# Isolation, Molecular Detection and Antibiotic Susceptibility Patterns of *Salmonella enterica* serovars enteritidis and *typhimurium* from Adama City Butcher houses, Oromia regional state, Ethiopia

By: Aschalew Kasu Gebre

A Thesis Submitted to Program of Applied Biology

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirements for the Degree of

Master's of Science in Applied Biology (Biotechnology)

Office of Graduate Studies

Adama Science and Technology University

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**ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY**

**SCHOOL OF APPLIED NATURAL SCIENCE**

**APPROVALSHEET**

As member of the examining board of the final MSc open defense, we certify that we have read and evaluated the thesis entitled **Isolation, Molecular Detection and Antibiotic Susceptibility Patterns of *Salmonella enterica* serovars *enteritidis* and *typhimurium* from Adama city butcher houses Oromia, regional state, Ethiopia**, prepared by Aschalew Kasu and recommend that it be accepted as fulfilling the requirement for the degree of Master of Sciences in Applied Biology (Biotechnology).

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| Advisor | Signature | Date |
| Internal examiner | Signature | Date |
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| Chair Person | Signature | Date |
| Department Head | Signature | Date |

## DECLARATION

I declare this MSc thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this thesis have been duly acknowledged.

Name: Aschalew Kasu

Signature: _____

Date: _____

## DECLARATION BY THE SUPERVISOR

We confirm that the work in this thesis was carried out by the candidate under our supervision. Name:

Dr. Hunduma Dinka

Signature: _____

Date: _____

# DEDICATION

This work is dedicated to my father Kassu Gebre and my mother Etenesh Adeye, Mammo Fulle and Asnakech Hassen, Dinku Assefa and Alemayehu Kifle for their guidance and support in my life.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| BPW | Buffered Peptone Water |
| CFU | Colony Forming Units |
| CLSI | Clinical and laboratory Standards Institute |
| DNA | Deoxyribonucleic Acid |
| EDTA | Ethyl Demine Tetra Acetate |
| $H_2S$ | Hydrogen Sulphide |
| IOS | International Organization for Standardization |
| MB | Methyl Blue, |
| NTS | Non-typhoidal *Salmonella* |
| PCR | Polymerase Chain Reaction |
| SC | Simmons's Citrate |
| Sp | Species |
| TSA | Tryptone Soya Agar |
| VP | Vogues Proskauer |
| WHO | World Health Organization |
| XLD | Xylose Lysine Deoxycholate |
| µg | Microgram |

# Abstract

*Non-typhoidal Salmonella has been found to be the major cause of foodborne diseases and a serious public health problem in the world, with an increasing concern for the emergence and spread of antimicrobial resistant strains. The common Salmonella enterica serovars Enteritidis and Typhimurium represent the major serovars associated with human salmonellosis. Contamination of meat products with these serovars is considered the main source of infection. Therefore, this study is aimed to isolate and detect Salmonella enterica serovars enteritidis and typhimurium by molecular tool from Adama City butcher houses and to assess the antibiotics resistance profile of the isolates. A total of 48 samples consist of liver stomach tissue and raw meat samples were investigated for the presence of Salmonella species. The isolates were identified based on cultural, biochemical methods and polymerase chain reaction. The polymerase chain reaction was applied to test the presence of sdfI and spy specific target genes suspected to have a role in infection. In the present investigation, 7 isolates of Salmonella serovars were isolated from 48 bovine tissue samples (the raw meat, liver and stomach) collected from the butcher house of Adama city. From the total isolates of Salmonella serovars 5(71.43%) were Salmonella Typhimurium, while 2(28.57%) of the isolates were Salmonella Enteritidis which using the target genes by polymerase chain reaction. All Salmonella serovars were sensitive to Ciprofloxacin, Ceftraxene and Gentamycin. But 71.41% and 100% of the isolates were found resistant to Tetracycline and Azethromycin respectively. The commonly used antibiotics and misuse use of them may have contributed to the development of resistance to Salmonella serovars. Since the use of Ceftraxene, Gentamycin and Ciprofloxacin should be selected for treating Salmonella rather than Azethromycin and tetracycline. Therefore, further molecular characterization of pathogenic serovars with detection of antibiotics resistant gene and virulence gene can have paramount role for diagnosis of Salmonella direction for action to minimize food contamination and resistance gene development.*



Keywords: Antibiotics, Isolation, Meat, Molecular, *Salmonella* serovars

# 1. INTRODUCTION

## 1.1. Background of the study

The *Salmonella* bacterium was first described by Theobald Smith (1859-1934) and then in 1885, by two American veterinarians, Salmon and Smith isolated the bacterium causing hog cholera from infected pigs. The name *Salmonella* was subsequently adopted in honor ofDoctor Salmon (Salmon and Smith, 1886).The genus consists of two species: the first is S. Enterica which is divided into six subspecies. *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae* and *S. enterica* subsp. indica; and the second is S. Bongori (formerly called S. *enterica* subsp. *bongori*) (WHO., 2003).*Salmonellae* are Gram negative, short rod shape, nonsporeforming, noncapsulated, aerobic and facultative anaerobic organisms and classified under the family *Enterobacteriaceae*(Mondal *et al*., 2008).The organism is mesophilic with optimum growth temperature 37°C but capable of growth within a wide temperature range of 8– 48$^{o}$C (Mastroeni and Maskell, 2006).

*Salmonella* is highly distributed in the environment originating from the gastrointestinal tracts of domesticated and wild animals and can be present without causing apparent illness. Most infections result from the ingestion of foods of animal origin contaminated with *Salmonella* species such as beef, chicken, turkey, pork, eggs, and milk (Joseph *et al*., 2012).

Food borne diseases are one of the most widespread global public health problems of recent times, and their implication for health and economy is increasingly recognized. *Salmonella* species are joined with food-borne diseases worldwide. Foods of animal origin, such as poultry meat, eggs and their products, are frequently involved in outbreaks. In the period of 1999–2008,*Salmonella*species were responsible for 1408 (23.2 %) of the 6062 outbreaks investigated in Brazil, 267 (43.8 %) of the 609outbreaks occurring in Parana State between 2000 and2005 (Luciana *et al*., 2011).

According to FAO (2006) report, every year a huge number of people suffer from foodborne diseases worldwide due to contaminated food and water consumption. A wide range of pathogens play a role in foodborne disease, most of which have a zoonotic origin and have carriers in healthy food animals from which they spread to an increasing variety of foods of animal origin and are considered as major vehicles of foodborne infections. Among the common diseases causing pathogens, *Salmonella* is considered the most prevalent foodborne pathogen worldwide and has long been recognized as an important zoonotic bacterium of economic significance in animals and

humans especially in the developing countries (Carrasco *et al*., 2012). Non-typhoid *Salmonella* is a common cause for foodborne diseases, representing an important public health problem worldwide. Underdeveloped and also technologically developed countries are struggling with foodborne disease outbreaks which result in illness, death and large economic losses. In underdeveloped countries, there are more than one billion cases of gastroenteritis and up to 5 million deaths annually (Gould and Russell, 2003).

In Ethiopia, as in other developing countries, it is difficult to evaluate the burden of Salmonellosis because of the limited scope of studies and lack of coordinated epidemiological surveillance systems. In addition, under reporting of cases and the presence of other diseases considered to be of high priority may have overshadowed the problem of Salmonellosis. The real situation of antibiotic resistance is also not clear since *Salmonella* are not routinely cultured and their resistance to antibiotics tested. Surveillance for the prevalent *Salmonella* serovars and the assessment of antimicrobial susceptibility is essential to control the spread of the pathogen (Beyene *et al*., 2008).

Studies conducted in Ethiopia reveled the prevalence of several serotypes of Salmonella in various food animals, food products (Addis et al., 2011; Molla et al., 2006; Zewdu and Cornelius, 2009) and high level of drug resistance has also been reported. For instance, 75%, 59.4%, 46.9%, 40.6%, and 40.6% of NTS isolates from food items and personnel in Addis Ababa were resistant to streptomycin, ampicillin, tetracycline, spectinomycin and sulfisoxazole, respectively (Zewdu and Cornelius, 2009). Limited researches were done in Ethiopia on isolation and molecular detection of *Salmonella enterica* serovars *enteriti*dis and *typhimurium* from animal meat (Tadesse, 2016). Knowledge on the identification of sources and points of contamination are essential for implementation of effective strategies for prevention of contamination of meat with *Salmonella* (Asrat, 2008). Therefore, the aim of the present study is to isolate and detect *Salmonella enterica* serovars *enteritidis* and *typhimurium* by using cultural, biochemical and PCR based molecular techniques from cattle meat in Adama butcher houses, as well as to determine the antibiotic resistant profiles of commonly available drugs to the isolated *Salmonella.*

## 1.2. Statement of the problems

Salmonellosis is the major public health problem worldwide (Carrasco *et al*., 2012). It contributes negative effects because of the cost of surveillance, prevalence and treatments of illness (Gómez-Aldapa, *et al*., 2012).In Ethiopia, like in other developing countries; it is difficult to evaluate the burden of food-borne diseases, due to the limited scope of studies. In addition, underreporting of cases and the presence of other diseases considered to be of higher priority may have overshadowed the problem of food-borne diseases due to salmonellosis (Flint *et al*., 2005). *Salmonella* can causes of zoonotic diseases, which can be transmitted to human when consumed contaminated animal food products (Sanchez-Vargas *et al*., 2011). Probability of transmission of non-typhoidal is high, because most Ethiopian people are consuming raw meat. Isolation and molecular detection of *S. enterica* serovars from food can have basic achievement to know the possible important serovars for risk of salmonellosis and to control the virulent serovars with correct treatment. Although there are few studies on *Salmonella* in humans, animals and food of animal origin in Ethiopia, there is no surveillance and monitoring on non-typhoidal *Salmonella* serovars using molecular technique.

## 1.3. Objectives of the study

### 1.3.1. General objective

- To isolate, molecular characterize and antibiotic susceptibility patterns of *Salmonella enterica* serovars *enteritidis* and *typhimurium* from meat samples collected from Adama city butcher house

### 1.3.2. Specific objectives

- To isolate *Salmonella enterica* serovars *enteritidis* and *typhimurium* from bovine raw meat, liver, and stomach tissue sdfI and spy target gene respectively samples collected from Adama City butcher houses.
- To detect *Salmonella enterica* serovars *enteritidis* and *typhimurium* using molecular techniques
- To assess the antibiotics resistance profile of the *Salmonella* isolates.

## 1.4. Significant of the study

The study will provide information on the presence of *Salmonella enterica* serovars in butcher houses in Adama city which are possible important food borne pathogen having risk of salmonellosis. Thus,

- ✓ It emphasizes on the method and techniques used for detection of any food contaminant by using molecular methods.
- ✓ It helps to identify the most common antimicrobial resistant *Salmonella enterica* serovars and the effective drug for treatment strategies.
- ✓ It will serve as baseline information for extensive application of such molecular diagnosis technology along food chain.

# 2. LITERATURE REVIEWS

## 2.1. Overview of food hygiene and food safety

In both developed and developing countries, food borne diseases remain as a real and formidable problem that causing great human infection and significant economic losses for treating illness and prevention or to control before outbreak. The problem occurred more likely widespread in developing countries, where food and water-borne diarrheal diseases kill an estimated 2.2 million people each year, most of them are Children (FAO, 2006). The problem is severe in developing countries due to difficulties in securing optimal hygienic food handling practices. In developing countries, up to an estimated 70% of cases of diarrheal disease are associated with the consumption of contaminated food (Knife and Abera, 2007; Beyene *et al*., 2011).In sub-Saharan Africa, S. Typhimurium is the most common serotype and it is highly distributed (Okoro et al., 2012). Study in the Congo reported that 79% of the serotypes causing invasive non-typhoidal *Salmonella* recovered from blood of young children were Typhimurium (Lunguya *et al*., 2013).

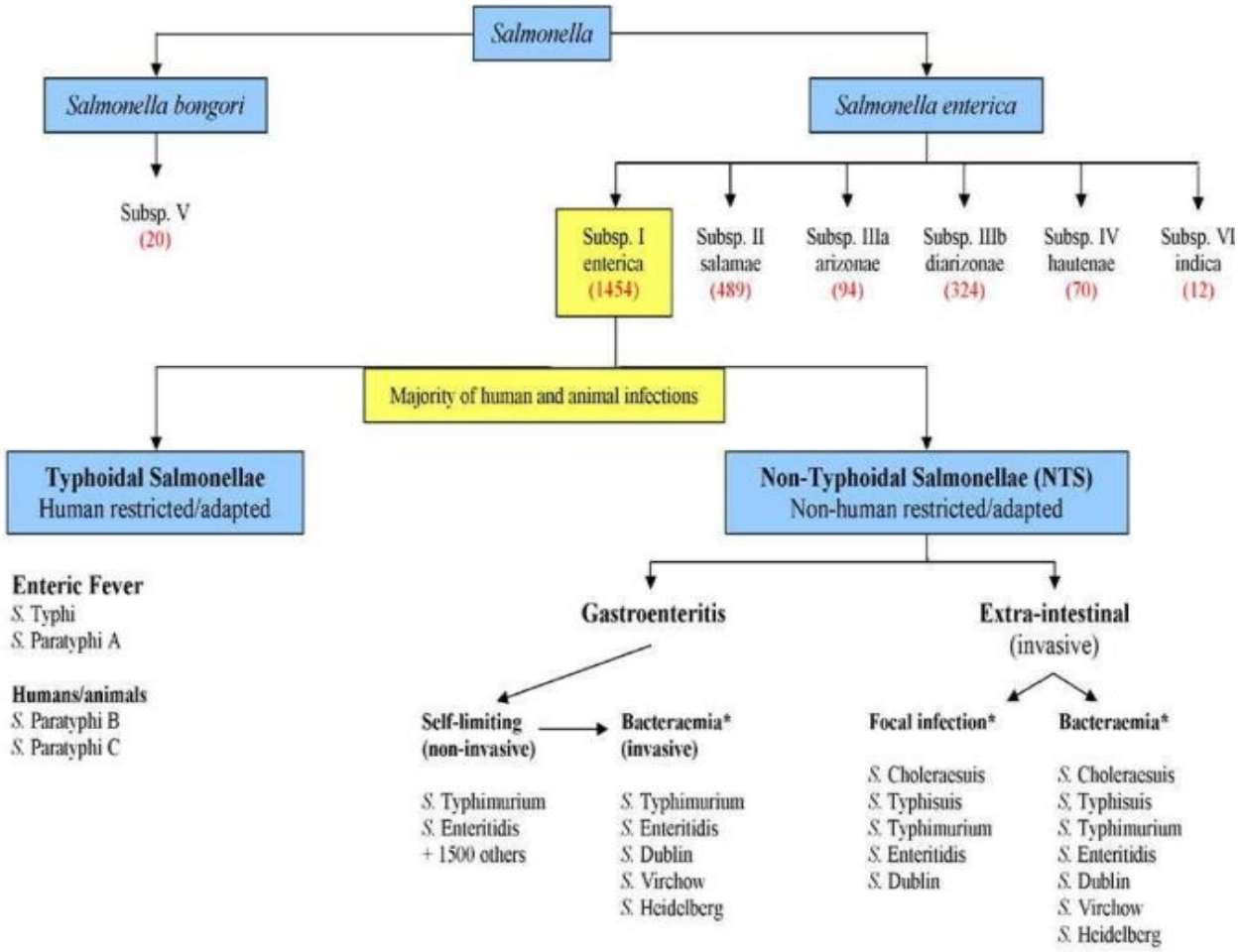
## 2.2. Taxonomy of *Salmonella*

Mondal et al. (2008) describe that *Salmonella* belongs to the family Enterobacteriaceae. The genus *Salmonella* has two species; S. Enteric and S. Bongori, which was formerly subspecies V. Subspecies *Salmonella* Enteritis differentiated in to six subspecies based on their biochemical and genomic characteristics. Roman numeral and a name are used for the designation of these six subspecies as (Table 1) follows: I, *S. enterica* subsp. *enterica*; II, *S. enterica* subsp. *salamae*; III,*S. enterica* subsp. *arizonae*; III, *S.* enterica subsp. *diarizonae*; IV, *S. enteric* subsp. *houtenae*, and VI, *S. enterica* subsp. *Indica* (Brenner *et al*., 2000). With regard to food safety *S. enterica* subsp *enteric*is the subspecies of most concern because the strains within these serogroups are known to cause 99% of *Salmonella* infections in humans (Figure 1) (Brenner *et al*., 2000).

**Table 1**: Species and subspecies of the genus *Salmonella's (*Card, 2009).

| ***Salmonella* species** | **Subspecies** | **Number of Serovars** |
|---|---|---|
| S. Enterica | enterica | 1,478 |
| | salamae | 498 |
| | arizonae | 94 |
| | diarizonae | 327 |
| | houtenae | 71 |
| | indica | 12 |
| S. Bongori | | 21 |
| **Total** | | **2,501** |

**Figure 1:** Classification of the genus *Salmonella* (Achtman *et al*., 2012).(Note: Numbers in the brackets indicate the total number of serotypes included in each subspecies)



## 2.3. General characteristics of *Salmonella*

*Salmonella* make up a large genus of gram-negative bacilli and are highly adapted to both humans and animals causing a wide spectrum of infectious (Achtman *et al*., 2012). The growth of Salmonella Typhi and Salmonella Paratyphi is restricted only to human hosts, to whom they cause enteric (typhoid) fever. But other types of *Salmonella* serovars, which is called non-typhoidal *Salmonella*, that can colonize the gastrointestinal tracts of a broad range of animals, including mammals, reptiles, birds, and insects (Kumar, 2012). More than 200 of these serotypes are pathogenic to humans causing gastroenteritis and but also associated with localized infections and/or bacteremia (Fuaci and Jameson, 2005).

*Salmonella* are facultative anaerobic with ranges from 8°C to 45°C, strains can able to exist pH between 4 to 9. *Salmonella* is heat sensitive and inactivated at normal cooking temperatures (> 70 °C) although the cooling time and values for temperature and time could change depending on the serotype and the food matrix and also *Salmonella* has been exhibited to tolerate up to 20% salt concentration (González, 2010). Under freezing conditions (from -23°C to -18°C), this microorganism can be survive as long as seven years. The biochemically *Salmonella* can reduce nitrates to nitrites, produce hydrogen sulfide on triple-sugar iron agar, and they are usually able to use citrate as the sole carbon source (Mondal*et al*., 2008).

## 2.4. Source and means of Salmonella infection

*Salmonella* can be acquired from contaminated food, water, contact with infected animals, human to human contact and from the environment. The main source for *Salmonella* contamination is the intestinal tract of a wide range of food animals and various food of animal origin (Bartholomew *et al*., 2014; Bouchrif *et al*., 2009). Plant products contaminated with intestinal content of food animals are potential sources of *Salmonella* infection (Bayer *et al*., 2014; Lienemann *et al*., 2011). The regional outbreak and global spread of *Salmonella* was significantly contributed by which the people travels between distant countries and the global trade of food items (Davis *et al*., 2002). Feco-oral route is the primary ways of *Salmonella* infection in humans and other animal species. The minimum required infectious dose varies from 30 to more than $10^9$ colony forming units (CFU) depending on the food type and serotype concerned. *Salmonella* in food with high fat content such as cheese or ice cream needs low infectious dose (Foley and Lynne, 2008).

## 2.5. Global of non-typhoidal *Salmonella* Species

Non-typhoidal *Salmonella* (NTS) species is everywhere in every corner of the world with varying level of prevalence and serotype distribution. Globally, NTS species are a leading bacterial cause of acute gastroenteritis causing estimated 93.8 million cases of gastroenteritis and 155,000 deaths annually (Majowicz *et al*., 2010). The NTS causes most illnesses among the major bacterial enteric pathogens in young children and was shown to be the top among the seven leading foodborne pathogens in USA in causing disability adjusted life year (Scallan *et al*., 2015). A five-year surveillance in China involving 5 viral, 8 bacterial and 3 protozoan pathogenic etiologies of diarrhea in under five children involving 213participating hospitals have shown that NTS accounted for 4.3% of the cases. NTS was the 5$^{th}$ pathogen preceded by rotavirus (29.7%), norovirus (11.8%), diarrhogenic *E.coli* (5%) and adenovirus (4.8) (Li *et al*., 2014).

In general, the epidemiology of NTS is characterized by the temporal dominance of certain successful clones followed by a decline and replacement with another clone (Lan *et al*., 2009). Different serotypes have been reported to be dominant in different countries at different times. For example, the dominant serotypes in *Shangai*, China were S.Enteritidis followed by S. Typhimurium during 2010 and 2011 in children with acute gastroenteritis (Li *et al*., 2014).

## 2.6. Epidemiology of non-typhoidal *Salmonella* species in Africa and Ethiopia

In sub-Saharan Africa, *S. typhimurium* is the most widespread serotype and it is highly invasive (Okoro *et al*., 2012). Study in the Congo indicated that 79% of the salmonella serotypes causing invasive NTS recovered from blood of young children were *S. typhimurium* (Lunguya *et al*., 2013). Predominant NTS reported from Ethiopia are S. Concord and. S. Typhimurium serotypes isolated from patients with diarrheal illness (Beyene *et al*.,2011; Asrat, 2008). Prevalence of *Salmonella* was 11.5% among diarrheic patients in Harrar hospital, east Ethiopia (Reda *et al*., 2011), 10.5% among diarrheic patients in Butajira health center, west Ethiopia (Mengistu *et al*., 2014). In study conducted in pediatric diarrheic outpatients in Addis Ababa and Jimma (west Ethiopia), prevalence of 5.3% was reported (Beyene *et al*., 2011) and 4.5% among diarrheic adult outpatients from various hospitals and clinics. The major problem in ascertaining the real situation of *Salmonella* in a country and especially in developing countries like Ethiopia is the lack of coordinated surveillance. Even where there is a laboratory-based surveillance system, it provides only trend information underestimating disease burden (Flint *et al*.,

2005).*Salmonella* isolates in Ethiopia may have similar phenotypic and genotypic characteristics with isolates elsewhere in the world and non-typhoidal *Salmonella* enterica infection in children in Ethiopia is a major health problem and is caused by similar serovars to these reported from elsewhere in Africa: S. Typhimurium and S. Enteritidis (Getenet, 2008).

### 2.7. Impacts Non-typhoidal *Salmonella*

#### 2.7.1. Economical importance

Historically, *Salmonella Typhimurium* was the most common agent of foodborne disease in humans. However, in the past decades, *Salmonella Enteritidis* has been most regularly involved in salmonellosis outbreaks (Berchieri and Freitas, 2009). It causes millions of dollars in losses to the industry, mainly in cattle, swine and poultry production, both in local and international trade. Salmonellosis represents a significant cost to society in many countries. Very few countries though report data on economic cost of the disease. Estimated costs of medical expenses, sick leaves and loss of productivity related to the high incidence of salmonellosis in the US range from US$1.3 to US$4.0 billion a year (Taitt *et al*., 2004). In Denmark, the annual estimated cost of food borne salmonellosis was US$ 15.5 million in 2001, representing approximately 0.01% of the country's Gross Domestic Products (GDP) (WHO, 2005).

#### 2.7.2. Public health impact

Salmonellosis is an important global public health problem causing substantial morbidity. 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 (Antunes et al., 2003). Infections are also acquired via direct or indirect animal contact in homes, zoological gardens, veterinary clinics, farm environments or other public, professional or private settings (Hoelzer *et al*., 2011). Food-borne diseases have been estimated to infect up to 76 million people in the united state annually. *Salmonella* is overall the most common food borne pathogen in the United States, in some other states *Campylobacter* is more prevalent than *Salmonella* (Anonymous, 2009). Of the non-typhoidal Salmonellosis, S. Enteritidis and S. Typhimurium are common in Ethiopia (Tadesse and Tessema, 2014).

## 2.8. Clinical signs of Salmonellosis

The NTS serotypes can cause protean manifestations in humans, including acute bacteremia, gastroenteritis and extra intestinal localized infections involving many organs (Chiu *et al*., 2004). The clinical course of human salmonellosis is usually characterized by acute onset of fever, abdominal pain, nausea and diarrhea and sometimes vomiting. In some cases, particularly in the very young and in the elderly, the associated dehydration can become severe and life-threatening. In such cases, and also in cases where *Salmonella* causes bloodstream infection, effective antimicrobials are essential drugs for treatment. Serious complications occur in a small proportion of cases (WHO, 2005). Animal salmonellosis is characterized clinically by septicemia, acute enteritis or chronic enteritis. However, the clinically normal carrier animal is a serious problem in all host species. Cardiovascular collapse and shock can occur, as can systemic infection, generally in pediatric or immune compromised patients (Marks and Kather, 2003). The acute phase of the infection may last from four to ten days. Chronic intermittent diarrhea for three or four weeks may be the sequel (Sanchez *et al*., 2011).

## 2.9. Isolation and identification of *Salmonella*

### 2.9.1. Culture method

Swati et al. (2015)stated that the standard protocol described in the Bacteriological Analytical Manual (BAM), U.S. Food and Drug Administration (USFDA), the method of Andrews and Hammack (2001), was adopted for the isolation of *Salmonella* species from meat samples. Briefly, 25 g of each type of sample was thoroughly triturated with a sterile mortar and pestle and transferred to 225 ml pre enrichment in lactose broth. Subsequently 0.1 and 1 ml of pre-enriched sample was transferred to enrichment in Rappaport-Vassiliadis Soybean Meal (RVSM) broth and Tetrathionate Broth (TTB), respectively, followed by 24h of incubation at 42 and 37$^{o}$C, respectively. The enrichments were streaked on Brilliant Green (BG) agar and xylose lysine deoxycholate (XLD) agar and incubated for 24 h at 37$^{o}$ C. Typical colonies on XLD were showed pink colonies with or without black centers, while colonies on the BGA were exhibits colorless or pink or opaque-white colonies often surrounded by pink or red zone. The pure cultures were streaked on Tryptone Soya agar (TSA) and incubated at 37$^{o}$ C for 18 h. Then, those producing typical reaction on TSI (red slant and yellow butt with H2S production-blackening of agar)

were further characterized by biochemical tests such as catalase, oxidase, production of Indole, methyl red test, Voges Proskauer test, utilization of citrate and urease test. The colonies identified as *Salmonella* were preserved in 20 percent glycerol broth at -20$^{o}$ C for further characterization. Muktaruzzaman et al. (2010) mentioned that *Salmonella* organisms exhibited various cultural characteristics in different cultural media. These were turbidity in Tetra Thionate broth, pink white color colonies in Brilliant Green agar, gray white colony in Nutrient agar, slightly grayish color colonies in *Salmonella Shigella* agar, black color colony in Tripple Sugar Iron agar, pale color colonies in MaConkey's agar, well defined glistening colonies in Blood agar.

### 2.9.2. Molecular methods

Camila et al. (2010) was done on the PCR-based identification and characterization of *Salmonella enterica* serovars *enteritidis* and *typhimurium* by using specific forward and reverse primers, *SdfI* - SE F 5′- TGT GTT TTA TCT GAT GCA AGA GG-3′ and SE R 5′- TGA ACT ACG TTC GTT CTT CTG G-3′ of Salmonella Enteritidis with the size of 304 (Yang *et al*., 2014). Other primers for Salmonella Typhimurium was Spy- typh F 5′ TTG TTC ACT TTT TAC CCC TGA A and typh R 5′ CCC TGA CAG CCG TTA GAT ATT with the of 401 used by Kumar et al. (2006) and Alvarez et al. (2004) for molecular detection of *Salmonella*. A 304 bp fragment of the SdfI gene, chromosomal region related to invasiveness and infection of poultry and eggs was selected for serotype enteritidis. For *S. Typhimurium* serotype, a 401 bp Spy gene fragment was selected, which encodes a specific per plasmatic protein. The reaction were standardized in thin walled PCR tubes in 25 µl reaction volumes (mastermix) with different concentrations of reactants under different annealing temperatures and cycling conditions. Finally, the reaction mixture was optimized to contain 12.5 µl 2X PCR master mixes, 10 pmol of each forward and reverse primer, 7.5 µl nuclease free distilled water and 3 µl of DNA template. Reaction conditions will employs: initial denaturation at 94$^{o}$ C for 5 minutes, followed by 35 cycles of 94$^{o}$C for 1 minute, 58$^{o}$ C for 1.5 minutes, and 72$^{o}$ C for 1.5 minutes.

### 2.10. Antibiotic susceptibility tests

There are three antibiotic susceptibility tests methods which are disk diffusion, broth dilution and agar dilution (CLSI, 2008). The extensive use of antimicrobials in human and animal has created selection pressure and promoted the emergence and spread of antimicrobial resistant pathogens worldwide. Resistant organisms and the genes responsible for the development of resistance can easily circulate among humans, animals, food, water and the environment in a given country and globally through trade, travel and migration at an unprecedented pace (WHO, 2014).The isolates were tested for their susceptibility to antimicrobial agents, which are currently used in animals and human therapy. Accordingly the majorities of antibiotic susceptibility tests were performed as per the method described by Clinical and Laboratory Standards Institute (CLSI, 2015) to find out the phenotypic antibiotic resistance pattern. Briefly, biochemically confirmed isolates including *Salmonella* were grown in Muller-Hinton broth for 6 hr and Muller Hinton agar plates were seeded with the cultures. Different antibiotic disks impregnated with antimicrobial agents like ampicillin, ceftraxene, ciprofloxacin, gentamycin, tetracycline and azethromycin and otherwere placed on the inoculated medium. The antibiotic sensitivity plates were incubated at $37^{o}$C for 24 h. The diameter of inhibition zones were measured and zones were graded as sensitive, intermediate and resistant (CLIS, 2015).

# 3. MATERIALS AND METHODS

## 3.1 Study area

The study was conducted in Adama city located at about 99km South East of the Ethiopian capital city, Addis Ababa (National Meteorological Service Agency, 2006). The city is located in the Rift valley, $08^{0}32'29"$N $39^{0}16'08"$E at an elevation from 1712 meters above sea level. Its annual rainfall ranges from 400-800 mm and annual mean temperature is 22ºC (Mengistu *et al*. 2009). In Adama there is cattle market which is called Rob Gebeya (Wednesday market). On this day thousands of cattle come to the market from different directions to Adama.

## 3.2. Sample collection and sample size

By using simple random sampling techniques, a total of 48 meat samples were collected from 16 different butcher houses. The carcass samples were collected in five non-consecutive sampling days in Adama by using the sampling procedure done by Gebeyehu *et al*. (2013) to determine sample size. Briefly, 48 fresh samples of animal origin from different portion of the carcass raw meat, liver and stomach tissue samples were collected aseptically in separate sterile plastic bags in the morning hours as they are offering for sale to the public at different retail meat shops located in Adama city, and transported to the Research Laboratory of the National Veterinary Institute in Bishoftu. Collected samples were put in sterile plastic bags identified by date of collection and sample type and kept in an ice box containing ice packs until the samples reaches to laboratory. Upon arrival at the laboratory, the samples were stored at 4°C in the refrigerator for microbiological analysis within 24 h of sampling.

## 3.3. Culture and biochemical identification

### 3.3.1 Bacterial enrichment and isolation

Isolation of *Salmonella* species was carried out according to the techniques recommended by the International Organization for Standardization (IOS 6579, 2002). Briefly, 25 g each of raw meat, liver and stomach tissue samples were weighed on electronic beam balance. The samples were minced and transferred in to 225 mL of per-enrichments media which is buffered peptone water (BPW), agitated for 2 min and incubated at 35ºC for 18-24 h. One mL of pre-enriched samples was inoculated in to 10 mL selective enrichment media which is Tetrathionate broth then, incubated at

37ºC for 18-24 h. After incubation, broth culture was streaked onto xylose lysine deoxycholate (XLD) agar plated, and then incubated at 37ºC for 18-24 h. The XLD plates were examined for the presence of *Salmonella* colonies. For those plates without typical colonies or slight growth of *Salmonella*, the plates were incubated again for a further of 18 to 24 hrs and reexamined for the presence of typical *Salmonella* colonies. Clear to pinkish red colonies with black center on XLD was considered presumptive *S. Salmonella* colonies. Finally the expected *Salmonella* was streaked on tryptone soya Iron (TSI) agar for gram's staining, biochemical confirmation and molecular conformation.

### 3.3.2. Gram stain and biochemical confirmation of isolates

Gram staining examination was used in the microscopic identification of bacterial contaminants. The reagents used were crystal violet solution, safranine (biological stain), grams iodine and absolute ethanol. A clean microscopic slide was labeled and one loopful of unknown bacteria culture was applied on the slide using an inoculating loop. Smeared on separate glass slide with a drop of distilled water and fixed by gentle heating. Crystal violate was then applied on each smear to stain for one minutes followed by washing with running water. Few drops of Gram's Iodine was then added, which acted as mordant for one minute and then washed with running water. Acetone alcohol was then added for few seconds. After washing with water, safranin was added as counter stain and allowed to stain for two minutes. The slides were then washed with water, dried in air and then examined under microscope with high power objective (100X) using immersion oil. Then, those with pink colored and short rods suspected to be *Salmonella* colony.

Each suspected colonies with typical *Salmonella* morphology were picked from the tryptone soya agar (TSA)media were tested using triple sugar iron (TSI) agar, methyl red-Voges-Proskauer (MR-VP) broth, Indole test, Simmons citrate agar, oxidase and catalase through incubating at 37ºC for 18–48 hours. Interpretationwas made according to the International Organization for Standardization guidelines (2002). Isolates which produced alkaline (red) slants and acid (yellow) butts, with production of $H_2S$ (blackening of agar) in TSI were positive for nontyphoidal *Salmonella* (NTS). NTS is positive for Methyl red, Simmons's citrate, catalase and whereas, negative to oxidase, Indole and Voges-Proskauer.

## 3.4. Molecular detection of *Salmonella* serovars

### 3.4.1. DNA extraction

Extraction of the bacterial DNA was conducted using the DNeasy Blood and Tissue kit extraction kit (Qiagen, Germany) following the manufacturer's instructions (www. qIagen.com/handbooks). Briefly, presumptive S. Enterica was streaked on tryptone soya agar overnight at 35°C. After incubation colonies were harvested and suspended in 200 µL of Phosphate buffered saline and centrifuge for 5 minute at 300xg (190rpm) then, 20 µL of proteinase K was added. 200 µl Buffer AL was added and mixed thoroughly by vortex and incubated at 56°C for 10 min. After incubation, 200 µl ethanol (96 -100%) was added and vortexes. The mixture was pipette to a DNeasy Mini spin column and placed in a 2mL of collection tube then, centrifuge at ≥ 6000xg (8000rpm) for 10 minute. After discarding the flow through the spin column was placed in a new 2 mL collection tube; where 500 µl Buffer AW1 and AW2 were added with different time and gravity. The spin column was transferred into 1.5 mL of micro centrifuge tube and 200 µL of buffer AE (elute buffer) was added to the center of the spin column membrane, incubated for 1 minute at room temperature (15-25°C) and centrifuged for 1 minute at ≥ 6000g with 8000 rpm. The eluted DNA was used as the template DNA for PCR.

### 3.4.2. PCR-based detection of *Salmonella* serovars

PCR based identification of *Salmonella enterica* serovars *enteritidis* and *typhimurium* was done by using gene specific forward and reverse primers as shown in Table 2.

**Table 2**: Primers used in PCR for identification of S. Enteritidis and S. Typhimurium Yang *et al*. (2014) and Kumar *et al*. (2006).

| **Primers Name** | **Forward and reverse primers sequence (5'-3')** | ***Salmonella* serovars** | **PCR product size (bp)** |
|---|---|---|---|
| *SdfI* | SE F 5′- TGT GTT TTA TCT GATS. GCA AGA GG 3'<br>SE R 5′- TGA ACT ACG TTC GTT CTT CTG G 3' | Enteritidis | 304 |
| Spy | Typh F 5′ -TTG TTC ACT TTT TAC CCC TGA A 3'<br>Typh R 5′ -CCC TGA CAG CCG TTA GAT ATT 3' | S. Typhimurium | 401 |

The reaction was standardized in thin walled PCR tubes in 25 µl reaction volumes (master mix) with different concentrations of reactants under different annealing temperatures and cycling conditions. Finally, the reaction mixture was optimized to contain 10 µl IQ supermix, 2 µl of each forward and reverse primer, 5 µl nuclease free distilled water and 6 µl of DNA template. The following reaction conditions was employed: initial denaturation at 94$^{o}$C for 5 minutes in 1 cycle, followed by 35 cycles a three-step process of 94$^{o}$C for 1 minute, at a specific annealing temperature of 55$^{o}$C for 1minutes, and 72$^{o}$C for 1.3minutes of extension time, final elongation at 72$^{o}$C for 7 minutes in 1 cycle (Qiagen protocol). The PCR products were checked by electrophoresis on a 2% agarose gel in 1×TAE (Tris- acetate EDTA) buffer for 120 minute at 120 V. The gel was stained with GelRed (Biotium) and the gel image was taken using the gel documentation system (SynGene, Gene Genius Bio Imaging System, UK).

### 3.5. Antimicrobial susceptibility test

*Salmonella* isolates were tested for susceptibility to 6 common generic antibiotics based on the availability and common usage. Those generic antibiotics were selected based on their availability on the market and easily affordable to peoples. These antibiotics are easily affordable with low cost rather than brand name antibiotics, therefore they are commonly used and they may cause for development of antibiotics resistance bacteria (OXOID, England). Ampicillin (10$\mu$g), Ceftriaxone (30$\mu$g), Gentamycin (10$\mu$g), Azithromycin (15 $\mu$g), Ciprofloxaicin (5$\mu$g) and Tetracycline (30$\mu$g) were used for the disk diffusion method according to guidelines set by the Clinical Laboratory Standards Institute (CLSI, 2012).

Susceptibility of the isolates to antimicrobials was tested using the disk diffusion method. The antibiotics disk was prepared using C1V1=C2V2 formula based on antimicrobial disk potency (Vineetha *et al*., 2015). The presumptive S. Enterica isolates were sub cultured on tryptone soya agar (Becton, Dickinson and Company, USA) from which 3 to 4 pure colonies were inoculated to a tube containing 5 ml of tryptone soya agar (TSA) (Becton, Dickinson and Company, USA) and mixed by vortex. Then it was incubated at 37°C for 4-5 hours. The turbidity of the suspension was adjusted to the optical density of a McFarland unit of 0.5 using sterile saline to standardize the inoculums size. It was inoculated to Mueller Hinton Agar plate (Oxoid, Ltd) by sterile streaking the swab over the entire surface of the plate at room temperature to dry for 5-10 minutes. Antimicrobial discs were placed by pressing on the plate with sterile forceps and the plates were inverted and incubated at 37°C overnight after which diameters of the zone of inhibition was measured to the nearest millimeter using a plastic ruler in Table 3.

**Table 3:** Inhibition zone diameter breakpoint's for Enterobacteriaceae according to clinical and laboratory standard institute (CLSI, 2017).

| Name of Antibiotics | (Sensitive Cm) | Intermediate (Cm) | Resistant (Cm) |
|---|---|---|---|
| Ampicillin 10 $\mu$g | ≥17 | 14-16 | ≤13 |
| Azethromycin 15 $\mu$g | ≥ 13 | 12-13 | ≤12 |
| Tetracycline 30 $\mu$g | ≥15 | 12-14 | ≤11 |
| Ciprofloxacin 5 $\mu$g | ≥21 | 16-20 | ≤15 |
| Ceftraxene 30 $\mu$g | ≥23 | 20-22 | ≤19 |
| Gentamycin 10 $\mu$g | ≥15 | 13-14 | ≤12 |

## 4. DATA ANALYSIS

Presence of S. Enterica serovars in collected samples were indicated the contamination of the end products. The total contaminated end products in Butcher houses were compared and the values were expressed in percentages, tabulation and graph. The data of bacterial contamination collected from 16 butcher house was analyzed using Microsoft Excel 2013.

# 5. RESULTS

## 5.1. Occurrence of *salmonella* and antimicrobial susceptibility

A total of 48 samples were collected during the study period. Out of the total 48 samples such as liver, stomach tissue and raw meat, 7 (14.58%) were positive for *Salmonella enterica* serovars *enteritidis* and *typhimurium*. From the isolated *Salmonella* species 5 were Salmonella Typhimurium while the remaining two were Salmonella Enteritidis were all isolates identified from raw meat and stomach but *Salmonella* serovars was not isolated from liver samples. Antibiotics sensitivity test for isolated *Salmonella* serovars shows highest level of resistance for Azethromycin (100%). For ampicillin, intermediate 3 (42.86%) and sensitive 4 (57.14%), whereas tetracycline was observed resistance 5 (71.43%), sensitive 1 (14.3%) and intermediate 1 (14.3%). The remaining three antibiotics were 100% effective for isolated *Salmonella* species.

## 5.2. *Salmonella* colonial growth on elective Xylose lysine deoxycholate and Tryptone soya agar media

The nature of the growth of the recovered bacterial isolates on Xylose lysine deoxycholate agar (XLD) was pink or red colonies with black center (Figure 2).Seven of the isolates were suspected *Salmonella* species since they were non-lactose fermenters and the colonies characteristics were appeared pink or red colonies with black center due to production of $H_2S$.XLD was used as selective media for *Salmonella*, due to its sodium deoxycholate which can inhibit other grams positive bacterial growth (Figure 2).

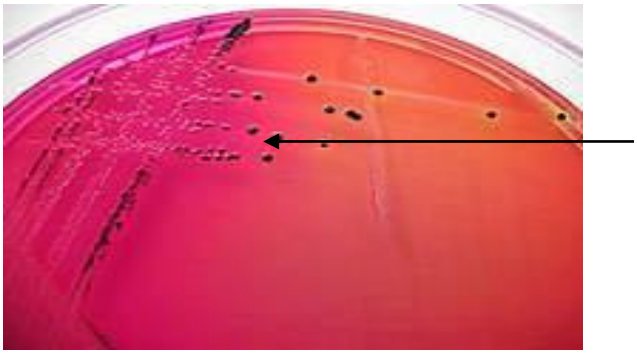



**Figure 2:** The growth of *Salmonella* species on XLD form pink or red colonies with black center.

Typical colonies on xylose lysine deoxycholate (XLD) (pink colonies with black centers) were picked and streaked further on Tryptone soya agar (TSA) for purification suspected *Salmonella* (Figure 3). The pure cultures were streaked on Tryptone soya agar and incubated at 37$^{o}$C for 18 to 24h. The TSA haven't any inhibitory chemicals, therefore any bacteria can row on it. Then, the pure culture was subject for grams staining, biochemical and molecular identification (Figure 3). In this study salmonella were grams positive bacteria which showed pink colonies after addition of Safranine.

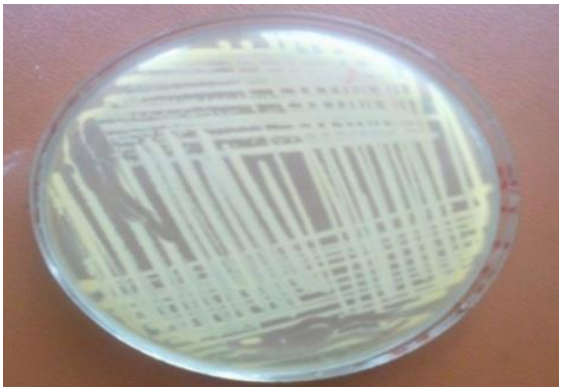


**Figure 3**: Pure isolated *Salmonella* growth on Tryptone soya agar plates.

### 5.3. Biochemical test of isolated *Salmonella*

Biochemical tests result of catalase test, oxidase test, Indole production, Simmons citrate test, triple sugar iron test, Voges Proskauer test and methyl red for *Salmonella* serovars according to the procedure described by Tokuyasu et al. (2012), those shows in (Table 4).Totally 7 Salmonella Enteritidis and Salmonella Typhimurium were isolated from raw meat and stomach tissue (Figure 4).

**Table 4:** Biochemical test results of presumptive Salmonella Enteritidis and Salmonella Typhimurium.

| Sample | Growth characteristics | | | | Grams stain | Indole Test | MR | VP | Simmon's Citrate | Oxidase | Catalase |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | XLD | TSI | | | | | | | | | |
| | | Butt | Slant | $H_2S$ | | | | | | | |
| ASTU1 | Pink with block center | yellow | Red | +ve | -ve | -ve | +ve | -ve | +ve | -ve | +ve |
| ASTU2 | " | Yellow | Red | +ve | -ve | -ve | +ve | -ve | +ve | -ve | +ve |
| ASTU3 | " | Yellow | Red | +ve | -ve | -ve | +ve | -ve | +ve | -ve | +ve |
| ASTU4 | " | Yellow | Red | +ve | -ve | -ve | +ve | -ve | +ve | -ve | +ve |
| ASTU5 | " | Yellow | Red | +ve | -ve | -ve | +ve | -ve | +ve | -ve | +ve |
| ASTU6 | " | Yellow | Red | +ve | -ve | -ve | +ve | -ve | +ve | -ve | +ve |
| ASTU7 | " | Yellow | Red | +ve | -ve | -ve | +ve | -ve | +ve | -ve | +ve |

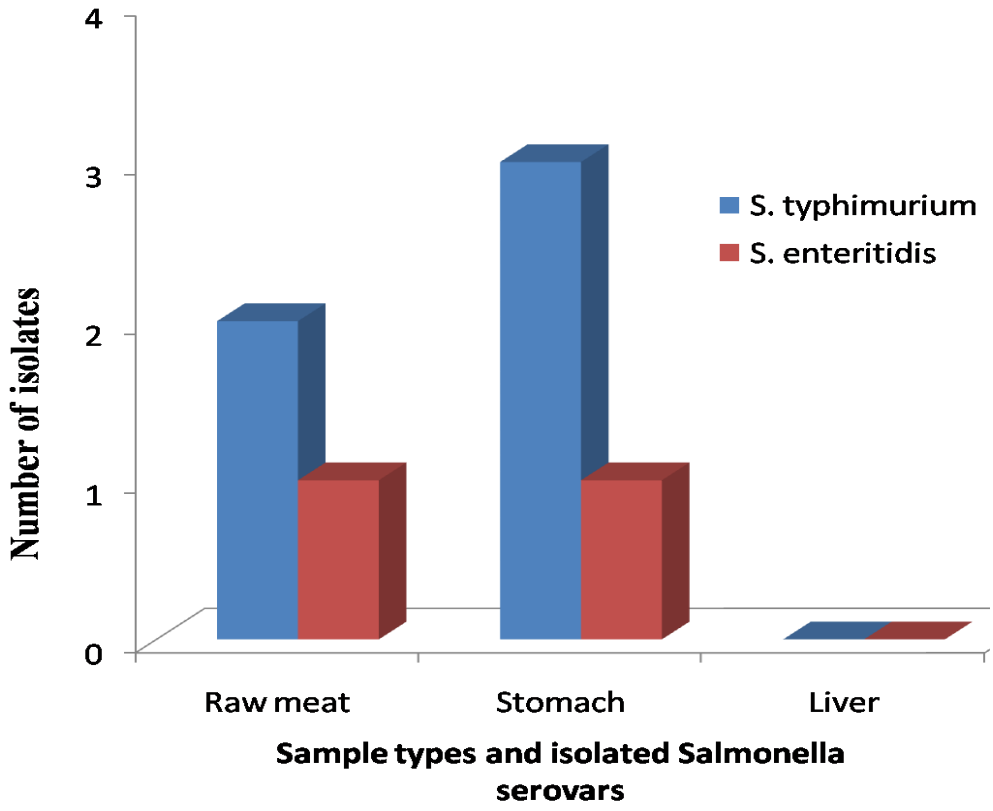



**Figure 4:** Number of isolated S. Typhimurium and S. Enteritidis from different samples

## 6. PCR based molecular detection of Salmonella enterica serovars enteritidis and typhimurium

In the present study all the 7 isolates of isolated *Salmonella* serovars from 48 butcher house samples such as raw meat, stomach and liver were subject to PCR assays for the detection of specific gene. All the 7 amplified products of detected *Salmonella* 304 bp and 401 bp, which were similar to that of reference strain of *Salmonella* using the primer pairs for *SdfI* and *spy* respectively.

The amplified PCR products were loaded on 2% agarose gel, each band represents the extracted DNA from seven bacterial samples which are 2 (28.57%) were *sdfI* of *Salmonella enteritidis* and 5(71.42%) were *Salmonella typhimurium.* Gel electrophoresis of PCR amplified products had seven bands for *salmonella enteritidis and typhimurium*. Bands on lane one and five were for *S. enteritidis* with 304 bp size and bands on lane two, three, four, six and seven were for *S. typhimurium* as shown in figure 5 and 6.

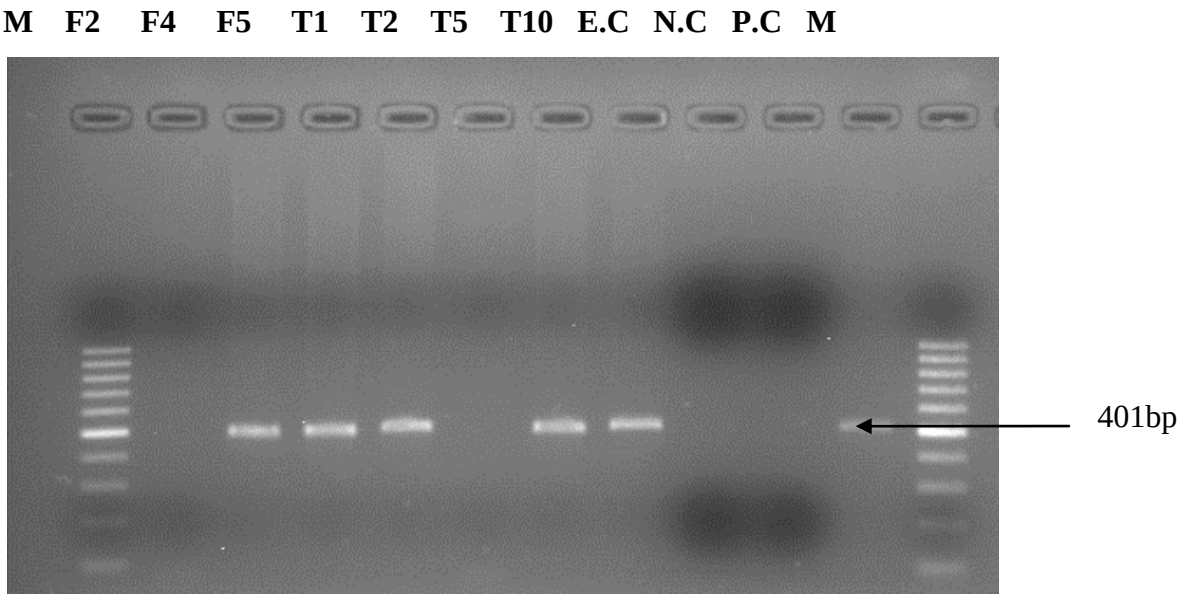



**Figure 5:** Gel electrophoresis in 2% Agarose gel for PCR amplified product of *Salmonella* Typhimurium spy gene (401bp) specific PCR products indicated by arrow.

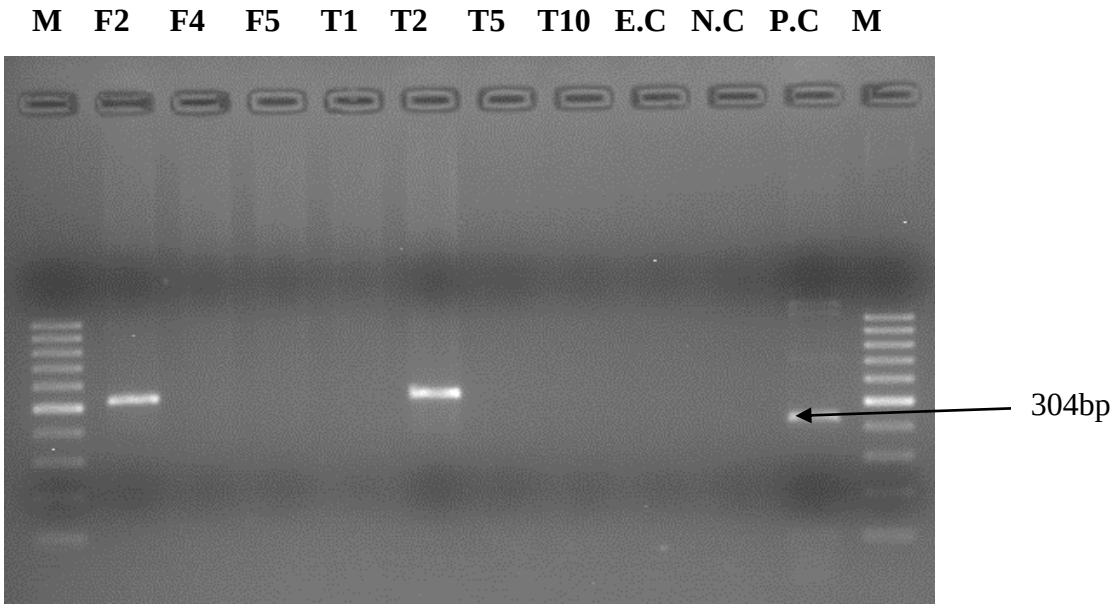



**Figure 6**: Gel electrophoresis using 2% Agarose of PCR amplified product of *S. enterica* serovars *enteritidis* by using *sdfI* primers.

**7. Antibiotic sensitivity test**

*In vitro* sensitivity testing of the *Salmonella* serovars for six antibiotics revealed a variety of result. Five isolate were resistant for tetracycline and all 7 isolates were resistance for Azethromycin, those were isolated from stomach tissue and raw meat sample. Three of the isolates were intermediate resistant for ampicillin that isolated from raw meat and stomach tissue and other four were susceptible for ampicillin, which isolate from raw meat and stomach tissue. The two Salmonella Typhimurium isolate from raw meat and stomach tissue were observed for both intermediate and sensitive result. All isolates from raw meat and stomach tissue were100%susceptible for Ciprofloxacin, Ceftraxene and Gentamycin (Table 6).

**Table 5:** Antibiotics susceptibility test result for isolated *Salmonella* serovars from raw meat and stomach tissue.

| Isolates | antimicrobials | Number of R. Isolates | Number of I. Isolates | Number S. Isolates |
|---|---|---|---|---|
| **S. Enteritidis** | Ampicillin 10µg | 0% | 1(14.3%) | 1 (14.3%) |
| | Tetracycline30µg | 1(14.3%) | 0% | 0% |
| | Azethromycin15µg | 2(28.6%) | 0% | 0% |
| | Ciprofloxacin 5 µg | 0% | 0% | 2 (28.6%) |
| | Ceftraxene 30 µg | 0% | 0% | 2 (28.6%) |
| | Gentamycin 10 µg | 0% | 0% | 2 (28.6%) |
| **S.Typhimurium** | Ampicillin 10 µg | *0%* | 2 (28.6%) | 3 (42.86%) |
| | Tetracycline 30 µg | 4(57.2%) | 1 (14.3%) | 1 (14.3%) |
| | Azethromycin15µg | 5(71.43%) | 0% | 0% |
| | Ciprofloxacin 5 µg | 0% | 0% | 5(71.43%) |
| | Ceftraxene 30 µg | 0% | 0% | 5 (71.43%) |
| | Gentamycin 10 µg | 0% | 0% | 5 (71.43%) |

Notes: R.M - Stands for raw meat

S.T - Stomach tissue

R – Resistant, I - Intermediate and S – Sensitive

# 8. DISCUSSION

Contaminated foods of animal origin are the primary source for transmitting non-typhoidal *Salmonella* to humans. *Salmonella* induced food borne illness needs more attention like other food borne pathogens. Contamination with *Salmonella* species is special public health significance intended for a country like Ethiopia, due to consumption of raw and undercooked meat product is common in most areas of Ethiopia (Mebrat *et al*. 2016).

In this study, out of 48 collected meat samples 7 (14.58%) samples were found positive for *Salmonella enterica* subspecies *enterica* serovars *enteritidis* and *typhimurium*. Two (28.57%) Salmonella Enteritidis and five (71.42%) Salmonella Typhimurium isolates were recovered from 48 of the samples. Gebeyehu et al. (2013) was studied on the evaluation of microbial load of meat in Adama but, he couldn't isolate *Salmonella* species from meat sample. But the result of this study showed contrast with the previous study of Gebeyehu et al. (2013). To identify *Salmonella* species, the use of molecular techniques is the basic methods for the identification of *Salmonella* serovars based on their specific gene. Salmonella Enterica is extremely diverse, isolated during outbreaks of foodborne salmonellosis whereS. Enteritidis, S. Typhimurium, S. Virchowand S. Infantis (Jones *et al*., 2008) were frequent. Salmonella Typhimurium and S. Enteritidis are highly predominant detected *Salmonella* serovars associated with the consumption of contaminated beef products. Contamination with *Salmonella* in beef products can happen at different steps along the food chain, including production, processing, distribution, retail marketing, handling, and preparation (Dookeran *et al*., 2012).

The result of the present study agrees with the findings of Jones et al. (2008) and Dookeran et al. (2012) on isolating of the most common *Salmonella* serovars which are S. Enteritidis and S. Typhimurium. In this study S. Typhimurium were recovered from five (71.42%) of the total isolates, where three were detected from raw meat sample and two from stomach. The remaining two were isolated from raw meat and stomach sample. *Salmonella* serovars were not detected from liver sample which agrees with the null *Salmonella* in liver (Swati *et al*., 2015). In present investigation S. Typhimurium were dominant isolate serovars from Adama butcher houses which is in line with the report of Reda et al. (2016) from Egypt, Tadesse (2016) from central part of Ethiopia and Kariuki et al. (2006) from Kenya.

In the present investigation all the 7 isolates of *Salmonella* serovars from 48 butcher house samples were subjected to PCR assays for the detection of a 304 bp segment of the *SdfI* gene. Which is chromosomal region related to invasiveness and infection of poultry (ENTF and ENTR primers) for Salmonella Enteritidis. For Salmonella Typhimurium serovars 401 bp *Spy* gene segment was selected, which is encodes a specific periplasmatic protein (TyphF and TyphR primers) (Camila *et al*., 2010). The 7 isolates of *Salmonella* serovars yielded desired amplified products of 401 bp and 304 bp were related to that of reference strain of *Salmonella* primer pairs for *SdfI* and *Spy* target gene respectively. Among the total isolates of *Salmonella* serovars 5 (71.42%) were positive for the *Spy* and 2 (28.57%) were positive for *SdfI*. Conventional methods for detection of *Salmonella* serovars is take a time and laborious, whereas molecular methods like PCR is not time consuming and testing a number of primers with a large number of serotypes, therefore it is sensitive and successful for the identification of *Salmonella* serovars.

In the present study, the antibiotic resistance result of the *Salmonella* serovars indicated that, 100 percent of *Salmonella* isolates were resistant to Azethromycin and 71.4% of isolated *Salmonella* serovars were resistance for tetracycline. From the total 28.57% of isolates were resistances for antibiotics, which is almost comparable with report Garedew et al.(2015). However, in this study observed resistance profile was high than what other studies reported in Ethiopia (23.5%) (Zewdu and Cornelius, 2009). The use of antimicrobial agents in food animals at sub therapeutic level or prophylactic doses which may encourage antimicrobial resistant strains and patently increase the human health risks associated with consumption of contaminated meat products (Zelalem et al. 2011) reported that the isolates of *Salmonella* from food items and workers from Addis Ababa were resistant to the commonly used antibiotics including streptomycin, ampicillin, and tetracycline.

Those antimicrobial resistances have the potential to affect human health by causing illness that is more difficult to treat due to their resistance profile of the microorganism. The higher resistance *Salmonella* isolates to tetracycline might be attributed to high level of utilization of this drug both in human medicines and veterinary medicine due to its moderately cheaper price and readily availability. While, the other antibiotics were such as ciprofloxacin, ceftraxene, and gentamycin were 100% therapeutically active for all 7 isolated *Salmonella* serovars, generally the selected antibiotics were 61.9% sensitive for *Salmonella* and 9.5% were showed intermediate result. In the present studies, all of the *Salmonella* isolates were susceptible to ceftriaxone, gentamycin and

ciprofloxacin which were in close agreement with that of Garedew et al. (2015) and Molla et al. (2006). The effectiveness of ciprofloxacin could be because of that they are not widely used in countries like Ethiopia and other African countries (Zelalem *et al*., 2011).

# 9. CONCLUSION AND RECOMMENDATION

Non- typhoidal *Salmonella* serovars are one of the common food contaminating bacteria which are recognized as major zoonotic pathogens for causing human salmonellosis. During the present study, 7 *Salmonella* serovars were recovered from 16 different butcher houses. This result revealed that the presence of *Salmonella* contamination in butcher houses. The detection and identification of the isolated *Salmonella* serovars was based on morphological, cultural, biochemical and PCR methods. The significant advantages of this universal PCR method are rapid detection and identification with higher sensitivity and specificity result. The two serovars of *Salmonella* specific gene (*Spy* and S*dfI*) were detected by using PCR. The sources of contamination of *Salmonella* may be due to the poor personnel hygiene, low sanitary standard of butcher shops and transportation of meat.

Besides, moderate to higher degree of drug-resistance was exhibited for antimicrobials test which are in frequent use for both human and veterinary medicine in Ethiopia.

Ceftraxene, ciprofloxacin and gentamycin were effective; drugs for treatment of S. Typhimurium therefore those three antimicrobials can be selective for *Salmonella* treatment.

Hence the problem of drug resistance should also be considered through using drug susceptibility tests before treating patients and give strong awareness to peoples about the proper use of antibiotics as prescribe by professionals.

Further molecular techniques have to be done to successfully fight the increasing number of drug resistant and food contaminant bacteria.

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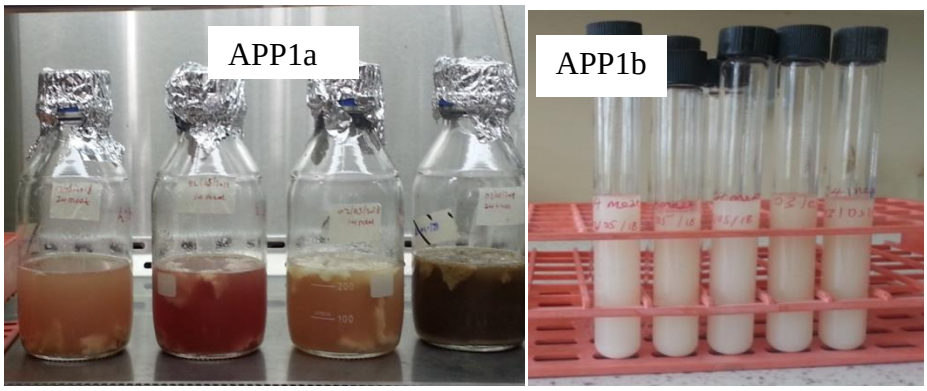
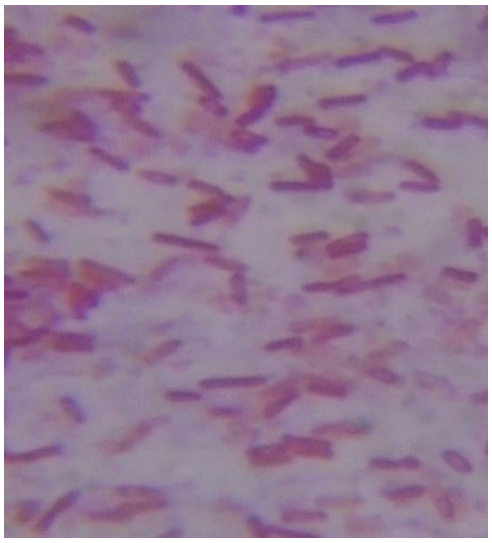
## 11. Appendices

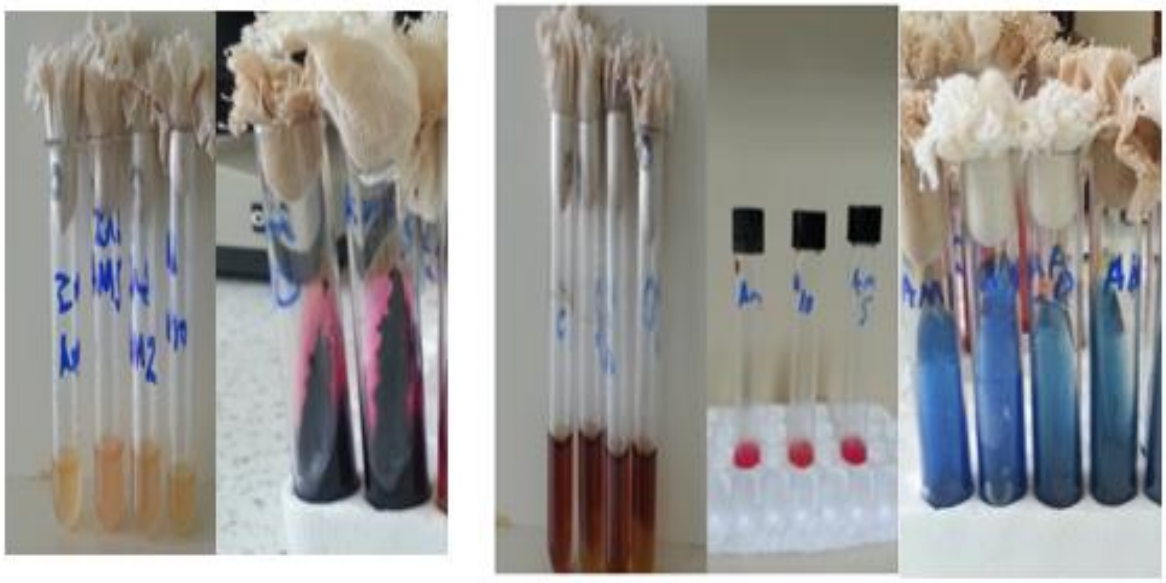



**Appendices 1a and 1b**. Recovery of *Salmonella* species by using buffered peptone water and Tetrathionate broth from raw meat, liver and stomach tissue sample.



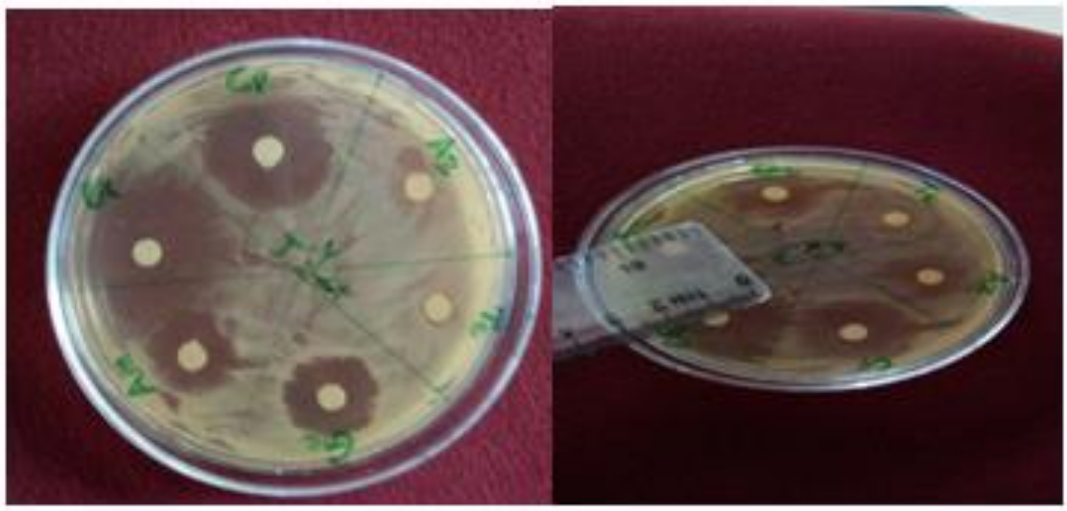
**Appendices 2:** Gram's staining of isolated *Salmonella* under microscopic observation

**A. Indole –ve** **B. TSI** **C. VP -VeD. MR +veE. S.C +ve**



**Appendices 3:** Biochemical tests result for non-typhoidal salmonella.

**(**Note: MB- Methyl blue, SC- Simmons's citrate, VP-Vogues Proskauer and TSI- Triple sugar Iron agar).



**Appendices 4:** Disk diffusion techniques of antibiotics sensitivity test.

**Appendices 5**: Serovars distribution of *Salmonella* isolates in Liver, raw meat and Stomach tissue within sample type.

| Sample type | No. examined | Serovars | No. of isolates |
|---|---|---|---|
| Raw meat | 16 | S. Enteritidis | 1 (14.3%) |
| | | S. Typhimurium | 2 (28.6%) |
| Stomach | 16 | S. Enteritidis | 1 (14.3%) |
| | | S. Typhimurium | 3 (42.9%) |
| Liver | 16 | No isolate | No isolate (0%) |
| **Total** | **48** | **7 (100%)** | |