ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
MSc THESIS

ISOLATION AND ANTI-MICROBIAL SUSCEPTIBILITY TESTING OF *SALMONELLA SPECIES* FROM FOODS OF CATTLE SOURCE IN MERKATO, ADDIS ABABA ETHIOPIA

By

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ABBREVIATIONS

API: Analytical Profile Index

AP-PCR: Arbitrary Primed-Polymerase Chain Reaction

BGA: Brilliant Green Agar

CDC: Center for Disease Control and Prevention

CIDRAP: Center for Infectious Disease Research Policy

DMIP: Department of Microbiology, Immunology and Parasitology

EFSA: European Food Safety Authority

ERS-USAD: Economic Research Service-United States Department of Agriculture

FDA: Food and Drug Administration

HACCP: Hazard Analysis Critical Control Point

ISO: International Standard Organization

LB: Luria Broth

LPS: Lip polysaccharide

MDR: Multi-Drug Resistant

MLVA: Multilocus VNTR Analysis

NCCLS: National Committee for Clinical Laboratory Standards

NTS: Non-typhoidal Salmonellosis

PFGE: Pulse Field Gel Electrophoresis

RVS: Rappaport-Vassiliadis Soya Peptone Broth

SPI: *Salmonella* Pathogen city Islands

SPSS: Statistical Package for Social Sciences

TSI/KIA: Triple Sugar Iron Agar/ Kliglers Iron Agar

VNTR: Variable-Number Tandem Repeats

WHO: The World Health Organization

ABSTRACT

Back ground: Infectious diseases are diseases that are transmitted mainly through food and still remain a common and persistent public health problem resulting high morbidity and occasional mortality in both developed and developing countries. Foods obtained from animals play an important role for food borne diseases particularly in developing countries like Ethiopia. Food borne diseases are caused by a variety of bacteria, viruses, parasites and fungi. Salmonellosis is one of those food borne diseases caused by *Salmonella species* which is becoming resistant to antimicrobials and causing serious diseases especially in developing countries because of consumption of raw foods such as meat, milk and cheese.

Objective: This study is aimed to determine the prevalence of *Salmonella species* from foods of cattle source and perform their anti- microbial susceptibility tests.

Materials and Methods: A cross-sectional study was conducted over a period of 4 months from November, 2011 to February, 2012 in Merkato, Addis Ababa Ethiopia. In this study, a total of 384 samples from meat, milk and cheese were collected randomly from the Market and transferred to the laboratory following the ISO-6597, 2002 procedure. In order to isolate *Salmonella species*, conventional culture method such as pre-enrichment, enrichment and selective plating were performed. Then, to confirm the identification of isolated colonies as *Salmonella Species*, TSI, motility and API 20E tests were used. Finally, anti-microbial susceptibility testing following the standard procedure was performed in order to know the pattern of drug resistant for each isolate and the results were analyzed and interpreted by using SPSS 16.0, 2007 computer Software.

Results: Out of 384 food samples consisting of 128 meat, 128 milk and 128 cheese samples, 13 (3.39%) were positive for *Salmonella spp.* Of the 128 meat, 128 milk and 128 cheese samples, 9 (7.03%), 3(2.34%) and 1 (0.78%) yielded *Salmonella spp.* respectively. Assay of antimicrobial resistance revealed that 100% of *Salmonella* isolates were resistant to one or more of the 10 antimicrobials tested. Generally, resistance for 9 different antimicrobial drugs was recognized. However, all of the isolates were sensitive to ciprofloxacin. The most common resistance was observed to amoxicillin (100%)

followed by ampicillin and tetracycline (76.9%) and most of *Salmonella spp.* isolates are multi-drug resistance (84.6%).

Conclusions and Recommendations: The findings of the present study ascertain that *Salmonella spp*. is isolated in food samples of cattle sources particularly in retail meat samples and all of them have developed resistance for routinely prescribed antimicrobial drugs and this may be a considerable risk to the consumers. So, prudent antimicrobial usage, adequate heat treatment, improvement of standards of hygiene and development and enforcement of suitable legislation which safeguard consumers are urgently instituted and must be practiced to combat the ever increasing situation of antimicrobial resistance.

Key Words: *Salmonella*, antimicrobial resistance, food items, Merkato, Addis Ababa, Ethiopia.

1: INTRODUCTION

1.1 Background

Infectious microbial diseases constitute a major cause of death in many parts of the world, particularly in developing countries**.** Food borne diseases are infectious microbial diseases which cause a public health problem each year in both developed and developing countries (Hussein *et al.*, 2010). More than 250 different food borne diseases have been described. Most of these diseases are infections caused by a variety of bacteria, viruses and parasites. Other diseases are poisonings caused by harmful toxins or chemicals like poisonous mushrooms (CDC, 2005).

Bacteria that cause food borne diseases include *Salmonella*, *Campylobacter, Listeria*, *Yersinia*, *Shigella*, *Enterobacter* and *Citrobacter* and Pathogenic *Escherichia coli*. In addition, food borne diseases can be caused by bacterial toxins. Bacterial toxins are toxins generated by bacteria and may be highly poisonous in many cases. These includes toxins from *Staphylococcus aureus*, *Clostridium botulinum* and *Bacillus cereus* (Plaut, 2000).

As well as being the cause of enteric (typhoid) fever, an important infectious disease, *Salmonella* is perhaps best known as a cause of bacterial food poisoning. Although typhoid fever has been largely eradicated in the developed world, *Salmonella* food poisoning has long been and continues to be an important global public health problem. In much of Europe and North America, *Campylobacter* is now the most frequent cause of food borne human infections, but *Salmonella* remains a very important and widespread pathogen. It is a major cause of concern for the food industry, where its control is vital for products ranging from cooked meats to chocolate and from fresh produce to peanut butter (Http://www.rapidmicrobiology.com/test-methods/Salmonella.php).

Salmonella serotypes continue to be a prominent threat to food safety worldwide. Infections are commonly acquired by animal to human transmission through consumption of undercooked food products derived from livestock or domestic fowl.

The emergence of *Salmonella* serotypes that became associated with new food sources and the emergence of *Salmonella* serotypes with resistance against multiple antibiotics are common. *Salmonella species* has been considered as one of the most important food borne pathogen around the world. Animals are the principal reservoir of this pathogen and foods with animal sources such as beef, poultry meat, egg, cheese and milk as well as animal fecal samples have been proven to carry this pathogen and causes food borne diseases in humans (Gillespie *et al.*, 2003). *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Enteritidis are the most frequently isolated serovar from food-borne outbreaks throughout the world (Herikstad *et al.*, 2002, Jamshidi *et al.*, 2010).

Infections due to *Salmonella spp.* remain a global problem. These infections may cause significant morbidity and mortality in humans and productive animals and cause considerable economic losses. *Salmonella spp*. is typically transmitted among humans and animals via a fecal-oral route, usually through the consumption of contaminated food or water. Timely identification and serotyping of *Salmonella* from clinical specimens facilitates outbreak detection and patient management while prompt and accurate detection of *Salmonella spp.* in contaminated food or water provides an opportunity to prevent the contaminated food from entering the food supply. Sensitive and specific laboratory methods for the isolation, identification, and serotyping of *Salmonella* are essential elements of *Salmonella* monitoring and control programmes. An ideal method will be rapid, inexpensive, easily reproducible, sensitive and specific. Currently, no single method meets all these criteria and the optimal method may vary depending on the source of specimen (e.g., human clinical specimens, different food matrices and environmental specimens) and the target serotype (e.g. typhoidal versus non-typhoidal *Salmonellae*). Additionally, new methods are being described regularly and comparison of current methodologies to new methodologies is highly recommended. To insure continuity of results, any new method must be validated and standardized prior to implementation (WHO, 2010).

Salmonella infections can be typhoidal or nontyphoidal. Serotypes such as *S.* Typhi, *S.* Paratyphi A and B, causes of typhoidal salmonellosis, are highly adapted to humans and do not cause disease in non-human hosts. The majority of *Salmonellae*, (e.g. *Salmonella*

Choleraesuis and *Salmonella* Enteritidis) are chiefly pathogenic in animals that constitute the reservoir for human infection such as poultry, pigs, rodents, cattle, pets (from turtles to parrots) and many others and cause nontyphoidal salmonellosis in both humans and animals throughout the world (Murray *et al.*, 2005).

1.2 Literature Review

1.2.1 Evolution of *Salmonella*

It is speculated that the genera of *Escherichia coli* and *Salmonella* diverged from a common ancestor about the time of the emergence of mammals and emerge as mammalian and avian pathogens through the acquisition of pathogenicity islands and of a virulence plasmid, through variation in lipopolysaccharide antigens, through development of mechanism for flagellar antigen phase shifting and in other ways. Some writers estimate *Salmonella* diverged from the genus *Escherichia* 120–160 million years ago. The close DNA relatedness among *Salmonella* serotypes is evidence for their clonal origin and based on the degree of sequence divergence, it can be estimated that a common ancestor of the genus existed about 25 to 40 million years ago (Bäumler *et al.*, 1998). In 1892, Loeffler described the causative agent of murine typhoid, (then known as *Bacillus* Typhi*)* that caused an epidemic typhoid fever-like disease in mice. Recently, *Salmonella* Typhi was identified in ancient skeletal material, thereby incriminating typhoid fever for the plague that devastated Athens in 430-426 B.C. It is hypothesized that accumulation of single mutations, insertions or deletions with the genome of modern-time *Salmonella* Typhi appears to have generated many pseudogenes, suggesting its recent evolutionary origin (Papagrigorakis *et al.*, 2007).

1.2.2 The Genus *Salmonella*

Salmonella organisms are facultative anaerobic gram-negative rods within the family of Enterobacteriaceae. Classically, the members of this genus are motile by peritrichous flagella except *Salmonella* Pullorum and *Salmonella* Gallinarum, which lack flagella. However, the long standing fact that *Salmonella* Pullorum is non motile has been disproved and it has been shown that the motility can be induced under special medium conditions (Holt and Chaubal, 1997). *Salmonella* grow optimally at $35^{o}C$ to $37^{o}C$, catabolize a variety of carbohydrates into acid and gas, use citrate as the sole carbon source and produce H_2S and decarboxylate lysine and ornithine to cadaverine and putrescine respectively. Historically, *Salmonella* catabolized glucose and lysine but failed to metabolize lactose, sucrose and urea. Many serotypes in the group are closely related to each other by somatic and flagellar antigens and most strains show diphasic variation of the flagellar antigens (John *et al.*, 1994).

The genus *Salmonella* comprises two species: (1) *Salmonella enterica*, which is divided into six subspecies: *Salmonella enterica* subspecies Enterica (I), *Salmonella enterica* subspecies Salamae (II), *Salmonella enterica* subspecies Arizonae (IIIa), *Salmonella enterica* subspecies Diarizonae (IIIb), *Salmonella enterica* subspecies Houtenae (IV) and *Salmonella enterica* subspecies Indica (VI); and (2) *Salmonella bongori* (formerly called *Salmonella enterica* subspecies Bongori V). Species and subspecies can be distinguished on the basis of differential characters and through antigenic formulae into 2501 serovars (Solari *et al.*, 2003). However, a recent report from the Centre for Infectious Disease Research and Policy classifies members of the *Salmonella species* into more than 2541 serotypes (serovars) according to their somatic (O) and flagellar (H) antigens (CIDRAP, 2006). The antigenic formulae of *Salmonella* serotypes are defined and maintained by the World Health Organization (WHO) Collaborating Centre and new serotypes are listed in the annual updates of the Kauffmann-White scheme (Brenner *et al.*, 2000).

1.2.3 Current Nomenclature

Several schemes based on biochemical characteristics, DNA homology and enzyme electrophoresis patterns have been used for the taxonomic classification of *Salmonella*. *Salmonella* nomenclature is complex and scientists use different systems to refer to and communicate about this genus. The current usage often combines several nomenclatural systems that inconsistently divide the genus into species, subspecies, subgenera, groups, subgroups and serotypes (serovars) which causes confusion (Brenner *et al.*, 2000). The genus *Salmonella* has two systems of nomenclature. One system proposed by Le Minor and Popoff in the 1980s which has received a wide acceptance although it does not conform to the rules of bacteriological code and the other which conforms to the bacteriological code but used by the minority. The present problem is that two systems of nomenclature are in use for the members of the genus *Salmonella* (Tindall *et al.*, 2005).

The nomenclature of the genus *Salmonella* has evolved from the initial one serotype-one species concept proposed by Kauffmann on the basis of the serologic identification of O (somatic) and H (flagellar) antigens. A capsular polysaccharide, the Vi antigen is present on *Salmonella* Typhi and few other serovars of *Salmonella*, including *Salmonella* Dublin (Heyndrickx *et al.*, 2005). The defining development in *Salmonella* taxonomy occurred in 1973 by DNA-DNA hybridization that all serotypes and subgenera I, II and IV of *Salmonella* and all serotypes of "Arizona" were related at the species level, thus belonging to a single species. The single exception subsequently described is *Salmonella bongori* previously known as subspecies V which by DNA-DNA hybridization is a distinct species. In 1986 the subcommittee of Enterobacteriaceae of the International Committee on Systematic Bacteriology at the XIV International Congress of Microbiology, unanimously recommended the change of type species of *Salmonella* to *Salmonella enterica*, a name coined by Kauffmann and Edwards in 1952. However, the Judicial committee denied with the fact that the *Salmonella* Typhi might be overlooked as it is one of the most important human pathogens (Brenner *et al.*, 2000).

The current nomenclature used by CDC is based on the recommendations from the WHO collaborating centre and it adequately addresses the concerns and requirements of clinical and public health microbiologists (Deb and Kapoor, 2005). The nomenclature is summarized in the Table 1.1 below.

Table 1.1: *Salmonella* Species, Subspecies, Serotypes and Their Usual Habitats, Kauffmann-White Scheme (Brenner *et al.*, 2000)

species and subspecies	No. of serovars within subspecies	Usual habitat
S. enterica subsp. Enterica (I)	1454	Warm blooded animals
S. enterica subsp. Salamae (II)	489	Cold blooded animals and the environment
S. enterica subsp. Arizonae (IIIa)	94	Cold blooded animals and the environment
S. enterica subsp. Diarizonae (IIIb)	324	Cold blooded animals and the environment
S. enterica subsp. Hautenae (IV)	70	Cold blooded animals and the environment
S. enterica subsp. Indica (VI)	12	Cold blooded animals and the environment
S. bongori (V)	20	Cold blooded animals and the environment

According to the CDC system, the genus *Salmonella* contains two species, each of which contains multiple serotypes (Brenner *et al.*, 2000) as shown in Table 1.2 below.

Table 1.2: *Salmonella* Nomenclature in Use at CDC, 2000.

Taxonomic position	**Nomenclature**
Genus (italics)	*Salmonella*
Species (italics)	• *enterica*, which includes subspecies I, II, IIIa, IIIb, IV and VI • *bongori* (formerly subspecies V)
Serotype (capitalized, not italicized)	•The first time a serotype is mentioned in the text; the name should be preceded by the word "serotype" or "ser". •Serotypes are named in subspecies I and designated by antigenic formulae in subspecies II to IV, and VI and S. *bongori* •Members of subspecies II, IV and VI and S. *bongori* retain their names if named before 1966

1.2.4 Morphology

Salmonellae are Gram-negative, straight rods not exceeding 1.5 micrometers in width. They are facultative anaerobes usually motile by peritrichous flagella. Most *salmonellae* form common fimbriae and most of them possess type-1 fimbriae associated with mannose-sensitive adhesive properties. These fimbriae are composed of fimbrillin subunits containing a high proportion of hydrophobic amino-acids (Old and Threlfall, 1998). *Salmonellae* are routinely classified by serotype on the basis of expression of three surface antigens, the somatic O antigen, the flagella H1 and H2 antigens and the capsular Vi antigen, according to the Kauffmann-White scheme (Scott *et al.*, 2002). The absence of flagella may consequently affect complete identification of the serotype; *Salmonella enterica* serovar Typhimurium exhibits morphological differences dependent on the peptone constituents of the culture medium. However, in media containing soy-based peptone as the primary nutrient *Salmonella* displays a normal flagellated morphology (Victoria *et al.*, 2006).

1.2.5 *Salmonella*: Disease and Pathogenesis

Salmonellae are well-known pathogens which are highly adaptive and potentially pathogenic for humans and/or animals. *Salmonella* infections are capable of producing serious infections that are often food borne and present as gastroenteritis. However, a small percentage of these infections may become invasive and result in bacteremia and serious extra intestinal disease (CIDRAP, 2006).

While certain serovars of *Salmonella enterica* cause disease in humans and a variety of animals, other serovars are highly restricted to a specific host. *Salmonella* infections range from gastrointestinal infections that are accompanied by inflammation of intestinal epithelia, diarrhea and vomiting to typhoid fever, a life threatening infection. The outcome of *Salmonella* infections is determined by the host and the status of the bacterium. Whereas, age, genetic and environmental factors mainly determine the status of the host, the status of the bacterium is determined by so-called virulence factors. *Salmonellae* avoid host defense in the stomach and reach the intestines and the bacteria interact with the non-phagocytic cells such as the epithelial cells of the intestinal mucosa. They adhere to the intestinal epithelial cells by adhesive structures (fimbriae) that promote binding and invade epithelial cells to provoke gastroenteritis. The organisms have virulence factors such as virulence-plasmids, toxins, fimbriae and flagella that help in establishing an infection. The mechanism of pathogenesis has been described in the following steps (Alphons *et al.*, 2005).

a) **Bacterial mediated endocytosis***:* A highly coordinated series of interactions between proteins released by *salmonellae* and proteins of the host cell causes host cellular surface membrane ruffling and engulfment of bacteria in cellular vacuoles.

b) **Neutrophil recruitment and migration***: Salmonellae* associated with gastroenteritis induce a secretory response in intestinal epithelium and initiate recruitment and transmigration of neutrophils into the intestinal lumen.

c) **Epithelial cell cytokine secretion***:* In tissue culture models of *Salmonella* Enteritidis, translocation of SPI-1 proteins into intestinal epithelial cells leads to synthesis and polarized secretion of inflammatory mediators and neutrophil chemo-attractants.

d) **Fluid and electrolyte secretion***:* Several translocated SPI-1 proteins contribute to intestinal inflammation and fluid secretion. Intestinal inflammation probably contributes to fluid secretion and diarrhea by disrupting the epithelial barrier and increasing water flux by an exudative mechanism. Innate immune system activation also contributes to intestinal inflammation.

e) **Systemic infection**: *Salmonella* Typhi invades macrophages and the migration of infected macrophages to reticuloendothelial organs via the lymphatic system and blood produces systemic illness with less diarrhea.

1.2.5.1 Salmonellosis in Humans

With respect to human diseases, *Salmonella* serotypes can be divided into three groups that cause distinctive clinical syndromes; typhoid fever, bacteremia and enteritis. The non-typhoid *Salmonella* serotypes can cause protean manifestations in humans, including acute gastroenteritis, bacteremia and extraintestinal localized infections involving many organs (Chiu *et al.*, 2004). Within *Salmonella enterica* subspecies I (*Salmonella enterica* subspecies Entericae), the most common O-antigen serogroups are A, B, C1, C2, D and E. Strains within these serogroups cause approximately 99% of *Salmonella* infections in humans and warm-blooded animals. Serotypes in other subspecies are usually isolated from cold-blooded animals and the environment but rarely from humans (Velge *et al.*, 2005).

Following ingestion of contaminated food or water, the pathogenesis of both typhoidal and non-typhoidal Salmonellosis begins in the intestine. Transmission of these diseases within the human population is generally a result of poor sanitation of water and food supplies in developing nations. The broad host-range *Salmonella* serovars are prevalent within warm-blooded animal populations that make up the bacterial transmission generally results from consumption of raw or undercooked food products. The vast majority of *Salmonellae* infections are transmitted from animals to humans through food and occasionally from person to person through the fecal-oral route. In general, *Salmonellae* cause one or more of four broad clinical syndromes such as gastro-enteritis, enteric fever, septicemia with associated focal lesions and asymptomatic long-term carriage (Jones, 2005).

1.2.5.2 Salmonellosis in Animals

Salmonella serotypes have a broad host range prevalent in the warm-blooded animal populations including rodents, snakes and free living terrestrial and aquatic turtles and the pathogenicity of *Salmonella* serovars is known to be specific for animal species. Some serotypes are highly adapted to animal hosts, such as *Salmonella* Gallinarum in poultry and *Salmonella* Abortus-ovis in sheep. Many nontyphoidal *Salmonella* strains such as *Salmonella* Typhimurium and *Salmonella* Enteritidis infect a wide range of animal hosts including poultry, cattle and pigs. These serotypes generally cause self limiting gastrointestinal infections usually less severe than enteric fever in humans. However, they also have the capacity to produce typhoid-like infections in mice and in humans or asymptomatic intestinal colonization in chickens (Velge *et al.*, 2005).

1.2.5.3 A Public Health Perspective and Economic Impact

Although most *Salmonella* infections cause mild to moderate self-limited diseases, serious infections leading to deaths do occur (Jong and Ekdahl, 2006). In spite of the improvement in hygiene, food processing, education of food handlers and information to the consumers, food borne diseases still dominate as the most important public health problem in most countries. Many foods, particularly those of animal origin, have been identified as vehicles for transmission of these pathogens to human beings and spreading them to the processing and kitchen environment. In developed countries, food is recognized as the most frequently implicated vehicle of transmission and causes heavy financial burden on health care systems (Jordan *et al.*, 2006). In the United States alone, an estimated 1.4 million non-typhoidal *Salmonellae* infections, resulting in 168, 000 visits to physicians, 15, 000 hospitalizations and 580 deaths occur annually and the total cost associated with *Salmonella* is estimated as 3 billion USD annually. Apart from the food borne infections, the other major problem in Salmonellosis is the emergence of MDR *Salmonellae*, particularly in the developing countries (WHO, 2005).

In United States, The Center for Disease Control and Prevention in 2003 has estimated that *Salmonella* infections were responsible for 1.4 million annual illnesses, resulting in nearly 600 deaths. More severe cases of Salmonellosis tend to occur in the very old, the very young and the immuno-compromised people. The estimated annual costs in dollars in 1998 and 2003 of medical care and lost productivity due to food borne *Salmonella* infections in the United States were 2.3 and 2.9 billion USD (ERS-USDA, 2003).

Food safety is a particular concern in developing countries not only because of the high prevalence of the food borne illnesses and other hazards associated with food but also the considerable economic and social costs that reflects prevailing levels of economic development. The majority of trade-limiting factors in developing countries relate to economics, poor infrastructure and lagging skills; food safety is still mainly the responsibility of the consumers. However, improving food safety along western standards may carry considerable costs of food out of reach of the poor (Veen, 2005).

1.2.6 **Epidemiology**

1.2.6.1 Global Overview

In many countries, incidences of human *Salmonellae* infections have been increased drastically over the years. The two most commonly isolated serotypes of concern and mostly implicated in disease outbreaks are *Salmonella enterica* serotype Typhimurium and *Salmonella enterica* serotype Enteritidis. The *Salmonella species* is one of the eight micro-organisms in the European Union Zoonoses Monitoring Directive which shows it is a disease considered to have a high impact on human health in the Union. The Enter-net surveillance program reported *Salmonella enterica* serotypes Enteritidis and Typhimurium are the most predominant organisms identified by the participating countries making up over 80% of all isolates during the period of 1998-2003 (Eurosurveillance, 2004).

Salmonella serovars are widespread in the environment and may contaminate a variety of food and food ingredients. Red meat and red-meat products are recognized as an important source of human salmonellosis. Only in 2007, for instance, beef and pork (and products thereof) contaminated with *Salmonella* serovars were responsible for 800 cases of human salmonellosis involving 37 outbreaks in Europe (EFSA, 2009).

In Denmark, an outbreak of human salmonellosis caused by *S.* Typhimurium DT 104 occurred between July and August 2005 and the comparison of strains from patients and food using PFGE, tandem repeat analysis and antibiogram tests showed that the source of the outbreak was imported beef served as carpaccio. Some months later, there was an increase in the number of *S.* Typhimurium DT 104 cases in the Netherlands. This occurrence was found to be epidemiologically associated with the disease in Denmark. A case-control study was conducted with 56 cases and 100 controls and logistic regression analysis was used for analyzing the results. From this investigation, beef was considered as the most probable vehicle of *S.* Typhimurium DT 104. Complementary assessment suggested that imported beef from a third European country had spread this *Salmonella* strain resulting in these outbreaks (Ethelberg *et al.*, 2007).

In the USA in 2004, an epidemiological investigation demonstrated that ground beef sold by a national chain of one supermarket was the source of food-borne salmonellosis due to *S.* Typhimurium in nine states. The PFGE patterns of *S.* Typhimurium isolates from patients and ground beef were found to be identical. Many other reports involving human salmonellosis outbreaks associated with consumption of red meat have been recorded in the literature. In most of the cases, the disease was associated with the consumption of contaminated meat or as a result of incorrect or inadequate cooking (CDC, 2006).

Dairy products may also cause human food-borne salmonellosis. Usually milk and milk products are submitted to pasteurization which kills *Salmonella* serovars. Milk-borne salmonellosis is therefore often related to the consumption of raw or inadequately pasteurized milk. However, *Salmonella* serovars may also contaminate dairy products after the pasteurization process (CDC, 2007).

S. Typhimurium caused an outbreak in Pennsylvania, USA, in 2007 and an epidemiological investigation confirmed that the source of *S.* Typhimurium was the raw milk and raw milk products. An outbreak of *S.* Newport in the USA in 2008 occurred predominantly among Hispanics in Northeastern Illinois. It was recovered from patients and from a Mexican-style aged cheese made with unpasteurized milk. The strains isolated from people and cheese had indistinguishable PFGE patterns (CDC, 2007). Another outbreak of human salmonellosis associated with cheese was described in Switzerland, Europe, where a nationwide outbreak of gastrointestinal disease caused by *S.* Stanley, a rare serovar in Europe, occurred from September 2006 to February 2007. *S.* Stanley strain was isolated from a local soft cheese and from 94% of affected people. The withdrawal of the cheese from the market resolved the problem (Pastore *et al.*, 2008).

1.2.6.2 Status in Developing Countries

With the increasing population in the developing countries, demand to livestock production increases together with the disease transmission risks. There is a multi-factorial risk of food borne hazards in the developing countries due to poor sanitation and inadequate access to potable water (Henson, 2003).

It is recognized that the prevalence of food-borne illnesses in developing countries is considerable. However, in most countries, there is limited data through which the incidence of particular diseases and trends can be assessed over time. The growing movement of people, live animals and food products across borders, rapid urbanization, increasing numbers of immune-compromised people, changes in food handling and consumption and the emergence of new or/and antibiotic-resistant pathogens all contribute to increasing food safety risks (Unnevehr, 2003).

1.2.6.3 ***Salmonella* in Ethiopia**

Even though *Salmonella* populations in different geographical areas or in different hosts and environmental niche may undergo different evolutionary change, their strains found in different countries of the world are believed to be clonally related (Winokur, 2003). *Salmonella* isolates in Ethiopia may have similar phenotypic and genotypic characteristics with isolates elsewhere in the world and non-typhoidal *Salmonella enteric* infection in children in Ethiopia is a major health problem (Getenet, 2008).

From 1974 to 1981, a study was conducted to identify the prevalent serotypes and their susceptibility to drugs in Addis Ababa, which serves as a base-line data for further surveillance of *Salmonella species* in Ethiopia. At that time, 216 *Salmonella* strains which were predominantly isolated from adult patients referred from different hospitals to the Central Laboratory and Research Institute, Addis Ababa between January 1974 and October 1981was studied. Of the 216 *Salmonella* strains, 48.6% were *S.* Typhi and most of the *Salmonella* isolates were from stool (54.6%) followed by blood (34.7%), pus (5.6%) and urine (5.1%). This base-line study identified the existence of 26 different serotypes among the 216 *Salmonella* isolates. In this findings, *S.* Concord (12.5%) and *S.* Typhimurium (11.1%) predominates the non-Typhi isolates. The high isolation rate of *S.* Concord in Ethiopia was unusual in contrast to high prevalence of *S.* Typhimurium elsewhere. The author also suggested that further study should be done to clarify the animal or food sources associated with its epidemiology (Gebre-Yohannes, 1985).

Following this suggestion, the prevalence of *Salmonella* in minced beef samples found in the previous studies made in Ethiopia by Nyeleti et al (2000), Gebremedhin (2004), Ashenafi (1994), Ejeta et al. (2004), Molla *et al.* (1999), Tegegne and Ashenafi (1998) was 7.9%, 8.5%, 9%, 14.4%, 40% and 42% respectively.

Cottage cheese or “ayib” is an Ethiopian traditional dairy product made from sour milk after the removal of the fat by churning and cooking the curd to a temperature of 40-70_{o}C. It comprises 79% water, 14.7% protein, 1.8% fat, 0.9% ash and 3.1% soluble milk constituents (O’Mahony, 1988). A study in Ethiopia stated that *Salmonella* prevalence in cottage cheese was low (2.1%) (Gebremedhin, 2004).

1.2.7 Diagnosis of *Salmonella*

1.2.7.1 Culture

The diagnosis of *salmonellae* requires bacteriologic isolation of the organisms from appropriate specimens. Feces, blood, or other specimens should be placed on several nonselective and selective agar media (blood, MacConkey, eosin-methylene blue, bismuth sulfite, *Salmonella-Shigella*, Xylose lysine desoxycholate and brilliant green agars) as well as into enrichment broth such as selenite, Rappaport-Vassiliadis Soy or tetrathionate. Any growth in enrichment broth is subsequently sub-cultured onto the various agars. The biochemical identification of *salmonellae* has been simplified by systems that permit the rapid testing of 10-20 different biochemical parameters simultaneously. The presumptive biochemical identification of *Salmonella* then can be confirmed by antigenic analysis of O and H antigens using polyvalent and specific antisera. Fortunately, approximately 95% of all clinical isolates can be identified with the available group A-E typing antisera. *Salmonella* isolates then should be sent to a central or reference laboratory for more comprehensive serologic testing and confirmation (Giannella, 2000).

New selective chromogenic media such as CHROMagar and COMPASS agar are more specific than other selective media and lower the need for confirmatory testing and time to identification. These are increasingly being used for primary isolation and identification of clinical stool specimens. Tetrathioneate- and selenite-base enrichment broths are often used to aid recovery of low numbers of organisms. Selenite with brilliant green medium is highly *Salmonella*-selective and should be reserved for suspected carriers and for use in special circumstances such as outbreaks (Perez *et al.*, 2003).

1.2.7.2 Serotyping

The Kauffmann-White scheme, first published in 1929, divides *Salmonella* into more than 2500 serotypes according to their antigenic formulae. Today, 57 O antigens and 117 H antigens have been identified. Some of the H antigens share common antigen factors. These antigens are clustered in five complexes, the E, G, L, Z4 and 1 complex. *Salmonella* H antigens are expressed in different phases. Most serotypes are diphasic, i.e. they express two flagellar antigens and a minor part are monophasic, i.e. express one flagellar antigen. *Salmonella* Gallinarum is the only serotype in the Kauffmann-White scheme that does not express any flagellar antigen and is therefore non-motile (Sonne-Hansen and Jenabian, 2005). *Salmonella* sero-typing methods recognize 63 distinct phase 1 flagellar antigenic factors and 37 phase 2 flagellar antigenic factors although the latter are not always present. Some antigenic factors may be present or absent without affecting serotype designation. Sero-typing methods are stable, reproducible and have high typability, yet there are several drawbacks, particularly the dependence on availability of antisera, considering the ethics, cost and quality control measures necessary to maintain such a supply (Mortimer *et al.*, 2004).

Salmonella isolates can be differentiated from one another in a wide variety of ways and the number of *Salmonella* continues to increase. Epidemiologically, it is important to be able to distinguish *Salmonella* isolates, because definitive typing of *Salmonella* isolates may assist in tracing the source of an outbreak and monitoring trends in antimicrobial resistance associated with a particular type (Yan *et al.*, 2003). In addition to the conventional antigen-based sero-typing, there are advanced techniques for sero-typing currently being used to enhance the tracing of the individual isolates. The following techniques are currently available for sero-typing.

(a) Conventional method

(b) Phage typing

(c) Molecular typing

a) Conventional Serotyping

Conventional sero-typing of *Salmonellae* is the most commonly used method to differentiate strains which are epidemiologically the smallest bacterial unit from which isolates share the same phenotypic and genotypic traits. In most clinical studies, initial sero-typing is done using polyvalent O antisera to allow *Salmonella* isolates to be grouped into different O groups designated in capitalized letters. Many *Salmonellae* show diphasic production of flagellar antigens and each strain can spontaneously and reversibly vary between these two phases with different sets of H antigens. In phase 1 or the specific phase, the different antigens are designated by small letters, and in phase 2 or the group phase, the antigens first discovered are numbered. According to this classification scheme, all *Salmonella* serotypes can be designated by the following antigenic formula: subspecies [space] O antigens [colon] phase 1 H antigen [colon] phase 2 H antigens. For example, the formula for *Salmonella* Typhimurium is I 4, 5, 12: i: 1, 2. In a single cell, usually only one antigen is expressed at a time (Yan *et al.*, 2003). Conventional sero-typing using the auto-agglutination method has some limitations, such as limitations in detection of Vi antigens and strains which are not typeable are; only allow detection of a single antigen-antibody reaction at a time, require well experienced technologists to perform, consume relatively high amount of reagents and takes a longer time (Cai *et al.*, 2005).

b) Phage Typing

In this technique, *Salmonella i*solates are characterized by their susceptibility or resistance to lyses by each member of a panel of bacteriophage. The power of resolution is limited, for example several distinct strains of *S.* Typhimurium can belong to the same phage type and the technique is largely empirical and doesn't reflect true evolutionary classification. Phage susceptibility may be relatively plastic and susceptibility changes can occur rapidly. Within six weeks, *S.* Enteritidis isolated from one patient changed from DT4 to DT7 and DT9a. Phage typing is often an important early step in an investigation but needs to be supplemented with other techniques. Phage typing is technically demanding and requires the maintenance of stocks of biologically active phage and control strains are conditions that relegate this technique to reference laboratories (Maslow *et al.*, 1993).

c) Molecular Typing

Molecular genotyping of *Salmonella* strains is fundamental in tracking disease associated and drug-resistant strains in various populations. Genotypic methods are those that are based on analysis of the genetic structure of an organism and include polymorphism in DNA restriction patterns based on cleavage of the chromosomes (DNA) into hundreds of fragments (frequent cutters) or into 10 to 30 fragments (infrequent cutters) and the presence and absence of extra-chromosomal DNA. They are less subjected to natural variation although DNA changes (insertions, deletions and random mutations) can have an effect on resulting fingerprinting. Molecular techniques which are commonly used in typing bacteria include: pulse field gel electrophoresis (PFGE), ribotyping, plasmid profile analysis, amplified fragment polymorphism, arbitrary primed PCR (AP-PCR) and repetitive sequence PCR (Winokur, 2003). For many bacterial species, combination of different methods or selection of the most discriminative methods is usually required in identifying a particular strain. For typing *Salmonella species* including *S.* Typhi, *S.* Typhimurium and *S.* Enteritidis, a combination of PFGE, ribotyping, and plasmid analysis are used by most researchers/laboratories (Tsen *et al.*, 2002).

1.2.8 Treatment

More than 60 to 90 percent of cases of typhoid fever are managed at home with antibiotics and bed rest. For the hospitalized patient, effective antibiotics, good nursing care, adequate nutrition, careful attention to fluid and electrolyte balance and prompt recognition and treatment of complications are necessary to avert death. The fluoroquinolones are well tolerated and more rapidly and reliably effective than the former first-line drugs; chloramphenicol, ampicillin, amoxicillin, and trimethoprim-sulfamethoxazole for the treatment of typhoid fever (WHO, 2003).

The fluoroquinolones attain excellent tissue penetration, kill *S.* Typhi in its intracellular attaining stage in monocytes/macrophages and achieve higher active drug levels in the gall bladder than other drugs. They produce a rapid therapeutic response, i.e. clearance of fever and symptoms in three to five days and very low rates of post-treatment carriage. Concern has been expressed about three main issues regarding the use of fluroquinolones in the treatment of typhoid fever: the potential for toxic effects in children, the cost and the potential emergence of resistance (Bethell *et al.*, 1996).

Chloramphenicol, amoxicillin and trimethoprim-sulfamethoxazole remain appropriate for the treatment of typhoid fever in areas of the world where the bacterium is still fully susceptible to these drugs and where the fluoroquinolones are not available or affordable. The disadvantage of using chloramphenicol includes a relatively high rate of relapse (5-7%); long treatment courses (14 days) may cause bone marrow depression and the frequent development of a carrier state in adults. The third generation cephalosporins (ceftriaxone, cefixime, cefotaxime and cefoperazone) and azithromycin are also effective drugs for typhoid. In general, in areas with high prevalence of MDR *Salmonellae* infections, all patients suspected of having typhoid fever should be treated with a quinolone or third-generation cephalosporin until the results of culture sensitivity studies become available (Miller *et al.*, 2000).

Gastroenteritis caused by non-typhoidal *Salmonella* is usually a self-limiting disease and therapy should be directed primarily to the replacement of fluid and electrolyte losses. Therefore, antimicrobials should not be used routinely to treat uncomplicated non-typhoidal *Salmonella* gastroenteritis or to reduce convalescent stool excretion. However, antimicrobial therapy should be considered for any systemic infection (Parry *et al.*, 2002).

1.2.9 Antimicrobial Resistance

Drug resistance in food borne bacterial enteric pathogens is almost inevitable consequence of the use of antimicrobial drugs in food-producing animals and specifically in the developing countries by use of medicines in humans. A major concern is that the high levels of antibiotic resistance as a result of the use of antibiotics in food animals. A recent estimate in the United States suggests that 24.6 million pounds of antibiotics are given to animals each year as growth promoters at sub-therapeutic amounts in their feed compared to 3 million pounds consumed by humans. In recent years, the emergence and global dissemination of MDR typhoidal strains has posed major public health problems in the developing countries (Okeke *et al.*, 2005).

Antimicrobial resistance among non typhoid *Salmonella* serotypes has been a serious problem worldwide. The identification of antimicrobial-resistant *Salmonellae* in food has raised concerns on treatment of food borne Salmonellosis especially the development of ceftriaxone and ciprofloxacin-resistant *Salmonellae,* as these are important in treating *Salmonellae* infections in children and adults respectively (Butaye *et al.*, 2006).

Conventional antimicrobial agents, such as ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole had been the drug of choice in the treatment of Salmonellosis before the 1980s. However, MDR with rates of resistance to these antimicrobial agents of more than 50% has been reported in many areas of the world. Extended-spectrum cephalosporins and fluoroquinolones are increasingly reported after 1991. The possible emergence and spread of *Salmonella* strains resistant to antibiotics commonly used as treatment are concerns. Because, these infections can be invasive and difficult to treat by the drugs of choice for invasive diseases (Paterson, 2006).

In developing countries, household subsistence farming is common, which means that a large proportion of the population has close contact with food animals; therefore, if resistant organisms are common in animals, the chance that they will be transmitted to human beings is more likely (Howard and Scott, 2005).

The emergence of *Salmonella* strains that are resistant to commonly used antimicrobials should be particularly noted by clinicians, microbiologists and those responsible for control of communicable diseases as well as food producers including the food industry. Control of drug resistant *Salmonella* is most efficiently achieved through the reduction of antimicrobial use. Prudent usage in food animals should be combined with good husbandry, good abattoir practice and good hygiene at all stages in the food production chain, from processing plants to food service establishments. The increased occurrence of drug-resistant pathogens in food of animal origin emphasizes the general need for cooking such foods thoroughly prior to consumption. Education of food handlers in the principle of safe food handling is an essential step towards reducing the incidence of food borne diseases resulting from cross-contamination during processing and preparation of foods (WHO, 2005).

1.2.9.1 Global Trends in Resistance Pattern

Antimicrobial resistance is one of the biggest challenges for global public health. Although antimicrobial drugs have saved many lives and eased the suffering of many millions; poverty, ignorance, poor sanitation, hunger and malnutrition, inadequate access to drugs, poor and inadequate health care systems, civil conflicts and bad governance, misdiagnosis, counterfeit drugs and lack of education in developing countries tremendously limit the benefits of these drugs in controlling infectious diseases (Walia, 2006).

Most non-typhoidal *Salmonella* infections manifest as potentially self-limiting diarrhea. Antimicrobial resistance is clinically relevant because 3-10% of these infections can progress to life-threatening bacteraemia, particularly in young and immuno-compromised patients. In Indonesia, *Salmonella* Paratyphi isolates recovered between 1995 and 2001 were universally susceptible to commonly used antimicrobials. A similar study in Zimbabwe, reported much lower rates of resistance among *Salmonella* Enteritidis and more than 50% of non-typhoidal *Salmonella* isolates from children in Kenya were MDR. One of the studies in Spain reported high percentages of resistance of *Salmonella* isolates to sulfadiazine, neomycin, tetracycline and streptomycin, which might be the result of use of antibiotics as a prophylaxis, growth promoter or treatment. A similar study in Alberta, Canada indicated high resistance of *Salmonella* isolates from food and food animals to ampicillin, streptomycin, sulfamethoxazole and tetracycline (Johnson *et al.*, 2005).

Over the past decade in Nepal, 35 multi-drug-resistant strains out of 132 strains of *Salmonella* Typhi were observed and showed simultaneous resistance to ampicillin, chloramphenicol and co-trimoxazole which lead to a shift of the antibiotics used from chloramphenicol and ampicillin to trimethoprim-sulfamethoxazole, fluoroquinolones and ceftriaxone. Although there were no isolates resistant to ciprofloxacin, 69.23% of 52 isolates tested for minimum inhibitory concentration of ciprofloxacin showed reduced susceptibility and 76% of 112 strains tested for nalidixic acid were resistant. There are reports of *Salmonella* resistant strains isolated from The Netherlands, France, Portugal and many other countries (Antunes *et al.*, 2003).

From 1999 to 2003, 34, 411 *Salmonellae* were isolated from animals in the USA. Of which, 10.9% were found to be resistant to ceftiofur, a third generation cephalosporin used in animals while only 0.3% were resistant to ceftriaxone, a third generation cephalosporin used in human medicine (Fluit, 2005).

In 1997, *S.* Typhimurium DT104 strain was resistant to five antimicrobials (ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline) and affected 110 people in two outbreaks in USA. A case-control study and laboratory investigations indicated that an unpasteurized Mexican-style cheese was responsible for these outbreaks (Cody *et al.*, 1999). In another study, 133 isolates of *Salmonella* serovars recovered from retail meats purchased in the USA and China were analyzed and the antimicrobial resistance of these strains were assessed. In this study, it was demonstrated that seventy-three (82%) of these *Salmonella* serovars isolates were resistant to at least one antimicrobial agent. Resistance to the following antibiotics was common among the USA isolates:

tetracycline (68%), streptomycin (61%), sulfamethoxazole (42%) and ampicillin (29%). Eight *Salmonella* isolates (6%) were resistant to ceftriaxone. Fourteen isolates (11%) from China were resistant to nalidixic acid and displayed decreased susceptibility to ciprofloxacin (Chen *et al.*, 2004).

Moreover, antimicrobial resistance of *Salmonella* serovars isolated from German foodstuffs was determined. A total of 319 epidemiologically independent MDR isolates comprising 25 different serovars were tested for their antimicrobial susceptibility. The results of this study shown that the most prevalent resistances found in the multidrug resistant *Salmonella* serovars from foods were: streptomycin (94%), sulfamethoxazole (92%), tetracycline (81%), ampicillin (73%), spectinomycin (72%), chloramphenicol (48%) and trimethoprim (27%) (Miko *et al.*, 2005).

1.2.9.2 Resistance Pattern in Ethiopia

From September 2003 to February 2004, antimicrobial resistance of 98 isolates of *Salmonella* serovars recovered from food in Addis Ababa, Ethiopia was assessed. The results revealed that 32 *Salmonella* isolates were resistant to one or more of the 24 antimicrobials tested. The most common resistance was: streptomycin (75%), ampicillin (59.4%), tetracycline (46.9%), spectinomycin (40.6%) and sulfisoxazole (40.6%) (Zewdu and Cornelius, 2009).

Moreover, the antimicrobial resistance to 10 drugs in 39 *Salmonella* serovars isolated from raw minced beef and chicken (gizzard, liver and heart) samples in Addis Ababa (Ethiopia) was assessed. Thirty four isolates (87.2%) were resistant to one or more drugs. The antibiotics to which isolated strains were resistant included nitrofurantoin (48.7%), furazolidone (48.7%) and streptomycin (46.2%). Only four antimicrobials (gentamycin, kanamycin, rifampicin, and sulphamethoxazole-trimethoprim) were effective against all isolates. About 80% of the *S.* Enteritidis strains showed multiple resistance to up to four antibiotics followed by *S.* Typhimurium (60%) and *S.* Dublin (33.3%) (Molla *et al.*, 1999).

Of the 98 *Salmonella* serotypes subjected to antimicrobial susceptibility test, using a panel of 24 different antimicrobials, 32 isolates (32.7%) were resistant to one or more of the antimicrobials used. In relation to the total *Salmonella* isolates tested, 24.5%, 19.4%, 15.3% and 13.3% were resistant to streptomycin, ampicillin, tetracycline, spectinomycin and sulfisoxazole respectively. Out of the 32 resistant *Salmonella* isolates, 23 (23.5%) were multidrug resistant (MDR). Among MDR, resistance to streptomycin, spectinomycin, sulfisoxazole, ampicillin and tetracycline was most often observed. None of the cottage cheese isolates showed resistance for more than three antimicrobials and only one serotype from minced beef showed resistance for 8 antimicrobials (Gebremedhin, 2004).

1.2.10 Prevention and Control of *Salmonella*

Theoretically, it is possible to eliminate *salmonellae* that cause enteric fever since the bacteria survive only in human hosts and are spread by contaminated food and water. The control and near elimination of typhoid fever in developed countries has been achieved largely because of improved sanitation, surveillance, contact tracing and successful therapy; this is also supported with vaccination. In developing countries, reducing the number of cases in the general population requires the provision of safe drinking water, effective sewage disposal and hygienic food preparation. In areas where the epidemic is high, mass immunization has been used successfully. Currently, three vaccine alternatives are available: 1) a heat–killed, phenol extracted, whole cell vaccine, 2) Ty21a, an attenuated *S.* Typhi vaccine, 3) Vi vaccine, consisting of purified Vi polysaccharide from the bacterial capsule. In developed countries, most cases are the result of travel to endemic areas. Travelers in such areas need to take particular care with water and food (Parry *et al.*, 2002).

Non-typhoidal *S. enterica* infections are a major public health problem world-wide and reduction of these diseases presents a serious and challenging problem. These diseases have several animal reservoirs and a large number of different *S. enterica* serovars cause gastroenteritis in humans which make vaccines very difficult to realize and/or use commercially. The incidence of nontyphoidal salmonellosis continues to rise along with rates of emergence of antibiotic resistant strains. Thus, it is important to monitor every step of food production, from handling of raw products to preparation of finished foods (Miller *et al.*, 2000).

Salmonellae are difficult to eradicate from the environment. However, the major reservoir for human infection is poultry and livestock. Therefore, reducing the number of *salmonellae* harbored in these animals would significantly reduce human exposure. In Denmark, for example, all animal feeds are treated to kill *salmonellae* before distribution, resulting in a marked reduction in salmonellosis. Other helpful measures include changing animal slaughtering practices to reduce cross-contamination of animal carcasses; protecting processed foods from contamination; providing training in hygienic practices for all food-handling personnel in slaughterhouses, food processing plants and restaurants; cooking and refrigerating foods adequately in food processing plants, restaurants and homes; and expanding of governmental enteric disease surveillance programs (Giannella, 2000).

Ensuring safe food production requires knowledge on the nature and origin of the animals, animal feed, the health status of animals at the farm, the use of veterinary medicinal products, the results of any analysis of the samples taken at the farm and slaughter data regarding ante-mortem and post-mortem findings and the risks associated with post-harvest production stages or no part of the food chain can be regarded alone but has to be seen as part of the whole. A holistic approach to fresh meat storage and retailing must therefore start with the living animal and cannot end with the sale of the meat. Additional measures to control secondary contamination could be prevention of contamination by cleaning and disinfection, hygiene of personnel and proper processing. Growth of micro-organisms in meat and poultry products can be controlled by maintaining a cold chain at 10°C, especially for *Salmonella* during transport and storage (Coleman *et al.*, 2003).

1.3 Significance of the Study

Food borne diseases are a public health problem in developed and developing countries. The world health organization (WHO) estimated that in developed countries, up to 30% of the populations suffer from food borne diseases each year where as in developing countries up to 2 million deaths are estimated per year. *Salmonella* infections are very common and an important public health problem in many parts of the world. More than 93 million cases of gastroenteritis due to non-typhoidal *Salmonella species* occur globally each year with 155,000 deaths and the existing estimate of the global burden of typhoidal *Salmonella* serovars such as *Salmonella enterica* serovars Typhi and Paratyphi cause 21million illnesses and 600,000 deaths annually (WHO, 2007).

In spite of the improvement in hygiene, food processing, education of food handlers and information to the consumers, food-borne diseases still dominate as the most important public health problem in most countries. Many foods, particularly those of animal origin such as milk, meat and cheese have been identified as vehicles for transmission of these pathogens to human beings. In developed countries, food is recognized as the most frequently implicated vehicle of transmission and causes heavy financial burden on health care systems (Jordan *et al.*, 2006).

In sub-Saharan Africa, there is very little direct data on strain type and antibiotic resistance. In Ethiopia like other sub-Saharan Africa, 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 overshadow the problem of Salmonellosis. The real situation of antibiotic resistance is also not clear since *Salmonellae* are not routinely cultured and their resistance to antibiotics cannot be tested (Getenet, 2008).

However, research to date, as well as unpublished reports from different health institutions in Ethiopia, has indicated that salmonellosis is a common problem and the extensive use of the first line drugs has led to the development of multiple drug resistance at a level which could pose a serious problem in the near future (Molla *et al.*, 2003, Asrat, 2008).

Ethiopia is the leading country in cattle population in Africa. However, there is limited surveillance for *Salmonella* in cattle. It is reported that *Salmonella* is very common in foods of animal origins such as meat, milk, cheese and other types of food world-wide. Given the long history of food borne salmonellosis, *Salmonella* infections begin with the ingestion of organisms in contaminated food or water. Therefore, it is not surprising that this study is designed to perform prevalence testing of *Salmonella* from food of cattle products which is very significant to:

- Give information for the food producers that large numbers of food ingredients and food products must be routinely tested since the presence of *Salmonella* in any ready-to-eat food such as meat products, eggs, dairy products, chocolate, herbs and spices, fresh salads, fruits, seeds and nuts, flour and shellfish is not acceptable.

- Provide information on the bacterial load and will form the basis for future quality control of foods and food ingredients in order to control food borne diseases in the country.

- Recommend to establish systems for controlling of *Salmonella* infection and to develop national and local guidelines for antibiotic treatment.

2: OBJECTIVES OF THE STUDY

2.1 General Objective

- To determine the prevalence of *salmonella species* from foods of cattle source and perform antimicrobial sensitivity testing.

2.2 Specific Objectives

- To isolate *salmonella* from meat, milk and cheese
- To perform antimicrobial sensitivity testing
- To determine the relative distribution of *Salmonella species*

3: MATERIALS AND METHODS

3.1 Study Design, Study Area and Study Period

The Cross-sectional study was performed in Merkato over a period of 4 months since November, 2011 to March, 2012. Merkato is located in the Addis Ketema district of Addis Ababa, Ethiopia. It is the largest open air market in Africa, covering several square miles and employing an estimated 13,000 people in 7,100 business entities

3.2 Sample Size Determination

Sample size was determined based on the prevalence rate of 50%, 5% level of significance, 95% confidence interval and 5% degree of precession by using the following formula:

$$N = \frac{(Z\alpha/2)^2 \times P(1 - P)}{d2}$$

$$= \frac{(1.96)^2 \times 0.5(1-0.5)}{(.05)^2}$$

$$^{=}384$$

Where N = Number of food sample

Zα = the standard normal deviation corresponding, 95% of Confidence level = 1.96

P = prevalence (50%)

d = the degree of accuracy desired (5%= 0.05)

Based on the above formula the calculated sample size was 128meat sample+128 milk sample+128 cheese sample =384.

3.3 Sampling Strategy, Sample Collection, Handling and Transport

A total of 384 samples; 128 meat, 128 milk and 128 cheese samples were collected randomly from the market of Merkato. Therefore, a total of 128 samples (10 samples per week) for each type of sample were collected from the market over a period of four months. Approximately, 25g of blended meat and 25g of cheese and 25ml of milk samples were collected in sterile universal bottles from each sampling place in order to insure accurate representation and to increase the sensitivity of detection. Then, the collected samples were transported to Addis Ababa University Bacteriology Laboratory within two hours. Therefore, 25g of blended meat, 25g of cheese and 25ml of milk samples were mixed within 225 ml of buffered peptone water (Oxoid) to perform pre-enrichment tests needed to isolate *Salmonella species* by following the ISO-6579, 2002 procedure (Microbiology of food and animal feeding stuffs horizontal method for the detection of *Salmonella spp.*).

3.4 *Salmonella* Isolation Procedure

The isolation of *Salmonella* from food samples may be complicated. Because, *Salmonella* populations in food samples may be affected due to heat, pH or salt content or unevenly distributed through the food matrix (WHO, 2010). Therefore, the study was conducted utilizing the conventional methods for the detection of *Salmonella* following the standard guide lines from ISO-6579:2002 as follows:

- A large sample volume (25g or 25 ml) was used. This helps to insure accurate representation of the entire matrix.
- A pre-enrichment step, such as growth in buffered peptone water before direct plating for *Salmonella* was done. This allows stressed or injured *Salmonellae* to recover before exposure to selective enrichment media.
- Selective medium known as selenite F broth (Oxoid) was used for pre-enrichment to preferentially recover *Salmonella*. Following incubation, the selective broth was inoculated onto Xylose-Lysine Desoxycholate agar (XLD) (Oxoid).

3.4.1. **Preparation of Non-Selective Pre-Enrichment**

To do this procedure, 25 g or 25ml of food samples were weighed with a sterile aluminum foil and pipettes respectively and placed into an Erlenmeyer flask of 225 ml buffered peptone water to obtain 1 part sample + 9 part buffer. Then, it was mixed and the sample mixture was shaken approximately for 2 minutes and incubated at 36°c overnight (16-20 hours).

3.4.2 **Preparation of Selective Enrichment**

1ml of the pre-enrichment broth after incubation was mixed and transferred into 10 ml of selenite F broth by using a pipette and incubated at 36°c for overnight (18-24 hours).

3.4.3 Plating out and Identification

A loop full of the inoculated and incubated selenite F broth was spread on XLD agar plates and incubated in an inverted position at 36.0°c overnight (18-24 hours) and after incubation; the plates were checked for growth of typical *Salmonella* colonies.

Fig. 3.1: *Salmonella spp.* Grown on XLD Media

XLD medium is used for the isolation of *shigellae* and *salmonellae* from foods and clinical specimens. *Shigella*, *Providencia* and *Edwardsiella* do not ferment xylose, sucrose or lactose. Therefore, they alter the pH to alkaline and produce red colonies. *Salmonella spp*. ferments xylose, but at the same time decarboxylates lysine in the medium causing an alkaline pH and thus producing red colonies. The hydrogen sulphide producers, *Salmonella* and *Edwardsiella*, grow colonies with a black centre because the medium contains an iron salt. Fermentation of sucrose and/or lactose produces higher acid levels, thus preventing sucrose and/or lactose positive bacteria from changing the pH to alkaline through decarboxylation of lysine. Non-pathogenic hydrogen sulphide-producing bacteria do not decarboxylate lysine. At the same time, the level of acid produced by fermentation prevents blackening of the colonies until after 18 or 24 hours. Sodium desoxycholate is an inhibitor which at the concentration used in this medium inhibits coliforms but not *salmonellae* and *shigellae*.

3.4.4 Biochemical Identification and Confirmation

By means of an inoculating loop, two suspected colonies/plate from XLD agar were transferred and inoculated on TSI (Oxoid) and motility media (Oxoid) and incubated overnight at 35^{o}c. These biochemical tests help to reduce the number of suspected *Salmonella* colonies for further API 20E (BioMerieux) tests. Finally, API 20E was used for further confirmation which is a standardized identification system for Enterobacteriaceae with 20 micro-tubes containing dehydrated substrates

3.5 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing was done by the agar disk diffusion method as described by the The National Committee for Clinical Laboratory Standards (NCCLS) (NCCLS, 2000). The pure *Salmonella* isolates confirmed by the biochemical testing procedure as described in ISO 6579: 2002 were tested for antimicrobial susceptibility. For antimicrobial susceptibility tests of *Salmonella* isolates, ten antimicrobial drugs (Oxoid) (amoxycillin 25µg (AMX25), ampicillin 10µg (AM10), ceftriaxone 30µg (CRO30), chloramphenicol 30µg (C30), ciprofloxacin 5µg (CIP5), gentamycin 10µg (CN10), kanamycin 30µg (K30), nalidixic acid 30µg (NA30), trimethoprim sulfamethoxazol 25 µg (SXT25) and tetracycline 30 µg (TTC30) were used and performed on Mueller-Hinton agar (Oxoid) by disc diffusion (Kirby-Bauer) method following the standard procedures. In brief, a Mac-Farland 0.5 standardized suspension of the bacteria in 0.85% sterile saline was prepared and swabbed over the entire surface of Mueller Hinton agar with a sterile cotton swab. Then, 10 rings of disks each containing single concentration of each antimicrobial agent per medium were placed uniformly onto the inoculated surface by pressing gently down to ensure even contact with the medium and incubated at 37°C overnight and clear zones produced by antimicrobial inhibition of bacterial growth was measured in mm using a straight line ruler from the undersurface of the plate without opening the lid. The diameter of the zone was read using an interpreting chart for zone sizes of NCCLS. Finally, Findings of antibiotic resistance testing was recorded as susceptible, intermediate and resistant.

Fig.3.2: Antimicrobial susceptibility testing done on Mueller-Hinton agar

3.6 Quality Controls

-Standard operational procedures were followed during processing of each sample.

-All instruments used for sample processing were checked for proper functioning.

- *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *S. enterica* serotype Typhimurium (ATCC 13311), *S. enterica* serotype Enteritidis (ATCC 13076) were taken from EHNRI and used as a quality control throughout the study.

3.7 Data Analysis and Interpretation

Data recorded in the result section were organized, summarized, analyzed and interpreted. Tables and figures were used to describe the findings. Tables were used to examine the prevalence of *Salmonella species*. All components of the recordings were entered and analyzed by using SPSS, version-16.0, 2007 computer software. Finally, the interpreted and summarized data were discussed based on the requirements of this research objective.

3.8 Ethical Consideration

The ethical clearance and permission was given by the Department of Microbiology, Immunology and Parasitology (DMIP) Ethical Review Committee. The owner of food samples were informed that the study process including taking small pieces of food samples (25g of meat, 25mL of milk and 25g of cheese) has no harm or has minimum risk to them and their food. Rather, it is helpful to design methods needed to reduce the prevalence of *Salmonella species* that causes salmonellosis because of consumption foods of animal products. In addition, informed consent was made with each volunteers and subjects who were not volunteer allowing the collection of these food samples was not enforced to be included in the study.

4: RESULTS

4.1 Sources and Characteristics of Samples

The source of retail milk and cheese samples to Merkato, Addis Ababa were Sululta, Sheno and Chancho and retail meat samples were obtained from Kera Slaughter Houses. The sample collection was done randomly from the destination. Weekly, 30 samples were collected and analyzed.

4.2 Prevalence of *Salmonella* in the Samples

A total of 384 samples were processed for isolation of *Salmonella* organisms during November, 2011 to March, 2012. Of the 384 samples tested, *Salmonella* was detected from 13 (3.39%) samples using the method described by ISO 6579:2002. From all isolates; 9 (69.3%), 3 (23.08%) and 1 (7.69%) were obtained from meat, milk and cheese samples respectively. For each type of food samples, a total of 128 samples were taken and tested for the presence of *salmonella spp.* and 9 (7.03%), 3 (2.34%) and 1 (0.78%) were detected from meat, milk and cheese samples respectively.

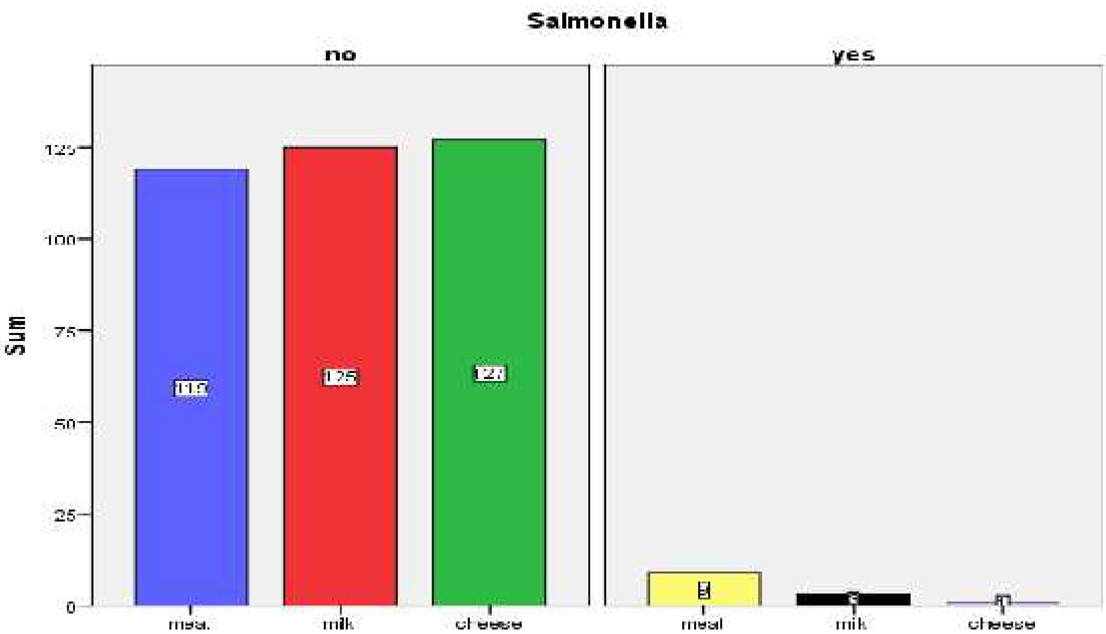

Figure 4.1: Bar chart for the N<u>o</u> of *Salmonella species* that was present or absent in meat, milk and cheese samples

4.3 Results of Antimicrobial Resistance Testing

A total of 13 isolates (n=13) were tested against ten commonly used antimicrobials. Among all the antimicrobials tested, amoxicillin was the most resisted drug (100%) followed by ampicillin and tetracycline (76.9%). Ciprofloxacin showed maximum sensitivity (100%) and was the only antimicrobials not resistant to any of the isolates tested.

Table 4.1: **A**ntimicrobial resistance profile of *Salmonella* isolated from this study (n=13).

Antimicrobial Agent	**Resistant**		**Intermediate**		**Sensitive**	
	No	**Percent**	**No**	**Percent**	**No**	**Percent**
AMx	**13**	**100**	**0**	**0**	**0**	**0**
AM	10	76.9	0	0	3	0
CRO	3	23.1	3	23.1	7	53.8
C	6	46.2	0	0	7	53.8
CIP	0	0	0	0	13	100
CN	2	15.4	3	23.1	8	61.5
K	6	46.2	4	30.7	3	23.1
NA	6	46.2	3	23.1	4	30.7
SXT	6	46.2	1	7.6	6	46.2
TTC	10	76.9	3	23.1	0	0

-No. is the number of isolates resistant, intermediate and sensitive
- AMX (amoxycillin), AM (ampicillin), CRO (ceftriaxone), C (chloramphenicol), CIP (ciprofloxacin), CN (gentamycin), K (kanamycin), NA (nalidixic acid), SXT (trimethoprim sulfamethoxazol) and TTC (tetracycline)

Table 4.2: MDR exhibited by *Salmonella* spp. of each meat, milk and cheese isolates

Isolates	Resistance pattern	No of resisted antimicrobials
Go 37	AMX+AM+C+TTC+SXT+NA+K	7
Go40	AMX+AM+TTC	3
Go41	AMX+AM+C+CN+TTC+SXT+NA+K	8
Go45	AMX+AM+C+CN+TTC+SXT+NA+K	8
Go57	AMX+AM+CRO+C+TTC+SXT+NA+K	8
Go59	AMX+AM+CRO+C+CN+TTC+SXT+NA+K	9
Be81	AMX+TTC	2
Se118	AMX+AM+C+TTC+SXT+NA+K	7
Se128	AMX	1
AbMi12	AMX+AM+TTC	3
AbMi22	AMX+AM+TTC	3
AbMi55	AMX+AM	2
Ga Ch7	AMX	1

- Go, Be and Se with Nos= code of meat samples

-AbMi with Nos=codes of milk samples

-Gach7=code of cheese sample

5: DISCUSSIONS

It is widely known that *Salmonella species* have been considered as one of the most important food borne pathogens and continue to be a prominent threat to food safety worldwide. Infections are commonly acquired by animal to human transmission through consumption of undercooked food products derived from livestock or domestic fowl such as beef, poultry meat, egg, cheese and milk. The emergence of *Salmonella* serotypes that became associated with new food sources and the emergence of *Salmonella* serotypes with resistance against multiple antibiotics are common (Gillespie *et al.*, 2003). Food of animal products are contaminated with harmful, pathogenic and spoilage bacteria by infected stocks, cross contamination, improper handling and storage or improper cooking which can lead to human food borne illnesses. Epidemiological data are needed to inform public health authorities about the nature and magnitude of the problem and to monitor trends over time (Myint, 2004). Therefore, this study is conducted to assess the prevalence and antimicrobial resistance profile of *Salmonella species* from foods of animal products.

This study found an overall *Salmonella* prevalence of 3.39% with prevalence of 7.03%, 2.34% and 0.78% from meat, milk and cheese samples respectively. The prevalence of *Salmonella* in minced beef samples found in the previous studies done in Ethiopia by Nyeleti *et al.* (2000), Gebremedhin (2004), Ashenafi (1994), Ejeta *et al.* (2004), Molla *et al.* (1999), Tegegne and Ashenafi (1998) was 7.9%, 8.5%, 9%, 14.4%, 40% and 42% respectively. Therefore, in this study, prevalence of *Salmonella* in meat samples is low in contrast to many of the studies done in Ethiopia although a prevalence of 7.03% in this study is almost similar with 7.9% reporting by Nyeleti *et al.* (2000). The low level of detection in this study could probably be due to the improvement of living standards or it may be due to the difference in the tests used.

A study by Gebremedhin (2004) stated that *Salmonella* prevalence in cottage cheese was low (2.1%). However, the prevalence of cottage cheese in this study is very low (0.78%). The low level of detection in this study could probably be due to the prevalence of this pathogen in milk is also low (2.34%). It could also be due to the fact that the methodology of cooking used in preparing cottage cheese from the curd might have destroyed the pathogen of interest originally present. The low pH of cottage cheese, which varies from 3.3 to 4.6, was an important limiting factor which was not favorable for survival, growth and multiplication of this pathogen thereby contributing significantly for the low recovery rate of *Salmonella*.

The use of antimicrobials in food of animal products has resulted in the development of antimicrobial resistance through mutation and acquisition of resistance encoding genes (Fluit, 2005). The situation in Ethiopia may be exaggerated by uncontrolled use of antibiotics to humans as well as animals. In this study, 100% of the isolates were found to be resistant to amoxicillin followed by (76.9%) to ampicillin and tetracycline; (46.2%) to chloramohenicol, kanamycin, nalidixic acid and trimetoprim-sulfamethxazole; (23.1%) to ceftriaxone and (15.4%) to gentamycin. The level of resistance to amoxicillin (46.9%) and (19.4%) and tetracycline (46.9%) and (15.3%) in the previous studies done in Ethiopia by Zewdu and Cornelius (2009) and Gebremedhin (2004) respectively is lower than this study. The level of resistance in this study is also higher than the previous studies done in other countries such as: *Salmonella* serovars isolated from German foodstuffs was resistant to amoxicillin (73%). However, the level of resistance to sulfamethoxazole (92%), tetracycline (81%), chloramphenicol (48%), and trimethoprim (27%) (Miko *et al.*, 2005) is higher than the level of resistance in this study. Only ciprofloxacin in this study was sensitive to all of the isolates tested which is an indication that the level of sensitivity is lower than the previous study done by Molla et al. (1999) that four antimicrobials (gentamycin, kanamycin, rifampicin, and sulphamethoxazole-trimethoprim) were effective against all isolates. However, it is similar with a study in Nepal that 35 multi-drug-resistant strains were detected out of 132 strains of *Salmonella* Typhi and no isolates were resistant to ciprofloxacin (Antunes *et al.*, 2003).

In the present study, 100% of meat samples were found resistant to amoxicillin, 88.9% to tetracycline and 77.8% to ampicillin 66.7% to chloramphenicol, kanamycin, nalidixic acid and trimethoprim-sulfamethoxazole, 33.3% to ceftriaxone and 22.2% to gentamycin and 100% were sensitive to ciprofloxacin followed by 55.6% to gentamycin. However, two antibiotics, amoxicillin (100%) and tetracyclin (66.7%) are resistance for *Salmonella species* isolated from milk samples and only amoxicillin (100%) is resistance in

Salmonella species isolated from cheese samples. Therefore, antibiotic resistance has been more common in *Salmonella species* isolated from meat samples than the other types of samples and lowest in cheese samples.

Of the 13 *Salmonella* isolates subjected to antimicrobial testing, none were sensitive and 13 (100%) were resistant to one or more drugs tested. From these, 15.4%, 15.4%, 23.1% and 46.2% of the isolates were resistant to 1, 2, 3 and more than 3 types of antimicrobials respectively. Therefore, MDR (which were reported as resistant to two or more drugs) in this study is higher than the previous studies done in Ethiopia by Molla *et al.* (1999) (87.2%) and Gebremedhin (2004) (32.7%) and other countries, USA and China, by Chen *et al.* (2004) (82%). Overall, eight resistance patterns were observed among 13 *Salmonella* isolates. One isolates of *salmonella species* was resistant to nine of the ten antimicrobials tested with simultaneous MDR to up to seven antimicrobials of three other patterns. The resistance pattern of this study was higher than the previous study done in Ethiopia. In 2004, *Salmonella* Enteritidis was resistant to five of the seven antimicrobials tested with simultaneous MDR to up to three antimicrobials. None of the mutton and cottage cheese isolates showed resistance for more than three antimicrobials and only one serotype from minced beef showed resistance for 8 antimicrobials (Gebremedhin, 2004).

Limitations of the Study

The study was done only on limited foods of cattle sources from Merkato and may not represent a true reflection of *Salmonella* prevalence in the whole community of Ethiopia and in other food sources. In addition, Serotyping and molecular analysis was not done due to limited resources.

CONCLUSIONS

Microbiological analysis of food items from retail outlet for food borne organisms like *Salmonella* is of paramount importance in ensuring the supply of safe food for the consumers. The information collected in this cross-sectional survey coupled with other similar studies can be used as a basis to undertake qualitative microbiological risk assessment and provide information to design monitoring and surveillance programs. In view of the fact that most samples investigated presently are consumed raw or under cooked by significant proportion of the population and the existence of immense export potential, further monitoring of their microbiological quality must be needed to limit further spread and ensure public health standards by using the following specific and important conclusions from this cross-sectional survey.

- ✓ A total of 13 *Salmonella species* out of 384 food samples were identified. It was prevalent in food items of meat (7.03%), milk (2.34%) and cheese (0.78%) in Merkato, Addis Ababa and the magnitude of the problem is especially high in meat samples as compared to others.
- ✓ Those *Salmonella* isolates might arise from post-preparation contamination of the products from one or more of the following; unclean environment, equipment, poor hygiene of handlers, starting from site of preparation up until the site of retail selling. There is also a possibility that the heat treatment of cottage cheese may not be adequate to kill the *Salmonella* present or *Salmonella* may tolerate the heat treatment and hence appear in the product tested.
- ✓ Although the prevalence of *Salmonella species* in this study is low in contrast to many of the studies done in Ethiopia and other countries, further monitoring and control programme is needed to reduce its prevalence more and more.
- ✓ All *Salmonella* isolates were resistant to one or more of the antimicrobials tested. Although all of them were susceptible to ciprofloxacin, most of them were MDR. This could make treatment of humans' clinical salmonellosis difficult.

- ✓ There is a marked difference in the case of antimicrobial resistance of *Salmonella species* isolated from meat, milk and cheese samples. Unlike cheese and milk samples, most antimicrobial drugs in meat samples are resistant except ciprofloxacin.

RECOMMENDATIOS

This study reveals the presence of *Salmonella species* in meat, milk and cheese samples in Merkato, Addis Ababa and all of them were resistant to one or more of the antimicrobial drugs and most of them were MDR. In view of this research finding, there is a need to develop comprehensive policies to ensure safe food practices by Public Health, Veterinary and Food Regulatory Authorities. The following recommendations should be useful to ensure the microbiological quality of these and other food samples.

- ❖ Although there is a general practice of cooking food products before consumption in Ethiopia, eating raw foods such as meat and other types of foods are very common. Therefore, awareness campaigns and notifications as and when necessary should be organized and incorporated in the routine process by the Public Health Department and Food Regulatory Authority and should also emphasize on educating the consumers, food handlers and all others who have access to food about the importance of hygiene and cooling system.
- ❖ Adoption of HACCP (Hazard Analysis Critical Control Point) principle at various critical points from farm to table, as a tool to determine the precise sources of contamination, is of paramount importance in designing appropriate strategies to substantially reduce the prevalence and associated risk for consumer and trade partners.
- ❖ Encouraging prudent and judicious use of antimicrobial drugs in veterinary and public health sectors must be needed.
- ❖ Establishment and enforcement of legislation which prohibit handling of food unless free from *Salmonella* or excretion (shedding) is terminated.
- ❖ Improve method and hygiene of meat transport from slaughterhouse to retail markets such as use of refrigerated transport vehicle.

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ANNEXES

Annex I: Record Sheets for Laboratory Results

Record sheet I: Isolation of *Salmonella* from food by using Morphology on selective agar plates

Date:___________________

Type of Sample and number: _________________________

Sample number and type will be recorded for each mixed food sample by using the following record sheet format.

	colour	results	comments
Morphology on XLD			

Record Sheet II: Biochemical Tests for *salmonella species* conformation

Date:_______________

Type of Sample and number: _________________________

Record for biochemical tests will be done for each type of sample and number by using the following record sheet format.

			Salmonella spp.			
Test						
TSI Acid from glucose						
TSI gas from glucose						
TSI acid from lactose						
TSI acid from sucrose						
TSI H_2S produced						
Motility						

Record Sheet III: Antimicrobial susceptibility for *salmonella species*

Date:_______________, Type of Sample and number: __________________________

Record for Antimicrobial susceptibility tests will be done for each type of sample and number by using the following record sheet format.

Antimicrobials	Levels of sensitivity	*Salmonella spp.*					
Ampicillin	resistant						
	intermediate						
	sensitive						
Amoxicillin	resistant						
	intermediate						
	sensitive						
Ceftriaxone	resistant						
	intermediate						
	sensitive						
Chloramphenicol	resistant						
	intermediate						
	sensitive						
Ciprofloxacin	resistant						
	intermediate						
	sensitive						
Gentamicin	resistant						
	intermediate						
	sensitive						
Kanamycin	resistant						
	intermediate						
	sensitive						
Tetracycline	resistant						
	intermediate						
	sensitive						
Trimethoprem-sulfamethoxazole	resistant						
	intermediate						
	sensitive						

Annex II: Subject Information and Consent Form

This study is aimed to assess the prevalence of *Salmonella species* from foods such as milk, cheese and meat as well as to perform anti-microbial susceptibility testing. *Salmonella* is one of the bacteria which cause a serious food borne diseases known as salmonellosis throughout the world particularly in developing countries like Ethiopia.

Therefore, we are requesting you to voluntarily participate in this study by allowing giving 25g or 25mL of the above food samples to the sample collector. Giving those food samples does not harm you and any of your property. Rather, the finding in the laboratory examination may help you to give information about the disease prevalence and to develop the different methods that are needed to reduce this disease.

Since participation in this study is entirely voluntary, you can refuse to participate in this study at any time. Your refusal to participate will not affect any of your benefit.

Contact Address

If you have any question or any information, you can contact:

-Ayalew Lemma

-Addis Ababa University, School of Medicine, Department of Microbiology, Immunology and Parasitology

-Tel: 0912329855

-E-mail: ayuledesta@yahoo.com/ ayuldesta@gmail.com

I, the undersigned, confirm that I give consent to participate in this study by allowing the above food samples after I got a clear understanding of the objectives of the study and with recognition of my right to withdraw from participation in the study at any time if I change my mind.

I----------------------------do interestingly give consent to Mr./Mrs./Miss--------------- to participate voluntarily in the proposed research. The proposal has been explained to me in the language I understand.

Name of participant/owner---------------------------------------

Participant's signature---

Name of sample collector--

Sample collector signature---------------------------------------

Type of food sample and number--------------------------------

Date---

Amharic Version of Subject Information and Consent Form

መረጃ መስጫ እና ፈቃድ መጠየቂያ ቅፅ

ሳልሞኔላ የሚባለው ተህዋስ በተበከሉ የእንስሳት ምግቦች ውስጥ በብዛት በመገኘት በሰዎችም ሆነ በእንስሳት ላይ በሽታ በማምጣት ይታቃወል። ስለዚህ የዚህ ጥናት ዋነኛ አላማ ሳልሞኔላ የተባለ በሽታ አምጭ ተህዋስን ከእንስሳት ምግቦች ማለትም ከወተት፣ ከአይብ እና ከከብት ስጋ ለይቶ ማውጣት ስለሆነ ከላይ የተጠቀሱትን የእንስሳት ውጤቶች ከእርስዎ ለመውሰድ እና ጥናቱን ለማካሄድ የእርስዎ ፈቃደኝነት አስፈላጊ ነው። ይህንን በማድረግዎ በእርስዎ ላይም ሆነ በንብረትዎ የሚደርስ ጉዳት አይኖርም። ይልቁንም በሽታውን አምጭ ተህዋስ የትኞቹ የእንስሳት ውጤቶች ውስጥ በብዛት እንደሚገኝ በላቦራቶሪ ከተለየ በኋላ መከላከያ መንገድ ለማግኘት ጥረት ይደረጋል። እርሶም ሆኑ ሌላው የህ/ሰብ ክፍል ከዚህ ጥናት ተጠቃሚ ይሆናሉ ብለንም እናምናለን።

የጥናቱ ተሳታፊ መሆን በእርስዎ ፈቃደኝነት ላይ የተመሰረተ በመሆኑ በየትኛውም ሰዓት ተሳትፎዎን ማቋረጥ ይችላሉ። ባለመሳተፍዎም የሚያጡት ነገር አይኖርም።

<u>አድራሻ</u>

<u>ጥያቄ ወይም አዲስ ሃሳብ ካለዎት ይህንን አድራሻ ይጠቀሙ፣</u>

-አያሌው ለማ

-አዲስ አበባ ዩኒቨርስቲ፣ የህክምና ት/ቤት፣ የማይክሮባዮሎጂ፣ ኢሚውኖሎጂ እና ፓራሳይቶሎጂ ት/ክፍል

-ስልክ፡- 0912329855

-ኢ-ሜይል፡- ayuledesta@yahoo.com/ayuldesta@gmail.com

እኔ_______________________ የተባልኩ የጥናቱ ተሳታፊ የጥናቱን አላማ እና ጥቅም በዝርዝር ከተብራራልኝ እና ከተረዳሁ በኋላ አቶ(ወ/ሮ/ሪት)________________ ለሚያካሂዱት ጥናት ተሳታፊ ለመሆንና የእንስሳት ውጤቶችን ለመስጠት ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

-የተሳታፊው/ዋ (የባለንብረቱ/ዋ) ስም____

-ፊርማ____

-የእንስሳት ውጤቶችን ሰብሳቢ ስም____

_ፊርማ____

-የምግቡ አይነት እና ቁጥር____

-ቀን____

Annex III: Flow diagram for isolation of *Salmonella* from Foods

Non-selective pre-enrichment

25 g foods in 10% phosphate buffer (36° +/-1°C, 24 h.)

↓

Selective enrichment *

0.1 ml in 10 ml Rappaport-Vassiliadis Soy Broth (41.5° +/- 0.5°C, 24 h.)

1 ml in 10 ml Tetrathionate broth (Müller-Kauffman) (36° +/-1°C, 24 h.)

↓

Isolation

XLD with an inoculation loop (36° +/-1°C, 24 h.)

BGA with an inoculation loop (36° +/-1°C, 24 h.)

↓

Suspect colonies to nutrient agar (36° +/-1°C, 24 h.)

↓

Biochemical confirmation (36° +/-1°C, 24 h.)

Triple Sugar Iron Agar (TSI) or Kliger Iron Agar (KIA)

Urea broth

Lysine Iron Agar (LIA)

Motility-Indol-Ornithine (MIO)

Citrate

↓

Serotyping

O-antigens

H-antigens

Phase I (37°C, overnight)

Phase II

If *Salmonella* serovars Typhi or Paratyphi A are suspected: inoculate 1mL of pre-enrichment broth into 10mL of Selenite Cystine (or Selenite F) broth and incubate at 36° C (+/-1°C) for 18-24 h. Following incubation, it is advisable to inoculate the selective broth onto bismuth sulphate agar (in addition to XLD and BGA) (WHO, 2010).

Annex IV: Materials, Equipments and Reagents

-Erlenmeyer flasks (500 ml) sterile (for pre-enrichment)

-Disposable inoculation loops (1 µl and 10 µl)

-Plastic petridishes (9 cm diameter) sterile

- Glass slides

-Balance

-Incubator

-Bunsen burner

-Pipettes

-Wood spatulas

-Ruler

Media and Samples

-Buffered peptone water

-Rappaport Vassiliadis soy peptone broth

-Xylose Lysine Desoxycholate (XLD) agar

- Luria broth

-Nutrient agar plates

- Mueller Hinton agar

-TSI agar/KIA

- Urea agar

- Medium for indole reaction

-Food samples (milk, cheese, meat)

-10 anti-microbials

Annex V: Declaration

I, under signed, declare that this M.Sc. thesis is my original work, has not been presented for a degree in any other University and that all sources of materials used for this thesis have been duly acknowledged.

M.Sc. Candidate: Ayalew Lemma (Bsc)

Signature: ________________

Date and place of Submission:________________

Addis Ababa, Ethiopia

Supervisor: Dr. Solomon Gebre-Selassie (Associate Professor)

Signature ________________

Date and place of Submission________________

Addis Ababa, Ethiopia