

**ADDIS ABABA UNIVERSITY**  
**SCHOOL OF GRADUATE STUDIES**  
**MSc THESIS**



**ISOLATION OF SALMONELLA SPECIES AMONG  
APPARENTLY HEALTHY FOODHANDLERS OF ADDIS  
ABABA UNIVERSITY STUDENTS' CAFETERIA, ADDIS  
ABABA, ETHIOPIA**

**BY**

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**June 2011**  
**Addis Ababa, Ethiopia**

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**A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES OF Addis Ababa UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIRMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICAL MICROBIOLOGY**

**June 2011**

**Addis Ababa, Ethiopia**

## **ACKNOWLEDGEMENTS**

First of all, I would like to acknowledge Addis Ababa University, department of Microbiology, Immunology and Parasitology, Faculty of Medicine for its facilitation and Aklilu Lemma Institute of Pathobiology for unreserved material and reagent supply that makes the study possible, and school of graduate studies of the University for Financing and allowing me to carry out my research. My heartfelt gratitude also goes to Jigjiga University for sponsoring of my graduate study and providing me with monthly salary during my stay in graduate school.

My deepest and heartfelt gratitude forwarded to my advisor, Dr. Solomon Gebre-Selassie for his unreserved scientific guidance, comments, encouragement, support and patience starting from date of topic selection till the accomplishment of this research thesis.

My cordial appreciation and heartfelt gratitude also forwarded to my advisor, Dr. Nigatu Kebede for invaluable scientific Guidance, material support and persistent encouragement. Furthermore, I would like also to acknowledge for his politeness, hospitality, friendly approach while I was seeking his support.

My deepest gratitude also extends to Mr. Haile Alemayehu, ALIPB microbiology laboratory personnel, for his cooperation, instructing and training me the laboratory techniques, daily follow up, encouragement and his supportive nature without hesitation.

All friends and ALIPB staffs are greatly acknowledged for their unreserved and friendly support. Special thanks goes to Dr. Legesse Garedew & Mr. Gizachew Andualem for sharing their scientific knowledge unreservedly and giving me a hand in the laboratory work at ALIPB and Mr. Selam Yirga, Semera university, for his scientific comments, support and reviewing of my paper.

My gratitude also extends to student service director, all prospective faculty student deans and cafeteria chairpersons of AAU for their cooperation, support and organizing the preconditions of data and sample collection, and the foodhandlers of AAU not only for their willingness to participate but also for their cooperation, friendly approach and funny jokes. In general, the study would be impossible without them.

Finally, I would like to express my deepest & heartfelt gratitude to God and my family for their help and making me hopeful. My mother, Manaye Ewunetu, I thank her very much for her special, unconditional, deepest and limitless love, moral support and encouragement throughout my life.

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## **ABBREVIATIONS**

AAU:	Addis Ababa University
ASM:	American Society for Microbiology
ALIPB:	Aklilu Lemma Institute of Pathobiology
CDC:	Center for Disease Control and Prevention
CI:	Confidence Interval
DMIP:	Department of Microbiology, Immunology and Parasitology
DNA:	Deoxyribo-Nucleic Acid
LIA:	Lysine Iron Agar
MDR:	Multi Drug Resistant
OD:	Odds Ratio
TMP-SMX:	Trimethoprem sulfamethoxazole
TSI:	Triple Sugar Iron Agar
WHO:	World Health Organization
XLD:	Xylose Lysine Desoxycholate Agar

## ABSTRACT

**Background:** Food is an important vehicle for spread infectious agents causing disease resulting appreciable morbidity and mortality. Food handlers play an important role in ensuring food safety. However, in developing countries like Ethiopia the proportion of certified food handlers and their carrier status is not well studied. Salmonellosis is one of such diseases that can be transmitted from chronic asymptomatic *salmonella* carriers especially the food handlers.

**Objective:** To isolate the *Salmonella* species among food handlers of Addis Ababa university students' cafeteria, Addis Ababa, Ethiopia

**Methods:** A cross sectional study was conducted to isolate *Salmonella* among food handlers of AAU students' cafeteria, from December 2010 to February 2011. A structured questionnaire was used to collect socio-demographic data & predisposing factors. Stool samples collected from 233 food handlers were put in selenite cystine broth for *Salmonella* enrichment, and then cultured on macConkey & XLD for primary culture and purification. The isolate were identified by biochemical tests and drug susceptibility tests were done. All components of data were entered using EPI-INFO 3.5.1 and analyzed using SPSS version 16 computer software. Fisher's exact test was applied and p-value of less than 0.05 was considered as statistically significant.

**Results:** Eight *Salmonella* species were isolated among 233 foodhandlers giving an isolation rate of 3.4%, all were females. Of these; two *S.typhi*, one *S.paratyphi* A and five unidentified *Salmonella* species were isolated. Among the risk factors associated with salmonellosis, hand washing habit after toilet with or without soap had a statistically significant association with isolation of Salmonellae,  $p = 0.003$  (OD= 0.07, 95% CI= 0.008–0.58). The antimicrobial susceptibility profile showed all except one were resistant to Ampicillin and all isolates were resistant at least to one of antimicrobials tested.

**Conclusion:** A 3.4% isolation rate of *Salmonella* species was obtained and all isolates were resistant at least to one of antimicrobials tested. Accordingly, foodhandlers could be a source of salmonellosis unless carriers treated after periodic screening and other preventive measures taken. Antimicrobial resistance profile also reflects it would be a serious problem in near future.

**Key words:** Antimicrobial, Cross sectional study, Foodhandler, Isolate, *Salmonella*

# CHAPTER I: INTRODUCTION

## 1.1 Background

Food borne diseases are a public health problem in developed and developing countries. The World health organization (WHO) stated that, most of the populations suffer from food borne diseases each year, both in developing and developed countries up (WHO, 2007).

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 food borne diseases can be poisonings, caused by harmful toxins or chemicals like poisonous mushrooms and enterotoxins of some bacteria (CDC 2005).

Bacteria that cause food-borne diseases include among others are *Salmonella*, *Campylobacter*, *Listeria*, pathogenic *Escherichia coli*, *Yersinia*, *Shigella*, *Enterobacter* and *Citrobacter*. 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 include toxins from *Staphylococcus aureus*, *Clostridium botulinum* and *Bacillus cereus* (European Union, 2009).

*Salmonella* is a bacterium that is widespread in the intestines of birds, reptiles and mammals and is one of the main causes of foodborne disease in humans. There is also concern about increased antibiotic resistance when treating Salmonellosis in humans. It can spread to humans through a variety of different foods of animal origin. (Todar, 2005).

Salmonellosis is a major cause of bacterial enteric illness in both humans and animals. *Salmonella* are a group of bacteria that can cause diarrheal illness in people. This constitutes a major public health burden and represents a significant cost to society in many countries. One species, *Salmonella enterica* has more than 2,000 serovars with *Salmonella typhimurium* and *Salmonella enteritidis* most commonly encountered globally. *Salmonella* are inhabitants of the feces of many types of animals including poultry, eggs, dairy products and foods prepared on contaminated work surfaces. Therefore, *Salmonella* plays significant role in foodborne disease worldwide (Mead *et al*, 1999; WHO, 2005).

*Salmonella* infections can be typhoidal or non-typhoidal. Serotypes such as *S.typhi*, *S.paratyphi A* and *S.paratyphi B*, causes of typhoidal salmonellosis, are highly adapted to humans and do not cause disease in non-human hosts. The vast majority of *Salmonellae* (e.g., *salmonella choleraesuis* and *salmonella enteritidis*), however, are chiefly pathogenic in animals that constitute the reservoir for human infection: poultry, pigs, rodents, cattle, pets (from turtles to parrots), and many others (European Union, 2009; Boyle *et al*, 2007).

Non-typhoidal salmonellosis is a worldwide disease of humans and animals. Animals are the main reservoir, and the disease is usually food borne, although it can be spread from person to person (Todar, 2005; Brooks *et al*, 2004).

Typhoid fever is a systemic disease characterized by fever and abdominal pain caused by dissemination of *S. typhi* or *S. paratyphi*. The disease was initially called typhoid fever because of its clinical similarity to typhus. In 1869, given the anatomical site of infection, the term enteric fever was proposed as an alternative designation to distinguish typhoid fever from typhus. However, to this day, the two designations are used interchangeably (Todar, 2005).

The *Salmonellae* that cause Typhoid fever and other enteric fevers spread mainly from person-to-person via the fecal-oral route and have no significant animal reservoirs. Asymptomatic human carriers ("typhoid Marys") may spread the disease. Such infections may occur when food or water contaminated by infected food handlers is ingested (Wikipedia, 2010; Murray *et al*, 2003).

## **1.2 Statement of the problem**

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, whereas in developing countries up to 2 million deaths are estimated per year (WHO, 2007).

More than 93 million cases of gastroenteritis due to Nontyphoidal *Salmonella* species occur globally each year, with 155,000 deaths. Of these, the estimated 80.3 million cases were foodborne. *Salmonella* infection represents a considerable burden in both developing and

developed countries. Efforts to reduce transmission of *salmonellae* by food and other routes must be implemented on a global scale (WHO, 2007; Majowicz et al, 2010).

Typhoid fever is a global health problem. Its real impact is difficult to estimate because the clinical picture is confused with those of many other febrile infections. Additionally, the disease is underestimated because there are no bacteriology laboratories in most areas of developing countries. These factors are believed to result in many cases going undiagnosed (WHO, 2003). However, the existing estimate of the global burden of Typhoidal *Salmonella* serovars, such as *Salmonella enterica* serovars *Typhi* and *paratyphi*, is 21 million illnesses and 600 000 deaths annually (Brooks et al, 2004; Crump et al, 2004; WHO, 2003).

The risk of disease is highest in developing countries and remains a significant threat to the health of individuals in developing countries. Although its prevalence varies across regions, diseases caused by *S. enterica* serovars are especially prevalent in developing areas, such as Southeast Asia, Africa, and South America that leads to an estimated 20 million cases and 200,000 deaths each year. Challenges such as antibiotic-resistant *Salmonella* strains also pose a significant threat to the development of reliable therapies (Brooks et al, 2004; WHO, 2003). The emergence of MDR strains has reduced the choice of antibiotics in many areas. There are two categories of drug resistance: resistance to antibiotics such as chloramphenicol, Ampicillin and trimethoprem-sulfamethoxazole (MDR strains) and resistance to the fluoroquinolone drugs. (MacDonald et al, 1987; WHO, 2003).

Typhoid fever (enteric fever) caused by *S. typhi* is an endemic disease in the tropic and sub-tropic areas and has become a major public health problem in developing countries of the world with an estimated annual incidence of 540 per 100,000 (WHO, 2003; WHO, 2008).

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 cannot be tested. As in a developed country, however, to control the

spread of salmonellosis, surveillance for *Salmonella* serovars and the assessment of antimicrobial susceptibility is essential (Beyene *et al*, 2008).

Persistent excretion following both symptomatic and asymptomatic salmonellosis may result in prolonged intestinal carriage. *Salmonella* carriers have caused or perpetuated outbreaks of salmonellosis but documentation of this phenomenon is poor. Although chronic carriage is uncommon, identifying temporary carriers, especially among food handlers and those having contact with infants or hospitalized patients, creates an important management for public health workers (Buchwald *et al*, 1984).

Diseases spread through food still remain a common and persistent problems resulting in appreciable morbidity and occasional mortality, for instance, an outbreak of typhoid fever occurred in Adama University (unpublished). Food handlers play an important role in ensuring food safety throughout the chain of production, processing, storage and preparation. However, in Ethiopia the proportion of certified food handlers and their carrier for salmonellosis is not studied except one study in Bahir Dar (Abera *et al*, 2010). Therefore, this study is designed to isolate the *Salmonella* species from food handlers and to determine the antibiotic sensitivity profile of the isolates among food handlers of AAU students' cafeteria, Addis Ababa, Ethiopia.

### **1.3 Literature Review**

*Salmonella* is a genus of rod-shaped, Gram-negative, non-spore forming, predominantly motile enterobacteria with diameters around 0.7 to 1.5 µm, lengths from 2 to 5 µm, and flagella which project in all directions (i.e. peritrichous). They are chemoorganotrophs, obtaining their energy from oxidation and reduction reactions using organic sources, and are facultative an-aerobes (Ryan *et al*, 2004).

*Salmonella* have a complex antigenic structure. They are classified by heat-stable somatic O (lipopolysaccharide) antigens, heat-labile K (capsular) antigens, and H (flagellar) antigens. In *Salmonella typhi*, the capsular antigens are called Vi antigens (Todar, 2005; Ryan *et al*, 2004).

*Salmonellae* constitute a genus of more than 2500 serotypes that are highly adapted for growth in both humans and animals and that cause a wide spectrum of disease. A new classification for *Salmonella* has been adopted based on DNA relatedness. This new nomenclature recognizes only

two species: *Salmonella bongori* and *Salmonella enterica*, with all human pathogens regarded as serovars within the subspecies of *S. enterica*. For example, the proposed nomenclature would change *S. typhi* to *S. enterica* serovar *Typhi*, abbreviated *S. Typhi*, and *Salmonella enterica* serovar *Enteritidis* would be referred to as *S. Enteritidis* instead of *S. enteritidis* (CDC, 2010).

Salmonellosis is an infection with *Salmonella* bacteria transmitted through feco-oral route. Most people infected with *Salmonella* develop diarrhea, fever, vomiting, and abdominal cramps 8 to 72 hours after infection. In most cases, the illness lasts 4 to 7 days and most people recover without treatment. However, in some persons the diarrhea may be so severe that the patient becomes dangerously dehydrated and the *Salmonella* infection may spread from the intestines to the blood stream, and then to other body sites and can cause death unless treated. Salmonellae produce three main types of disease in humans, but mixed forms are frequent. These are enteric fever (typhoid), septicemia and enterocolitis and rarely chronic carriers may develop gall bladder stone (Boyle *et al*, 2007; Books *et al*, 2004).

Some patients may harbor *Salmonella* species in stool or urine for periods of 1 year or longer but remain asymptomatic. Approximately 3% of patients with typhoid fever and 0.2–0.6% of persons with non-typhoid *Salmonella* gastroenteritis will have positive stool cultures for more than 1 year where the organism present in the gallbladder, biliary tract, or rarely the intestine or urinary tract (Vandepitte *et al*, 2003).

Mary Mallon (1869 –1938), also known as Typhoid Mary, was the first person in the United States to be identified as a healthy carrier of typhoid fever. Over the course of her career as a cook, she is known to have infected 53 people, three of whom died from the disease (Wikipedia, 2010).

*Salmonella enterica* serovar *Typhi* can establish a chronic, asymptomatic infection of the human gallbladder, suggesting that this bacterium utilizes novel mechanisms to mediate enhanced colonization and persistence in a bile-rich environment by forming a biofilm. Bacteria reaching the gallbladder can induce an active local infection (cholecystitis) or exist asymptotically in a chronic carrier state (Crawford *et al*, 2008).

The chronic typhoid carrier state can occur following symptomatic or subclinical infections of *Salmonella typhi*. Among untreated cases, 10% will shed bacteria for three months after initial onset of symptoms and 2-5% will become chronic carriers. The chronic carrier state occurs most

commonly among middle age women (CDC, 2010). Bacteria shed by asymptomatic carriers contaminate food and water and account for much of the person-to-person transmission of serovar Typhi in underdeveloped countries (Vandepitte *et al*, 2003; Crawford *et al*, 2008).

In Namakkal, India, screening of *Salmonella typhi* in asymptomatic typhoid carriers among suspected food handlers reported that among 35 samples, 6(17.14%) yielded a positive result. Out of these 4 (20.0%) were women and 2 (13.33%) were men. Five isolates were having the multidrug resistant character for conventional antibiotics: four (66.66%) multidrug resistant isolates were found to have plasmids, while one (16.66%) multidrug resistant isolate had no plasmid and the chromosome encoded the resistance and only one strain (16.66%) showed single antibiotic resistance in the study and had no plasmid DNA (Senthilkumar *et al*, 2005). This study shows that food handlers can be a source of drug resistant strains.

In Kyushu, Japan, *Salmonella* were isolated from 0.032% of fecal samples from food handlers in Japan to determine the incidence and features of *Salmonella* serovars among food handlers. *S. enterica* subspecies *enterica* serovar *Infantis* (*S. serovar infantis*) was the dominant serovar (accounting for 48.1%), followed by *S.corvallis*, which showed poor genetic diversity, and *S.enteritidis* among food handlers (Murakami *et al*, 2007).

A survey for *Salmonella* carriers in the Chinese army recruits was made. *Salmonella* was detected in 1.83% of the rectal swabs collected from 1,150 recruits and 50 cooks. Among the 22 isolates 5, 1, 5, and 6 strains were identified as *Salmonella* groups: B, C1, D and E1 respectively and 5 strains were ungroupable. *Salmonella* isolates were found to be susceptible to chloramphenicol, kanamycin, Ampicillin and tetracycline (Show *et al*, 1982).

A study in Kumasi, Ghana, to determine the prevalence of chronic typhoidal salmonellae among food Vendors, Typhoidal Salmonellae were isolated from six people, giving a carriage rate of 2.3%. Three of the Salmonellae isolated were *S. typhi* (Feglo *et al*, 2004)

Around 12.9% food handlers were suffering from intestinal parasitic infestation, out of which 42.81% were contributed by *Entamoeba histolytica*. Only one person (0.47%) was found to have *S. typhi* in stool sample while 28(13%) were Vi Reactors by agglutination test. The main deficiencies in personal hygiene were poorly kept nails, dirty working clothes, lack of foot-wear,

irregular bathing & not brushing teeth amongst Food Handlers in Amritsar City, India (Mohan *et al*, 2006).

A survey of food handlers in a restaurant in Lagos, Nigeria about typhoid fever found more than half (62.2%) washed their hands with water only before eating while 27.7% did not wash their hands always before preparing food. After using toilets, 71.9% washed their hands with soap and water while 28.1% washed their hands with only water (Smith *et al*, 2010).

Screening of asymptomatic typhoid carriers from nail samples of roadside foodhandlers in Tamilnadu, India, showed that all the five *S.typhi* isolated were resistant to Ampicillin, four to amoxicillin and one to chloramphenicol but none to gentamycin (Valli *et al*, 2010).

In Ethiopia, there have been several studies conducted on salmonellosis which suggest an increase in the antibiotic resistance of *Salmonella* to commonly used antimicrobials in both the public health and veterinary sectors (Gebre-Yohannes, 1985).

A study to identify the prevalent serovars and their susceptibility to drugs in Addis Ababa between January 1974 and October 1981 indicates that, of 216 *Salmonella* isolates studied, 54.6% were from stools and 45.4% from invasive sites: blood 34.7%; pus 5.6%; and urine 5.1%. There were 26 different serovars, of which *S. typhi* (48.6%) was the most common, followed by *S. concord* (12.5%), *S. typhimurium* (11.1%) and *S. paratyphi B* (5.6%). The high isolation rate of *S. concord* in Ethiopia is unusual and is in contrast to the other regions in Africa where *S. typhimurium* or *S. enteritidis* are more common (Beyene *et al*, 2008).

By 1995, 28.6% of *S. typhi* isolates were resistant to chloramphenicol (Ryan *et al*, 2004) and in 2000, the most recent study, reports that 30.8% of the isolates of *S. typhi* in Jimma were resistant to chloramphenicol, 54% to Ampicillin, and 38% to co-trimoxazole (Mache, 2002). These data show clearly the emergence of a significant resistance problem in the last decade in *S. Typhi* isolated in Ethiopia, especially in Jimma.

Fingernail contents of both the hands and stool specimens were collected from all the 127 food-handlers working in the cafeterias of the University of Gondar and the Gondar Teachers Training College, Gondar, Ethiopia. The samples were examined for bacteria and intestinal parasites following standard procedures. Coagulase-negative staphylococci were the predominant bacteria

species (41.7%), followed by *Staphylococcus aureus* (16.5%), *Klebsiella* species (5.5%), *Escherichia coli* (3.1%), *Serratia* species (1.58%), *Citrobacter* species (0.8%), and *Enterobacter* species (0.8%). *Shigella* species were isolated from stool samples of four food-handlers (3.1%). None of the food-handlers was positive for *Salmonella* species and *Shigella* species in respect of their fingernail contents (Andargie *et al*, 2008).

Among 384 food handlers working in different food services establishments, such as hotels, restaurants and snack bars in Bahir Dar, 158 (41.1%) food handlers had intestinal parasites and 6 (1.6%) were found positive for *S. typhi* where 33.3%, 16.6%, 83.4%, 66.7%, 33.3% and 100% were resistant to chloramphenicol, Norfloxacin, Cotrimoxazole, tetracycline, gentamycin Ampicillin respectively. Of these, 25 (6.5%) were suffering from diarrhea (Abera *et al*, 2010).

Among 206 apparently health foodhandlers working in bukkas in Lagos, Nigeria, *Salmonella* species were isolated from 17% of the stool samples obtained from the food handlers in which *S. typhi* (6.8%), *S. enteritidis* (5.3%), *S. choleraesuis* (2.9%), *S. paratyphi* A (1.5%) and *S. arizona* (0.5%). The organisms were completely resistant to tetracycline, Ampicillin and amoxicillin (Smith *et al*, 2009).

#### **1.4 Significance of the study**

In Ethiopia, the proportion of certified food handlers and their carrier for *Salmonella* species is not well studied. Therefore, this study was designed to isolate the *Salmonella* species from apparently healthy food handlers and the antibiotic susceptibility profile of the isolates among food handlers of AAU students' cafeteria, Addis Ababa, Ethiopia.

Therefore, the results of this study will contribute in provision of concrete evidence about the Salmonellae isolation rate among foodhandlers and antibiotic sensitivity profile of the isolates in the study area, designing strategies for prevention and control of Salmonellosis in the university as well as helps to create awareness among study participants with regard to Salmonellosis and their associated health problems. Finally, it will serve as base line data to conduct further studies.

## CHAPTER II: OBJECTIVE

### General objective:

- This study is designed to isolate *Salmonella* species among apparently healthy food handlers of Addis Ababa University students' cafeteria

### Specific objectives:

- to determine the prevalence of *Salmonella* among apparently healthy food handlers
- to determine the antibiotic susceptibility pattern of *Salmonella*
- to determine the relative distribution of *Salmonella* serovars
- to assess the risk factors associated with *Salmonella* infection

## CHAPTER III: METHODS AND MATERIALS

### 3.1 Study design

A cross sectional study was conducted to assess the isolation rate of *Salmonella* among apparently healthy food handlers working in Addis Ababa university students' cafeteria

### 3.2 Study area and study period

The study was conducted among food handlers in Addis Ababa university students' cafeteria from December 2010 up to February 2011, Addis Ababa, Ethiopia. Addis Ababa is the capital city of Ethiopia. It is the largest city in Ethiopia, with a population of 3,384,569 according to the 2007 population census. It lies at an altitude of 7,546 feet (2,300 meters) at the foot of Mount Entoto to the north of city and is a grassland biome, located at 9°1'48"N 38°44'24"E 9.03°N 38.74°E Coordinates.

It is home to Addis Ababa University founded On March 20, 1950, by Emperor Haile Silassie I as the University College of Addis Ababa, which includes the faculties of Arts and Science. At the time there were only 33 students enrolled compared to the current number of more than 400,000 students in six campuses in Addis Ababa and one in Debre Zeit.

### 3.3 Source population

All individuals of the Addis Ababa University community

### 3.4 Study population

Study subjects are individuals working as a food handlers in students' cafeteria from different campus of the university except Commerce Business College.

### 3.5 Sample size determination

Sample Size is determined by the following formula:

$$n = \frac{Z_{\alpha/2}^2 P(1-P)}{d^2}$$

Where:

- p is the estimated proportion in the population believed to be salmonella carrier.
- The prevalence rate of *Salmonella* among the population of AAU is not known.

So in this study the estimate of 50% ( $p=0.5$ ) which will yield the maximum value for  $n$  is used.

-  $Z$  reflects the confidence interval; we used 95 % confidence interval so the value of  $z_{\alpha/2}$  will be 1.96

-  $d$  is the margin of error, here it is 0.05

$\alpha$  = Is the level of error one is willing to tolerate

$$n = \frac{(1.96)^2 0.5(1-0.5)}{(0.05)^2} = 384$$

Since the study population is less than 10,000 that is the total number of foodhandlers is ( $N=594$ )

$$- N=594 \quad \text{So, } n/N=384/594=0.65$$

Since 65% is greater than 10%, the final sample size correction will be:

$$n_f = \frac{n}{1 + \frac{n}{N}}$$

Where  $n_f$  is the final sample size

$$n_f = \frac{384}{1 + 0.65} = 233$$

So the sample size was 233

### 3.6 Sampling technique

Probability simple random sampling technique was used, and data was collected based on lottery method after the complete list of food handlers obtained from human resource management office.

### 3.7 Eligibility criteria

#### Inclusion criteria

- All food handlers without a clinical symptom of salmonellosis such as diarrhea, fever, gastroenteritis, vomiting and abdominal cramp during the study

#### Exclusion criteria

- Food handlers who were infected during the study and the preceding two weeks with salmonellosis
- Food handlers taking antibiotic treatment

### 3.8 Definition of terms/ standard or working terms

- **Apparently healthy:-** an individual who seems healthy but may be a carrier of *Salmonella* species
- **Carrier:-** a person that is infected with *Salmonella* but without displaying any of the symptoms of salmonellosis and excrete *Salmonella* species in stool or urine
- **Feco-oral route:** - the way by which *Salmonella* is transmitted by ingesting food and water contaminated with faeces which contain it.
- **Food handler:-** a person who is responsible for preparing, handling, transporting of food, food supplements and materials.
- **Prevalence:-** the percentage of a population that is affected by salmonellosis at a given time.
- **Risk factor:-** a factor whose presence is associated with an increased probability of salmonellosis

### 3.9 Variables

#### Independent variables

Sex

Age

Hand washing habit

Finger nail status

Medical check up

Educational level

Source of drinking water

Certified in Food training

Latrine usage

#### Dependent variables

Salmonella carrier status

### **3.10 Data collection and Laboratory Processing**

The foodhandlers were informed about the study and its objectives and asked for consent. After food handlers signed informed consents, they were interviewed for risk factors associated with salmonellosis and Stool samples were collected from each study subject after provided with stool container, tissue paper and clean applicator stick. After stool samples were transported to the ALIPB laboratory inside an ice box within 2-4 hours, were processed for enrichment to isolate *Salmonella* species.

#### **3.10.1. Data and Specimen Collection**

After foodhandlers signed informed consent, structured questionnaire interviewed by data collector or principal investigator was used:

- for demographic data like age, sex, educational background & service year
- Predisposing factors for salmonellosis like hand washing habit, finger nail status, medical checkup, certification in food training, source of drinking water and latrine usage.
- 2-5 grams of stool was collected from each participant using sterile specimen cups(ANNEX II)
- Specimens diluted with urine and formed stools were rejected.

#### **3.10.2. Culturing and Identification procedure**

- 1-2 grams of each stool sample was added to a tube containing 10 ml of selenite cystine broth (OXOID, England and HIMEDIA, India) and incubated at 37°C for 18-24 hours.
- A loopful of each Selenite cystine broth enriched stool samples was inoculated on selective media MacConkey and XLD agar(OXOID, England) and incubated for 24 hours at 37°C.
- Isolated colorless transparent colonies on macConkey (seen as medium color) and red colonies with or without black centers on XLD were subcultured on XLD agar for purification.
- Isolated organisms from XLD were further identified by TSI(DIFCO™, France), urea(OXOID, England and HIMEDIA, India), motility(BBL™ DIFCO, USA), citrate utilization(OXOID, England), and LIA(DIFCO™, France) biochemical tests

- Antibiotic susceptibility pattern was performed on Muller Hinton agar using disc diffusion method for nine common antibiotics including Ciprofloxacin, Chloramphenicol, Norfloxacin, Ampicillin, Tetracycline, Trimethoprem-sulfamethoxazole, Ceftriaxone, Gentamycin and amoxicillin/clavulinic acid (BD BBL™ Sensi-Disc™ antimicrobial susceptibility test discs, USA). (The detailed for the above on ANNEX III)

### **3.11 Data processing & Analysis**

All components of data were entered and cleaned using EPI-INFO version 3.5.1 computer software. The data was analyzed using SPSS version 16 and EPI-INFO version 3.5.1 computer software. Data was organized and summarized in simple descriptive Statical methods. Fisher's exact test and binary logistic regression test results were used and p-value of less than 0.05 was considered as statistically significant. Data was described based on age, sex, food training certification, educational level & service year, and was also be analyzed for hand washing habit, finger nail status, source of drinking water, latrine usage variables and their association with salmonella carrier status.

### **3.12 Quality controls**

To manage the quality of the work standard operational procedures were followed during processing of each sample. All the instruments used for sample processing were checked for proper functioning as far quality control strains of *E.coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14028 were used. Completeness of the questionnaire was checked whether the necessary information was properly full filled or not. The sterility of prepared media was checked by incubating one of the prepared media for 24 hours at 37°C.

### **3.13 Source of funding**

This study was performed by the financial support obtained from AAU, school of graduate studies in partial. However, the most crucial and necessary material and media support was from ALIPB microbiology laboratory.

### **3.14 Ethical consideration**

Initially, the research proposal was approved by the Department of Microbiology, Immunology & Parasitology (DMIP), ethically cleared by the department ethical committee. Permission was obtained from the Addis Ababa university student service director's office.

The study participants were informed that the study process had no harm to them and their work, and is important and will help to design intervention strategies so that Confidentiality was kept. In addition, informed consent to participate in study was made by each volunteer. Any subject who was not volunteer to participate was not enforced to be included in the study.

Finally, the copy the result was given to the student service director's office of the University with recommendations to take appropriate measures.

## CHAPTER IV: RESULTS

### 4.1. Sociodemographic Data

A total of 233 foodhandlers from all campuses of Addis Ababa University students' cafeterias except the College of Commerce were examined for *Salmonella* species by stool culture and a structured questionnaire was used to assess risk factors associated with salmonellosis. Of these, 193(82.8%) females and 40(17.2%) males were included giving 14:3 female to male ratio. Their mean and median ages were 36.28 with a standard deviation of 10.66 and 35 years respectively, ranging from 18-60 years. The majority of foodhandlers, 195(79.4%) were from grade 5-12 (table 1).

Table 1: A Sociodemographic data of food handlers working at AAU students' cafeteria from December 2010 to February 201

Characteristic	Frequency	Percent	
<b>Age group</b>	18-27	59	25.3
	28-37	77	33.1
	38-47	50	21.4
	48-57	42	18.1
	≥58	5	2.1
	Total	233	100
<b>Educational</b>	Illiterate	8	3.4
	Read and write	6	2.6
<b>Background</b>	Grade 1-4	17	7.3
	Grade 5-8	88	37.8
	Grade 9-12	97	41.6
	Above 12	17	7.3
	Total	233	100
	<b>Sex</b>	Female	193
Male		40	17.2

<b>Service year in cafeteria</b>	Less than one year	51	21.9
	One to two years	10	4.3
	More than two years	172	73.8
	Total	233	100

## 4.2 Salmonellae Isolation Rate

Of the 233 food handlers screened, *Salmonella* species were isolated from 8 foodhandlers; giving a Salmonellae isolation rate of 3.4%. Among the eight Salmonellae positive results, two *S.typhi*, one *S.paratyphi* A were isolated and the other five, belong to unidentified *Salmonellae* species that could not be serotyped. None of foodhandlers was diagnosed for multiple *Salmonella* species.

All of the eight *Salmonella* species were isolated from females. In other words, 8(4.1%) of female foodhandlers examined were positives of the *Salmonella* species.

### 4.2.1. Isolation of *Salmonella* by Age Group

The isolation rate of *Salmonella* species was highest at the age group Of 18–37 in which 6(62.5%) percent of the positives belong.

Table 2: the isolation rate of salmonella species identified at different age groups out of all examined foodhandlers of AAU students' cafeteria from December 2010 to February 2011.

Age Group	Stool culture Result for <i>Salmonella</i>		Total	P value
	Positive (%)	Negative (%)		
<b>18-27</b>	3(1.27)	56(24)	59(25.27)	<b>P=0.10</b>
<b>28-37</b>	3(1.27)	74(31.7)	77(32.97)	
<b>38-47</b>	0(0)	50(21.4)	50(21.4)	
<b>48-57</b>	2(0.84)	40(17.1)	42(17.94)	
<b>≥58</b>	0(0)	5(2.2)	5(2.2)	
<b>Total</b>	8(3.4)	225(96.6)	233(100)	

#### **4.2.2. Isolation of *Salmonella* by Educational Background**

Majority, 201(86.3%) of the foodhandlers were above grade 5. Among foodhandlers having a positive stool culture result for *Salmonella* species, 5 of 88(5.7%), 2 of 97(2.1%) and 1 of 17(5.9%) had an educational level of grade 5-8, grade 9-12 and grade above 12 respectively. However, lower educational level foodhandlers were free of any *Salmonella* species, may be due to low number of isolates.

#### **4.2.3. Isolation of *Salmonella* by Service Year**

Out of 51 foodhandlers served in the students' cafeteria for a period of less than one year, 3(5.9%) were positive for *Salmonella* species and only 7(13.7%) of them had a medical checkup for salmonellosis. The rest, 5 of 182 (2.9%) positives were detected from those served for more than two years in which 146(84.9%) had been medically checked. This indicates that the rate of isolation is highest among foodhandlers served for less than one year since most did not have a medical checkup.

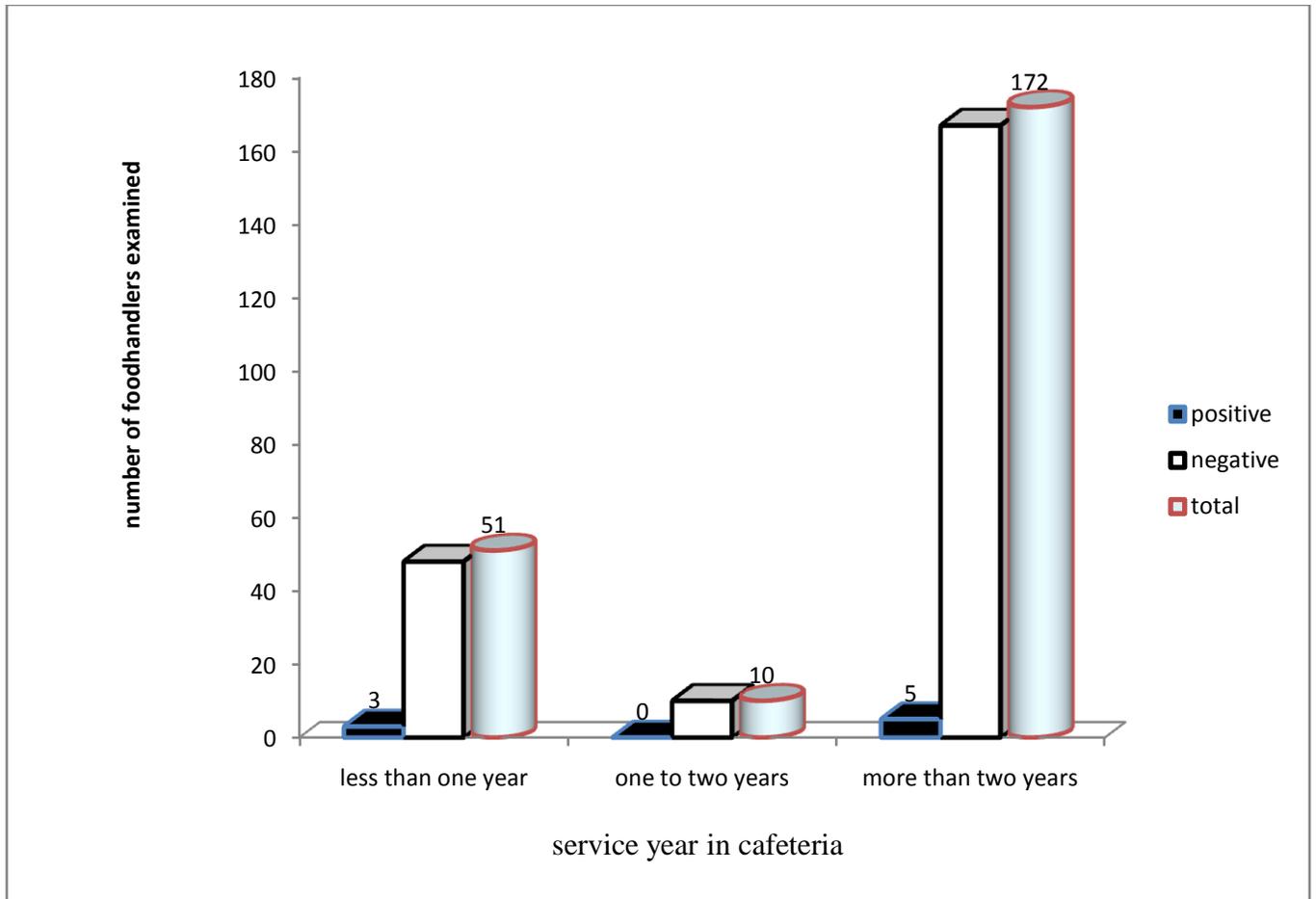


Figure 1: the isolates of *Salmonella* among foodhandlers of AAU students' cafeteria according to their service year in the cafeteria from December 2010 to February 2011.

#### 4.2.4. Isolation of *Salmonella* by Food Training

Of the total, only 28(12%) of foodhandlers were trained & certified in food handling and preparation from different food training institutions, and 136(58.4%) were trained but not certified after a few days training organized by the university last year. The remaining 69(29.6%) foodhandlers have never had a training related with food safety. Although *Salmonella* species had been isolated from certified, trained but not certified and untrained foodhandlers, it had no statistical association between isolation rate and their level of training ( $p=0.09$ ,  $DF=2$ ).

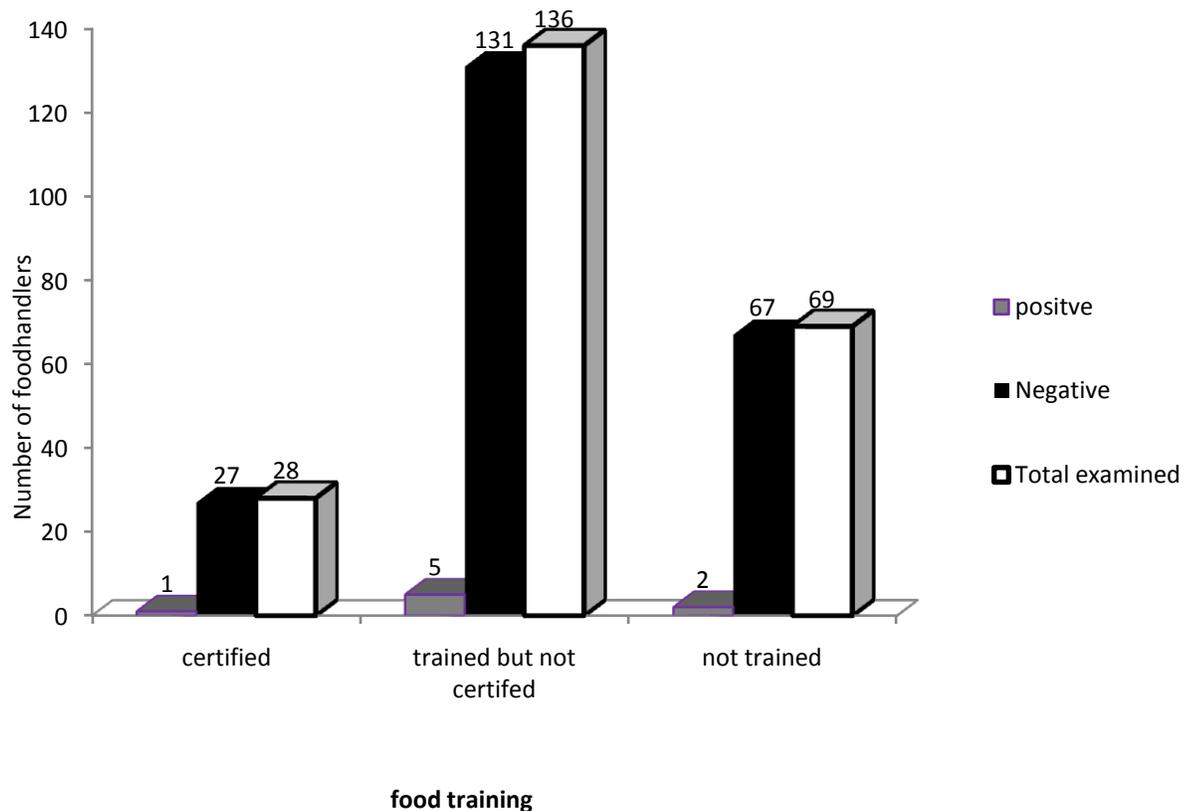


Figure 2: the isolates of *Salmonella* species among foodhandlers in accordance with their level training in food preparation, December 2010 to February 2011.

### 4.3 Risk factors Associated with Salmonellosis

All of the foodhandlers' working at AAU students' cafeteria had a habit of latrine usage. In addition, all of them used pipe water as a source of drinking.

Among the 233 foodhandlers examined, 62(26.6%) had been diagnosed for typhoid fever at different times in their life. Of these, 3(4.8%) foodhandlers were positives for *Salmonella* species in this study, in which two were diagnosed in the last one year and the other one was before fourteen years. Therefore, the isolation rate was higher among diagnosed than never diagnosed for salmonellosis which is 5(2.9%) out of 171 foodhandlers.

In this study, 158(67.8%) foodhandlers have been found to have a history of serological medical checkup inconsistently for typhoidal *Salmonella* organized by the university. However, it has been done before three years in some campuses like Debrezeit College of veterinary medicine and south technology campuses of AAU.

Table 3: the history of previous medical checkup for typhoidal *Salmonella* species versus the number of *Salmonella* isolates among foodhandlers of AAU students' cafeteria from December 2010 to February 2011

Medical checkup for <i>Salmonella</i>	positive for <i>Salmonella</i>		Total	Fisher's exact test P value = 0.505 P value > 0.05
	Yes	No		
Yes	5	153	158	
No	3	72	75	
Total	8	225	233	

Statistical analysis of medical checkup versus isolation rate for *Salmonella* indicated that there is no statistically significant difference in isolation rate of *Salmonella* between those medically checked for typhoidal *Salmonella* and not checked (p=0.505) though it was assumed that isolation rate would be decreased among previously diagnosed because of treatment given for positives.

All of the foodhandlers working at AAU students' cafeteria had a habit of hand washing with or without soap after toilet covering 65.2 and 34.8 percent respectively.

Table 4: the hand washing habit after toilet and the number of *Salmonella* isolates among the foodhandlers of AAU students' cafeteria from December 2010 to February 2011

Hand washing habit after toilet	positive for <i>Salmonella</i>		Total	Fisher's exact test P value = 0.003 OR=0.07 (0.008,0.58) P < 0.05
	Yes	No		
With soap and water	1	151	152	
Only with water	7	74	81	
Total	8	225	233	

Statistical analysis of hand washing habit after toilet shows that there is a statistically significant difference in isolation rate of *Salmonella* between foodhandlers washed their hands with soap and water and only with water after toilet,  $p=0.003$ . Therefore, the chance of getting *Salmonella* infection after washing hands with soap and water is 93% lesser than washing hands only with water after toilet (OR=0.07; with a 95% CI = 0.008–0.58).

Of the 233 foodhandlers interviewed, all had a trend of hand washing after touching dirty materials with and without soap.

Table 5: hand washing habit after touching dirty materials and number of *Salmonella* isolates among foodhandlers of AAU students' cafeteria from December 2010 to February 2011.

Hand washing after touching dirty materials	Positive for <i>Salmonella</i> species		Total	Fisher's exact test P value= 0.601 P > 0.05
	Yes	No		
With soap and water	6	172	178	
Only with water	2	53	55	
Total	8	225	233	

This statistical analysis shows that there is no statistically significant association between hand washing habit with and without soap after touching dirty materials and isolation rate of *Salmonella* among foodhandlers of AAU students' cafeteria,  $p=0.601$ .

Out of 233 foodhandlers interviewed, 198(85%) washed their hands with soap and water but the remaining washed only with water before food handling.

Table 6: hand washing habit before food handling and number of *Salmonella* isolates among foodhandlers of AAU students' cafeteria from December 2010 to February 2011.

Hand washing habit before food handling	Positive for <i>Salmonella</i> species		Total	Fisher's exact test P value = 0.65 p>0.05
	Yes	No		
With soap and water	7	191	198	
Only with water	1	34	35	
Total	8	225	233	

Statistical analysis shows that there is no statistically significant difference in the isolation rate of *Salmonella* among foodhandlers having a habit hand washing with soap and water and those only with water, p=0.65.

Based on the result of interview, 198(85%) of foodhandlers in AAU students' cafeteria had a totally trimmed finger nail. Of these, 7(3.5%) foodhandlers were positive to one of the *Salmonella* species during the study period.

Table 7: The fingers nail status and number of *Salmonella* isolates among foodhandlers of AAU students' cafeteria from December 2010 to February 2011.

Finger nail status	positive for <i>Salmonella</i> species		Total	Fisher's exact test P value=0.65 P >0.05
	Yes	No		
Trimmed	7	191	198	
Semi-trimmed	1	34	35	
Total	8	225	233	

This statistical analysis showed that there is no statistically significant association between the isolation rate of *Salmonella* among foodhandlers and their fingernail status, p = 0.65.

#### 4.4 Antimicrobial Susceptibility Profile of *Salmonella* Isolates

Even though no MDR *Salmonella* was identified, all of the isolates were resistant at least to one of tested antimicrobials and one isolate was resistant to fluoroquinolone drugs, both to ciprofloxacin and Norfloxacin. Strains that develop resistance to antibiotics such as chloramphenicol, Ampicillin and trimethoprem-sulfamethoxazole are categorized as MDR *Salmonellae*.

Table 8: antimicrobial activity profile of antimicrobial agents against *Salmonella* isolates identified from foodhandlers of AAU students' cafeteria from December 2010 to February 2011.

Antimicrobial Agent( $\mu$ g)	<i>Salmonella</i> Isolate							
	S.T-1	S.T-2	S.P	O.S-1	O.S-2	O.S-3	O.S-4	O.S-5
Amoxicillin/ clavulanic acid(30)	S	S	R	R	S	S	S	I
Ampicillin(10)	R	R	R	R	R	I	R	R
Ceftriaxone(30)	S	S	S	S	S	S	S	S
Chloramphenicol(30)	R	S	S	S	S	S	S	S
Ciprofloxacin(5)	S	S	S	S	R	S	S	S
Cotrimoxazole(1.25)	S	I	S	S	R	S	S	S
Gentamycine(10)	S	S	S	S	S	S	S	S
Norfloxacin(10)	S	S	S	S	R	S	S	S
Tetracycline(30)	I	S	S	I	R	I	I	I

**Key:** S.T-1 = *S.typhi* isolate 1, S.T-2 = *S.typhi* isolate 2, S.P = *S.paratyphi* A, O.S-1= other *Salmonella* species isolate 1, O.S-2 = other *Salmonella* species isolate 2, O.S-3 = other *Salmonella* species isolate 3, O.S-4 = other *Salmonella* species isolate 4, O.S-5 = other *Salmonella* species isolate 5

S = sensitive, I = intermediate, R = resistant

All *Salmonella* species isolated were resistant to Ampicillin except one isolate with intermediate susceptibility. Only 2(25%) *Salmonella* isolates (one *S.typhi*, *S.paratyphi* A) were sensitive to

tetracycline while five had intermediate and one resistant result. However, all isolates were sensitive both to Ceftriaxone and Gentamycine.

Of the two *S.typhi* isolates, one was resistant to chloramphenicol and intermediate to Tetracycline while the other one had an intermediate susceptibility to Cotrimoxazole in addition to their resistance for Ampicillin. Whereas, the single *S.paratyphi* A isolate had a resistance both to Ampicillin and Amoxicillin clavulanic acid.

One non serotyped *Salmonella* isolate was resistant to five antimicrobial agents including Ampicillin, ciprofloxacin, Cotrimoxazole, Norfloxacin and Tetracycline.

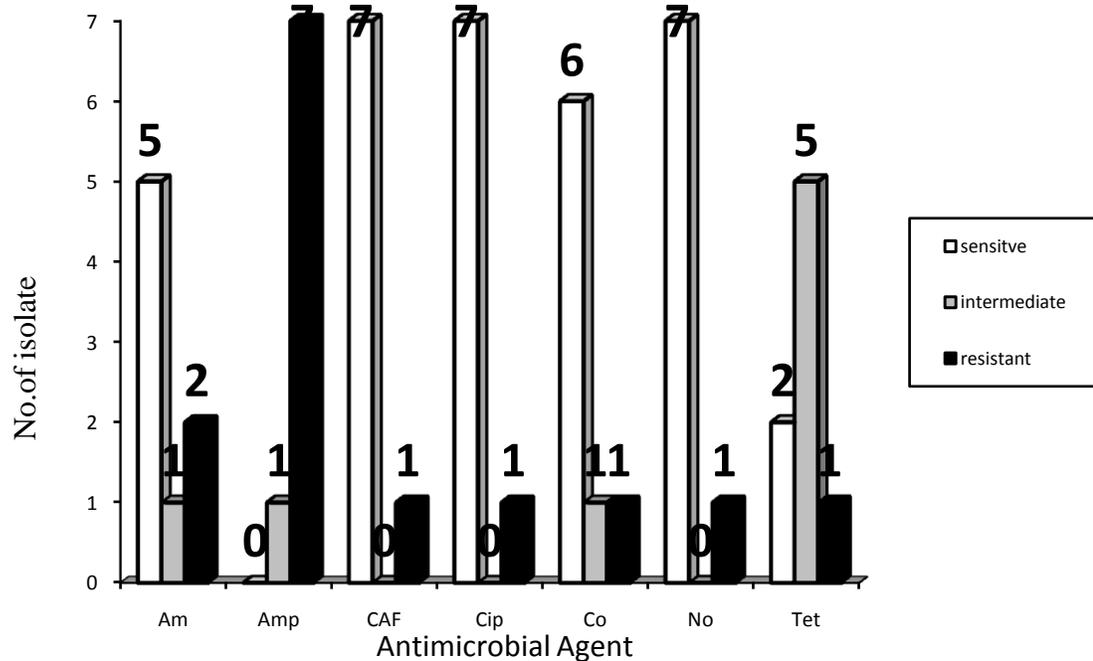


Figure 3: the number of Salmonella isolates showing resistance to antimicrobial agents from December 2010 to February 2011.

**Key:** Amp = Ampicillin, Am = Amoxicillin clavulanic acid, CAF = Chloramphenicol, Cip = Ciprofloxacin, Co = Cotrimoxazole, No = Norfloxacin, Tet = Tetracycline

None of the Salmonella isolates identified was sensitive to all antimicrobial discs tested. Therefore, all the isolates were resistant at least to one of the nine antimicrobial agents tested.

Table 9: the Salmonella species identified and their antimicrobial resistance profile among foodhandlers of AAU students' cafeteria from December 2010 to February 2011.

<i>Salmonella</i> species	No. of isolate	Antimicrobials resistance observed	No. of anti-microbials to which resistance observed
S.typhi	2	Amp., CAF	2
S.paratyphi A	1	Amp, Am	2
Other salmonella species	5	Amp, Am, Cip, Co, No, Tet	6

**Key:** Amp = Ampicillin, Am = Amoxicillin clavulinic acid, CAF = Chloramphenicol, Cip = Ciprofloxacin, Co = Cotrimoxazole, No = Norfloxacin, Tet = Tetracycline

## CHAPTER V: DISCUSSION

This cross sectional study was designed to isolate *Salmonellae* among a population of foodhandlers at AAU students' cafeteria. An isolation rate of 3.4% was detected.

This rate of isolation is lower than 17% among apparently healthy foodhandlers working in bukkas (type of local restaurant) for *Salmonella* species in Lagos, Nigeria (Smith et al, 2009) and 17.14% among suspected asymptomatic foodhandlers for *S.typhi* in Namakkal, India (Senthilkumar et al, 2005) but higher than a study from Japan among food workers in hotels, supermarket, food factories, and restaurants in which only 0.032% of the faecal samples harboured *Salmonella*, and the most common serovars were *S.agona*, *S.corvallis*, *s.infantis* and *S.enteritidis* (Murakami et al, 2007). Furthermore, none of the food-handlers was positive for *Salmonella* species in a study done on food-handlers working in the cafeterias of the University of Gondar and the Gondar Teachers Training College, Gondar, Ethiopia (Andargie et al, 2008).

However, it is interestingly similar to a study from Kumasi, Ghana, where a 2.3% *Salmonellae* carriage rate among food vendors was detected, in which three of the six *Salmonellae* isolates were *S. typhi* and the other three belong non-typhoidal *Salmonella* species (Feglo et al, 2004), like in our study: two *S.typhi*, one *S.paratyphi* A and five non-typhoidal *Salmonellae* among eight isolates.

We found that isolation rate of *S.typhi* among foodhandlers was 0.85%. This finding disagrees with a study done in Bahir Dar town, North West Ethiopia, where 1.6% food handlers were found to be infected with *S. typhi* (Abera et al, 2010). This may be due to that foodhandlers in AAU had a better habit of hand washing after toilet, after touching materials and before food handling and also more foodhandlers trained in food handling and preparation than food handlers working in the restaurants, cafeterias and hotels of Bahir Dar town.

However, it coincides with a study from Amritsar, India by Mohan et al only one person (0.47%) was found to have *S. typhi* in the stool sample (Mohan et al, 2006).

Many factors may contribute to the difference in the prevalence of *Salmonellae* as well the carriage rate among asymptomatic carriers at different times, places and conditions as well in different population. The possible factors that favor the transmission and prevalence of

salmonellosis may include environmental and personal sanitation, socio-economic and living standards, microbial quality and availability of water supply and awareness of safe food handling and preparation among individuals.

The chronic *Salmonellae* carrier state occurs most commonly among middle age women (CDC, 2010). In our study, all asymptomatic *Salmonellae* positive foodhandlers were females as previous studies in Ghana, where all six positives were females (Feglo *et al*, 2004)) and In Namakkal, India, four out of six *S.typhi* carriers were females (Senthilkumar *et al*, 2005) even though the small number of organisms isolated does not permit any reliable conclusions on the isolation rate of both sexes.

However, this overall isolation rate of *Salmonella* species from female foodhandlers in our study may be due to high number of females participated, they usually involved cleaning of houses, toilets, household materials etc as well as low number of isolates.

In this study, most of foodhandlers working in students' cafeteria of AAU were females, young adults and had low educational levels; which is in line with a study from Bahir Dar and Gondar town (Andargie *et al*, 2008; Abera *et al*, 2010).

It is expected that all foodhandlers at university, military, hospitals etc cafeterias to have a medical checkup for foodborne pathogens. Despite this fact, the interview result of our study showed that only 67.8% of foodhandlers working in AAU students' cafeteria had a medical checkup for typhoidal *Salmonellae*. However, in Bahir Dar town, it was found that none of the foodhandlers had ever a medical checkup (Abera *et al*, 2010).

Hygienic assessment of the foodhandlers revealed they had a good habit of hand washing after toilet, touching dirty materials and before food handling. Hand washing habit after toilet with and without soap had a strong association with the isolation rate of *Salmonellae*,  $p=0.003$  (OD = 0.07 with a 95% CI = 0.008–0.58). The result indicates that 65.2% of foodhandlers had a habit of hand washing with soap after toilet while 34% washed only with water. This is in parallel with a study in Lagos, Nigeria, in which 71.8% washed their hands with soap and water while 28.2% washed their hands with only water after visiting toilets (Smith *et al*, 2010). However, a study

from Gondar university and Bahir Dar town found that only 89% and 90.6% of foodhandlers had a habit of hand washing after toilet respectively (Andargie *et al*, 2008; Abera *et al*, 2010).

Around 85% of foodhandlers had a trimmed finger nail. Even though it had no association with the isolation rate of *Salmonellae* in this study, finger nails serve as a vehicle for transport of microorganisms from their source to the foods or/and directly in to the body.

According to this study, sex and hand washing habit after toilet were found to be predisposing factors for *Salmonella* among the foodhandlers in which isolation rate would be higher among females and wash hands only with water.

Inadequate or absence of treatment and asymptomatic cases of salmonellosis directly contribute in the increment of *Salmonellae* carriers. Therefore, carriage of *Salmonellae* following inadequate treatment may increase the probability of caring a drug resistant strain. Even though no MDR *Salmonella* was detected, this study indicated that there was an increase in antimicrobial resistance of *salmonellae* isolated from foodhandlers especially to Ampicillin and tetracycline. Seven of eight *Salmonellae* isolated (87.5%) were resistant to Ampicillin, where one was intermediate. It agrees with a finding from Bahir Dar, Ethiopia, and Tamil nadu, India, where 100% of isolates were resistant to Ampicillin (Abera *et al*, 2010; Valli *et al*, 2010). However, it differs from a result found from Jimma, Ethiopia; only 54% were resistant to Ampicillin (Mache, 2002).

Ceftriaxone & gentamycin at all and fluoroquinolones were highly effective. Ciprofloxacin is effective for the treatment of salmonellosis and carrier state according to the current guidelines for the treatment of salmonellosis. However, one of the isolates in this study showed resistance to ciprofloxacin.

The two *S.typhi* isolates showed a resistance only to Ampicillin and chloramphenicol which unmatched with study from Namakkal, India, reported that five out of six *S.typhi* isolates were multi-drug resistant (Senthilkumar *et al*, 2005). Furthermore, a study from Bahir Dar reported that *S.typhi* developed a resistance to Ampicillin, Cotrimoxazole, tetracycline, and chloramphenicol, genentamicin and Norfloxacin (Abera *et al*, 2010). These differences might be due to low number of isolates in our study.

## **Limitation of the study**

The isolated salmonella species were not serotyped due to unavailability of the serotyping kits in the laboratory as well in the suppliers and scarcity of budget to import. The few numbers of compressive documents regarding *Salmonella* among foodhandlers in Ethiopia was also limited our discussion.

## **CONCLUSIONS**

In general, many studies proved that foodhandlers can be a source of food outbreak infections especially bacterial infections. Salmonellosis is one of the most food and water outbreak infections following contamination of food or water by the organism which can be obtained from symptomatic or asymptomatic carriers. In this study, therefore, the 3.4% isolation rate of *Salmonella* species among apparently healthy foodhandlers serving in AAU students' cafeteria can be a possible source of salmonellosis for the students unless positives treated or other preventive measures taken.

Food handlers play a prominent role in food safety and transmission of *Salmonella* and so it is important that the food handlers are well informed about their hygiene status and the causes of salmonellosis transmission and ways by which its spread is prevented. Therefore, foodhandlers at public sectors like universities, hotels, restaurants should be trained and medically checked for possible pathogens like *Salmonella*.

All *Salmonellae* positives and most of foodhandlers (82.8%) in students' cafeteria were females. This may also increase the risk of transmission to the students they feed unless intensive preventive measures undertaken.

As a whole, foodhandlers in AAU students' cafeteria had a good habit of hand washing with or without soap. It was found that washing hands with soap reduces the chance of *Salmonellae* isolation rate by 93% than washing only with water ( $p = 0.003$ ).

Antimicrobial sensitivity of *Salmonella* organisms is crucial for treatment of *Salmonella* infections. Increasing antimicrobial resistance of *Salmonella* with no doubt may result in higher death to case ratios for resistant *Salmonella* infections than for infections with sensitive strains.

In whatever way, this study shows that all of the *Salmonellae* isolates had a resistance at least to one of the antimicrobials tested.

## **RECOMMENDATIONS**

Finally, we recommended the university to:

- ☞ Provide constant & periodic focused medical checkup for *Salmonella* species; preferable if twice a year
- ☞ Provide immediate treatment for diagnosed foodhandlers and check again after treatment
- ☞ Arrange further training and health education concerning personal and food hygiene
- ☞ Provide all foodhandlers with soap and other sanitary items for maintenance of personal hygiene and aware them to use it always especially after toilet

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## **ANNEXES**

### **ANNEX I: SUBJECT INFORMATION & CONSENT FORM**

This study is aimed for investigating principally the carrier status of salmonella, drug sensitivity and to assess the associated risk factors among food handlers of AAU students' cafeteria.

We are requesting you and others to voluntarily participate in this study. What we expect from everyone is to be examined for salmonella as well as to answer a few questions regarding risk factors. The laboratory examination involves collection of stool samples that should be collected using a sterile and disposable plastic container.

Giving stool doesn't have any harm to your health and any other aspects like your job rather you will be benefited. That is, if there is a positive finding in laboratory examination, we will communicate with the university's administration to provide treatment and health education.

Any information that we collect about you during this research will be kept in secret. Information about your identity will be put away after re-coding your file; and kept in a secured place. Only the principal investigator will be able to link your identity with the code number.

At the end of the study of this study we will write a report about the results of the study through publication or any other means. We assure you that the reports will not bear any information of your personality like name and identity.

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 benefits.

#### **Contact Address**

If you have any question and in case of urgency you can contact:-

Fentabil Getnet

Addis Ababa University, faculty of Medicine, department of microbiology, immunology and Parasitology

Tel: - 0913289380

E-mail:- [getinfen@yahoo.com](mailto:getinfen@yahoo.com)/ [b.infen4ever@gmail.com](mailto:b.infen4ever@gmail.com)

I, the undersigned, confirm that, as I give consent to participate after a clear understanding of the objectives and conditions of the study & with recognition of my right to withdraw from the study if I change my mind.

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

Name of Participant: \_\_\_\_\_

Participant's signature: \_\_\_\_\_

Name of data collector: \_\_\_\_\_

Signature of data collector: \_\_\_\_\_

Date: \_\_\_\_\_

## ANNEX II: QUESTIONNAIRE

A descriptive study of the carrier status of salmonella among food handlers of AAU students' cafeteria from December 2010 to February 2011, Addis Ababa, Ethiopia

### 1. Eligibility checking questions

1.	Did you have a diarrhea, nausea, fever, malaise and loss of appetite in the last 2 weeks?	Yes.....1 No.....2
2.	Are you taking antibiotic drug?	Yes.....1 No .....2
3.	If yes for the above question	When.....?

### 2. Questionnaire

		Date ____/____/____
		Label number _____
1.	Name	_____
2.	Sex	Female..... 1 Male.....2
3.	Age	_____
4.	Ethnicity	Oromo .....1 Amhara ..... 2 Tigray ..... 3 Others (specify) _____
5.	Religion	Orthodox..... 1 Muslim ..... 2 Protestant .....3 Others (specify)_____

6.	Educational background	Illiterate.....1 Only read and write ....2 Grade 1-4.....3 Grade 5-8.....4 Grade 9-12 .....5 >12.....6
7.	Hand washing habit	1, After toilet Yes with water and soap.....1 Yes only with water.....2 No.....3 2, After touching dirty materials yes with water and soap .....1 Yes only with water.....2 No.....3 3, before food handling Yes with water and soap .....1 Yes only with water.....2 No.....3
8.	Finger nail status	Trimmed.....1 Semi trimmed.....2 Not trimmed.....3
9.	Service year	<1 year .....1 1-2 years .....2 >2 years .....3
10	Medical check up	yes .....1 no .....2
11.	Certified in Food training	Yes.....1 Trained but not certified.....2 No.....3
12.	Have you ever diagnosed for salmonellosis?	Yes.....1 No .....2

13.	If yes for the above question	When.....?
14.	Latrine usage	Yes.....1 No.....2
15.	Source of drinking water	Pipe.....1 Bottled.....2 Others(specify).....3
	Stool culture result	

## **ANNEX III: PROCEDURES**

### **A. Collection of stool samples**

1. The food handlers provided with small wooden sticks and a suitable labeled plastic container with a leak proof clean, dry, disinfectant-free suitable wide-necked container in which to pass a specimen, toilet tissue and applicator stick.
2. The foodhandlers were instructed to collect the stool specimen on a piece of toilet tissue and transfer a portion (about a spoonful) of the specimen especially that which contains mucus, pus, or blood, into a clean, dry, leak proof container, using applicator sticks. The specimen should contain at least 5g of faece to the container.
3. The specimens were transported to ALIPB microbiology laboratory with a request form within 2-4 hours of collection inside ice box

### **B. Reagents Used**

Reagents used include all broth, semisolid slant and plate agars from enrichment of stool samples to final antimicrobial susceptibility and serotype testing, antimicrobial discs as well serotyping reagents. These are:

- Selenite cystine broth: OXOID, England and HIMEDIA, India
- MacConkey agar: OXOID, England
- XLD agar: OXOID, England
- Triple sugar iron Agar: DIFCO™, France
- Lysine iron agar: DIFCO™, France
- Motility test medium: BBL™ DIFCO, USA
- Urea agar base: OXOID, England and HIMEDIA, India
- Simmons citrate medium: OXOID, England
- BD BBL™ Sensi-Disc™ antimicrobial susceptibility test discs, USA

### **C. Inoculation of stool on enrichment media**

- 1.a faecal suspension was prepared by suspending approximately 1g of the stool sample in a tube containing 10 ml of selenite cystine broth.
- 2.Incubated selenite cystine broth for 18 hours.

3. Incubated selenite cystine broth suspension was subcultured by streaking a loopful of broth on selective media like MacConkey agar and XLD.

#### **D. Procedure for inoculation of primary isolation media**

1. A loopful of broth suspension was inoculated on both MacConkey agar and XLD. The inoculum was placed in the middle of the agar plate and streaked up and down and across the plate. This procedure will maximize the number of isolated colonies.

2. After inoculation, the agar plates incubated at 37 °C in an aerobic incubator (without CO<sub>2</sub>)

3. Discrete red transparent colonies on MacConkey and red colonies with or without black center on XLD colonies found in the peripheral portion of the plate were subcultured on XLD for purification.

#### **E. Inoculation and reading of UREA**

1. Using an inoculating loop, non-lactose-fermenting colonies from the primary plates were transferred to a tube containing UREA.

2. Incubated in the tubes for 2–4 hours at 37 °C and observe for a change in color to pink (urease-positive). Discard the urease-positive tubes.

3. Growth from the urease-negative tubes subcultured to Motility test, Simmons citrate, LIA and to TSI, and incubated for 24 hours but 48 hours for citrate utilization test in an aerobic incubator.

#### **F. Inoculation and reading of motility test, citrate utilization LIA and TSI**

1. All tubes containing media were labeled

2. Purified colonies inoculated in to the motility test medium by inserting a straight inoculating needle to 2mm above the bottom of the tube. Withdraw the needle along the same line.

3. Inoculation in to the Simmons citrate, LIA and TSI was by stabbing the agar butt with a straight inoculating needle and streaking the slant in a zigzag.

4. Incubated overnight at 37°C.

5. The motility medium was examined for motility. Motile organisms spread out into the medium from the line of inoculation and produce diffuse growth. Non-motile organisms grow only along the line of inoculation.

6. A positive lysine reaction is indicated by an alkaline reaction (purple color) at the bottom of the medium, and a negative reaction by an acid reaction (yellow color) at the bottom of the medium (caused by fermentation of glucose).

7. A positive growth (citrate utilization) produces an alkaline reaction and changes the color of the medium from green to bright blue whilst in a negative test the color of medium remains unchanged.

8. Examination of the TSI medium. All Enterobacteriaceae ferment glucose, producing acid and gas or acid only, which gives a yellow slant. If gas is produced, bubbles or cracks are seen throughout the medium; the medium may even be pushed up in the tube if a large amount of gas is produced. If lactose is simultaneously fermented, both the agar butt and the slant become acid, i.e. yellow (e.g. in the case of *E. coli*). If lactose is not fermented (e.g. in the case of *Shigella* and *Salmonella* spp.), the agar butt is yellow but the slant becomes alkaline, i.e. red. Blackening along the stab line or throughout the medium indicates the production of hydrogen sulfide.

Suspected colonies obtained on the above media are screened by means of the following media/tests:

Table 10: biochemical test characteristics of Enterobacteriaceae

Organism	Triple Sugar Iron Agar			Motility	Urea	Citrate	Lysine Iron Agar		
	Slant	Butt	H <sub>2</sub> S				Slant	Butt	H <sub>2</sub> S
<i>S.typhi</i>	Alk	Acid	Wk	+	-	-	Alk	Alk	+
<i>S.paratyphi A</i>	Alk	AG	-	+	-	-	Alk	Acid	-
Other <i>Salmonella</i> spp.	Alk	Acid/ AG	V	+	-	V	Alk	Alk	+
<i>E.coli</i>	Acid	AG	-	+	-	-	Alk	Acid	-
<i>Klebsiella</i> spp.	Acid	AG	-	-	+	+	Alk	Alk	-
<i>Citrobactor</i> spp.	V	AG	+++	+	-	+	Alk	Acid	+
<i>Proteus</i> spp.	Acid	AG	+	+	++	V	Red	Acid	-
<i>Shigella</i> spp.	Alk	Acid	-	-	-	-	Alk	Acid	-

Note:

Alk: means alkaline

AG: Acid & gas

V: variable

Wk: weak

#### **G. Procedure for antimicrobial sensitivity testing**

1. To prepare the inoculum from the a purified colonies of XLD, touch with a loop the tops of each of 3–5 colonies of the organism to be tested
2. Were transferred & suspended to a tube of saline.
3. The tube compared with the 0.5 McFarland turbidity standards and the density of the test suspension adjusted to that of the standard by adding more bacteria or more sterile saline. Proper adjustment of the turbidity of the inoculum is essential to ensure that the resulting lawn of growth is confluent or almost confluent.
4. The plates inoculated by dipping a sterile swab into the suspension. Excess inoculum Remove by pressing and rotating the swab firmly against the side of the tube above the level of the liquid.
5. The swab streaked all over the surface of the medium three times, rotating the plate through an angle of 60°c after each application. Finally, pass the swab round the edge of the agar surface. Leave the inoculum to dry for a few minutes at room temperature with the lid closed.
6. The BD BBL™ Sinsi-Disc™ antimicrobial discs were placed on the inoculated plates using a pair of sterile forceps uniformly. A maximum of five discs were placed on a 9–10 cm plate.
7. Each disc was gently pressed down to ensure even contact with the medium. The plates were placed in an incubator at 37 °C within 30 minutes of preparation.
8. After overnight incubation, the diameter of each zone (including the diameter of the disc) was measured and recorded in mm. The results were interpreted according to the critical diameters. The measurements were made with a ruler on the under-surface of the plate without opening the lid.

# ANNEX IV: LABORATORY REPORT

## 1. Result

<b>ADDIS ABABA UNIVERSITY, FACULTY OF MEDICINE</b>	
<b>DEPARTMENT MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY</b>	
<b>A descriptive study of the carrier status of salmonella among food handlers of AAU students' cafeteria, Addis Ababa, Ethiopia</b>	
<b>LABORATORY REQUEST FORM</b>	
Label number:	Time of sample collection
Age	____/____
Sex      M <input type="checkbox"/> F <input type="checkbox"/>	Date of sample collection
	____/____/____
<b>Laboratory Results</b>	
Stool culture	
_____	_____
Name of lab personnel	signature

## 2. Antibiotic Susceptibility Profile

Table 11: antibiotic susceptibility pattern of salmonella

Antibiotic	Resistance		
	susceptible	intermediate	resistant
Ciprofloxacin			
Chloramphenicol			
Norfloxacin			
Cotrimoxazole			
Tetracycline			
Ampicillin			
Gentamycin			
Amoxicillin			
Ceftriaxone			