficult to differentiate clinically from other causes of fever, because its clinical presentation consists of non-specific symptoms such as fever, chills, headache, malaise, anorexia, nausea, abdominal discomfort, a dry cough or myalgia. In the later phase of illness, more specific physical signs such as rose spots and splenomegaly may be observed (Vollaard *et al*., 2005). Although rare, there may also be different localizations such as infections of joints, lungs, pleura, endocardium, abdominal organs, central nervous system, bone, urinary and genital

tracts (Lesser and Miller, 2001). The average mortality rate is 4.1%, varying from 5.8% during the first year of life, to 2% between the first and 50th year, and 15% in persons over 50 (Jay, 2000).

The frequency rankings of *Salmonella* serovars found in feed, live pigs, pig carcasses and humans are not the same (Wollin, 2007). Pig meat and pig meat products are increasingly recognized as an important source of human Salmonellosis. However, as *Salmonella* Typhimurium is also found in other foods including beef, dairy, lamb and poultry products as well as some herbs and spices, it is difficult to specifically quantify the proportion of *Salmonellosis* cases that are directly related to contaminated pig products (Snary *et al.*, 2010; DEFRA, 2007).

Nevertheless, the role of pork and pork products in causing human *Salmonellosis* has been quantified for other countries. For instance in Germany five large outbreaks related to pig meat were reported from 2001 to 2005 (Jansen *et al.*, 2007). The consumption of contaminated pig meat or processed products was found to be associated with 20% of human Salmonellosis in Germany, whereas *S.* Typhimurium, especially DT 104, was the most frequently isolated *Salmonella* serotype from pig meat in 1999. Also in the Netherlands, on average 23% of all Salmonellosis cases for the period 2001 to 2008 were estimated to be associated with the consumption of pig meat (Van Pelt *et al.*, 2009). It is reasonable to assume that similar proportions of human *Salmonellosis* can be attributed to pork and pork products (EFSA, 2006).

2.4.2. Economic significance

An estimated 1.2 million cases of *Salmonella* occur annually in the United States; of these, approximately 42,000 are laboratory-confirmed cases reported to CDC. *Salmonella* serotypes Enteritidis, Typhimurium, and Newport account for about half of culture-confirmed *Salmonella* isolates reported. From the estimated 400 fatal cases each year; a few cases are complicated by chronic arthritis (CDC, 2008). The number of deaths from foodborne disease like salmonellosis is likely to be underestimated as most estimate of mortality are short

term and do not take into account coexisting illnesses. Infections with *Salmonella* were associated with increased long-term mortality (Helms *et al.*, 2003). Cost estimates per case of human Salmonellosis range from approximately US$ 40 for uncomplicated cases to US$ 4.6 million for cases ending with hospitalization and death (WHO, 2005).

Salmonellosis is also estimated to cost nations billions of dollars annually thereby draining funds that could have been used for development. Financial costs are not only associated with investigation, treatment and prevention of human illness but may affect the whole chain of food production. In the USA alone, non-typhoidal Salmonellosis is estimated with costs of $1.1billion to $1.5 billion annually (CDC, 2008). Salmonellosis has a serious economic impact on the livestock industry worldwide. Mortality, treatment costs, abortion, reduced production, discarded milk and reduced consumer confidence all contribute to the cost of *Salmonella* to livestock industries (Randell, 2001). However, very few countries report data on the economic cost of *Salmonella;* data related to the cost of foodborne disease are generally not available from developing countries (WHO, 2005).

2.5. Antimicrobial Resistance

Drug resistance is the decreased sensitivity or the complete insensitivity of microbes to drugs that cause cell death or inhibition of growth (Sharma *et al.*, 2005; NCCLS, 1999). Antimicrobial resistance of *Salmonella* is emerging global challenge, especially in developing countries where there is an increased misuse of antimicrobial agents in humans and animals (Kasper *et al.*, 2005). The injudicious use of antibiotics in many developing countries such as Ethiopia has led to an increased antibiotic resistance and in turn reduced therapeutic efficacy in these countries (Okeke *et al.*, 2007). Even though information is not available on the amount of Veterinary antimicrobials used in Ethiopian and no strict regulations exist on the use of drugs in food animals, and antimicrobials mainly used for treatment purposes (Sibhat *et al.*, 2009).

3. MATERIALS AND METHODS

3.1. Description of the study area

The investigated specimens and epidemiological data from pigs were collected from two abattoirs located in Addis Ababa and Bishoftu/Debre-Zeit. Both cities are located in the central part of Ethiopia. Addis Ababa is the capital and largest city of the country, while Bishoftu is a rapidly growing and industrializing town located 46 km south-east of Addis Ababa. Apart from Addis Ababa and Bishoftu/Debre-Zeit, Pigs were brought for slaughter to these abattoirs from the surrounding Oromia Zone; including, Adama/Modjo, Sebeta, and Zeway/Meki which are at most within 100-200 km radius from Addis Ababa having different geographic features, as shown below (Table 3) (CSA, 2008; NMSA, 1999).

Table 3: Geographic description of origin of Pigs (Source: CSA, 2008)

Origin of Pigs	Location(altitude)	Temperature (average)	Annual rain fall	Humidity
Bishoftu	9^0N and 4^0 E (1880m a.s.l.)	17.5^0C	1800mm	61.3%
Addis Ababa	$9^0$1'48''N and $38^0$44'24"E (2355m a.s.l.)	17^0C	1800mm	73%
Sebeta	$8^0$55'N and $38^0$37'E (2356m a.s.l.)	19.5^0C	1030mm	72%
Adama	8.54^0N and 39.27^0E (1712m a.s.l.)	20.8^0C	400 – 800mm	60%
Zeway/Meki	$7^0$56'N and $38^0$43'E (1712m a.s.l.)	20^0C	650 – 750mm	60%

3.2. Study design

The study was cross sectional and carried out from March, 2014 to May, 2014. Additional information's about the slaughtering practices was collected by on site observation (observational study) in the slaughter houses. Pigs located in Addis Ababa and the surrounding towns were considered as a target population and the study populations were apparently healthy slaughtered Pigs that originated from different farms in and around Addis Ababa. Pigs were transported on trucks in small groups from farms to the abattoirs and kept on concrete floor and open-air holding pen for an average three hours without feed and water. Samples were taken using a systematic random sampling. The sample size was calculated as; n = [1.962Pexp (1-Pexp)/ d2] (Thrusfield, 2005); expected 4% carcass contamination rate (Aragaw et al., 2007), d (absolute precision) = 0.05: n = unadjusted sample size; the adjusted sample size, nadj = 58; which is doubled for mesenteric lymph node, ceacal content and carcass swab samples. A total of 583 samples were examined of which, 120 mesenteric lymph node, 120 ceacal content, 100 carcass swab samples and 243 environmental samples from Personnel's hand (n=58), Eviscerating knife (n=58), Skin surface (n=58), Dehairing material (n=58) and Scalding water (n=11).

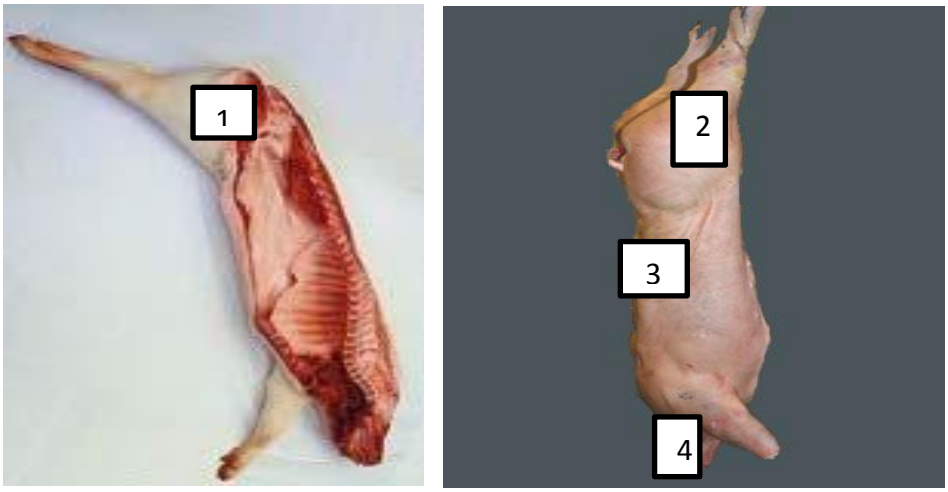
3.2.1. Sampling, Transport and Storage

Lymph node and Ceacal content samples

On sampling day, from each slaughtered animal sufficient amount (about 25gm) of samples were collected. Samples from Mesenteric lymph node by removing it from the intestines by using sterile blade and Ceacal contents were collected by piercing the cecum using sterile blade after evisceration. It was then put in to separate sterile plastic bags, and transported in an ice box to the Microbiology Laboratory of the CVMA, AAU and Institute of Biodiversity, Addis Ababa, Ethiopia, for microbiological analysis on the same day of sample collection for isolation and identification of *Salmonella.*

Carcass swabs

Sample swabbing of the carcasses was performed after evisceration at the final stage of the slaughtering process. Selected carcasses were swabbed using the method described in ISO-17604 (2003); by placing sterile template (10 x 10 cm) on specific sites of a carcass. A sterile cotton tipped swab, (2X3 cm) fitted with shaft, was first soaked in 10 ml of buffered peptone water (Oxoid Ltd., Hampshire, England) rubbed first horizontally and then vertically several times on the carcasses to maximize uptake of surface material the swab were rotate between the thumb and forefinger; a second complete sweep was made at right angles to the first sweep. The surface to be sampled were Ham, flank (belly, lateral abdomen), and jowl as shown in (figure 3). On completion of the rubbing process, swab were placed in to the tube, properly tighten the cap. Finally, the samples were transported to the Laboratory, using ice box in cold chain for microbiological analysis. Up on arrival, the samples were stored in refrigerator at 4 ^{0}C for 24 hrs before until being processed for isolation.



a) Medial b) Lateral

Figure 3: Swab sampling sites on the carcass
(Source: ISO 17604:2003)

Environmental samples

Environmental samples in slaughterhouse were collected from contact surfaces with the carcass; from eviscerating knives/carcass splitter (from the tip to the base, twice on both sides of the blade), personnel's hand, bedding/de-hairing materials during shaving, and from the surface of the skin at stunning-bleeding level in the holding pen with a surface of 100 cm^2 was sampled with a sterile Swab. Swabs were immediately placed in sterile universal bottle with an identification label on them. 25 ml of scalding water were taken with a sterile collection tube once in every sampling day. All samples were transported to the laboratory in a cooled container (ice box) and processed within 24 hours.

3.2.2. Microbiological method

Isolation and identification

Isolation and identification of *Salmonella* organisms was carried out by using the standard guidelines given by ISO-66579-3 (ISO, 66579-3: 2003) (Annex 1).

Sample processing and pre-enrichment on non-selective media

Samples were allowed to thaw for 3 – 5 hours at room temperature before analysis. The bacteriological media used for the study were prepared following the instructions of the Manufacturers (Annex 2). For the lymph node samples, first, all the mesenteric lymph nodes were separated from connective tissues and mesenteric fats then immersed for 10 seconds in 95% ethanol followed by flaming to decontaminate the surface. Each sample was put in a sterile stomacher bag and 225 ml of buffered peptone water (BPW, HiMedia, India) was added, and homogenized using a laboratory blender (Stomacher 400, Seward, England) for 2 minutes. Samples from ceacal content were pre-enriched in BPW in a ratio of 10-25 g of sample to 90-225 ml of BPW (1:9 ratios). The swab samples from the carcass and abattoir environment were kept at room temperature for few minutes and following this all the samples were incubated for 24 hours at 37 ^oC.

Enrichment in selective liquid media

Rappaport-Vassilliadis broth (HiMedia Laboratories Pvt. Ltd, Mumbai, India) and Selenite-F broth (Titan Biotech Ltd, Rajasthan, India) were used for selective enrichment. One ml and 0.1 ml aliquot of the pre-enrichment broths was transferred aseptically into a tube containing 10 ml of selenite-F broth and 10 ml of Rappaport-Vassilliadis (RV) broth, mixed and then incubated for 48 hours at 37 ^{o}C and 41.5 ^{o}C, respectively.

Plating out and identification

A loop full of each culture was streaked onto one plate of Xylose Lysine Desoxycholate (XLD) agar (HiMedia Laboratories Pvt. Ltd, Mumbai, India) and Brilliant Green Agar (BGA) (Titan Biotech Ltd, Rajasthan, India) medium and incubated at 37 ^{o}C for 24 to 48 hours. After incubation the plates (BGA and XLD) were examined for the presence of *Salmonella* colonies. Typical colonies of *Salmonella* grown on XLD-agar have a black center and a lightly transparent zone of reddish color due to the color change of the indicator and appears pink on BGA, with 1 mm to 2 mm in diameter, and cause the color of medium changed to red. H_2S negative variants grown on XLD agar are pink with a darker pink center and lactose-positive once are yellow with or without blackening (OIE, 2010; Quinn, 2004; Spencer, 2001; FDA, 2001). The suspected *Salmonella* positive colonies was transferred/plated on nutrient agar and incubated at 37^{o}C for 24 hrs.

Biochemical confirmation

The isolated typical *Salmonella* positive colonies on XLD and BGA agars was further examined using recommended biochemical tests (listed on annex 3), after growing overnight on nutrient agar at 37 ^{o}C. The biochemical tests were conducted by using TSI agar, Urea broth, L-lysine agar, Voges Proskauer and Indole tests based on the ISO-6579 standard. Colonies with alkaline slant, acid production (yellow), H_2S production (black), gas bubbles in the butt on TSI agar, positive for lysine (purple color), negative for urea hydrolysis (colorless), negative for tryptophan utilization (indole test) (yellow-brown ring), negative for Voges-Proskauer, and

positive for citrate utilization were considered to be *Salmonella*-positive (Annex 3) (ISO 6579, 1998; Wray and Wray 2001).

3.2.3. Antimicrobial susceptibility test

For the determination of antimicrobial susceptibility of the isolates, the Kirby-Bauer disk diffusion method was performed according to the recommendations of Clinical and Laboratory Standards Institute (CLSI, 2006). 167 *Salmonella* isolated from CC, MLN, Caracas swab and environmental samples were tested for 8 antimicrobials listed on table 4 below. To standardize bacterial suspension (in 0.8% NaCl), the density of suspension was adjusted to 0.5 McFarland and spread over the entire surface of Mueller Hinton agar (MHA) plates using a sterile cotton swab. Antimicrobial discs were placed on the agar surface followed by incubation of the plates at 37°C for 24 hours. Inhibition zones were measured by Venire Caliper and interpreted accordingly by CLSI recommendations. Inhibition were recorded to the nearest millimeter and classified as resistant, intermediate, or susceptible according to published interpretive chart (CLSI, 2006).

Table 4: Antimicrobials and Diameter of zone of inhibition (mm) used to test susceptibility of *Salmonella* isolates (Source: CLSI, 2006).

Type of antimicrobial	abbreviation	Disc potency	**Diameter of zone of inhibition (mm)**		
			Resistant	Intermediate	Susceptible
Ampicillin	AMP	10ug/ml	≤13	14-16	≥17
Amoxicillin/clavulanic acid	AMC	10ug/ml	≤13	14-17	≥18
Ceftriaxone	CRO	30ug/ml	≤13	14-20	≥21
Kanamycin	K	30ug/ml	≤13	14-17	≥18
Nalidixic Acid	NA	30ug/ml	≤13	14-18	≥19
Oxy-tetracycline	OT	30ug/ ml	≤11	12_14	≥15
Streptomycin	S	10ug/ml	≤11	12_14	≥15
Sulfamethoxazole/Trimethoprim	SXT	25ug/ml	≤10	11_15	≥16

3. 3. Data management and analysis

The coded data was entered in MS Excel and then analyzed using SPSS version 20.0. Descriptive statistics such as percentage, proportion, range, median, and mean were applied to compute some of the data. The occurrence of *Salmonella* was calculated by dividing the number of positive for *Salmonella* infection by the total number examined. Difference among and between proportions of the groups with certain determinant factor was determined by chi-square (χ^2) test. A p-value <0.05 was considered indicative of a statistical significant difference.

4. RESULT

4.1. Occurrence of *Salmonella*

The overall Occurrence of *Salmonella* in carcass swab, mesenteric lymph node, caecal content samples and environmental samples was 28.6% (167). Out of which 16% (16) were from carcass swab, 48.3% (58) from mesenteric lymph node and 41.7% (50) from caecal content samples and 17.6% (43) from environmental samples.

Of the total mesenteric lymph node samples ($n = 120$) examined, a higher occurrence was recorded from pigs that originated in Debre Zeit 36(53.7%) followed from pigs that originated in Sebetta and Addis Ababa. Of the caecal content samples the proportion of positive samples ranged from 6% in Adama to 50% in Debre Zeit. *Salmonellae* were found more prevalent in Debre Zeit (50%) compared to Zeway/Meki (16%), Addis Ababa (14%), Sebeta (14%) and Adama (6%); (Table 5).

Table 5: Occurrence of *Salmonella* by origin of pigs

Origin of pigs	Number examined	Positive (%)	
		MLN	CC
Debre Zeit	67	36(53.7)	25(50)
Zeway	16	7(43.8)	8(16)
Addis Ababa	16	6(37.5)	7(14)
Sebetta	13	6(46.2)	7(14)
Adama	8	3(37.5)	3(6)
Total	120	58 (48.3)	50(41.7)

MLN= Mesentric lymph node, CC= Ceacal content

The recovery rate of *Salmonella* was apparently higher from samples taken from mid-land pigs than from highland and lowland pigs; from young animals than older animals and from females

than males. There was no statistically significant association in the occurrence of *Salmonella* among the different origins, sex and age group (p>0.05) (Table 6 and 7).

Table 6: Occurrence of *Salmonella* by risk factors

Risk factor		**Number examined**	**Positive (%)**	
			MLN	**CC**
Altitude	Highland	29	12(41.4)	14(48.3)
	Mid-land	67	36(53.7)	25(37.3)
	Lowland	24	10(41.7)	11(45.8)
Age	Young	71	35(49.3)	34(68.0)
	Old	49	23(46.9)	16(32.0)
Sex	Female	57	33(57.9)	20(40.0)
	Male	63	25(39.7)	30(60.0)

MLN= Mesentric lymph node, CC= Ceacal content

Table 7: Association in occurrence of *Salmonella* by risk factors (MLN, CC)

Risk factors	**X^2**	**df**	**p-value**
Altitude	1.77, 1.21	2	0.412, 0.545
Sex	3.97, 1.933	1	0.046, 0.164
Age	0.18, 2.768	1	0.66, 0.96

X^2 = chi-square test; df = degree of freedom; p-value = significant difference

4.2. Occurrence of *Salmonella* in the slaughtering line

Out of 100 Caracas swabs from slaughtered swine the overall contamination level was 16(16%). Of the 243 environmental samples from the slaughtering line *Salmonella* was recovered from 43 (17.7%), the highest rate of *Salmonella* were recovered from the skin surface 20 (34.5%), followed by Bedding/de-hairing materials, personnel's hand 8 (13.8%) and Eviscerating knife 7 (12.1%), respectively (table 8). The rate of *Salmonella* contamination in samples taken from the slaughtering line (n=58, each samples) ranged from 0 % (*Salmonella* not detected in any of the

samples) to 100 % (all the samples are positive for *Salmonella*). Association of carcass contamination with the risk factors in the slaughtering line were assessed using chi-square test and no statistical significant result were observed except with eviscerating knife, which was found to be significantly associated with carcass contamination (table 9).

Table 8: Occurrence of *Salmonella* on Carcass and environmental samples

Sample type	**No. of samples**	**No. of *Salmonella* positive (% of raw)**
Carcass Swab	100	16 (16.0)
Personnel's hand	58	8 (13.8)
Eviscerating knife	58	7 (12.1)
Skin surface	58	20 (34.5)
De-hairing material	58	8 (13.8)
Scalding water	11	0
total	343	59 (17.2%)

Table 9: Association of carcass contamination with the risk factors in the slaughtering line

Environmental samples	**X^2**	**df**	**p-value**
Eviscerating knife	16.38	1	0.00
Personnel's hand	0.391	1	0.532
Skin surface	1.122	1	0.29
De-hairing material	2.66	1	0.102
Scalding water	0.008	1	0.927

X^2 = chi-square test; df = degree of freedom; p-value = significant difference

4.3. Antimicrobial resistant profile of *Salmonella* isolates

4.3.1. Mono drug resistance

The mono-drug resistance feature of the isolates is presented in Table 10. The highest resistance was recorded for Oxy-tetracycline (74.2%) followed by Streptomycin (69.4%) and Ampicillin (65.2%). The lowest resistance was observed for Sulfamethoxazole/Trimethoprim (3.6%). The proportion of isolates resistant to ceftriaxone and Nalidixic acid were 10.2% and 40.1% respectively. The majority of the isolates was susceptible to Sulfamethoxazole/Trimethoprim (68.9) followed by Kanamycin (65.8%) and Ceftriaxone (55.7%).

Table 10: Proportions of resistant and susceptible isolates by antimicrobials (n = 167).

Type of antimicrobial	Number of isolates		
	Resistant (%)	Intermediate (%)	Susceptible (%)
AMP	109 (65.2)	43 (25.7)	15 (8.9)
AMC	29 (17.3)	47 (28.1)	91(54.6)
CRO	17 (10.2)	57 (34.1)	93 (55.7)
K	11 (6.5)	46 (27.5)	110 (65.8)
NA	67 (40.1)	54 (32.3)	46 (27.5)
OT	124 (74.2)	33 (19.7)	10 (5.9)
S	116 (69.4)	42 (25.1)	9 (5.3)
SXT	6 (3.6)	46 (27.5)	115 (68.9)

4.3.2. Multi drug resistance

Of the total 167 *Salmonella* isolates 142 (85%) were multidrug resistant of which 53 (31.7%) isolates were resistant to 2 antimicrobials; 41 (24.5%) were resistant to 3 antimicrobials; 32 (19.2%) isolates were resistant to 4 antimicrobials and 16 (9.5%) was resistant to 5 antimicrobials. Overall 36 MDR patterns were recorded. The most common MDR phenotype

was the Oxy-tetracycline and Streptomycin phenotype (14/142) followed by the Nalidixic acid Oxy-tetracycline and Streptomycin phenotype (10/142). Results of the MDR profiles of the isolates are shown in Table 11, below.

Table 11: Multi-drug resistance patterns of *Salmonella* isolates

Number of antimicrobials	Resistance pattern (number of isolates)	Number of isolates (%)
Two	AMP, AMC (8) OT, S (14) AMC, S (3) AMP, S (5) S, SXT (1) AMC, OT (4) NA, K (2) AMP, OT (5) AMC, N (2) AMC, SXT (1) NA, OT (5) CRO, SXT (2) NAL, STR (1)	53 (31.7%)
Three	AMP, AMC, OT (6) AMC, OT, S (4) NA, OT, S (10) AMP, NA, S (4) AMP, OT, S (5) AMP, NA, OT (3) AMC, CRO, S (2) AMP, NA, OT (1) AMP, AMC, NA (4) AMP, AMC, S (1) CRO, OT, S (1)	41 (24.5%)

Four	AMP, AMC, OT, S (5) AMP, AMC, NA, OT (2) AMP, NA, OT, S (6) CRO, NA, OT, S (4) NA, K, OT, S (1) AMP, K, OT, S (1) AMC, NA, OT, S (7) AMP, AMC, CRO, OT (4) AMP, K, NA, OT (2)	32 (19.2%)
Five	AMP, AMC, K, OT, S (5) AMP, AMC, NA, OT, S (7) AMP, CRO, NA, OT, S (4)	16 (9.5%)

5. DISCUSSION

In the present study, a total of 583 samples were examined from apparently healthy slaughtered pigs and abattoir environment. Of which, 120 mesentric lymph nodes and caecal content samples were taken immediately at slaughter, almost perfectly indicates the infection rates of pig herds at farm level. The reason of taking samples from the lymph nodes is that *Salmonella* is often present in the mesenteric lymph nodes in carrier animals and that lymph nodes are the tissues most consistently colonized in infected animals (Berends *et al.,* 1997; Carlson and Blaha, 2001). Although, the presence of *Salmonella* in the mesenteric lymph nodes mostly refers to an infection originating from the pig herd (Fravalo *et al.,* 1999; Swanenburg *et al.,* 2001b). The presence of *Salmonella* from surfaces of carcasses after evisceration is an indication of cross-contamination under slaughtering hygiene by feces, infected tissues, and equipment's (contact surfaces) in slaughterhouse environment.

In the present study, the occurrence of *Salmonella* was 48.3% in the mesenteric lymph nodes of apparently healthy slaughtered pigs. This finding was agreed with earlier observation by Molla *et al.,* (2003) and Aragaw *et al*., (2007); who reported 41.6% and 43% *Salmonella* prevalence in Addis Ababa municipal abattoirs and that of 40% prevalence rate of mesenteric lymph node by Amaechi and Ezeronye (2006). In contrast, the present finding was higher than reported 34.8% prevalence of *Salmonella* from lymph nodes in Hanoi, Vietnam (Nguyen Phu Thai, 2007). The finding of 41.7% of *Salmonella* from caecal contents in the present study was varied with 21.8%, and 22.7% prevalence of caecal contents reported by Molla *et al.*, (2003) and Aragaw *et al*., (2007) in Debre Zeit and Addis Ababa abattoir, Ethiopia. The estimate was also higher than the previous reports of 8.6%, 19%, 22.2%, 7.9%, 24.6% and 23% prevalence of the faecal samples in Kenya, Belgium, Danish, Canada, USA and Great Britain commercial slaughterhouses, respectively (Gideon *et al*., 2010; Botteldoorn *et al*., 2003; Baggesen *et al.* 1998; Letellier *et al.*, 1999; Davies *et al.,* 1997; and DEFRA, 2003). In this study, there was statistical significant difference of *Salmonella* occurrence in caecal content and lymph nodes ($p < 0.05$). This could be due *Salmonella* might not shed in faceses of the chronic carrier pigs.

The difference in the reported results could be associated with the sampling plan, sample type, the bacteriological techniques employed in detecting *Salmonella* in the study population regardless of test samples and methods of detection. Furthermore, the management systems and the epidemiology of *Salmonella* across countries might influence on the infection rate. It is also known that keeping animal to be slaughtered in crowded waiting pens at abattoir could facilitate the excretion and transmission of infection among them (Watson, 1975; Radostits *et al*., 1994). In general there is no standard set and estimates ranging from 0.2% in Sweden to 48% in Hungary were recorded (Rowe *et al.,* 2003).

In the present study, although the potential risk factor for the occurrence of *Salmonella* among Swine's was assessed and no statistically significant association could be demonstrated between the occurrence of *Salmonella* in the examined animals based on origin, sex and age. However, relatively higher recovery rate of *Salmonella* was recorded in Debre Zeit (50% - 53.7%), in females than males and in young pigs than elder once. The difference observed in the occurrence of *Salmonella* between origins, sex and age could be attributed to the differences in management practices in the farms, immune status, and stress factors. Based on some study findings *Salmonella* occurrence is highly variable between batches within a herd and other factors (Corrégé and Guyomard, 2007).

The current study finds out *Salmonella* in 16 % of carcass swab samples at slaughterhouses. This finding is agreed with that of Gideon *et al*., (2010) in Kenya who reported 19.0% prevalence of *Salmonella* from the carcass swabs. However, it was lower than that of previous studies who found 37%, 48.9% and 95.7% prevalence of *Salmonella* from carcass swab (Botteldoorn *et al.*, 2003; Nguyen Phu Thai, 2007; and Cédric *et al.,* 2006). In contrast the present find is higher than that of Aragaw *et al*., (2007) who reported 4% prevalence of *Salmonella* on pig carcass in the same study area. It might be due to the fact that the hygiene level of slaughterhouses was varied or actual prevalence of *Salmonella* is increased.

The high recovery rate of *Salmonella* from the carcass swabs could be due to cross contamination associated with the potential risk factors in the slaughtering line. Based on some study findings the process of evisceration, dressing area, poor disinfection of knives/dehairing

machines and other equipment, poor sanitation of the slaughterhouse may contribute to high level of contamination of carcass. Carcass contamination also directly linked to the skin contamination of live pigs before stunning, and cleanness of the holding pen. Based on some studies for instance, the level of carcass contamination decreased from 59% to 35% when the skin was contaminated or not. On the other hand, skin contamination was connected to the contamination of lairage pens. Some studies suggests that the conditional probability of skin contamination decreased from 70% to 36% when the floor of the lairage pens was contaminated or not (Giovannini *et al.*, 2004; Rossel, 2009).

The present study revealed that the holding pen/lairage surface was not cleaned frequently and both the stunning and bleeding practice were conducted in the holding pens/lairage which is contaminated with feaces. Out of 58 of swab samples from the surface of the skin of pigs in the holding pen 34.5% were found to be *Salmonella* positive. In contrast, the present finding was lower than the finding of 62.5% and 90.3% positive results in two abattoirs (Hurd *et al.,* 2003). However, it is higher than 20.0% prevalence of *Salmonella* from holding pens reported by Amaechi and Ezeronye, (2006).

In addition to our findings, other studies also reported high rates of *Salmonella* contamination in holding pens from two abattoirs in Europe (Swanenburg *et al.,* 2001a). Similar results were reported for the holding pens of cattle and sheep abattoirs in the United States (Small *et al.,* 2002). In the study abattoirs, pigs from several farms spent pre-slaughter holding time in these pens. This common practice in swine abattoirs allows a buildup of *Salmonella* populations in the holding pen environment, which constitutes a potential infection source. The exposure time, in trailers or pens, was sufficient to allow infection or contamination of the gastrointestinal tracts (Hurd *et al.,* 2003). Stunning-bleeding and scalding are also important steps in relation to *Salmonella* contamination (ESFA, 2010).

The present study revealed that the Scalding operations range from 57.7^{o} C – 61^{o} C (136^{o} F – 142^{o} F) for three to eight minutes and the scalding water was used for all of the pigs slaughtered without a change. All the samples from scalding water were free of *Salmonella*, this agreed with other findings in which no *Salmonella* was recovered from 16 scalding water samples at Addis

Ababa abattoir, Ethiopia (Aragaw *et al.*, 2007) and 5 Belgian slaughterhouses (Botteldoom *et al.*, 2003). Based on the microbiological data the absence of *Salmonella* in the scalding water is as a result of the majority of *Salmonella* would not survive a scald process of 58.8 ^{o} C for six minutes. This indicates the scalding operation significantly reduces the overall microbial population on the skin of hog carcasses, this suggests that cross-contamination with *Salmonella* between carcasses in the scald tank would be an unlikely event. However, other data suggests that scald tanks may harbor viable *Salmonella* and may be a potential source of cross contamination. In some studies *Salmonella* were detected on 2% (scalded) and 6.7% (skinned) carcasses (Arguello *et al*., 2012; Botteldoorn *et al.*, 2003).

The potential for carcass contamination during the de-haring process is illustrated in this study by taking samples from bedding/de-haring materials (n= 58) and *Salmonella* was recovered from 12.1%. The present study revealed that the de-hairing practice was conducted manually and this could introduce bacteria into the skin surface by scratching. The potential for contamination during the de-hairing process is illustrated in other study which showed that the mesophilic bacterial populations on hog carcasses increased after the de-hairing operation, when compared to the populations before de-hairing (Gill and Bryant, 1992). Other studies reported an increase in the incidence of *Salmonella* positive carcasses immediately after scalding (Davies *et al*., 1999; Pearce *et al.*, 2004). In a similar study, Nerbrink and Broch (1989) reported increases in Enterobacteriaceae populations on hog carcasses after de-hairing.

The occurrence of *Salmonella* in eviscerating knife in this study was 13.8%, which is comparable with 15.4% prevalence in another study undertaken on evisceration knifes (Amaechi and Ezeronye, 2006). In contrast, it is higher than that of 7.4% prevalence of *Salmonella* reported by Akafite (2008), and 5% in Queensland, Australia (Peel and Simmons, 1978). However, it is lower than the reported 26.7% prevalence of evisceration knife in Botswana (Motsoela *et al.*, 2002). In related to this evisceration is one of the operations which has the greatest potential for *Salmonella* contamination (Alban and Stark, 2005; Arguello *et al*., 2012). Specific attention must be given to the serialization of knives. As clearly indicated by different workers, it is crucial to note that knives must be immersed in water for 2 minutes at 82^{0}c to reduce the number of

contaminating microorganisms (Watson, 1975; Smeltzer *et al.,* 1980b. and Motsoela *et al.,* 2002).

In addition, *Salmonella* isolate were recorded from the hands of the workers in all stages along the slaughtering line from 13.8% of the hand swab samples. This finding is comparable with 8.9% prevalence of *Salmonella* from evisceration hand swabs at mojo abattoir reported by Akafete, (2008). In contrast, it was lower than 30% and in a study undertaken in Queensland (Smeltzer *et al.,* 1980a). Amaechi and Ezeronye, (2006) also reported 26.7% prevalence of *Salmonella* from workers hands. Some studies suggest that washing of the hands is needed to reduce cross contamination of carcass with S*almonella* (Watson, 1975; Smeltzer *et al.,* 1980a). Therefore, the variation obtained in this study could be due to absence of frequent washing of hands, difference in the slaughtering capacity, slaughtering practice and implementation of HACCP system in the abattoirs.

The final carcass wash is a "whole carcass" treatment by the use of hot water or hot water in combination with organic acids has been shown to be an effective method of decontaminating hog carcasses (Barkate *et al.,* 1993; Gill *et al.,* 1995; Dickson, 1998; Eggenberger-Solorzano *et al.,* 2002). In this survey, it was observed that the final washing was done by normal pipeline water and after washing the carcass using pressurized water there is a close contact between the carcasses during hanging. This situation might considerably contribute to the cross contamination of carcasses resulting in relatively high prevalence of *Salmonella* on carcasses.

Since bleeding, evisceration, splitting, and hanging/signing of the carcass as it is commonly practiced is a manual operation, contamination is of a random nature, and typically would affect only the specific carcass in which the break occurred through improperly sterilized knives and other personal equipment. The finding of high proportion of *Salmonella* in the skin surface, bedding/dehairing materials, personnel's hand and eviscerating knifes in this study illustrates that they could be the potential risk factors which results cross contamination of the carcass in the slaughtering line.

Other researches also show that equipment and knives have the potential to become contaminated by contact with the hide and then this contamination may be spread to the edible

tissue. *Salmonella* may be carried on improperly sterilized knives and other personal equipment (Peel and Simmons, 1978; Berends *et al.,* 1997). In a similar manner, contamination from one carcass may be spread to tissue from another uncontaminated carcass by contact with common surfaces; such as hands, knives, processing equipment or conveyor belts (Arguello *et al*., 2012; Botteldoorn *et al.,* 2003).

In the present study, Antimicrobial resistant profiles of *Salmonella* isolates to commonly used antimicrobials were detected and all the isolates were resistant at least for one antimicrobial. This finding was agreed with a study conducted in US who reported 78.6% resistance for OxyTTC, but higher than the reported 53.3% of Streptomycine and 38.7% of Ampicilin (APHIS, 2009). The finding of a study by Tesfaw *et al.,* (2013), on cottage cheese and Zewdu (2004), on various animal source foods has also a comparable result with the present finding, who reports 83.3%, of resistant isolates to tetracycline and 50% - 59.4% of resistant isolates to Ampicillin, respectively. In contrast, Argaw *et al.,* (2007) reported 88.6% resistance to tetracycline on isolates from pigs, 82.1% and 64.5% were reported from North Carolina (Gebreyes *et al.,* 2000) and on isolates from pork carcass in Northeast Georgia (Epling and Carpenter, 1990) which is higher than the present finding. Different studies in Ethiopia also reported that *Salmonella* has been resistant to first line antibiotics such as ampicillin, tetracycline and chloramphenicol (Mache, 2002; Alemayehu *et al*., 2003; Asrat, 2008; Alemu and Molla, 2011; Beyene *et al.,* 2011).

The finding of 85% Multidrug resistance *Salmonella* isolates in this study; support other reports in which most of *Salmonella* species usually resistant to five drugs: Ampicillin, Chloramphinicol, Streptomycin, Sulfonamides, and Tetracyclines (Soyer *et al.,* 2009). In comparable with the present finding a much higher prevalence of multi-drug resistance (85.1%) was observed in *Salmonella* isolates from pigs in North Carolina (Gebreyes *et al.,* 2004), Spain (83.3-97%) (Agustin *et al*., 2005) and Germany (90.5%) (Schroeter *et al.,* 2004). In contrast, the present finding was higher than 57 (32.9%) and 69 (73.4%) multi-drug resistant (MDR) isolates from Swines reported by Aragaw *et al.,* (2007) and Molla *et al.,* (2006). This might be associated with the common use of these antimicrobials in food animals including pigs in Ethiopia. The present proportion is comparable with the MDR estimates in previous studies in other food animals including chicken, cattle and camels (Tibaijuka *et al*., 2002; Alemayehu *et al*., 2003; Molla *et al*., 2004) and humans (Mache *et al*., 1997; Mache, 2002) in Ethiopia.

The significantly high frequency of resistant *Salmonella* for these antimicrobials was probably an indication of their frequent usage both in the livestock and public health sectors. Furthermore, it could be attributed to the indiscriminate and sub-therapeutic dose of commonly available and relatively cheaper antimicrobials in Ethiopia. In general, antimicrobial use is the key driver of resistance. This selective pressure comes from a combination of overuse in many parts of the world particularly for minor infections, misuse due to lack of access to appropriate treatment and under use due to inadequate dosing, poor adherence, substandard antimicrobial and lack of financial support to complete treatment courses (WHO, 2001). Use of antimicrobials for treatment, prophylaxis and growth promotion in food animals rearing has contributed for the wide spread use of these compounds and thought to further encourage the emergence and spread of resistance (WHO, 1999).

The widespread use of antibacterial substances for human and animal therapy resulted into development of resistance by bacteria towards antimicrobial substances. The high percentage of multiple resistant *Salmonella* isolates to the commonly used antimicrobials observed in this study could therefore pose significant health hazards. Most antimicrobial resistant *Salmonella* infections were acquired from eating contaminated foods of animal origin (Angulo *et al.*, 2000). Therefore, efforts should be directed towards reducing the magnitude of the problem at various levels through prudent use of antimicrobials.

6. CONCLUSION AND RECOMMENDATIONS

The finding of high proportion of drug resistant *Salmonella* in apparently healthy slaughtered pigs and slaughtering line indicates that *Salmonella* infection was widespread at farm level and a risk of cross contamination in the abattoirs (harvest stage), unless the hygienic practice (implementation of GMP, HACCP) in the abattoir was improved. In adequate Management system in pig farms like poor hygienic status of farms, feed and water supplies, herd level (overcrowding) and other factors could be the potential risk factors.

The following recommendations for hygiene improvement in the pig farms and slaughterhouse standard are given in order to reduce the level of antimicrobial resistant *Salmonella* in pig production chain.

- More emphasis should be given on strategies of *Salmonella* control at farm level by implementing appropriate management practices to reduce prevalence of *Salmonella* from farm origin. It is useful to study more about surveillance on *Salmonella* at farm level.
- Epidemiological data of *Salmonella* in pigs and pork products should be available to inform public health authorities about the level and magnitude of the problem for improving the current situation.
- Improving sanitary practice and system standards strictly in the slaughterhouse such as HACCP, find out the adapted CCPs where the reverse pathogens can be reduced to acceptable level carry out regular training for people handling in slaughterhouse.
- Encouraging prudent and judicious use of antimicrobial drugs in veterinary and public health sectors.
- Further large scale studies should be carried out to describe the risk factors associated with the emergence of drug resistant *Salmonella* and control of their spread across the country.
- It is useful to improve the public awareness, suggest consumers and food business handlers to follow safe practices on food handling such as appropriate storage and consumption cooked pork products.

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APPENDICES (ANNEXES)

Annex 1: Flow diagram for isolation and identification of *Salmonella* (ISO, 66579-3: 2003)

Non-selective pre-enrichment

Add 25 g samples in a 225 ml of buffered peptone water and incubated at 37°C for 24 h

⇩

Selective enrichment

0.1 ml in 10 ml Rappaport-Vassiliadis Soy Broth at 37°C, 24 h

1 ml in 10 ml Selenite F broth at 37°C, 24 h

⇩

Isolation/plating

Inoculate a loop full of inoculums on Xylose-Lysine-Desoxycholate (XLD) and Brliant green agar (BGA) and incubate at 37°C for 24 h to 48hrs

⇩

Streaking on nutrient agar at 37°C, 24 h

⇩

Biochemical confirmation at 37°C, 24h

(TSI, Urease Test, L-Lysine Decarboxylation Test, MR-VP Test, Indole Test

⇩

Serotyping (O-antigens, H antigins)

Annex 2: Media used for bacteriological isolation

1. Buffered Peptone Water (HiMedia Laboratories Pvt. Ltd, Mumbai, India), *REF*= M1494I-500g

Ingredients/Composition	gm/l
Casein enzymic hydrolysate	10.00
disodium hydrogen phosphate	9.00
sodium chloride	5.00
monopotasium hydrogen phosphate	1.50

Final PH at 25^0C 7.0 ±0.2

Directions:

1. Suspend 20.07gm of dehydrated medium in 1000ml distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs pressure (121^0C) for 15 minutes.

2. Selenite F broth (Titan Biotech Ltd, Rajasthan, India), *REF*= TM 389 *Part I)*

Ingredients	(gm/l)
Sodium phosphate	10.00
Lactose	4.00
Casein enzymic hydrolysate	5.00

Final $_P$H at 25^0C 7.0 ±0.2

Directions:

1. Dissolve 19.0gm in 750ml distilled water.
2. Mix part I and part II.
3. Warm to dissolve the medium completely.
4. Dispense in sterile test tubes.
5. Sterilize in boiling water bath or free flowing steam for 10 minutes. DO NOT AUTOCLAVE. Excessive heating is detrimental.

3. Selenite F broth (Titan Biotech Ltd, Rajasthan, India), *REF*= TM 389 *Part II*

Ingredients (gm/l): Sodium hydrogen selenite (4.00).

Directions:

1. Dissolve 4gm of part II in 250ml distilled water.
2. Mix part I and part II.
3. Warm to dissolve the medium completely.
4. Dispense in sterile test tubes.
5. Sterilize in boiling water bath or free flowing steam for 10 minutes. DO NOT AUTOCLAVE. Excessive heating is detrimental.

4. Rappaport-Vassiliadis (HiMedia Laboratories Pvt. Ltd, Mumbai, India), *REF*= M880-500g

Ingredients	gm/l
Papaic digest of soyabean meal	4.50
Sodium chloride	7.20
Monopotasium phosphate	1.44
Magnesium chloride	36.00
Malachite green	0.036

Final PH at 25^0C 5.2 ±0.2

Directions:

1. Suspend 49.2gm in 1000ml distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Dispense in sterile test tubes and sterilize by autoclaving at 10lbs pressure (115^0C) for 15 minutes.

5. Xylose Lysine Deoxycholate (HiMedia Laboratories Pvt. Ltd, Mumbai, India), *REF*= M031-500g

Ingredients	gm/l
Yeast extract	3.00)
L-lysine	5.00
lactose	7.50
sucrose	7.50
xylose	3.50
sodium chloride	5.00
sodium deoxycholate	2.50
sodium thiosulphate	6.80
ferric ammonium citrate	0.80
phenol red	0.08
and agar	15.00

Final PH at 25^0C 7.4 ±0.2

Directions:

1. Suspend 56.68gm in 1000ml distilled water.
2. Heat with frequent agitation until the medium boils. DO NOT AUTOCLAVE OR OVER HEAT.
3. Transfer immediately to water bath at 50^0C. After cooling, pour into sterile petri plates.

6. Brilliant Green Agar (Titan Biotech Ltd, Rajasthan, India); *REF*= TM951

Ingredients	gm/l
Agar	20.00
proteose peptone	10.00
lactose	10.00
sucrose	10.00
sodium chloride	5.00
yeast extract	3.00
phenol red	0.08
brilliant green	0.0125

Final PH at 25^0C 6.9 ±0.2

Directions:

1. Dissolve 58.09gm in 1000ml distilled water.
2. Boil to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs pressure (121^0C) for 15 minutes.
4. Mix well before pouring into sterile petri plates.

Media used for Biochemical Tests

1. Triple Sugar Iron Agar (OXOID, UK)

Ingredient/composition	gm/l
Lmb-Lemco powder	3.0
Yeast extract	3.0
Peptone	20.0
Sodium chloride	5.0

Lactos	10.0
Sucrose	10.0
Glucose	1.0
Ferric citrate	0.3
Sodium thiosulphate	0.30
Phenol red	0.024
Agar	12.0

PH 7.4 ± 0.2 at 25°C

Directions:

1. Suspend 65g in 1 liter of distilled water.
2. Bring to the boil to dissolve completely.
3. Mix well and distribute.
4. Sterilized by autoclaving at 121°C for 15 minutes.
5. Allow the medium to set in sloped form with a butt about 1 inch deep (5ml).

2. Tryptone Broth (Tryptone Water) (HiMedia Laboratories Pvt. Ltd, Mumbai, India)

Ingredients/ compositions	**gm/l**
Casein enzymic hydrolysate	10.0
Sodium chloride	5.0

PH at 25°C 7.5±0.2

Directions:

1. Suspend 15 grams in 1000 ml distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Dispense into tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

3. Lysine iron agar (Oxiod Ltd, Wade Road, UK)

Ingredients/compositions	Gm/l
Bacteriological peptone	5.0
Yeast extract	3.0
Glucose	1.0
L-lysine	10.0
Ferric ammonium citrate	0.5
Sodium thiosulphate	0.04
Bromocresto purple	0.02
Agar	14.5

pH 6.7 ± 0.2 at 25°C
Adjusted as required to meet performance standards

Directions:

1. Suspend 34g in 1 litre of distilled water.
2. Bring to the boil to dissolve completely.
3. Dispense into tubes and sterilise by autoclaving at 121° C for 15 minutes.
4. Cool the tubes in an inclined position to form slants with deep butts.

4. MR-VP broth (HiMedia Laboratories Pvt. Ltd, Mumbai, India)

Ingredients/ compositions	gm/l
Enzymatic Digest of Casein	3.5
Enzymatic Digest of Animal Tissue	3.5
Dextrose	5
Potassium Phosphate	5

Final pH: 6.9 ± 0.2 at 25°C.

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Directions:

1. Dissolve 17 g of the medium in one liter of purified water.
2. Mix thoroughly and autoclave at 121°C for 15 minutes.

6. Urea Broth (Filter Sterilizable) (HiMedia Laboratories Pvt. Ltd, Mumbai, India)

Compositions/ingrdients	gm/l
Monopotassium phosphate	9.100
Yeast extracts	0.100
Dipotassium phosphate	9.500
Urea	20.000
Phenol red	0.010

Final pH (at 25°C) 6.8±0.2

Directions:

1. Suspend 38.7 grams in 1000 ml distilled water.
2. Mix well and sterilize by filtration.
3. DO NOT AUTOCLAVE OR HEAT the medium.
4. Dispense in sterile tubes.

4. Brain-heart infusion broth (Oxoid Ltd, Wade Road, UK)

Ingredients/composition	gm/l
Brain Heart Infusion	17.5
Enzymatic Digest of Gelatin	10.0
Dextrose	2
Sodium Chloride	5.0

PH 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions:

1. For Laboratory Use.
2. IRRITANT; Irritating to eyes, respiratory system, and skin.

Directions:

1. Dissolve 37 g of the medium in one liter of purified water.
2. Heat with frequent agitation to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

5. **Mueller-Hinton Agar (Oxoid Ltd,** Wade Road**, UK)**

Ingredients/compositions	gm/l
Beef, dehydrated infusion from	300.0
Casein hydrolysate	17.5
Starch	1.5
Agar	17.0

PH 7.3 ± 0.1 at 25°C

Adjusted as required to meet performance standards

Directions:

1. Add 38g to 1 litre of distilled water.
2. Bring to the boil to dissolve the medium completely.
3. Sterilise by autoclaving at 121°C for 15 minutes.

Annex 3: Interpretation of biochemical test reaction

Biochemical test indicators	Test reactions(color) /observation		Typical *Salmonella* reaction	Percent
	Positive	Negative		
TSI acid(glucose)	Butt yellow, slant red	Both butt and slant are red	++	100
TSI gas	Gas pocket in the mediu m	No gas	+	91.
TSI lactose	Slant turns yellow	Color of slant unchanged	-	99.
TSI sucrose	Slant turns yellow	Color of slant unchanged	-	99.
TSI H_2S	Black butt and/or slant	No blackening	+	91.
Urea splitting	Medium changes to pink /red	Color of mediu m unchanged	-	99
L-lysine decarboxylation	Butt remains purple	Butt turns yellow	+	94.
β-galactosidase rxn	Yellow	Colorless after 24hrs	-	98.
Voges-Proskauer rxn	Red	Colorless	-	100
Indole reaction	Reagent layer deep red (tryptose water)	Reagent layer yellow	-	98.

Annex 4: GENERIC DATA RECORD SHEET

Date----------Species ---------- ----------Enumerator ----------

Date	Sample number	Breed e/l	Sex m/f	Age y/a	Comment

Annex 5: Plating media record sheet

Sample ----------Place----------Enumerator ----------

Date	Sample number	BGA agar		XLD agar		Sample (+/-)	Comments
		number of		number of			
		SE	RV	SE	RV		

Annex 6: BIOCHEMICAL TESTS RECORD SHEET

Sample ---------- place----------Enumerator----------

Date	Sample and colony identification †	Test colors /gas									Sal. Positive /negative (+/-)	Comments
		TSI butt	TSI slant	TSI gas	TSI H_2S	Urea	L-lysine	β-galac	VP	indole		