

**Prevalence of Salmonella and Shigella among Food Handlers
in Catering Establishments in Hawassa University, Hawassa,
Ethiopia**

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<u>TABLE OF CONTENTS</u>	<u>PAGE</u>
Acknowledgements	I
Table of contents	II
List of Tables	IV
Abbreviations	V
Abstract.....	VI
CHAPTER I: INTRODUCTION.....	1
1.1. General Introduction.....	1
1.2. Literature Review	1
1.2.1. Definitions	1
a.Salmonellosis.....	1
b.Shigellosis.....	1
c.Intestinal parasitosis.....	2
1.2.2. Etiologic agents	2
a. <i>Salmonella</i>	2
b. <i>Shigella</i>	2
c. Intestinal parasites	2
1.2.3. Epidemiology	3
a. Salmonellosis.....	3
b. shigellosis	4
c. Intestinal parasitosis.....	4
1.2.4. Pathogenesis and Pathology	5
1.2.5. Clinical features and complications.....	5
a. Salmonellosis.....	5
b. Shigellosis.....	6
c. Intestinal parasitosis.....	6
1.2.6. Diagnosis	7
a. Salmonellosis and Shigellosis.....	7
b. Intestinal parasitosis	7
1.2.7. Treatment.....	8
1.2.8. Prevention.....	9

1.2.9. Significance of the proposed study.....	10
1.2.10. Hypothesis	11
1.3. Objectives of the study	12
CHAPTER II: MATERIALS AND METHODS.....	13
2.1. Study area and design.....	13
2.2. Study Population	13
2.3. Sample collection and Handling.....	14
2.4. Culture and identification of <i>Salmonella</i> and <i>Shigella</i> spp	14
2.5. Stool examination for ova and parasites.....	14
2.6. Widal test.....	14
2.7. Reference Strain	14
2.8. Statistical Analysis	14
2.9. Ethical consideration	15
CHAPTER III: RESULT.....	16
3.1. Study population.....	16
3.2. Etiologic agents	17
CHAPTER IV: DISCUSSION	20
Limitations of the study.....	22
Conclusions and Recommendations.....	22
References	23
Appendix I: Questionnaire	27
Appendix II: Patient information sheet (English and Amharic version).....	29
Appendix III: Patients consent form (English and Amharic version)	32
Declaration	34

<u>List of Tables</u>	<u>Page</u>
Table 3.1. Sociodemographic characteristics of food-handlers, Hawassa University, Hawassa, Ethiopia	16
Table 3.2. Type and prevalence of intestinal parasites isolated from stool specimens of food handlers, Hawassa University, Hawassa, Ethiopia.....	17
Table 3.3. Frequency of parasitic infection among food handlers by sex, age, educational status, service year and hand hygiene, Hawassa University, Hawassa, Ethiopia..	18

Abbreviations

LPS	Lipopolysacchrude
NCCLS	National Committee for Clinical Laboratory Standard
NTS	<i>Non Typhoidal Salmonella</i>
PCR	Polymerase Chain Reaction
Sd1	<i>Shigella dysenteriae type 1</i>
SNNPR	South Nations and Nationalities People Region

Abstract

Background: Food borne diseases such as salmonellosis, shigellosis and intestinal parasitosis remain a major public health problem across the globe. The problem is severe in developing countries due to difficulties in securing optimal hygienic food handling practices. Food handlers may be infected by a wide range of enteropathogens and have been implicated in the transmission of many infections to the public in the community and to patients in hospitals. In Ethiopia, published information about prevalence of enteric pathogens among food handlers is very scarce.

Objective: To determine the prevalence of *Salmonella*, *Shigella* and Intestinal parasites among food handlers in catering establishments in Hawassa University, Hawassa, Ethiopia.

Methodology: Cross sectional study was conducted from February 2010 through April 2010 at different catering establishments, in Hawassa University, Hawassa town, Ethiopia. Stool and blood specimens were collected from 272 food handlers. Microscopic examination and culture were performed on stool specimens for isolation of intestinal parasites and *salmonella* and *shigella*, respectively. In addition, all food handlers were screened for *S. typhi* using Widal test.

Results: Of the total 272 food handlers screened for enteric pathogens, 20.6% were found to be positive for different types of intestinal parasites. Among the parasites, *A. lumbricoides* was the most prevalent parasite (9.6%), followed by *S. stercoralis* (2.2%) and *E. histolytica /dispar* (2.2%). *Shigella* spp. was detected in 0.4% of food handlers. No *salmonella* was detected in stool cultures. Twenty-two (8.1%) of food handlers were positive for Widal test.

Conclusion: The findings of high prevalence of enteric pathogens among food handlers in the present study indicate the hygiene practice of the food-handlers working in catering establishments of the study site is very poor. Education and training in good-hygiene practices should be provided to all food-handling personnel which are effective means of preventing the transmission of enteric pathogens from food-handling personnel via food to consumers.

Key words: Food handlers, *salmonella*, *shigella*, intestinal parasites, Hawassa, Ethiopia

CHAPTER I: INTRODUCTION

1.1. General Introduction

Food borne diseases remain a major public health problem across the globe. 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 (Zeru and Kumie, 2007).

Transmission of intestinal parasites and enteropathogenic bacteria is affected directly or indirectly through objects contaminated with faeces. These include food, water, nails, and fingers, indicating the importance of faecal-oral human-to-human transmission (Gashaw *et al.*, 2008). Over 70 species of protozoan and helminthic parasites can infect humans through food and water contamination (Ayeh *et al.*, 2009).

The risk of food borne disease is substantially heightened by biological and chemical contamination of areas where food is produced, processed and consumed (Käferstein and Abdussalam, 1999).

Food handlers may be infected by a wide range of enteropathogens and have been implicated in the transmission of many infections to the public in the community and to patients in hospitals (Khurana *et al.*, 2008). Published information about food borne pathogens among food handlers is scarce in Ethiopia. Therefore, this study is important to assess prevalence of *Salmonella*, *Shigella* and Intestinal parasites among food handlers in catering establishment in Hawassa University, Hawassa, Ethiopia

1.2. Literature Review

1.2.1. Operational Definitions

a. Salmonellosis

Clinical syndromes caused by *Salmonella* infection in humans are divided into typhoid fever, caused by *Salmonella typhi* and *Salmonella paratyphi*, and a range of clinical syndromes, including diarrhoeal disease, caused by a large number of *non-typhoidal salmonella* serovars (NTS)(Gordon, 2008).

b. Shigellosis

Shigellosis is an acute invasive enteric infection caused by bacteria belonging to the genus *Shigella*; it is clinically manifested by diarrhea that is frequently bloody. Shigellosis is endemic in many developing countries and also occurs in epidemics causing considerable

morbidity and mortality. Among the four species of *Shigella*, *Shigella dysenteriae type 1* (*Sd1*) is especially important because it causes the most severe disease and may occur in large regional epidemics. Major obstacles to the control of shigellosis include the ease with which *Shigella* spreads from person to person and the rapidity with which it develops antimicrobial resistance (WHO, 2005).

c. Intestinal parasitosis

Intestinal parasitic infections are among the major problems of public health in developing countries. Parasitic infection is associated with stunting of linear growth, physical weakness and low educational achievement in patients, especially children (Akhlaghi *et al.*, 2009).

1.2.2. Etiologic agents

a. *Salmonella*

Salmonella species are Gram-negative, flagellated facultative anaerobic bacilli characterized by O, H, and Vi antigens. Currently, there are over 2,500 identified serotypes of *Salmonella* (Foley and Lynne, 2008).

b. *Shigella*

Shigellae are Gram-negative, non motile, facultative anaerobic, non-spore-forming rods. *Shigellae* are differentiated from the closely related *Escherichia coli* on the basis of pathogenicity, physiology (failure to ferment lactose or decarboxylate lysine) and serology. The genus is divided into four serogroups with multiple serotypes: A (*Sh. dysenteriae*, 12 serotypes); B (*Sh. flexneri*, 6 serotypes); C (*Sh. boydii*, 18 serotypes); and D (*Sh. sonnei*, 1 serotype) (Thomas and Gerald, 2000).

c. Intestinal parasites

Protozoa are microscopic unicellular eukaryotes that have a relatively complex internal structure and carry out complex metabolic activities. Some protozoa have structures for propulsion or other types of movement. Most parasitic protozoa in humans are less than 50 µm in size. The smallest (mainly intracellular forms) are 1 to 10 µm long, but *Balantidium coli* may measure 150 µm. Protozoa are unicellular eukaryotes. Helminth is a general term meaning worm. The helminths are invertebrates characterized by elongated, flat or round bodies. In medically oriented schemes the flatworms or platyhelminths (platy from the Greek root meaning "flat") include flukes and tapeworms. Roundworms are nematodes (nemato from the Greek root meaning "thread"). These groups are subdivided for

convenience according to the host organ in which they reside, e.g., lung flukes, extraintestinal tapeworms, and intestinal roundworms (Thomas and Gerald , 2000).

The most common forms of helminthiasis are infections caused by intestinal helminthes, ascariasis, trichuriasis and hookworm, followed by schistosomiasis and the major protozoan species that affect humans are *Entamoeba histolytica*, *Acanthamoeba sp.*, *Neogleria sp.* *Giardia intestinalis*, *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Toxoplasma gondii*, *Isospora/Sarcocystis sp.* *Encephalitozoon intestinalis* and *Enterocytozoon bieneuisi*. These parasites exist in the environment as oocyst, cysts or spores, which are the transmissive stages in many environmental conditions, e.g. water, soil, food as well as being infective stages to subsequent generation of hosts ((Siński, 2003; Wang *et al.*,2008).

1.2.3. Epidemiology

a. Salmonellosis

Salmonellosis is a worldwide health problem. Approximately 95% of cases of human salmonellosis are associated with the consumption of contaminated products such as meat, poultry, eggs, milk, seafood, and fresh produce (Foley and Lynne, 2008). Risk factors for salmonellosis include gastric hypoacidity, recent use of antibiotics, extremes of age, and immunosuppressive conditions (Crum-Cianflone, 2008). During a one-year period, 283 food handlers in Irbid, Jordan were investigated for the presence of potential enteropathogens in their stools. The isolation rate of *salmonella* was 6% (Al-Lahham *e t al.*, 1990). Another study showed in two hospitals in Winchester, Southern England showed that Faecal screening of asymptomatic catering staff demonstrated 12.3% *salmonella* (Dryden *et al.*, 1994). Prevalence of chronic typhoidal *Salmonellae* carriers among food vendors in Kumasi, Ghana showed that Typhoidal *Salmonellae* were isolated from six people out of 258, giving a carriage rate of 2.3%.and three of the *Salmonellae* isolated were *S. typhi*, and the other three were non-typhoidal *Salmonellae* (Feglo *et al.*, 2004). Another study done in Nigeria showed that *Salmonella spp.* (three *S. typhi* [5.7%], three *S. enteritidis* [5.7%] and one *S. choleraesuis* (1.9%) were recovered from seven (13. 2%) of the 53 stool samples processed (Smith *et al.*, 2008).A study conducted in Gonder town showed that *Salmonella spp* was not detected in stool sample of food handlers. (Gashaw *et al.*, 2008)

On the other hand Prevalence study in Bahir dar town showed that six (1.6%) food handlers were found positive for *S. typhi* (Abera *et al.*, 2010).

b. Shigellosis

Annually, there are 165 million cases of shigellosis resulting in 1.1 million deaths in the developing world (Michael *et al.*, 2008). The most frequently reported factor associated with the involvement of the infected worker was bare hand contact with the food followed by failure to properly wash hands, inadequate cleaning of processing or preparation equipment or utensils, cross-contamination of ready-to-eat foods by contaminated raw ingredients. Many of the workers were asymptomatic shedders or had infected family members and/or used improper hygienic practices (Todd *et al.*, 2007). During a one-year period, 283 food handlers in Irbid, Jordan were investigated for the presence of potential enteropathogens in their stools. The isolation rate of *shigella* was 1.4% (Al-Lahham *et al.*, 1990).

Study done in Gonder town showed that *Shigella* species were isolated from stool samples of four food-handlers (3.1%) out of 127 food handlers (Gashaw *et al.*, 2008).

c. Intestinal parasitosis

About one third of the world, more than two billion people, is infected with intestinal parasites (Mehraj *et al.*, 2008). The majority of food- and water-borne infections of parasitic origin are influenced by many factors, but the most important are poverty, food habits, high population growth and low sanitation, and lack of health awareness (illiteracy) (Todd *et al.*, 2007).

Intestinal parasites detected in the stools of food handlers in Irbid, Jordan included *Ascaris lumbricoides* (4.9%), *Giardia lamblia* (3.9%), *Schistosoma mansoni*, (2.8%), hookworm (2.5%), *Hymenolyepis nana* (1.8%), *Trichuris trichiura* (1.1%), *Entamoeba histolytica/dispar* (0.7%), and *Taenia saginata* (0.4%)(Al-Lahham *et al.*, 1990).

The prevalence of intestinal parasites detected in the stools of the food-handlers in Gonder town was 29.21% and identified parasites were *Ascaris lumbricoides* (18.11%), *Strongyloides stercoralis* (5.5%), *Entamoeba histolytica/dispar* (1.6%), *Trichuris trichiura* (1.6%), hookworm species (0.8%), *Giardia lamblia* (0.8%), and *Schistosoma mansoni* (0.8%); 1.6% of the study subjects were positive for each of *A. lumbricoides*, *T. trichiura*, hookworm, and *G. lamblia*. The findings emphasize the importance of food-handlers as potential sources of infections and suggest health institutions for appropriate hygienic and sanitary control measures (Gashaw *et al.*, 2008).

1.2.4. Pathogenesis and Pathology

Pathogenic *salmonellae* ingested in food can survive passage through the gastric acid barrier and invade the mucosa of the small and large intestine and produce toxins. Invasion of epithelial cells stimulates the release of pro-inflammatory cytokines which induce an inflammatory reaction. The acute inflammatory response causes diarrhea and may lead to ulceration and destruction of the mucosa. The bacteria can disseminate from the intestines to cause systemic disease.

Shigellosis is initiated by ingestion of *shigellae* (usually via fecal-oral contamination). An early symptom, diarrhea (possibly elicited by enterotoxins and/or cytotoxin), may occur as the organisms pass through the small intestine. The hallmarks of shigellosis are bacterial invasion of the colonic epithelium and inflammatory colitis. These are interdependent processes amplified by local release of cytokines and by the infiltration of inflammatory elements. Colitis in the rectosigmoid mucosa, with concomitant malabsorption, results in the characteristic sign of bacillary dysentery: scanty, unformed stools tinged with blood and mucus.

Protozoal infection results in tissue damage leading to disease. In chronic infections the tissue damage is often due to an immune response to the parasite and/or to host antigens as well as to changes in cytokine profiles. Alternatively, it may be due to toxic protozoal products and/or to mechanical damage. Many infections by helminthes are asymptomatic; pathologic manifestations depend on the size, activity, and metabolism of the worms. Immune and inflammatory responses also cause pathology (Thomas and Gerald, 2000).

1.2.5. Clinical features and complications

a. Salmonellosis

Salmonellosis in older children and adults is usually a self-limited disease, but the risk of complications in infants is not well-defined. A retrospective review of 52 patients, 90 days of age or less, seen at the St. Louis Children's Hospital between 1975 and 1981 with stool cultures positive for *Salmonella*. Sixteen were 30 days old or less (neonates), 21 were 31- 60 days of age, and 15 were 61-90 days old. Among patients in whom blood cultures were done initially, bacteremia was most frequent in neonates: 5/11 (45%), compared to 2/18 (11%) in older infants. All seven infants presenting with bacteremia received 10 or more days of antibiotic therapy; yet complications (osteomyelitis, fatal meningitis or chronic diarrhea)

developed in three of five neonates and one of two older infants. Complications also developed in seven of 22 patients who initially had negative blood cultures, including two infants in whom sepsis later developed and two infants who required intravenous hyperalimentation because of chronic diarrhea and malnutrition. The group of 23 patients who did not have blood cultures all did well. Salmonellosis is not necessarily a self-limited infection in young infants. Even in the absence of bacteremia, clinicians would appear to be justified in using antimicrobial therapy in infants 3 months of age or less with salmonella gastroenteritis. (Nelson and Granoff, 1982)

Typhoid fever is difficult to differentiate clinically from other causes of fever, because its clinical presentation consists of non-specific symptoms such as fever, chills, headache, malaise, anorexia, nausea, abdominal discomfort, a dry cough or myalgia. In the later phase of illness, more specific physical signs such as rose spots and splenomegaly may be observed (Vollaard *et al.*, 2005).

b. Shigellosis

Clinical presentations of shigellosis are watery diarrhea occurring in younger children and associated with a shorter duration of illness and with more vomiting and dehydration and dysentery with stool blood and abdominal pain. These different presentations may reflect two mechanisms in the pathogenesis of shigellosis or different stages of the disease. The most useful signs and symptoms for the diagnosis of shigellosis were stool with blood and abdominal pain in all patients and the absence of watery diarrhea and vomiting in patients over one year old. Simple visual inspection of stool for blood correctly identified 44% of all patients infected with *Shigella* (Stoll *et al.*, 1982).

c. Intestinal parasitosis

Clinical features of helminthiases vary a lot depending on the helminth species, intensity of infection, and host age. *Taenia solium* can cause neurocysticercosis with mass lesions in brain. Ingested eggs of *Echinococcus granulosus* will lead to cysts in the liver and cause life-threatening anaphylaxis if antigens are released from the cysts. Chronic infection with *schistosoma* causes granulomas, fibrosis, and inflammation of the spleen and liver. Hookworm and *schistosoma* can infect pregnant women, cause neonatal prematurity and increased maternal morbidity and mortality (Wang *et al.*, 2008).

1.2.6. Diagnosis

a. Salmonellosis and Shigellosis

In laboratory, the diagnosis of salmonellosis depends on demonstrating the pathogen in blood, bone marrow, stool or urine cultures. However, bacteriological methods are time consuming and usually require 5-11 days. Additionally, in developing countries like Pakistan sensitivity of blood culture is lowered due to irrational use of antibiotics. The Widal test; a serologic test has a number of limitations including failure to diagnose *S. Paratyphi A* infection. Polymerase chain reaction (PCR), in addition to analysis of foods, has also been successfully applied to the detection and identification of pathogenic organisms in clinical and environmental samples. It has been successfully used for diagnosis of *S. Typhi* and proved superior to conventional methods. A similar approach for diagnosis of *S. Paratyphi A* can be of great help (Ali *et al.*, 2008).

The most useful signs and symptoms for the diagnosis of shigellosis are stool with blood and abdominal pain in all patients and the absence of watery diarrhea and vomiting in patients over one year old (Stoll *et al.*, 1982). The four *Shigella* species (*Sh. dysenteriae*, *Sh. flexneri*, *Sh. boydii*, and *Sh. sonnei*) are classically identified by culture of fecal specimens on selective media and testing of isolates for agglutination in species-specific antisera (Echeverria *et al.*, 1991).

The presence of many enteropathogens which are not easily detectable by routine stool culture has led to the development of alternative diagnostic methods. One of these techniques, nucleic acid probe hybridization, has been used to identify *Shigella spp.* and entero invasive *Escherichia coli* (EIEC) in stool specimens through the detection of genetic material encoded by a specific large approximately 200-kbp virulence-related plasmid (Oberhelman *et al.*, 1993).

b. Intestinal parasitosis

Most helminthes and protozoa exit the body in the fecal stream. Because of the cyclic shedding of most parasites in the feces, a minimum of three samples collected on alternate days should be examined. When delays in transport to the laboratory are unavoidable, fecal samples should be kept in polyvinyl alcohol to preserve protozoal trophozoites. Refrigeration will also preserve trophozoites for a few hours and protozoal cysts and helminthic ova for several days. Analysis of fecal samples consists of both a macroscopic

and a microscopic examination. Watery or loose stools are more likely to contain protozoal trophozoites, but protozoal cysts and all stages of helminths may be found in formed feces. Fewer antibody assays are available for the diagnosis of infection with intestinal parasites. *E. histolytica* is the major exception. Sensitive, specific serologic tests are invaluable in the diagnosis of amebiasis.

Commercial kits for the detection of antigen by enzyme-linked immunosorbent assay or of whole organisms by fluorescent antibody assay are now available for several protozoan parasites. DNA hybridization with probes that are repeated many times in the genome of a specific parasite and amplification of a specific DNA fragment by the polymerase chain reaction (PCR) is promising techniques for the diagnosis of parasitic infections (Charles, 2004).

1.2.7. Treatment

The antimicrobials most widely regarded as optimal for the treatment of salmonellosis in adults is the group of fluoroquinolones. They are relatively inexpensive, well tolerated, have good oral absorption and are more rapidly and reliably effective than earlier drugs. The earlier drugs chloramphenicol, ampicillin and amoxicillin and trimethoprim-sulfamethoxazole are occasionally used as alternatives (WHO, 2005).

Shigellosis is known to produce protracted epidemics and pandemics and is usually multi-drug resistant. Antibiotics are the mainstay of therapy of all cases of shigellosis. Antibiotics such as tetracycline, ampicillin and co-trimoxazole, were previously highly effective. Newer fluoroquinolones such as norfloxacin, ciprofloxacin, ofloxacin, azithromycin and ceftriaxone are effective. Although single dose of norfloxacin 800 mg and ciprofloxacin 1 g have been shown to be effective, they are currently less effective against *S. dysenteriae type 1* infection. Oral rehydration salt should be given concurrently to prevent or correct dehydration. Antimotility agents are contraindicated. Feeding during and after shigellosis is emphasized. Hand-washing practices with plenty of water and soap help to prevent the transmission of infection from person to person. A search is on for an effective vaccine against *shigella* (Bhattacharya and Sur, 2003).

Intestinal parasitic infections rank among the most significant causes of morbidity and mortality in the world, yet economic and other factors have contributed to a lack of innovation in treating these diseases. Nitazoxanide, a pyruvate ferredoxin oxidoreductase

inhibitor, is a new antiparasitic drug notable for its activity in treating protozoan infections, including *Cryptosporidium*. Importantly, studies have shown that nitazoxanide is also effective in treating common intestinal helminths.

The availability of a product with this spectrum of activity raises interesting new possibilities for treating intestinal parasitic infections. In a field characterized by lack of innovation, a new broad-spectrum antiprotozoal and anthelmintic drug offers interesting possibilities for managing intestinal parasitosis (Gilles and Hoffman, 2002).

1.2.8. Prevention

In many urban centers, eating and drinking in public establishments, such as Hotels, Restaurants, and Snack bars is a common practice in many countries. These establishments prepare, handle, and serve large quantities of food and drink to large groups of people within a short period of time implying a possible risk of infections if sanitary and hygienic norms are not strictly followed. The world health status review indicates that the health problem of developing nations is mainly linked to inadequate sanitation (Kumie *et al.*, 2002).

Better education of food industry workers in basic food safety and restaurant inspection procedures may prevent cross-contamination. Food handling errors can lead to outbreaks. Improvements in farm animal hygiene, in slaughter plant practices, and in vegetable and fruit harvesting and packing operations may help prevent salmonellosis caused by contaminated foods. Pasteurization of milk and treatment of municipal water supplies are highly effective prevention measures that have been in place for decades. Wider use of pasteurized egg in restaurants, hospitals, and nursing homes is an important prevention measure. In the future, irradiation or other treatments may greatly reduce contamination of raw meat (CDC, 2008).

Prevention of dysentery caused by *Shigella* relies primarily on measures that prevent spread of the organism within the community and from person to person. These include: hand-washing with soap, ensuring the availability of safe drinking water, safely disposing of human waste, breastfeeding of infants and young children, safe handling and processing of food, and control of flies. These measures will not only reduce the incidence of shigellosis, but of other diarrheal diseases as well. In all cases, health education and the cooperation of the community in implementing control measures are essential (WHO, 2005).

Intestinal parasitic infections are more prevalent among the poor sections of population. They are closely associated with low household income, poor personal and environmental sanitation, and overcrowding, limited access to clean water, tropical climate and low altitude. The world health status review indicates that the health problem of developing nations is mainly linked to inadequate sanitation (Gilles and Hoffman, 2002). Education on personal and environmental hygiene should be taken into account to reduce the prevalence of intestinal parasitosis (Mengistu *et al.*, 2007).

1.2.9. Significance of the proposed study

Annually, there are 165 million cases of shigellosis resulting in 1.1 million deaths in the developing world (Michael *et al.*, 2008). About one third of the world, more than two billion people, is infected with intestinal parasites (Mehraj *et al.*, 2008).

Approximately 95% of cases of human salmonellosis are associated with the consumption of contaminated products such as meat, poultry, eggs, milk, seafood, and fresh produce (Foley and Lynne, 2008). Study done in two hospitals in Winchester, southern England showed that faecal screening of asymptomatic catering staff demonstrated 12.3% *salmonella* (Dryden *et al.*, 1994). Prevalence of chronic typhoidal *Salmonellae* carriers among food vendors in Kumasi, Ghana showed that *Typhoidal Salmonellae* were isolated from six people out of 258, giving a carriage rate of 2.3% and three of the *Salmonellae* isolated were *S. typhi*, and the other three were *non-typhoidal Salmonellae* (Feglo *et al.*, 2004).

Other study done in Nigeria showed that *Salmonella spp.* three *S. typhi* [5.7%], three *S. enteritidis* [5.7%] and one *S. choleraesuis* (1.9%) were recovered from seven (13. 2%) of the 53 stool samples processed (Smith *et al.*, 2008).

Study done in Ethiopia, Gonder town showed that *Shigella* species were isolated from stool samples of four food-handlers (3.1%) out of 127 food handlers and the prevalence of Intestinal parasites detected in the stools of the food-handlers was 29.21% and identified parasites were *Ascaris lumbricoides* (18.11%), *Strongyloides stercoralis* (5.5%), *Entamoeba histolytica/dispar* (1.6%), *Trichuris trichiura* (1.6%), *hookworm species* (0.8%), *Giardia lamblia* (0.8%), and *Schistosoma mansoni* (0.8%); 1.6% of the study subjects were positive for each of *A. lumbricoides*, *T. trichiura*, *hookworm*, and *G. lamblia* (Gashaw *et al.*, 2008).

The findings mentioned above emphasize food-handlers serves as potential sources for food borne pathogens and suggest health institutions for appropriate hygienic and sanitary control measures. Therefore, this study was undertaken to assess prevalence of *Salmonella*, *Shigella* and Intestinal parasites among food handlers in catering establishments in Hawassa University, Hawassa, Ethiopia.

1.2.10. Hypothesis

The prevalence of the pathogens isolated from food handlers in catering establishments in Hawassa, Ethiopia could be similar to those isolated in other food establishments elsewhere.

1. 3. Objectives of the study

General objective

- To determine the prevalence of *Salmonella*, *Shigella* and Intestinal parasites among food handlers in catering establishments in Hawassa University, Hawassa , Ethiopia

Specific Objectives

- To identify *Salmonella*, *Shigella* and intestinal parasites among food handlers working in cafeterias and kitchens of Main Campus and Health Science College of Hawassa University.
- To study the antibiotic sensitivity of the isolated *Salmonella* and *Shigella* spp. to certain selected antimicrobial agents.

CHAPTER II: MATERIALS AND METHODS

2.1. Study design and area

Cross sectional study was conducted from February 2010 through April 2010 at different catering establishments in Hawassa University, Hawassa town, Ethiopia.

Hawassa town is the capital of SNNPR, is about 275 Km away from Addis Ababa. The total population of Hawassa town is 130,579 with 1:1 male to female ratio.

Hawassa University enrolls a total of 13,653 students in four campuses; Main campus, Health Science college, Agricultural college and Wondogenet forestry college. The majority of students (88%) of the university are in Main campus and Health Science College. Eight food-service establishments are found in Main Campus and Health Science College of Hawassa University including cafeterias' of the Hawassa referral Hospital.

2.2. Study Population

During the study period all 272 food handlers working in catering establishments in Main Campus and Health Science College of Hawassa University were screened for enteric pathogens.

The sample size (n) is calculated, assuming that the prevalence of shigella in food handlers was 13.3% (Khurana et al., 2008). The expected margin of error (d) was 0.05 and the confidence interval ($Z\alpha/2$) was 95%. The formula used to calculate the sample size was:

$$n = \frac{(Z\alpha/2)^2 * P(1-P)}{d^2} = \frac{(1.96)^2 * 0.13(1-0.13)}{(0.05)^2} = 173.8$$

Where; n= Sample size; Z= confidence interval; α = level of significance; d= tolerable error; P= prevalence.

The minimum sample size with 10% contingency with unknown circumstance was 191.

Food-handlers who did not take treatment for any intestinal ailment within the three months prior to the study were included.

Written informed consent was obtained from every study participant. Demographic and laboratory data were obtained by principal investigator and transferred to the questionnaire prepared for this study (see appendix I).

2.3. Sample Collection and Handling

a. Stool

Stool specimens were obtained from food handlers and put into screw capped containers and transported to Microbiology Laboratory of Hawassa Referral hospital for microscopic examination and culture.

b. Blood

Two ml of venous blood was collected by principal investigator from food handlers for Widal test.

2.4. Culture and identification of *Salmonella* and *Shigella* spp.

All stool specimens were inoculated into selenite F broth (Oxoid, UK) and incubated for 24 hours at 37°C followed by subculture on xylose-lysine-deoxychocolate agar (XLD) (Oxoid, UK) at 37°C for 24 hours for isolation of *Shigella* and *Salmonella* species. The bacteria were identified by their characteristic appearance on their respective media and confirmed by the pattern of biochemical reaction used for identification of enterobacteriaceae.

2.5. Stool examination for ova and parasites

Microscopic examination of stool specimens were done using direct wet mount at collection sites and formol-ether concentration method for detection of ova and parasites.

2.6. Widal test

Widal test was done using *S. typhi* O and H antigens according to the manufacturer's instruction. In brief, the test was done by mixing one drop of serum with one drop each of O and H antigens separately on glass slide. After rocking the slide back and forth, the mixture was observed for macroscopic agglutination. If there was agglutination within one minute it was reported as positive, otherwise as negative.

2.7. Reference Strains

Reference strain of *E. coli* (ATCC 25922) was used as a quality control for culture.

2.8. Statistical Analysis

Data entry and analysis was done using computer with SPSS version 15 software. Prevalence figures were calculated for the total study population and separately by sex and age groups. Chi-square test was used as to compare results between the sexes and with the previous findings from the literature. A p-value less than 0.05 was considered statistically significant.

2.9. Ethical consideration

This M.Sc. research project proposal was approved by the Department Research and Ethical Review Committee and ethically cleared by Institutional Review Board (IRB), Faculty of Medicine; Addis Ababa University. Official permission from the study site was obtained. Written informed consents were obtained from study participants (see appendix III). Food handlers who were found to be positive for enteric pathogens were referred to Hawassa University Referral Hospital for appropriate antimicrobial treatment and advised on good hygienic practice when handling food.

CHAPTER III: RESULTS

3.1. Study population

The age and sex distribution of food handlers are presented in Table 3.1. The study included 272 food-handlers. Majority of the food-handlers (81.3%) were young adults aged 20-40 years. The number of female food handlers (68.8%) was almost twice compared to the male food handlers (31.3%). One hundred eighty one (66.5%) food handlers had education above elementary school and only nineteen (7%) of the food-handlers did not have the habit of hand-washing before touching food. The majority (60.3%) of food handlers had served for 1-5 years.

Table 3.1. Sociodemographic characteristics of food-handlers, Hawassa University, Hawassa, Ethiopia (February -April 2010)

Characteristics	Frequency	%
Age(years)		
<20	20	7.4
20-40	221	81.3
>40	31	11.4
Sex		
Male	85	31.3
Female	187	68.8
Educational Status		
Illiterate	14	5.1
Read and Write	11	4.0
1-6 grade	66	24.3
7-10 grade	145	53.3
11-12 grade	26	9.6
>12 grade	10	3.7
Service year		
<1year	56	20.6
1-5 years	164	60.3
6-10years	36	13.2

11-20years	12	4.4
>20years	4	1.5
Hand washing practice before touching food		
Yes	253	93
No	19	7

3.2. Etiologic agents

a) *Shigella and Salmonella*

Out of 272 food-handlers screened, stool cultures revealed only one (0.4%) *Shigella* species. No *Salmonella* species was isolated from any of the stool samples obtained from food handlers. Widal test result showed that 22(8.1%) food handlers were positive for O or/and H antigens of *Salmonella typhi*.

b. Intestinal parasites

Direct microscopic and concentration techniques were used for identifying intestinal parasites from the 272 stool specimens. Fifty-six (20.6%) stool specimens were positive for different intestinal parasites. *A. lumbricoides* was the most prevalent parasite (9.6%), followed by *S. stercoralis* (2.2%) and *E. histolytica/dispar* (2.2%). Five (1.8%) stool specimens were positive for two parasites each (Table 3.2.). Frequency of parasitic infection among food handlers by sex, age, educational status, and service year and hand hygiene is presented in Table 3.3.

Table 3.2. Type and prevalence of intestinal parasites isolated from stool specimens of food handlers, Hawassa University, Hawassa, Ethiopia (February -April 2010)

Parasites	Frequency	%
<i>Ascaris lumbricoides</i>	26	9.6
<i>Strongyloides stercoralis</i>	6	2.2
<i>Giardia lamblia</i>	3	1.1
<i>Entamoeba histolytica/dispar</i>	6	2.2
<i>Hook worm</i>	4	1.5
<i>Taenia spp.</i>	1	0.4

<i>Schistosoma mansoni</i>	5	1.8
<i>Ascaris lumbricoides</i> and <i>Trichuris trichiura</i>	2	0.7
<i>Ascaris lumbricoides</i> and <i>Strongyloides stercoralis</i>	1	0.4
<i>Ascaris lumbricoides</i> and <i>Taenia spp.</i>	1	0.4
<i>Ascaris lumbricoides</i> and <i>Schistosoma mansoni</i>	1	0.4
No ova or parasite	216	79.4

Table 3.3. Frequency of parasitic infection among food handlers by sex, age, educational status, service year and hand hygiene, Hawassa University, Hawassa, Ethiopia (February -April 2010)

Variable	Number of examined	Number of infected	%
Sex			
Male	85	15	17.6
Female	187	41	21.9
$\chi^2=0.65, df = 1, P =0.419$			
Age(years)			
<20	20	6	30.0
20-40	221	45	20.4
>40	31	5	16.1
$\chi^2=1.47, df = 2, P =0.48$			
Educational status			
Illiterate	14	1	7.1
Read & Write	11	7	63.6
1-6 grade	66	22	33.3
7 -10 grade	145	25	17.2
11-12 grade	26	1	3.8
Above 12	10	0	0
$\chi^2=28.62, df = 5, P =0.000$			
Service year			
< 1year	56	9	16.1

1-5 year	164	40	24.4
6-10 year	36	6	16.7
11-20 year	12	1	8.3
>20 year	4	0	0
$\chi^2=4.63, df = 4, P =0.328$			
Hand washing before touching food			
Yes	253	53	20.9
No	19	3	15.8
$\chi^2=0.29, df = 1, P =0.592$			

CHAPTER IV: DISCUSSION

Food handlers may be infected by a wide range of enteropathogens and have been implicated in the transmission of many infections to the public in the community and to patients in hospitals (Khurana *et al.*, 2008). The spread of disease via food handlers is a common and persistent problem worldwide. Published information about food born pathogens among food handlers is scarce in Ethiopia (Gashaw *et al.*, 2008). Therefore, this study was undertaken to assess prevalence of *Salmonella* and *Shigella* and intestinal parasites among food handlers in catering establishment in Hawassa University, Hawassa, Ethiopia.

In this study, 0.4%, 8.1% and 20.6% of the 272 food handlers were positive for *Shigella*, *Salmonella typhi* and intestinal parasites, respectively (Table 3.2). These indicate the hygiene practice of the food-handlers working in catering establishments of the study site is poor.

The high 20.6% prevalence of intestinal parasites in the stools of the food-handlers in this study was in agreement with the findings of other studies conducted elsewhere e.g. in Accra, Ghana (21.6%) (Ayeh *et al.*, 2009), Irbid, Jordan (18.1%) (Al-Lahham *et al.*, 1990), Jeddah, Saudi Arabia (13.5%) (Salem, 1998), Aydin, Turkey (29.31%) (Yazici *et al.*, 2007) Gonder, North west Ethiopia (29.1%) (Gashaw *et al.*, 2008), Khartoum, Sudan (30.5%) (Babiker *et al.*, 2009) and Egypt (19%) (Sadek, *et al.*, 1997) Studies in different part of the world also showed higher and lower prevalence of intestinal parasites in the stools of food handlers compared to the present study. Higher prevalences of intestinal parasites were reported in Hawassa, Ethiopia (63%) (Teklemariam *et al.*, 2000), Abeokuta, Nigeria (97%) (Idowu and Rowland,2006), Uberlândia, Brazil (47.1%)(Costa *et al.*, 1995) and Sanliurfa, Southeastern Anatolia(52.2%)(Simsek *et al.*, 2009). Lower prevalences were reported in Omdurman, Sudan (6.9%) (Saeed and Hamid , 2010), Manisa, Turkey (8.8%) (Gündüz *et al.*, 2008). High prevalence of intestinal parasites is largely due to poor personal hygiene practices and environmental sanitation and ignorance of health-promotion practices.

Among intestinal parasites, *A. lumbricoides* was the most common parasites isolated alone or in combination with other parasites (11.4%) from food handlers. Similar finding has been reported in previous study conducted in Ethiopia (18.11%) (Gashaw *et al.*, 2008).

Another study conducted in Irbid, Jordan, *A. lumbricoides* was the leading intestinal parasite detected in stool samples of food handlers (4.9%) (Al-Lahham *et al.*, 1990).

In the present study only one *Shigella* spp. (0.4%) was isolated from stool culture of food handlers. Low prevalence of *Shigella* spp. in food handlers was also reported in some studies e.g. in Irbid, Jordan (1.4%) (Al-Lahham *et al.*, 1990), Omdurman, Sudan (1.3%) (Saeed and Hamid, 2010) and Ethiopia (3.1%) (Gashaw *et al.*, 2008). In other studies, no *shigella* recovered from stool specimens of food handlers' (Malhotra *et al.*, 2006; Simsek *et al.*, 2009). However, *Shigella* was the most common bacteria isolated among food handlers in a tertiary care hospital of North India (13.3%) (Khurana *et al.*, 2008)

In this study, no *salmonella* spp. recovered from stool culture of all food handlers, even though 8.1% of them were positive for Widal test. Similar finding has been reported in previous study conducted in Ethiopia (Gashaw *et al.*, 2008), North India (Malhotra *et al.*, 2006), Southeastern Anatolia (Simsek *et al.*, 2009). Studies done elsewhere showed the prevalence of salmonellosis among food handlers ranges from 0.032-11% (Murakami *et al.*, 2007, Abera, *et al.*, 2010, Saeed and Hamid, 2010, Vaeteewootacharn *et al.*, 2008).

No statistically significant association was found between the frequency of parasite infection and age, sex, service year and hand washing before touching food described in Table 3.3. Similarly, finding in Khartoum, Sudan showed that no statistical significant association was observed particularly parasitic infection comparable for age, sex and service year (Babiker *et al.*, 2009). The present study showed an equal distribution of parasitic infection among all ages, both sexes and among all service years of food handlers.

The prevalence of parasite infections among illiterate (7.1%) food handlers was lower than compared to the prevalence of parasite infections among food handlers grouped under read and write (63.6%), 1-6 grade (33.3%) and 7-10 grade (17.2%). This may be due to low sample size in each categories of educational status.

Limitations of the Study

- Sero-grouping on *Shigella* isolate was not done and Vi antigen for Widal test was not used.
- The study was conducted on two campuses (Main campus and Health Science College) out of four campuses of Hawassa University.
- Finger nails samples were not collected. Testing of samples from finger nails supports the idea of contamination by enteropathogens due to inadequate hand-washing of the food-handlers.

Conclusion and Recommendations

In the present study, 272 food handlers working in catering establishments in Main Campus and Health Science College of Hawassa University were screened for enteric pathogens. Majority of the food-handlers (81.3%) were young adults aged 20-40years. Stool cultures revealed *Shigella* species in 0.4% food-handlers. No *Salmonella* species was isolated from any of stool culture obtained from food handlers. However, 8.1% food handlers were positive for Widal test. 20.6% stool specimens were positive for different intestinal parasites. In conclusion the finding of high prevalence of intestinal parasites among food handlers in the present study indicates the poor hygiene practice of the food-handlers working in catering establishments at the study site. Such infected food handlers can contaminate food and drinks and serve as source of infection to consumers via food chain.

Based on the findings of the present study, the following recommendations are made: -

- Good personal hygiene and hygienic food-handling should be exercised by food handlers.
- Education and training in good-hygiene practices should be provided to all food-handling personnel.
- Food handlers should be screened for enteric pathogens at regular basis for proper management.

All are effective means of preventing the transmission of enteric pathogens from food-handling personnel via food to consumers.

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Appendix I: Questionnaire

Questionnaire for investigation of the prevalence of Salmonella, Shigella and Intestinal parasites among food handlers in catering establishment in Hawassa University, Hawassa, Ethiopia

A. participant Identification

1. Full name : _____ Code _____
2. Sex: Female Male
3. Age (in years) : <20 0 – 40 > 4
4. Educational status:
 Illiterate read and write 1-6 grade 7-10 grade 11-12 grade > 12 grade.
5. Job position in the catering establishment
 Cook cleaning utensils and waiter others/specify _____
6. Year/s of service:
 <1 1-5 6-10 11-20 >20
7. Hand-washing practice before touching food: es No
8. History on previous antibiotic and anti parasitic treatment es No
If yes, mention antibiotic/s or anti parasitic _____

B. Laboratory Data

1. Parasite isolated from stool:
 A.lumbricoid *stercoralis* *Giardia lamblia*
 T.trichuria *hookworm species* *Entamoeba histolytica/dispar*
 Others/specify _____
2. Culture Result of stool _____
3. Widal test Result _____
4. Antimicrobial susceptibility testing

	S (mm)	I (mm)	R (mm)
• Ampicillin	-----	-----	-----
• Ciprofloxacin	-----	-----	-----
• Ceftriaxone	-----	-----	-----
• Erythromycin	-----	-----	-----
• Gentamicin	-----	-----	-----
• Norfloxacin	-----	-----	-----
• Sulfamethoxazole-Trimethoprim	-----	-----	-----

Appendix II: Patient information sheet (English and Amharic version)

1. purpose

Prevalence of *Salmonella*, *Shigella* and Intestinal parasites among food handlers in catering establishments in Hawassa University, Hawassa, Ethiopia.

2. procedures to be carries out

- 2ml of blood is collected from the arm with sterile syringe & needle.
- Stool sample is collected

3. There is no discomfort or risk while collecting stool sample.

There is minor pain during collection of venous blood sample.

4. expected benefit of the study

- the subject can be diagnosed for food borne pathogens
- The study gives information for Hawassa University to work hard to prevent the food borne pathogens among students and people who get food service in the university food service establishments.

5. confidentiality of your information

- records are kept confidential
- You are not mentioned by name
- Your specimens are used only for this study purpose.

6. Voluntary participation

- Your participation is by willingness.
- The participant can with draw study at any time and he/she can't be asked the reason.
- Withdrawal from study doesn't have impact on participant health service.

7. Contact address

- PI : Moges Desta, Mobil 0911-744891
- IRB : Addis Ababa University, Faculty of Medicine,
Phone : 251-011-551-28-765 , P.O.Box 9086

Addis Ababa

... .. ለጥናቱ ተሳታፊዎች

1. የጥናቱ ዓላማ:-

... .. የኢቮርሲቲ በ... .. ላይ በሰውነቶቹ ውስጥ የሳልሞኔላ፣ጂጌላና የሆድ ትላትል መጠን ማወቅ።

2. የተሳትፎ ሁኔታ

ሀ. ሁለት ሚሊ ሊትር ደም ከክንድ ላይ በአዲስ መርፌ መውሰድ።
ለ. ናሙና መውሰድ።

3. ሊከሰቱ ስለሚችሉ ስጋቶችና የምቶት መጓደሎች

... .. ናሙና በሚወሰዱበት ወቅት ምንም ዓይነት ችግር አይከሰትም።
የደም ናሙና በሚወሰድበት ወቅት ግን ደም ለመውሰድ ሲባል በመርፌው ምክንያት ከሚፈጠር አነስተኛ ህመም በስተቀር ሌላ ምንም ዓይነት የምቶት መጓደሎች የሉትም።

4. ጥቅሞች

- የምግብ ወለድ በሽታ መኖሩን ማረጋገጥ።
- የጥናቱ ውጤት ለየኢቮርሲቲው ስለ ሳልሞኔላ፣ጂጌላና የሆድ ትላትል መረጃ በመስጠት የየኢቮርሲቲው ተማሪዎችና ሌሎች ተጠቃሚዎች በምግብ ወለድ በሽታ እንዳይጠቁ አስቀድሞ ለመከላከል ትኩረት ሰጥቶ እንዲሰራ ያደርገዋል።

5. ምስጢር ስለመጠበቅ

- ሊ... .. ይገ...።
- ዱት ዎች •ዚህ ሌሎች ናቸው።
- ።

6. በጥናቱ ያለመሳተፍ ወይም ራስን የማግለል መብት:-

- የርስዎ ተሳትፎ በፈቃደኝነት ላይ የተመሰረተ ነው።
- እርስዎ በጥናቱ ላይ መሳተፍዎትን ለመሰረዝ ከፈለጉ በማንኛውም ሰዓት መሰረዝ ይችላሉ። ይህንንም ከወሰኑ ማንም ምክንያቱን እንዲገልጹ ሊያስገድድዎት አይችልም።
- በጥናቱ ውስጥ መግባት አለመፈለግዎ በጤና ተከታታይነት ላይ ምንም ዓይነት ተፅዕኖ አይኖረውም።

7. መረጃ ስለማግኘት

የተመራማሪው- ሞገስ ደስታ
ሞባይል-0911744891
የኢንስቲትዩሽናል ሪቪው ቦርድ-

.....
..... 9086
... 251-011-551-28-765
.....

Appendix III: Patients consent form (English and Amharic version)

(To be translated in to the patient's language)

Name of study participant: _____

I have read the information sheet (or it has been read to me); I have understood that it involves the study about Prevalence of Salmonella, Shigella and Intestinal parasites among food handlers in catering establishments in Hawassa University, Hawassa, Ethiopia. I will be asked to provide information and samples such as stool and blood for laboratory examination. I have asked some questions and clarification has been given to me. I have given my consent freely to participate in the study.

I _____ hereby give my consent for giving of the requested information and specimens as the medical doctor and the researcher find best for me.

Participants signature _____ Date _____

Investigators signature _____ Date _____

Witness signature 1. _____ Date _____

2. _____ Date _____

.....

..//./.. -----

ስለጥናቱ አስፈላጊ የሆኑትን መረጃዎች አንብቤ ወይም ተነቦልኝ አላማዉም
..... ዩኒቨርሲቲ በ..... የሳልሞኔላ ፤ሺጌላና የሆድ ትላትል መጠን ማወቅ ሲሆን ለጥናቱ አስፈላጊ የሆኑትን መረጃዎችና ናሙናዎች ማለትም

ስገራና ደም ብንደምስጥ ተረድቻለሁ። ከጥናቱ ጋር የተያያዙ ጥያቄዎችንም ጠይቄ ማብራሪያዎችንም ተስጥተዋል።

•• •• _____ •••• •••• •••
•••••ና ••••• ••••• ••••• ••••• •••ና ለተመራማሪው ••••• •••••
•••••
••••• ••••• ----- •••••-----

••••••• ••••• ----- ••••• -----

••••••• ••••• 1. ----- ••••• -----

2. ----- ••••• -----

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been

presented for a degree in any other University and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Moges Desta B. Sc

Signature _____

Date and place of submission _____

Addis Ababa, Ethiopia

1. Supervisor: Dr. Daniel Asrat, MD, M.Sc, PhD

Signature: _____

Date and place _____

Addis Ababa, Ethiopia.

2. Supervisor: Dr. Yimtubezinash W/Amanuel, MD, M.Sc, PhD

Signature: _____

Date and place _____

Addis Ababa, Ethiopia.