PREVALENCE OF *SALMONELLA*, *SHIGELLA* AND INTESTINAL PARASITES AMONG APPARENTLY HEALTHY FOOD HANDLERS OF ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY STUDENTS' CAFETERIA, ADAMA, ETHIOPIA

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THESIS SUBMITTED TO THE SCHOOL OF MEDICAL LABORATORY SCIENCE, FACULTY OF HEALTH SCIENCES, INSTITUTE OF HEALTH, JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER IN MEDICAL MICROBIOLOGY.

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ABSTRACT

Background: *Food borne infections is a common problem among developed and developing countries like Ethiopia. It is estimated that about 30% of the world population is affected by food born disease annually and 2 million deaths were reported per year by World Health organization (WHO). The common etiologic agents of food borne disease are bacteria like Salmonella and Shigella species as well as intestinal parasites. There is a limited data on the causative agents of food born disease as well as the associated risk factors in Adama town, Ethiopia.*

Objective: *To assess prevalence of Salmonella, Shigella and intestinal parasites among apparently healthy food handlers working in Adama Science and Technology University (ASTU) students' cafeteria in Adama town, Southeast of Addis Ababa.*

Method*: A cross sectional study was conducted among 210 apparently healthy individuals, from April to June 2018. Socio-demographic and related data were collected using structured questionnaire. Stool samples were collected and examined for intestinal parasite using wet mount and formol ether concentration technique. In addition stool was processed for culture to isolate and identify Salmonella and Shigella species using MacConkey and XLD medias and standard biochemical testing media. Antimicrobial susceptibility test was performed using disk diffusion method. Statistical analysis was made for calculating the prevalence and associated risk factors.*

Result: *From the total of 210 participants 200 were females. No Salmonella was isolated and five Shigella species were isolated. All isolates were susceptible to Ciprofloxacin and all of them were resistant to Ampicilin and Cotrimoxazole. Thirteen study participants were positive for different types of intestinal parasites. E. histolitica, G.lamblia, A. lumbricoid ,Tainea spp. were the identified parasites. Hand washing habit before touching/preparing food and hand washing with only water or with water and soap after using toilet were significantly associated with Shigella species infection. But none of risk factors was associated with prevalence of intestinal parasite.*

Conclusion: *Prevalence of Shigella species and intestinal parasites were observed on few apparently healthy food handles. To reduce bacterial and intestinal parasitic infection as well as drug resistance isolates, periodic medical checkup and follow up and rational use of drugs are recommended for food handlers working in ASTU students' cafeteria.*

Key words: *Salmonella, Shigella**,** prevalence, Drug susceptibility, intestinal parasites*

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Abbreviations

ASTU	Adama Science and Technology University
CLSI	Clinical Laboratory Standard Institute
H_2S	Hydrogen Sulfide
IRB	Institutional Review Board
MSc	Master of Science
NTS	Non Ttyphoidal Salmonelosis
SOP	Standard operation procedure
SPP.	Species
SPSS	Statistical Package for Social Science
TSI	Triple sugar iron agar
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate agar

CHAPTER ONE

1. INTRODUCTION

1.1. Background

Food borne disease is caused mostly by contaminated food and under cooked vegetable as well as contaminated water and not clearly enough washed utensil. Now a day it is a global and local burden to the community health problem. Globally it is estimated that about 30% of the world population is affected by food born disease annually and 2 million deaths are estimated per year as reported by WHO(1).

Food borne disease may cause outbreak in point source of epidemic disease. It is known that food borne disease comes from a common source like hotels and cafeteria. The epidemiology of food borne problems like Salmonellosis is complex and expected to vary with change in the pathogens themselves, industrialization, urbanization and change of lifestyles, knowledge, belief and practices of food handlers and consumers, demographic changes like increased susceptible population, international travel and migration, animal feed and in animals, and poverty and lack of safe food preparation facilities (2).

Food borne diseases are common in developing countries including Ethiopia due to poor handling of food inadequate water resource, inadequate food safety laws, weak regulatory systems, lack of personal hygiene and lack of education for food handlers also contribute to food borne disease. The etiologic agents of food born disease frequently reported are infectious caused by a variety of bacteria, virus and parasite. Among mostly reported bacteria which cause food born disease are *Salmonella* (causative agent of salmonellosis), *Campylobacter, Listeria,* some serotypes of *Escherichia coli, Yersinia* and *Shigella* (causative agent of shigellosis) are take the leading(3).

Defferent conducted studies in Ethiopia indicated the isolation of *Salmonella* and *Shigella* species from food handlers working in different food establishments (4-7). The isolated bacteria also showed resistance to different commonly used drugs in Ethiopia (4, 7, 8).

1.2. Statement of the problem

Globally, food borne diseases remain a major public health problem. The problem is severe in developing countries due to difficulties in securing optimal hygienic food handling practices. Evidently, in developing countries, about 70% of cases of diarrheal disease are associated with the consumption of contaminated food (9). Transmission of enteropathogenic bacteria is affected directly or indirectly through objects contaminated with faeces. These include food and water indicating the importance of faecal-oral human-to-human transmission(5). Among the Enterobacteriaceae genus *Salmonella* are widespread and important causative agent of food borne infections in human and are the most frequent etiologic bacterial agents of food borne disease outbreaks (5, 6).

In recent years, the number of research which reveals the prevalence of *Salmonella* in humans has conducted considerably in Ethiopia. Much more is known now about the extent of food borne illness and how severe it can be, not just in terms of acute illness, but also in terms of long term consequences. Studies indicated various percentages of *Salmonella* isolates in towns of Ethiopia from North to South, from East to West including the Capital Addis Ababa, Ethiopia (4, 7, 10).

In addition to *Salmonella, Shigella* species which is a causative agent of shigellosis also an important public health problem since communication in the world has become more frequent. Annually, there are 165 million cases of shigellosis resulting in 1.1 million deaths in the developing world. It is almost equivalently has the prevalence with *Salmonella*. Shigellosis is more difficult to be prevented because only a small number of *Shigella* (about 10 to 100) are required to cause infection specially to those of immune suppressed(11).

Here in Ethiopia Shigellosis and the emergence of antimicrobial resistant *Shigella* species also a major health problem(12). A few studies conducted previously in the country indicated high rate of resistance to commonly used antimicrobial agents, such

as Ampicillin, Tetracycline, Cotrimoxazole, Chloramphenicol among isolates(4, 7, 8).

Moreover, in the recent study conducted on prevalence and antimicrobial susceptibility patterns of *Shigella* and *Salmonella* Species among patients with diarrhea attending Gondar town health institutions, Northwest Ethiopia by Tesfaye (2014) reviled that strains of *Shigella* species were resistant to Ampicillin (94.1%), Tetracycline (88.2%), Amoxicillin (88.2%), but susceptible to Norfloxacin (100%) and Ciprofloxacin (100%). *Salmonella* isolate were also resistant to Ampicillin (75%), Amoxicillin (100%) and Tetracycline (75%), but highly susceptible to Norfloxacin (100%), Chloramphenicol (100%) and Ciprofloxacin (100%) (13).

In the study conducted by Beyene and Tasew (2014) in Ethiopia, indicated that *Shigella* species showed hundred percent resistances to Ampicillin, Amoxicillin and Cotrimoxazole. All *Salmonella* isolates were resistant against amoxicillin. All *Shigella* and *Salmonella* species were susceptible to Ceftriaxone, Ciprofloxacin (14).

The study conducted by Mengistu (2014) show that high frequency of resistance for both *Shigella* and *Salmonella* isolates was observed to tetracycline (82.4, 52.5%), Cotrimoxazole (76.5, 37.5%) and Ampicillin (47.1, 60%), respectively. All isolates were sensitive to Ceftriaxone except 6 intermediate level *Salmonella* isolates(13).

In study conducted in Bahir Dar north west of Ethiopia in 2009 on food handlers to detect the prevalence of *Salmonella* and intestinal parasite the prevalence of intestinal parasite was 41.1% on one or mixed infection was detected (9).

On other study conducted in Addis Ababa in 2013 on 172 cafeteria food handlers to detect the prevalence of intestinal parasite, *Salmonella* and *Shigella* the prevalence of intestinal parasite 78(45.3%) of them was positive. from those about 68(70.8%) was positive for *E. histolitica/dispar* (4).

In addition to those problems in the study area, there is a scarcity of up to date published data on the prevalence and antibiotic spectrum of *Salmonella* and *Shigella* species. No

current data about intestinal parasite prevalence in the study area. Thus, the purpose of this study was to identify the prevalence and antibiotic susceptibility of *Salmonella* and *Shigella* species and as well as the magnitude of intestinal parasites.

1.3. Significance of the study

Food handlers may possess potentially intestinal parasites and/or bacteria such as *Salmonella* or *Shigella* species or both as carrier and may serve as source of infection to the students of the University. This may result in progressive epidemic (outbreak) that may be complicated because of possibly drug resistant strains of *Salmonella* and/or *Shigella* species. Therefore this study was design to provide reliable data about the distribution of *Salmonella, Shigella* species and intestinal parasites among apparently healthy food handlers in Adama Science and Technology University (ASTU). Beside, this study also designed to know the data of drug susceptibility patterns of *Salmonella* and *Shigella* species in the study area.

Finally this study gives benefit to the University's administrative body to know the status of the food handlers of the University's students' cafeteria to take proper measure like early treatment of asymptomatically stage to prevent the transmission of the disease to the students.

CHAPTER TWO

2. LITERATURE REVIEW

Food borne disease is global and local problem of the community. Research are conducted in different time and place to solve the problem encountered with the food borne disease. Among the causative pathogen of food borne disease the most commonly mentioned bacterial species are *Salmonella* and *Shigella*.

Salmonellosis is an infection which is caused by *Salmonella* that can be transmitted through feco-oral route. Salmonellosis has been also caused by ingestion of contaminated beef, fish, and reptile meat, among others which is already infected animals(14).

Shigellosis is disease which caused by *Shigella* spp. initiated by ingestion of *Shigella*e usually via fecal-oral contamination. An early symptom, diarrhea, may occur as the organisms pass through the small intestine. Some *Shigella* produce Shiga toxin, which is not essential for disease, but does contribute to the severity of the illness(11).

2.1. Biology of *Salmonella*

This large genus within the family Enterobacteriaceae consists of two species, *S. enterica*, which contains six subspecies, and *S. bongori* which contain only one sub spp. Members of the seven *Salmonella* subspecies are classified into >2500 serotypes according to the somatic O antigen(15).

*Salmonella*e are gram-negative, non-spore-forming, facultatively anaerobic bacilli that measure 2–3 by 0.4–0.6 µm and most of them are non capsulated. *Salmonella*e, ferment glucose, reduce nitrates, and cytochrome oxidase negativ. In addition, except *S.gallinarum-pullorum* all are motile by peritrichous flagella, produce gas (H_2S) on sugar fermentation(15, 16). *Salmonella* species can cause disease like typhoid fever, paratyphoid fever, bacteriemia, gastroenteritis and local infection (16).

2.2. Biology *Shigella*

*Shigella*e are Gram-negative, non motile, facultative anaerobic, non spore forming rods. The genus is divided into four sero groups with multiple serotypes: A (*S. dysenteriae,* 12 serotypes); B (*S. flexneri,* 6 serotypes); C (*S. boydii,* 18 serotypes); and D (*S. sonnei*, 1 serotype)(16, 17).

Shigella is limited to only human pathogen. Shigellosis is endemic in developing countries where sanitation is poor. Typically it accounts 10 -20% of enteric disease; and 50% of the bloody diarrhea or dysentery of young children. In developed countries, single-source, food or water-borne outbreaks occur sporadically, and pockets of endemic shigellosis can be found in institutions and in remote areas with substandard sanitary facilities(7, 18).

2.3. Clinical feature of salmonellosis and shigellosis

Salmonellosis is a disease caused by bacteria genus of *Salmonella*. It is enteric fever with a feature of abdominal pain, anorexia, nausea, vomiting, diarrhea ,and constipation are some of them(11). Shigelloses it is a disease depend more on the immunologic status of the individual. The manifestations are fever, limited watery diarrhea, malaise, and anorexia. These symptoms range from mild to severe due to the individual immune status(11).

2.4. Prevalence of *Salmonella* and *Shigella* species among food handlers

Different researches were conducted across the world to evaluate the prevalence of *Shigella* and *Salmonella*. From those studies some of them are listed here. A study conducted in Iran 2004 to see the prevalence of *Shigella* species on 1850 stool samples 260 (14.05%) was positive by culture(19). Another study conducted in 2012 Central Africa Republic to see the prevalence and antibiotic resistant strain of *Salmonella* and S*higella*, on 2500 individuals, 72(2.9%) was positive for *Salmonella* and 182 (7.3) for *Shigella* (20).

A study conducted in asymptomatic carriers of enteric pathogens and the risk factors among food handlers in Nigeria the prevalence of *Shigella* was 2(2.22%), and 5 (0.93%) for *Salmonella* species (21).

A research conducted in food handling practices and the prevalence of food borne pathogens among food handlers in Kenya, a total of 242 stool specimens were analyzed. Among these 70 (28.9%) of the food handlers were infected with *Salmonella typhi* (22).

A study conducted in Southern Ethiopia in 2002 *Salmonella* carriage among asymptomatic of 107 food-handlers, only one *Salmonella* was isolated (10). A cross sectional research conducted in 2009 Bahir Dar, Ethiopia to see prevalence and drug susceptibility of *Salmonella* among 384 food handlers 6(1.6%) of them was positive for *Salmonella* species (9).

A study conducted in 2011 Addis Ababa University, to assess the prevalence of *Salmonella* in a total of 233 food handlers, 8 (3.4%) of them was positive for *Salmonella* (23), and another study conducted in Hawasa University the prevalence of *Shigella* was 0.4% and no *Salmonella* detected (24) A study conducted to assess the bacterial profile and drug susceptibility among 300 food handlers in University of Gondar student cafeteria, in 2012, the prevalence of *Shigella* species was 8 (2.7%) and 4 (1.3%) for *Salmonella* (25). Another study in 2013 the same University the prevalence of *Salmonella was* 13 (3.1%) (26).

A study conducted in 2013 in Addis Ababa University student cafeteria on apparently healthy 172 food handlers the prevalence of *Salmonella* was 3.5% and no *Shigella* was detected (4). Another study conducted in Bahir Dar University on 410 food handlers in 2014 the prevalence *Salmonella* 11 (2.7%) and *Shigella* species was 5 (1.2%) (27). A study conducted in 2015 Dire Dawa University student cafeteria the prevalence was 5 (1.9%) and 14(5.4%) , *Shigella and Salmonella* respectively (8). A cross sectional study conducted in Arba Minch Ethiopia on 376 food handlers in 2015 the prevalence of *Salmonella* and *Shigella* was 6.9% and 3% respectively(7).

2.5. Treatment of Salmonellosis and Shigellosis

Since salmonellosis and shigellosis are diseased caused by Enterobacteriaceae group the choice drugs are Ciprofloxacin, Ceftriaxone , Amoxicillin , Chloramphinicol and Cotrimoxazole are selected(11).

2.6. Drug resistant *Salmonella* and *Shigella* species isolated food handlers

A study conducted in Iran on *Shigella* was show high resistance rate to Cotrimoxazole (73.84%) and Ampicillin (73.84%), and show low resistance rate to Ciprofloxacin (2.69%) and Cefotaxim (2.69%)(19). A study conducted in Turkey to assess the prevalence and antibiotic susceptibility *Salmonella* and *Shigella* in 2014 among 2425 both isolates were resistant to Amoxicillin, Ampicillin and susceptible for Ceftriaxone and Cefixine(28).

Another study conducted in Central Africa Republic the isolated *Salmonella* was resistant to Tetracycline, Cotrimoxazole and Amoxicillin but sensitive for Ceftriaxone, Cefotaxine and Ciprofloxacin. likewise *Shigella* species also resistant to Cotrimoxazole, Amoxicillin and Chloramphinicol but sensitive to Ceftriaxone, Cefotaxime and Ciprofloxacin(20).

A study conducted in Kenya 2008 to assess the prevalence and antibiotic resistant of pathogens isolated from children diarrhea of 651 children the antibiotic susceptibility of *Shigella* isolates was, show high levels of resistance to Ampicillin, Cotrimoxazole and Tetracycline were found. Among the *Shigella* strains, none was resistant to Norfloxacin. Resistance of *Salmonella* to Nalidixic acid was 44%. but the isolated *Salmonella* were sensitive to Ciprofloxacin (29).

A research conducted in 2012 in Sudan to detect *Salmonella* was resistant to streptomycin (41.3%) followed by Tetracycline (31.9%), Ampicillin (25.4%), Nalidixic acid (22.1%), Cotrimoxazole (17.4%), Ciprofloxacin (8.9%), Chloramphinicol (8%), Norfloxacin (7.5%) and Apramycin (5.6%).and sensitive to Chloramphinicol (30).

A research conducted in 2015 Nigeria to know antibiotic susceptibilities of *Salmonella species* isolates screened were resistant to Ampicillin and Amoxicillin and of these, 0.9% was resistant to Amoxicillin-Clavulanic Acid and 45.5% were resistant to Nalidixic Acid. 90.9% were sensitive to Ciprofloxacin and Cefotaxime but 9.09% showed reduced susceptibility to Ciprofloxacin(31).

A cross sectional research conducted 2009 in Bahir Dar, the isolated *Salmonella* were resistant to Amoxicillin 6(100%) , Cotrimoxazole 5(83.4%) and Tetracycline 4(66.7%) but sensitive to Norfloxacilin 5(83.4) (9). A study conducted in Addis Ababa, all *Salmonella* isolated were resistant to Ampicillin but 100% sensitive to Ceftriaxone (23).

A study conducted in University of Gondar 2012 all isolated *Salmonella* were resistant to Chloramphenicol (100%). All *Shigella* species were sensitive to Ciprofloxacin (25). Another study conducted in University of Gondar to see the drug resistance of *Salmonella among* 423 food handlers in 2013, from the isolated 9(69.2), 8(61.5), 6(46.2) and 6(46.2) were resistant to Amoxicillin Ampicillin and Tetracycline respectively but all of them were sensitive to Ceftriaxone (26).

A study conducted in 2013 in Addis Ababa University student cafeteria on apparently healthy 172 food handlers, isolated *Salmonella* was 100% resistance to Amoxicillin , Clindamycin ,Ampicillin and Erythromycin and Cotrimoxazole and Cefotacime 16.7% but 100% were sensitive to Ciprofloxacin ,and Amikacine (4).

A study conducted in 2015 among asymptomatic 257 food handlers in Dire Dawa University student cafeteria all *Salmonella* and *Shigella* isolates were resistant to Ampicillin and sensitive to Ceftriaxone (8). Another cross sectional study conducted on 376 food handlers in Arba Minch University in 2015 all *Salmonella* and *Shigella* was resistant to Amoxicillin, followed by 41% to Clarithromycin and 35% to Amoxicillin-Clavulanic Acid. However, 100% isolated were susceptible to Cotrimoxazole, Ceftriaxone, Chloramphenicol, and kanamycin (7).

2.7. Laboratory diagnosis of *Salmonella* and *Shigella* species

There are different phenotypic and genotypic method for diagnosis of *Salmonella* and *Shigella.* Among these techniques culture, DNA probe assay, PCR, Culture, Serological tests listed (16, 32).

Culture is one of the tests to diagnose *Salmonella* and *Shigella* species. Typical Medias which we use to growth of them MacConkey agar, Deoxycholate Agar (DCA) and Salmonella Shigella (SS) agar, Xylose Lysine Deoxycholate (XLD) are common. On XLD agar, *Shigella* and *Salmonella* spp. produce small red colonies, most strains of *Salmonella* with a black centre. Since they are non lactose fermenter on MacConkey agar both of them have colorless colony appearance (16, 32).

2.8. Risk factors associated with enteropathogenic bacteria and parasitic infection

Some commonly reported risk factors that contribute to the prevalence of *Salmonella* and *Shigella* to human population were foods from animal origin like poultry, eggs, pork, and dairy products, consumption of beef and unpasteurized milk mentioned (33). Consuming of undercooked vegetables, unclean fruit, source of water and personal hygiene are associated risk factors to prevalence of *Salmonella* and *Shigella* infection(34).

Study in Nigeria shows no hand washing before food preparation, use of pit toilet and defecation and use of local stream river water for drink had significantly association with infection of *Salmonella* (21). Knowledge about food borne disease and how handling food and age of food handlers were significantly associated with *Salmonella* infection in Kenya(22).

Study in Bahir Dar hand washing after touching dirty materials has significant association with *salmonella* infection (9). Study in Addis Ababa show no any significant association with risk factors and *Salmonella* prevalence (4).

Hand washing habit and finger nail trimming status was significantly associated with

Salmonella and *Shigella* species infection in Dire Dawa (8). Study conducted in Arba Minch finger nail status, hand washing practice after toilet, and transferring food with bare hands were significantly associated with *Salmonella* and *Shigella* infection(7).

2.9. Intestinal parasitosis among food handlers

Intestinal parasite has different prevalence in food handlers according to different study conducted in different regions. Study conducted in Nigeria food handlers the total positive rate of parasite was 126 (41.2%) while conducted in Kenya food the prevalence was 30.4% (58/191) (35, 36).

Study conducted in parts Ethiopia shows there were significant prevalence of intestinal parasite among food handlers. Study conducted in Arba minch the prevalence of intestinal parasite was 123 (36 %) and another study conducted in Addis Ababa University 78(45.3%) of them was positive for different types of parasite. The study conducted in Aksum the prevalence of intestinal parasite was 14.5% (4, 7, 37). Study conducted in Hawasa University food handlers 20.6% was found to be positive for different types of intestinal parasites (24).

Another study conducted in Bahir Dar on food handlers 158(41.1%) food handlers had intestinal parasites (9). On other study conducted in Addis Ababa in 2013 on 172 cafeteria food handlers the prevalence of intestinal parasites was 78(45.3%) (4). The research conducted in Gondar town food handlers 37 (29.1%) stool specimens were positive for different intestinal parasites (5).

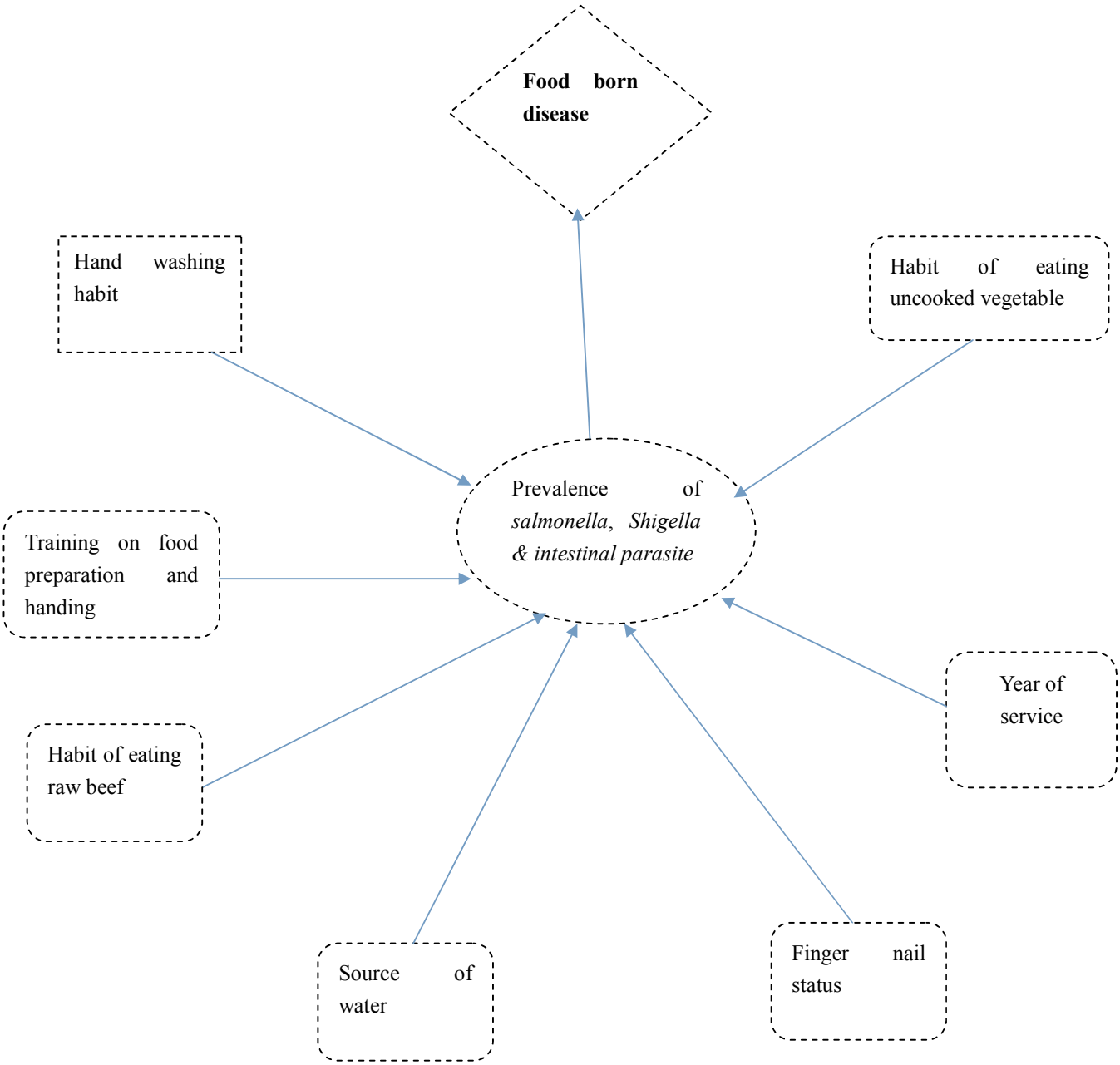

Figure 1: Conceptual framework of associated risk factors *Salmonella* and *Shigella* species infection.

(Source: - Adapted from different literature reviews)

3. Objective

3.1. General objective

- To assess the prevalence of *Salmonella*, *Shigella* and intestinal parasites among apparently healthy food handlers of Adama Science and Technology University students' cafeteria, from April to June 2018

3.2. Specific objectives

- To determine the prevalence of *Salmonella* and *Shigella* among apparently healthy food handlers
- To determine drug susceptibility of isolated *Salmonella* and *Shigella*
- To assess the prevalence of intestinal parasites among the ASTU food handlers
- To assess the risk factors associated with infection of *Salmonella*, *Shigella* and intestinal parasites

4. MATERIALS AND METHODS

4.1. Study area

The study was conducted in Adama Science and Technology University (ASTU) which is located in Adama town. Adama town is located in Oromia region East Shewa zone 100 km from Addis Ababa to South East. The town has about 324,000 estimated populations in 2015 national census. ASTU has generally about 225 food handlers working in three students' cafeteria which serve for more than 10,000 students (according to information from student service in 2018).

4.2. Study period and Study design

-Study period

The study was conducted from April to June 2018.

-Study design

A cross sectional study was conducted.

4.3. Population

Source population

The source population was all food handlers in ASTU students' cafeteria who were at work during the study period.

Study population

All apparently healthy individual working as food handlers in ASTU students' cafeteria, during study period and those fulfill inclusion criteria.

4.4. Eligibility criteria

Inclusion criteria

- ✓ All workers of the students' cafeteria that who prepare food, host students and cleaner of utensil in cafeteria

- ✓ Individuals who did not take any antibiotic and anti parasitic drugs within the last three weeks
- ✓ individuals who were apparently healthy

Exclusion criteria

- ✓ Food handlers who were ill (diseased) for various reasons.

4.5. Sample size determination

The data about the prevalence of *Salmonella* and *Shigella* species were not known in Adama town, but since this research was aimed to conduct apparently healthy food handlers which their number is already known no need to calculate the sample size. According to information obtained from ASTU student service, about 225 food handlers working in three students' cafeteria. So in order to make more representative all food handlers that fulfill inclusion criteria were enrolled in the study. From these 225 food handles 210 of them participated in the study, 15 of food handlers were not included in the study by their own different reasons.

4.6. Sampling technique

Purposive sampling method was used. Complete list of food handlers of the students' cafeteria was obtained from ASTU students' service.

4.7. Variable

Dependent variable

- ✓ Prevalence of *Salmonella* and *Shigella* isolates.
- ✓ Susceptibility pattern of *Salmonella* and *Shigella* isolates.
- ✓ Prevalence of intestinal parasite

Independent variable

- Sex ,age , monthly income, family size, Educational status,
- Service year, history of Medical checkup, knowledge about *Salmonella* and *Shigella* species as well as intestinal parasites, hand washing habit, finger nail

status, source of water for drink, training on food handling and preparation, eating raw beef, eating not enough cooked vegetable

4.8. Data Collection and Examination Technique

4.8.1. Socio demography

Data related to socio-demographic characteristics (age, sex, educational status, and etc) and personal hygiene practices of food handlers were collected using structured questionnaire. (Annex III)

4.8.2. Collection, handling and transport of clinical specimen

After proper instruction on how to collect, stool cups were given to food handlers to collect stool sample at the ASTU students' Health Center. From each 210 study subjects about 3 gm of fresh stool was collected. Each of the specimens was checked for its label, quantity and procedure of collection by inspection. Samples contaminated with other material such as urine and soil were rejected.

4.8.3. Microscopic examination of stool for intestinal parasite

All stool samples were examined for intestinal parasites at ASTU students' Health Center. The samples were examined using direct saline wet mount as well as formol ether concentration technique with iodine wet mount. All stages of intestinal parasites were examined using bright field microscope at 10X and 40X objectives. (Annex VI)

4.8.4. Culture and identification of *Salmonella* and *Shigella* species

All of 210 stool samples were also processed for bacteriological analysis at Oromia Public Health Research, Capacity Building and Quality Assurance Laboratory. Stool samples were inoculated in to Selenite F broth (Biomark Laboratories India) and incubated for 18 to 24 hour at 37 °C for recovery and increase the number of bacteria, then sub cultured on to Xylose Lysine Deoxycholate (XLD) (Biomark Laboratories India) agar plates which is selective for *Salmonella* and *Shigella* and MacConkey agar (Deben Diagnostic Ltd UK) to differentiate lactose fermenter from non fermenters and re incubated for 18 to 24 hour at 37 °C (32, 38). Examination of the plates for suspected colonies of *Salmonella* and *Shigella* was done. Colony appearance on media

was used as a clue for further identification of *Salmonella* and *Shigella* species using series biochemical tests.

4.8.5. Biochemical testing for identification of *Salmonella* and *Shigella* species

After getting pure colony of growth further definite identification was done using series biochemical testing. From the total 210 stool samples, 22 of them were selected to biochemical tests based on their colony appearance. Biochemical tests were carried out by inoculation on Simmons citrate agar (Biomark Laboratories India), Urea Agar Base (Biomark Laboratories India) TSI (Deben Diagnostic), lysine Iron Agar (Biomark laboratories India) and Sulfide Inodole Motility (S.I.M) (Oxoid England) for final identification(38).

4.8.6. Antibiotic susceptibility testing

For the isolated antimicrobial susceptibility test was carried out using disc diffusion method on Muller Hinton Agar (Biomark laboratories India) based on Clinical Laboratory Standard Institute (CLSI) guideline, 2016 (39). The inoculum was prepared by picking 3-5 similar colonies from pure culture with a sterile wire loop and was suspended in sterile normal saline. Then the turbidity was matched with 0.5 McFarland standards. The organisms was uniformly spread over the Mueller-Hinton agar surface and exposed to a concentration gradient of antibiotic diffusing from antibiotic-impregnated paper disk into the agar medium using disc dispenser or sterile forceps, and then was incubated at 37 ℃ for 16 to 18 hours. Diameters of the zone of inhibition around the discs was measured to the nearest millimeter using graduated caliper in millimeters ruler and classified as sensitive, intermediate, and resistant according to the standardized table supplied by CLSI, 2016(39)

The following antibiotics disks with their concentration were used for antimicrobial susceptibility testing, Ampicilin (10 µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Cotrimoxazole (25 µg), Tetracycline (30) Amoxicillin- Clavulanic Acid (20/10 µg), Ceftriaxone (30 µ g), . The reference strain *E. coli* (ATCC 25922) was used as a control strain.

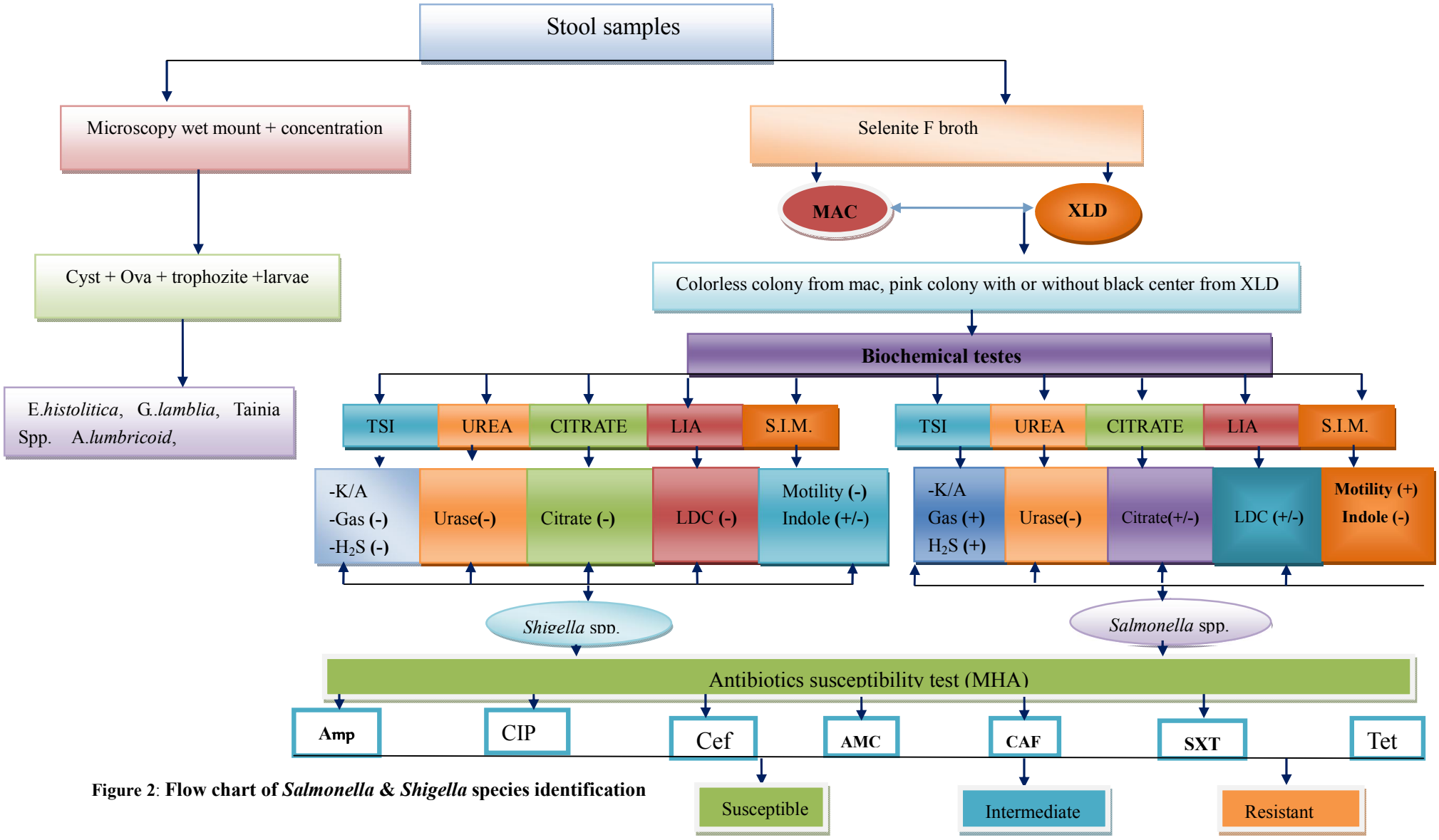

Figure 2: **Flow chart of *Salmonella* & *Shigella* species identification**

N.B MAC=MacConke, XLD= Xylose Lysine Deoxycholate, Amp=Ampicillin, CIP=Ciprofloxacin, Cef= Ceftriaxone, AMC=Amoxicillin-Claviculic Acid, CAF= Chloramphenicol, SXT= Cotrimoxazole, Tet =tetracycline

4.9. Data quality

Data quality was ensured at various activities of the study by following prepared standard operating procedure (SOP) of the Oromia Public Health Research, Capacity Building and Quality Assurance Laboratory for bacteriological testes. The questionnaire was translated to local language Amharic and Afaan Oromoo. Completed questionnaires was checked and corrected on daily base. Culture media were checked for expired date and storage condition met with manufacturers. The refrigerators and incubators were checked for their temperature twice daily. Culture Media was prepared according to manufacturer's instruction and sterility was checked by incubating representative of the batch at 35–37 °C overnight and observing for growth. A performance of all prepared media was also checked by inoculating standard-strains. The qualities of biochemical testing procedures were checked by these reference strains. *Escherichia coli* (ATCC 25922), was used (40). The culture media preparation and its performance was strictly followed SOP for media preparation and quality control (BACT -11) of Oromia Public Health Research, Capacity Building and Quality Assurance Laboratory.

4.10. Data analysis

Data were edited, cleaned, entered and analyzed using statistical package for social science (SPSS) version 20 for possible association. Finally the result was presented using tables. P-value less than 0.05 were considered statistically significant. Bivariate and multivariate analyses were done to see significance association of prevalence and risk factors.

4.11. Ethical clearance

The study was conducted after it was ethically reviewed and approved. Ethical approval was obtained from the Institutional Review Board (IRB), of Jimma University Institute of Health with written letter IHRPG1/146/2018 and from Oromia Health Bureau with written letter lakk/Ref.NoBefo/HBiPH/1-8/288. Permission was also obtained from ASTU students' service. Written informed consent was obtained from each participant after the purpose of the study was explained using the common language they speak and hear. Participants were notified about the purpose of the study, their right to refuse or participate in the study, and anonymity and confidentiality of the information gathered. Study participants were given detailed information concerning the purposes of study and they were asked about the study to check whether they have understood it correctly or not and their questions were cleared. Positive cases were linked to ASTU Health Center for further management.

4.12. Dissemination and utilization of results

The finding of the study will be presented to the school of medical laboratory sciences; Institute of Health Science, Jimma University, likewise copy of result will be given to Oromia Health Bureau, ASTU students' service and other stake holders after approval. An attempt will be made to publish the finding in peer reviewed journals.

4.13. Operational definition

-Food handlers => person who prepare, serve food and cleaner of all utensil to prepare food

-A**pparently healthy** = > person who is free from any sign and symptom of any disease

-Prevalence = > the positive rate of either *Salmonella* or *Shigella* species

5. RESULTS

5.1. Socio-demographic characteristics of food handlers at ASTU students' cafeteria in 2018

In this study a total number of 210 apparently healthy food handlers working at Adama Science and Technology University students' cafeteria were enrolled. Among these participants 200 (95.2%) participant were females. From the total, 172 (82%) of them were between 20-40 years old, and 110(52.4%) of them attended preparatory or college in their educational level **(Table 1).**

Table 1:- Socio-demographic characteristics of food handlers in 2018 ASTU students' cafeteria

S/No	Characteristics		Frequency	Percentage
1	Sex	Male	10	4.8
		Female	200	95.2
2	Age	20-40	172	82.0
		≥ 40	38	18.0
3	Educational status	1-10	100	47.6
		Preparatory and above	110	52.4
4	Monthly income in Birr	< 2000	72	34.3
		≥ 2000	138	65.7
5	Family size	<5	162	77.1
		≥5	48	22.9

5.2. Food preparation and hygienic related characteristics of food handlers ASTU students' cafeteria

As shown in table below, most of the food handlers 182(86.7%) had greater than five years experience in the cafeteria but most of them 175 (83.3%) had no certificate on food preparation or handling. One hundred eighty six (88.6%) of food handlers attended continuous medical checkup, and 170(81.0%) of food handlers trimmed their finger nails. From the total, 162 (77.1%) of them wash their hand with soap and water after toilet, and 204 (97.1%) of food handlers had use pipe water for drink. Eighty four (40.0%) responded that they were used under cooked vegetable **(Table -2).**

Table 2:- Food preparation and hygienic related characteristics of food handlers of ASTU in 2018

N0	Characteristics		Frequency	Percentage
1	Service year in catering establishment	<5	28	13.3
		≥ 5	182	86.7
2	Job position of study participants	Food preparation	122	58.1
		Cleaning utensils	6	2.9
		Hosting the students	82	39.0
3	Certificate or training in preparation of food	Yes	35	16.7
		No	175	83.3
4	Periodic medical check up	Yes	186	88.6
		No	24	11.4
5	Hand washing habit before preparation or touching of food	Yes	205	97.6
		No	5	2.4
6	Finger nail status of food handlers	Trimmed	170	81.0
		Untrimmed	40	19.0
7	Hand washing habit after using toilet	Only with water	48	22.9
		Soap and water	162	77.1
8	Habit of eating raw beef	Yes	59	28.1
		No	151	71.9
9	Source of water for drink	Pipe	204	97.1
		Other	6	2.9
10	Consuming uncooked vegetable	Yes	84	40.0
		No	126	60.0

5.3. Isolation rate of *Shigella* species with socio demographic characteristics of food handlers

From the total of 210 apparently healthy food handlers a total of 5(2.4%) *Shigella* species were isolated, but no *Salmonella* species were identified. Among participants who were positive for *Shigella* species, 4 of them were females. From these five positive participants, 2 of them were between 20- 40, and 3 of them were above 40 years old. Regarding to their educational level 3 of them were grade 1-10 and 2 of them were attended either preparatory or above in educational status **(Table -3).**

Table 3:- Isolation Rate of *Shigella* species with Socio-demographic distribution

	Characteristic		***Shigella* Frequency (%)**
1	Sex	Male	1(0.5%)
		Female	4(1.9%)
2	Age	20-40	2(1.0%)
		≥40	3(1.4%)
3	Educational status	1-10	3(1.4%)
		Preparatory and above	2(1.0%)
4	Monthly income	<2000	0(0.0%)
		≥2000	5(2.4%)
5	Family size	<5	3(1.4%)
		≥5	2(1.0%)

5.4. Isolation rate of *Shigella* species with food handling & related risk factors

From five positive apparently healthy food handlers, three of them were assigned in preparation of food and four of them were worked more than five years in the establishment. Three of them not certified how food is preparing and handled.**(Table 4).**

Table 4 :- Associated risk factor for prevalence of *Shigella* species

Associated risk factors for carriage rate of *Shigella*		***Shigella* Frequency (%)**
Service year in the establishment	<5 years	1(0.5%)
	≥5 years	4(1.9%)
Job position in catering establishment	Preparation	3(1.4%)
	Cleaning utensil	0(0.0%)
	Host the student	2(1.0%)
Certified on food handling and preparation	Yes	2(1.0%)
	No	3(1.4%)
Hand washing habit before preparation of food	Yes	4(1.9%)
	No	1(0.5%)
Ever diagnosis for *Salmonella* and *Shigella*	Yes	1(0.5%)
	No	4(1.9%)
Hands wash with water or soap after using toilet	Only with water	4(1.9%)
	With water & soap	1(0.5%)
Source of water for drink	Pipe	5(100%)
	Other	0(0.0%)
Habit of eating raw meat	Yes	2(1.0%)
	No	3(1.4%)
Consuming under cooked vegetable	Yes	3(1.4%)
	No	2(1.0%)

5.5. Magnitude of intestinal parasites among ASTU Food handlers

Four different types of intestinal parasites were isolated from the total apparently healthy study subjects. In this study no study subjects had mixed infection either bacterial or parasites infection.

Among the identified parasites, E. *histolitica*/ *dispar* was 7(3.3%), G. *lamblia 4(1.9%), A.lumbricoid* 1(0.5%) and Tainea *spp.* 1(0.5%) from the total study subjects **(Table-5).**

Table 5:- Distribution of intestinal parasite among food handlers

S/N	Types of parasites	Frequency	Percentage (%
1	E. *histolitica/ dispar*	7	3.3
2	G. *lamblia*	4	1.9
3	A. *lumbricoid*	1	0.5
4	*Tainea* species	1	0.5
	Total	13	6.2

5.6. Bivariate analysis of *Shigella* species against Socio-demographic characteristic

Isolation of *Shigella* species was not associated with socio-demographic; sex, age, educational status and family size of apparently healthy food handlers of ASTU students' cafeteria **(Table 6).**

Table 6: Bivariate analysis of socio demographic characteristics for prevalence *Shigella* species in ASTU food Handlers of 208

Characteristics of food handlers		*Shigella* N0 (%)		COR(95%CI)	P - value
		Positive	Negative		
Sex	Male	1(0.5)	9(4.3)	0.184 (0.019-1.82)	0.999
	Female	4(1.9)	196(93.3)		
Age	20-40	2(1.0)	170(81.0)	7.29(1.17-45.22)	0.520
	≥40	3(1.4)	35(16.7)		
Educational status	1-10	3(1.4)	97(46.2)	0.60(0.098-3.66)	0.296
	Preparatory& above	2(1.0)	108(51.4)		
Family size	<5	3(1.4)	159(75.9)	2.30(0.37-14.21)	0.219
	≥5	2(1.0)	46(21.9)		

5.7. Bivariate and Multivariate analysis of risk factor against *Shigella* species infection among ASTU food handlers

From the total study subjects, 204(97.1%) of them had used pipe water as source for drinking and 191 (91%) of them did not use unpasteurized milk. As indicated in table below, risk factor such as service year, having certificate, habit of eating raw beef, and habit of eating under cooked vegetable were not significantly associated with *Shigella*

species infection. But in this study hand washing habit before touching/preparing food was significantly associated with *Shigella* species infection, [AOR **(95%CI)** 33.774(1.142-998.636) and P- value =0.042] and hand washing with only water or with water and soap after using toilet was also significantly associated with *Shigella* species prevalence [AOR **(95%CI)** 0.043(0.003-0.599) and P- value =0.019] **(Table - 7).**

Table 7:- Multivariate analysis of associated risk factor for the prevalence of *Shigella* species

Characteristics		***Shigella* NO (%)**		**COR(95%IC)**	**p-value**	**AOR(95%CI)**	**P- value**
		Pos	Neg				
Service year	<5	1(0.5)	27(12.9)	0.61(0.65-5.63)	0.660	0.509(0.035-7.453)	0.622
	>/5	4(1.9)	178(84.8)				
Certification or training	Yes	2(1.0)	33(15.7)	0.288(0.046-1.790)	0.182	0.127(0.010-1.576)	0.108
	No	3(1.4)	172(81.9)				
Hand washing before touching/preparing food	Yes	4(1.9)	201(95.7)	12.56(1.14-139)	0.039	33.774(1.142-998.636)	0.042
	No	1(0.5)	4(1.9)				
Knowledge about *Salmonella* and *Shigella* species	Yes	2(1.0)	69(32.9	0.761(0.124-4.66)	0.768	0.507(0.044-5.827)	0.586
	No	3(1.4)	136(64.8)				
Ever diagnosed for either Salmonellosis or Shigellosis	Yes	1(0.5)	48(22.9)	1.22(0.133-11.20)	0.859	3.617(0.112-116.64)	0.468
	No	4(1.9)	157(74.8)				
Hand washing with water or soap after using toilet	Only water	4(1.9)	44(21)	0.68(0.007-0.63)	0.018	0.043(0.003-0.599)	0.019
	Water &soap	1(0.5)	161(76.7)				
Habit of eating raw beef	Yes	2(1.0)	57(27.1)	0.578(0.094-3.55)	0.554	0.532(0.049-5.777)	0.604
	No	3(1.4)	148(70.5)				
Eating not enough cooked vegetable	Yes	3(1.4)	81(38.6)	0.435(0.071-2.66)	0.368	0.467(0.046-4.744)	0.519
	No	2(1.0)	124(59.0)				

5.8. Bivarite analysis of socio demographic characteristics against intestinal parasite infection

Among 210 apparently healthy participants of this study 13(6.19%) of them were positive to four different types of intestinal parasites. From these positive participants 12 of them were females, and 11 of them were between 20-40 years old. From the positive participants eight of them attended preparatory or college in their educational status. In the current study the prevalence/infection of intestinal parasite did not associated with any socio demographic characteristics of the food handlers **(Table 8).**

Table 8 :- Bivariate analysis of socio demographic characteristics against intestinal parasite infection

Characteristics of food handlers		**Parasite N0 (%)**		**COR(95%CI)**	**p-value**
		Positive	Negative		
Sex	Male	1(0.5)	9(4.3)	0.574(0.067-4.916)	0.613
	Female	12(5.7)	188(89.5)		
Age	20-40	11(5.2)	161(76.7)	1.23(0.261-5.791)	0.794
	≥40	2(1.0)	36(17.1)		
Educational status	1-10	5(2.4)	95(45.2)	1.49(0.471-4.715)	0.497
	Preparatory & above	8(3.8)	102(48.6)		
Family size	<5	11(5.2)	151(71.9)	0.597(0.128-2.791)	0.512
	≥5	2(1.0)	46(21.9)		

5.9. Bivariate and Multivariate analysis of risk factor against intestinal parasites

As shown in the table **(Table-9)** below, Bivariate and Multivariate analysis showed no significance association between risk factor and intestinal parasitic infection. Even if there were no association, most positive participants 11(84.6%) were not certified for food handling and preparation [AOR (95%CI) 1.498(0.283-7.924), P-value =0.634]. Twelve of (92.3%) of positive participants had habit of eating raw beef [AOR (95%CI) 3.859(0.431-34.556), P-value =0.227], and 10(76.9%) of positive participant had habit of eating not enough cooked vegetables [AOR (95%CI) 1.834(0.426-7.905) P-value =0.416].

Table 9 :- Multivariate analysis of associated risk factor for the prevalence of intestinal parasite among ASTU food handlers in 2018

Characteristics		Parasite NO (%)		COR(95%IC)	P -value	AOR(95%CI)	P- value
		Pos	Neg				
Service year	<5	1(0.5)	27(12.9)	0.525(0.066-4.20)	0.543	1.719(0.202-14.588)	0.620
	≥5	12(5.7)	170(81.0)				
Certification or training	Yes	2(1.0)	33(15.7)	0.904(0.191-4.267)	0.898	1.498(0.283-7.924)	0.634
	No	11(5.2)	164(78.1)				
Hand washing with water or soap after using toilet	Only water	5(2.4)	43(20.5)	2.238(0.697-7.193)	0.176	0.335(0.097-1.159)	0.084
	Water &soap	8(3.8)	154(73.3)				
Drinking unpasteurized milk	Yes	1(0.5)	18(8.6)	0.829(0.102-6.746)	0.861	0.812(0.084-7.832)	0.857
	No	12(5.7)	179(85.2)				
Habit of eating raw beef	Yes	1(0.5)	58(27.6)	0.20(0.025-1.571)	0.126	3.859(0.431-34.556)	0.227
	No	12(5.7)	139(66.2)				
Eating not enough cooked vegetable	Yes	3(1.4)	81(38.6)	0.43(0.115-1.610)	0.210	1.834(0.426-7.905)	0.416
	No	10(4.8)	116(55.2)				

5.10. Antimicrobial drug susceptibility pattern

All the five *Shigella* isolates were tested for antimicrobial susceptibility pattern against seven antibiotic discs. All (100%) isolates were susceptible to Ciprofloxacin and most of them were also susceptible to Chloramphenicol (80%). But all (100%) isolates were resistant to Ampicillin and Cotrimoxazole. Most isolates showed resistance against Tetracycline (80%) and Amoxicillin- Clavulanic acid (60%), (**Table-10).**

Table 10: Isolates and their drug susceptibility

SR	Name of Drug	Susceptible N0 (%)	Resistance N0 (%)
1	Ampicillin	0(0.0)	5(100)
2	Chloramphenicol	4(80.0)	1(20.0)
3	Ciprofloxacin	5(100)	0(0.0)
4	Cotrimoxazole	0(0.0)	5(100)
5	Tetracycline	1(20.0)	4(80.0)
6	Amoxicillin- Clavulanic Acid	2(40.0)	3(60.0)
7	Ceftriaxone	3(60.0)	2(40.0)

6. DISCUSSION

In this study most of food handlers were females, 200 (95.2%), and 172(82.0%) of them were between 20-40 years old. This is in line with the study conducted in Dire Dawa University where 88.3% were females and 84.8% of them were between 20-40 years old (8). It is also agreed with study conducted in Hawasa University where 81.3% of them were between 20-40 years old (24). Out of the total food handlers, 110 (52.4%) were attended preparatory or above in their educational level which is higher than previous study conducted in Hawasa University where 13.3% of them were attended (24).

Regarding the medical checkup, 186 (88.6%) study participants were having regular medical checkup. This is similar with study conducted in Bahir Dar University where 83.5% of food handlers had medical checkup (9). This study also indicates ASTU food handles had the same hand washing habit with previous study conducted in Bahir Dar University 97% and 96% respectively (9).

In the current study no *Salmonella* species was isolated. This result is similar with the previous study conducted Hawasa University (24). However, different from other study *Salmonella* species were reported in Kenya 28.9% (29), in Arba Minch University 6.9% (7), in Dire Dawa University 5.4% (8), in Addis Ababa University 3.5% (4), in Bahir Dar University 2.7 % (9), in University of Gondar 1.3% (25) and in Nigeria 0.93% (21). This difference might be due to environmental factor, poor personal hygienic practice, work experience, educational status of food handlers, attending medical checkup and monitoring of the study subjects, and the methods used to assess the isolate.

In this study the prevalence of *Shigella* species was 5(2.4%). This is consistent with the previous study conducted in University of Gondar (25), and Nigeria (21), with prevalence of 2.7% and 2.22% respectively. However, it was higher than study conducted in Hawassa University (24), Bahir Dar University (9) and Dire Dawa

University (8) with prevalence of 0.4%, 1.2 % and 1.9% respectively. But This finding is less than study conducted in Arba Minch University with prevalence of 3% (7). This difference might be due to the sample size or the methods we used.

This study also tried to identify the associated risk factors with *Shigella* infection. Based on the finding, hand washing habit before touching/preparing food, and hand washing with only water or with water and soap after using toilet was significantly associated with *Shigella* infection with p-value [0.042] and [0.019] respectively. This is consistent with the previous study conducted in Arba Minch University (7), and Dire Dawa University where hand washing after toilet was significantly associated with the prevalence of *Shigella* species (8).

The antimicrobial susceptibility patterns showed that, all the isolates were susceptible to Ciprofloxacin. This is comparable with study conducted in Central Africa Republic and University of Gondar where all the isolated *Shigella* species were sensitive to Ciprofloxacin (20). In our study, *Shigella* species were 5(100%) resistant to Ampicillin. This is consistent with study conducted in Dire Dawa University where 100% of isolated *Shigella* species were resistant to Ampicillin (8).

In current study the prevalence of intestinal parasites was lower than most studies conducted in different countries. For instance study conducted in Nigeria reported 41.2% of food handlers were positive to intestinal parasites where as in Kenya 30.4% of the food handlers were positive for intestinal parasites. The variation might be due to geographical difference or socio demographic characteristics of study subjects (35, 36).

Similar studies were also conducted in Ethiopia in different University food handlers and the result was quite different. Study conducted in Addis Ababa University was showed 45.3% of study participants were positive to different types of intestinal parasites (7, 23), while study conducted in Bahir Dar University reported 41.1% (9),

Arba Minch University reported 36 % (7, 23), Hawasa University reported 20.6% (24), and Aksum University reported 14.5% (37) prevalence of intestinal parasites.

The difference might be due to socio demographic characteristics, difference in medical checkup and the habits of food handlers were different in pre exposing factors like consuming raw vegetables and hand washing habits. Moreover, the variation of our finding with the previously reported findings could be due to the methods that we have used to diagnose intestinal parasites.

7. Limitation of the research

- Small sample size to generalize the population of Adama town
- *Shigella* isolates were not serotyped because of absence of antisera

8. CONCLUSION AND RECOMMENDATION

8.1. Conclusion

In the current study no *Salmonella* spp. was isolated and five (2.4%) of *Shigella* species were isolated. Thirteen (6.19%) of the study subjects were positive for different types of intestinal parasites. Hand washing habit before food preparation and hand washing after toilet was significantly associated with the prevalence of *Shigella* species. However no risk factors were associated with the prevalence of intestinal parasite. All isolates *Shigella* species were susceptible to Ciprofloxacin and all of them were resistant to Ampicilin and Cotrimoxazole.

8.2. Recommendation

Based on my finding I forwarded some recommendation for stakeholders

- The ASTU has to give more training to the food handlers about how to handle and preparation of food
- Personal hygienic practice should be improved and ASTU has to provide sanitary items material like soap and detergents
- The medical checkup is not enough only for intestinal parasite it should have to include available bacterial tests for food handlers
- Rational use of drugs are recommended to prevent potential resistance
- Further study have to conduct related to this issue with advanced technique

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10. Annex

I. Annex : Participant information sheet: English, Afaan Oromoo and Amharic version

A. English Version:

Name of the Organization: Jimma University, Institute of Health Sciences, School of Medical Laboratory Science, Department of Medical Microbiology.

Title of the Research: 'prevalence of *Salmonella*, S*higella* and intestinal parasites and antibiotic susceptibility of isolates among apparently healthy food handlers of Adama Science and Technology University students' cafeteria, Adama.'

Name of researcher: Abiti Asamnew (MSc candidate)

Introduction

You are invited to participate in a study to be conducted by MSc student at Jimma University, Institute of health Science, and School of Medical Laboratory Science. It is aimed at determining the prevalence of *Salmonella, Shigella* intestinal parasites and their drug susceptibility pattern among apparently healthy food handlers of Adama Science and Technology University student's cafeteria, Adama, Ethiopia.

Purpose of the study

The main objective of this study will be to assess the prevalence of *Salmonella, Shigella* and intestinal parasites and their antibiotic susceptibility among apparently healthy food handlers of Adama Science and Technology University students' cafeteria. Participation in this study is exclusively voluntary. If you are not interested to participate or if you once decide to participate and with draw at any time, there will be no consequences on your duty. If you decide to participate, you have to sign on the consent form and you may obtain a copy of this information sheet.

What will be expected from you as a participant of the study?

As a participant of this study you will be expected to agree to give stool sample. In

addition you will be expected to give answers for some questions about your health and socio demographic conditions. You need to know that the results might be discussed with appropriate individuals who can give you appropriate consult if the result is significant. But your name, address and phone number will not be disclosed and rather than identification code will be used in such conditions. If you participate you will get your own code for identification.

How much time will I spent to participate in this study?

You will spend about 10-15 minutes until you give the specimen, the questionnaire will be filled and the consent will be signed.

What will be the risks of participating in this study?

There is no risk associated with the specimen collection since you give stool sample as natural way and these specimens would follow the routine procedures for the laboratory investigation.

How our information will be kept in confidential?

All information that you give and the results will be used for this study only. Only limited number of professionals will have access to the information. All the information will be encoded in a computer and will be password protected.

What will be the benefits from participation?

Since this study is MSc student research, there will be no payment for participants and you will be not asked to pay for the laboratory examination. The result will be given to you and if your result will be clinically significant, it will help you for further diagnosis and treatment.

What will be your rights as a participant of this study?

You have the right to withdraw from the study at any time for this no problem faced

you by withdrawing. You have also welcomed if you have any question for further explanations about the study.

What can I do if I have a problem or question?

Please direct any questions or problems you may encounter during this study to (investigator, advisors)

1. Abiti Asamnew (Msc candidate) investigator.
   Cell phone: +251-920-18-13-20 [Email: asamnewabity@gmail.com or asamnewabity@yahoo.com]
2. Dr. Getnet Beyene (PhD) Advisor.
   Cell phone: +251-911-64-40-93 [Email: rgetnet@yahoo.com]
3. Dr. Mulualem Tadesse (PhD) Advisor.
   Cell phone: +251-913-16-26-24 [Email: mulualem.tadesse@gmail.com]

Jimma University, Institute of health, School of medical laboratory science

B. Garagalcha Afaan Oromo

Maqaa dhaabbataa: Yuuniveersiitii Jimmaatti Muummee fayyaa kuuta meedikaal laabooratorii saayinsii.

Mataduree Qorannichaa: Baakteriyaa dhukkuba taayfoidi, shigelosis fidan adda baasuu fi raamolee garaa keessa qorachu fi qorannoo qoricha isaan balleessu hojatootaa mana nyaata Yuunivarsiitii Saaynisii fi Teekinoolojii Adaama irratti gochuu.

Maqaa qorataa: Abitti Asaaminaw

Seensa

Unka kun fedhii hirmaatootaa qorannoo kana irratti fedhii isaaniin, dhimma kana keessa beekuun irratti hirmaachuuf waadaa seenanii dha. Kaayyoon Unki kun qophaa'eef inni guddaan hirmaattootni qorannoo kana geggeffamu irrati namootni hirmaatan fedhii isaanii kan ittiin mirkanneffatanii fi oddeefanno itti argatanii dha.

Gahee hirmaatootaa irratti eggamu

Qorannoo kana irratti hirmaachuf wantii isiin irra eegamuu saamudaa sagaraa keenuu fi gaaffiwan dhihaatan deebisuu qoofaa dha. qorannon kun kan gaggefamuu dhuuma baruumsaaa maastarii waan taheef kafaltii hoomaa hin kafalamuf qorannoo

laabooraatoriif ilee waan kafaltani hinjiru.

Yeroo isiin irraa fuudhatu

Daqiiqaa 10-15 hangaa saamudaa keenitani fi gaaffiwan hawaassumaa fi fayya walqabatani deebistanii qofa dha.

Miidhaa

Qorannoo kanatti hirmaachuun miidhamni gama fayyaan mul'atuu fi isin irraa ga'uu danda'u tokko illee hin jiru.

Bu'aa

Bu'aan adda yookin kafaltiin hirmaachuun argamu hin jiru. Haa ta'uu malee qorannoon kun halaa fayya keessan akka beektan isiinif fayada. Bu'aa qoranno keessan yoo barbaadan isiinif kennama. Gorsaa barbaachisuu bu'aa keessan walqabatee issinnif kennama.

Iccitii

Mirgi sagalee keessan bilisan kennuu fi iccitiin isaa sirriitti eegama dha. Tarii dhoksaatti sagalee keessan kennu yoo barbaadan mirga guutuu qabaachuu keessan isinii mirkaneessaa odeeffannoon isin irra argamu lakk. Dhoksaa (koodii) waan funaanamuuf odeeffannoo isiin laattan eenyuu illee adda baasee beekuu hin danda'u.

Fedhii hirmaachuu

Qorannoo kana irratti hirmaachuu dhiisuuf mirga guutuu qabdu. Kana malees erga jalqabdan giddutti kutuuf mirgi keessan eegama dha.

Teessoo

Yoo gaaffii qabaattan amma bilisa taatanii nagaafachuu ni dandeessu. Kana malees gaaffii kamiyyuu yoo qabaattan namoota armaan gadi dubbisu dandeessu.

1. Abiti Asamnew (Msc candidate) qoorataa
   Lakk bil: +251-920-18-13-20 [Email: asamnewabity@gmail.com
2. Dr. Getnet Beyene (PhD)
   Lakk bil: +251-911-64-40-93 [Email: rgetnet@yahoo.com]
3. Dr. Mulualem Tadesse (PhD)
   Lakk bil: +251-913-16-26-24 [Email: mulualem.tadesse@gmail.com]

C. **የአማርኛ ግልባጭ፤**

የድርጅቱ ስም፡- ጂማ ዩኒቨርሲቲ የሕክምና ማእከል የሜዲካል ላቦራቶሪ ሳይንስ ትምሕርት የምዲካል ማይክሮባዮለጂ ክፍል

የጥናቱ ርእስ፡- የሳልሞኔላ (ታይፎይድ) ፤ ሺጌለሲሰ በሽታ አምጭ እና የሆድ ውሰጥ ጥገኛ ተህዋስያን እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን የስርጭት መጠን በአዳማ ሳይንስና ቴክኖሎጂ ዩኒቨረሲቲ የተማሪዎች ምግብ ቤት በሚሰሩ ሰራተኞች ለይ ለማወቅ፡፡

የተመራማሪ ስም፡ አቢቲ አሳምነው

የአማካሪዎች ስም፡ - ዶ/ር ጌትነት በየነ

ዶ/ር ሙሉዓለም ታደሰ

የጥናቱ ዓላማ

የጥናቱ አላማ የሳለሞኔላ እና ሺጌሎሲስ በሽታ አምጭ ተህዋስያን ስርጭትና የተህዋስያኑ መድሃኒት የመቋቋም አቅም ለማወቅ ነው፡፡

ጥናቱ የሚያስገኘው ጥቅም

በጥናቱ በመሳተፈዎ ምንም አይነት ክፍያ አይጠየቁም ወይም የሚያገኙት ገንዘብ አይኖርም ነገር ግን የምርመራ ዉጤቱ ህክምና የሚያስፈልገው ከሆነ ተጨማሪ ምርመራ እና ህክምና እንዲያገኙ የረዳዎታል፡፡ስለሆነም ከጥናቱ በሚገኘው እውቀት ስለራስዎ የበለጠ እንዲያውቁ የረዳዎታል፡፡

ስጋትና ጉዳት

የሰገራ ናሙና በሚሰጡበት ወቅት ምንም አይነት ጉዳት አያጋጥሞትም፡፡ የሚያሰጋ ምንም ነገር የለዉም ምክንያቱም ናሙና የሚሰጡት ልክ በተፈጥሮ መንገድ ስለሆነ፡፡

ምስጢራዊነት

የሚሰጡት መረጃ ምስጢራዊነቱ የተጠበቀ ነው፡፡በስም አይጻፍም የዚህ ኮድ መፍቻ በፋይል ተቆልፎ የሚቀመጥ ሲሆን የተፈቀደለት ሰው ብቻ ፋይሉን ማየት ይችላል፡፡ከዚህ ጥናት በሚወጡ ዘገባዎች ወይንም የህትመት ውጤቶች ላይ ስም ወይም ሌላ የእርስዎን ማንነት የሚገልጽ መረጃ አይኖርም፡፡ከምርመራ የሚገኘው ውጤት ወይም ሌላ መረጃ ለሚመለከታቸው አካላት ለምሳሌ፤ የጥናቱን ስነምግባር ጠብቆ መከናወኑን ለሚከተሉት የኮሚቴ አባላት ብቻ ይገለፃል፡፡ኮምፒውተር ላይ ያሉ መርጃዎች ምስጢራዊነታቸው የተጠበቀ ሲሆን በወረቀት ያሉመረጃዎችም ደህንነቱ በሚጠበቅ ቦታ የሚቆለፉና የተፈቀደለት ሰው ብቻ ሊያያቸው እንዲችል ተደርጎ

ይጠበቃሉ::

ከጥናቱ ስለ ማቋረጥ፣

በጥናቱየሚሳተፉትፈቃደኛከሆኑበቻነው::ስለዚህመሳተፍምአለመሳተፍምከጀመሩበኋላም ማቋረጥ ወይም መመለስ የማይፈልጉት ጥያቄ ከሆነ ይለፈኝ የማለት ሙሉ መብትዎ ነው::በጥናቱ መሳተፍ ወይም አለመሳተፍ በስራዎ ላይ ምንም አይነት ጥቅምም ሆነ ጉዳት አይኖረውም::ጊዜዎትን መሰዋት አድርገው ሰለተባበሩኝ ከልብ አመሰግናለሁ::በተጨማሪ መረጃ መጠየቅ ቢፈልጉ በሚቀጥለው አድራሻ ማግኘት ይችላሉ::

1. አቢቲ አሳምነው (Msc ዕጩ)

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II. Annex: Consent form for participant: English Afan Oromo & Amharic version

A. English Version

A. Participant Code Number_____

I am informed fully in the language I understand about the aim of above mentioned research. I understood the purpose of the study entitled with "*Salmonella*, *Shigella* and intestinal parasite and antibiotic susceptibility of isolates among apparently healthy food handlers working in ASTU". I have been informed this study which involves collecting stool sample. During collection of the specimen I have been told that there is no harm it is just as natural way. In addition I have been told all the information collected throughout the research process will be kept confidential. I understood my current and future duty will not be affected if I refused to participate or with draw from the study. I after being fully informed about the detail of this study, hereby give my consent to participate in this study and approve my agreement with signature.

Participant name _____ signature _____ Date_____

Investigator name _____signature _____Date_____

B. Unkaa waligaltee (Garagalcha Afaan Oromoo)

Lakkoofsa hirmaataaf kenname ……

Yommuun qorannoo kana irratti hirmaadhu afaan naaf galuun natti himameera ykn naaf ibsameera. Faayidaa qorannoo kanaatis ''Baakteriyaa dhukkuba Saalmonellosis (Taayfoyidi) fi Shigeelosiis fidan fi raamoole garaa keessaa adda baasuu fi qorannoo qoricha isaan balleessu irratti godhamu'' naaf galeera. Waa'ee dhukkubbii Taayifooidii fi Shiigeelosiis akkaa na gaafatamuu fi saamuda sagaraa akka kennamu naaf himameera. Odeeffannoo qorannoo kana irraa argamu hunduu iccitiin akka kaa'amus irratti walii galleerra. Qorannoo kana hirmaachuu yoon hin barbaadne ykn yoo addaan kute, ammas ta'ee fulduraaf fayyadamummaa kiyyarratti rakkoo tokkoollee akka hin uumnee naaf himameera. Anis erga naaf gale booda mallattoo kootin nan mirkaneessa.

Maqaa hirmaataa …….. Mallattoo …… Guyyaa…….

Maqaa qo'ataa……Mallattoo…… Guyyaa……

C. **የስምምነት ቅፅ (የአማርኛ ግልባጭ)**

የተሳታፊው መለያ ቁጥር _____

እኔ ስሜ ከታች የተጠቀሰው ተሳታፊ የሳልሞኔሎሲስ (ታይፎይድ) እና ሺጌሎሲስ በሽታ አምጭ ተህዋስያን ሥርጭት እንዲሁም የሆድ ውሰጥ ጥገኛ ተሕዋስ እና የተህዋስያኑ መድሃኒት የመቋቋም አቅም ምን ያህል እንደሆነ ለማውቅ የተዘጋጀ ጥናት ላይ እድሳተፍ ተጠይቄ ስለጉዳዩም ለመረዳት በቂ መረጃ በሚገባኝ ቋንቋ አግኝቻለሁ፡፡ስለሆነም የሰገራ ናሙና መሆኑን ስለተርዳሁ ናሙና ወስዶ መመርመር አስፈላጊ ስለሆነ ናሙናዉን በመስጠት ልተባበር ሙሉ ፈቃደኛ መሆኔን ገልጫለሁ፡፡ናሙና በምሰጥበት ሰዓት ምንም አይነት ጉዳት እንደሌለው ተነግሮኛል እንዲሁም ከመጠይቁ አንብቢያለሁ ወይም ተነቦልኛል፡፡በተጨማሪም የሚወሰዱ ማናቸውም መረጃዎች በሚስጢር እንደሚያዙ ተነግሮኛል፡፡እንዲሁም የምጠየቀውን መረጃ ያለመስጠትና በጥናቱ ያለመሳተፍ ከጥናቱ በማናቸውም ወቅት እራሴን ማግለል እንደምችል የተገለፀልኝ ሲሆን ይህንንም በማድረጌ ወደ ፊትም ሆነ አሁን በስራዬ ላይምንም ጉዳት እንደማይደርስ ተነግሮኛል፡፡እንዲሁም በጥናቱ ሂደት እንድሳተፍ ፍቃደኝነቴን በፊርማዬ አረጋግጠለሁ፡፡

የተሳታፊ ስም ----------ፊርማ ---------- ቀን ----------

የተመራማሪ ስም ----------ፊርማ ---------- ቀን ----------

III. Questionnaire: English version

	A Eligibility questioner	Answer
1	In the last 2 weeks, did you have any of the following sign and sympto	1. Diarrhea 2. Nausea 3.vomiting 4. Fever 5. abdominal pain
2	Do you take any antibiotic drug within the last three weeks?	1. Yes 2. No
	A. Questionnaire for the eligible Code _____	Date ----/--------/-------- EC.
	Socio demographic question	
1.	Sex	1 Male 2 Female
2.	Age in year ?	----------
3.	Educational status	1) 1-10 2) preparatory or college
4.	Monthly income in Eth. Birr	----------
5.	Family size (in Number)	----------
	B. Questionnaire related to food handling	
6.	Year of Service in this cafeteria?	1) < 5 yr 2) >5 yr
7.	Job position in the catering establishment?	.preparation **2**.cleaning utensil **3**. Hosting
8.	Do you have certification or training in food preparation and handling	1. Yes 2. No
9.	Do you attend continuous medical checkups?	1. Yes 2. No
10.	Do you wash your hands before touching/preparing food?	1. Yes 2. No
11.	Finger nail status	A. Trimmed B. not trimmed
12.	Do you know about salmonellosis and shigellosis and their transmission?	1. Yes 2. No
13.	Have you ever been diagnosed for either salmonellosis or shigellosis?	1. Yes 2. No
14.	If yes for the above question, did you follow all treatment?	1.Yes I finished 2. I discontinued treatment 3. I did not take treatment
15.	Do you wash your hands with water or soap after using toilet?	1. Only with water 2. With water and soap
16.	Source of drinking water	1. Pipe 2. Others
17.	Do you drink unpasteurized (un boiled) milk?	1. Yes 2. No
18.	Do you eat raw beef?	1. Yes 2. No
19.	Do you eat enough cooked vegetable?	1. Yes 2. No

Unka gaafiilee (garagalcha Afaan Oromoo)

Unki kun Hojjattoota mana nyaata barattootaa Yunivarsitii Saayiinsii fi Teekinoolojii Adaamaa keessaa dalaganiif qofa qopha'ee.

A. Gaaffiilee ulaagaa qo'annichaaf gahaa tahuu mirkaneessan.

1. Torbaan lamaan dabran kana keessatti dhukkubbin isiinitti mul'atee yoo jiraatee?

A. garaa kaasaa, B. fedhiin nyaata hir'achuu C. haqifataa D. boowoo E. dhukkubii garaa

2. Torbaan sadan darban keessaa qorichaa fudhattanii jirtu? A. Eyeen B. lakki

B. Gaaffii warra ulaagaa guutaniif dhihaate

Guyyaa --------/-------/-------- ALI lakk. / koodii ----------

C. Gaaffi hawaassumaa

1. Saala A) dhiiraa B) dubara
2. Umrii waggaan ----------?
3. Sadarkaa baruumsaa A) kuuta 1-10 B) qoophaa'iina fi koleejii
4. Galli ji'aa Birridhaan ----------
5. Baay'inaa maatii lakkoofsaan ----------

D. Gaafiilee qophii nyaata wajjin walqabatan

6. Baraa Tajaajilaa waggaan A. <5 B. 5 fi olii
7. Gahee hojii keessan? A. qoophii nyaata B.Meeshaalee qulquulessu C. baratootaa keesumeesuu
8. Sartafikeeti leenjii qoophii nyaata qabduu ? A. Eyyen B. lakki
9. Qorannaa fayyaa walitti fufaa ta'e ni gootu? A. Eyyen B. lakki
10. Nyaata tuquni fi qoopheesun duura harka keessan dhiqatuu? A. eyyen B. lakki
11. Qeensaa quuba ilaalchisee? A. Kan qorame B. Kan hin qoramne
12. Waa'ee dhukkubaa Saalmonolesisi (taayifoyidi) fi shiigeeloosisi dhagettanii beektu

A. Eyyen B. lakki

13. Qorannoo isaasnif keenamuu gootani beektuu ? A. eyyen B . lakki
14. ' Eyeen' yoo jatanii waldhaansaa fuudhatii ?

A. Fudhee Xuumureera B.Addaan kuuteraa C. Qorichaa hin fudhanee

15. Mana fincaanii eegaa fayyadamtan booda harka keessan akkamitti dhiqatu?

A. bishaaniin qoofa B. saamunaa fi bishaaniin

16. Bishaan dhugaatii eesa fayyadamtan? A.Birkaa B. Kan biraa(ibsa)
17. Anan hin affelamin dhugduu? A. eyyen B. lakki
18. Foon dhadhii ni nyaatuu? A eyyen B. lakki
19. Muuduraa fi fuuduraa hin affelamin nyaatu ? A. eyyen B lakki

ሀ) ለጥናቱ መስፈርት የማሙያ መጠይቅ	መልስ
በባለፉት ሁለት ሳምንታት ዉስጥ ከሚከተሉት ውስጥ የሚታይቦት የበሽታ ምልክት ካለ ይጥቀሱ፡፡	1. ትኩሳት 2. ማቅለሽለሽ 3. ተቅማጥ 4. ትውከት 5. የሆድ ህመም 6. የለም
በባለፉት ሶስት ሳምንተት ውስጥ መድሐኒት ወሰደው ነበር?	1. አዎ 2. የለም
በጥናቱ ለሚሳተፉ መጠይቅ መለያ ቁጥር	**ቀን ———/———/——— ዓ.ም.**
ፆታ	1) ወንድ 2) . ሴት
እድሜ (በዓመት)	----------
የትምሕርት ደረጃ	1)1-10 2) የምሰናዶ እና ከዛ ብላይ
የወር ገቢ (በብር)	----------
የቤተሰብ ብዛት (በቁጥር)	----------
ለ) ከምግብ ዝግጅት ጋር የተያያዘ ጥያቄ	
በምግብ ቤቱ ውስጥ ሰራ ከጀመሩ ምን ያህል ግዜ ሆኖት?	1. < 1ዓመት 2. 1-2 ዓመት 3. ከ5ዓመትበላይ
በምግብ ቤቱ ውስጥ ያሎት የስራ ድርሻ	1 ምግብ ዝግጅት 2.እቃ ማጠብ 3.መስተንግዶ
የምግብ ዝግጅት ስልጠና ሰርተፍኬት አሎት?	1. አዎ 2. የለም
ተከታታይ የሜዲካል ሕክምና ቼካፕ ያረጋሉ?	1. አዎ 2. የለም
ምግብ ከማዘጋጀት/ከመንካትዎ በፊት እጆን ይታጠባሉ?	1. አዎ 2. የለም
የእጅ ጣት ጥፍር ሁኔታ	1. የተቆረጠ 2. በትንሹ የተቆረጠ 3. ያልተቆረጠ
ስለ ሳለሞኔሎሲስ (ታይፎይድ) እና ሽጌሎሲስ በሽታ ያውቃሉ?	1. አዎ 2. የለም
ለሳለሞኔሎሲስ (ታይፎይድ) እና ሽጌሎሲስ በሽታ ታክመው ያው	1. አዎ 2. የለም
አዎ ካሉ መድሐኒት በተከከል ተከታትለዋል?	1. ጨርሻለሁ 2. አቋርጫለሁ 3. አልወሰድኩም
መጸዳጃ ከተጠቀሙ በኋላ በውሃ ወይም በሳሙና የመታጠብ ልምድ	1.በውሃ ብቻ 2.በውሃና ሳሙና 3.አልታጠብም
የመጠጥ ውሀ ከየት ይጠቀማሉ?	1. ከቢርካ 2. የታሸገ ውሀ 3. ሌላ(ይጠቀስ)
በደንብ ያለተፈላ ወተት ይጠጣሉ?	1. አዎ 2. የለም
ጥሬ ስጋ ይበላሉ ?	1. አዎ 2. የለም
በደንብ ያልበሰለ አትክልት ይመገባሉ	1. አዎ 2. የለም

IV. Annex: Laboratory data collection format

1. Participant ID. ---------- Sample ID ----------

2. Date of sample collection ----------/----------/----------

3. Type of specimen: stool. □ Other □

A) Bacteriological result

4. Culture growth: Yes □ No □

5. Gram stain result----------

7. Name of bacteria, if isolated----------

8. Biochemical identification test results----------

9. Antimicrobial susceptibility testing S (mm) I (mm) R (mm)

- Ampicillin (20µg) ---------- ---------- ----------
- CIP- Ciprofloxacin (5µg ---------- ---------- ----------
- Ceftriaxone (30µg ---------- ---------- ----------
- AMC-Amoxicillin-Clavulinic (20µg ---------- ---------- ----------
- CAF-Chloramphenicol (30µg ---------- ---------- ----------
- SXT-Trimethoprim- Sulfamethoxazole(25µg ---------- ---------- -------
- Tetracycline ---------- ---------- ----------

B) Parasitological result positive? Yes ☐ No ☐

-Tick the box for result

- ✓ A.*lumbricoid* ☐
- ✓ Hook worm spp. ☐
- ✓ E. *histolitica /dispar* ☐
- ✓ G. *lamblia* ☐
- ✓ S. *storcolaris* ☐
- ✓ T.*trichuria* ☐
- ✓ Tainia *spp* ☐
- ✓ Other specify -_____

V. Annex: Biochemical tests to isolate *Salmonella* and *Shigella* species

Types of Tests		*Salmonella* spp.	*Shigella* spp.
Triple Sugar Iron (TSI)	Lactose	**(-)**	**(-)**
	Glucose	**(+)**	**(+)**
	H2S	**(+)**	**(-)**
	Gas production	**(+)**	**(-)**
Simmons citrate agar		**(+/-)**	**(-)**
Urea agar base	Urease	**(-)**	**(-)**
Sulfide-indole-motility medium (S.I.M.)	Motility	**(+)**	**(-)**
	Indole production	**(-)**	**(+/-)**
Lysine Iron Agar		**(+/-)**	**(-)**

VI. Procedure of Formol ether Concentration technique (32)

1. Emulsify 1 gm. of feces in 7 ml of 10% formalin in a centrifuge tube
2. Strain the suspension through a brass wire sieve, and collect in beaker.
3. Pour the filtrate into a 15 ml conical tube and add 3 ml of ether, then mix well 15 sec on vortex or 1 min by hand.
4. Transfer the ether- formalin suspension back into the washed centrifuge tube, and centrifuge at 3,000 rpm for 1 min.
5. Loosen the fatty layer and debris at the top of the tube with an applicator stick and invert the tube quickly to discard the supernatant.
6. Few drops only should remain with the sediment, mix the sediment well and transfer one drop onto a glass slide and cover it with cover slip.
7. Scan the whole cover slip using 10X objectives, turning into 40X for confirmation of identification of parasites.
8. Report your finding

VII. Annex: Declaration Sheet

I hereby certify that I have read and evaluated this thesis prepared, under my guidance, by Abiti Asamnew entitled" **Prevalence of *Salmonella*, *Shigella* and Intestinal Parasites Among Apparently Healthy Food Handlers of Adama Science and Technology University Students' Cafeteria, Adama, Ethiopia"** I recommend that it can be submitted as fulfilling of the thesis requirement.

Name of principal investigator

Abiti Asamnew Signature _____ Date_____

Internal examiner

Dr. Tesfaye Kassa (PhD). Signature _____ Date _____