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College of Health Science

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Prevalence of Intestinal parasites, *Salmonella* and *Shigella*, Associated Risk Factors and Antibiotics Susceptibility Pattern of isolates amongfood handlers in Addis Ababa police commission camps, Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by Feleke Talegeta, entitled:

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List of abbreviations

AOR…….Adjusted odd ratio

AST…….Antimicrobial susceptibility testing

CDC ……..….. Centers for disease control and prevention

CI ……...…… Confidence interval

DCA ……..…… Deoxycholate citrate agar

KIA …….……..Kliger iron agar

IPs …….…...…….….. Intestinal Parasites

LIA……...…..…….. Lysine iron agar

NLF ……...…..…….. Non-lactose fermenting

SOP ……...…….. Standard operating procedure

WHO…….…….……..World health organization

XLD …….……Xylose Lysine Deoxycholate agar

Abstract:

Background: Diseases such as *Salmonellosis,Shigella,* and intestinal parasites remain a major public health problem worldwide. The problem is especially acute in developing countries due to the personal hygiene and handling practices of food processors. Food handlers have been caught by various pathogens and are being transmitted to the police. The purpose of this study was to examine the presence of intestinal parasites, *Salmonella* and *Shigella*, associated risk factors and antibiotics susceptibility pattern of isolates among food handlers in Addis Ababa police commission camps, Addis Ababa, Ethiopia.

Methods: From February to May 2021, cross-section study were conducted in ten sub-cities selected by Addis Ababa Police Commission Camps. Social demographic data and fecal samples were collected from 247 study participants. Stool specimens were tested by wet mount and floatation, and sedimentation with microscopic.The stoolculturewasdone onXylose Lysine Deoxycholate agar (XLD) and Deoxycholate citrate agar for(DCA) *Salmonella*andShigella identified on the biochemical tests(nutrient broth,KIA, Citrate, LIA, Urea, and Motility) and also Salmonella agglutinating test performed.Antimicrobial susceptibility test was doneon Muller Hinton agar plates against ampicillin (10 μg), ceftriaxone (30 μg), chloramphenicol (30 μg), and ciprofloxacin (5 μg). The Data were entered, coded and analyzedwith SPSS version 25.The associations between risk factor and intestinal parasite were tested using the Chi-square test. P values ≤ 0.05 were considered to indicate statistical Significance.

Results: The majority of food handlers (83%) were young people aged less than 26,the mean age of the respondents 22.7, standard division 5, and range 38. All the food handlers were women. One hundred and forty-three (57.9%) food handlers were educated beyond primary school. Most (54.3%) of food handlers were serve below one years. A 98.8% of the participants interviewed had a toilet in the workplaceand wash their hands before serving and serving any food. Similarly, most the respondents 179 (72.5%) said that when they suffer from diseases such as diarrhea, they do not prepare food.Twenty-nine (11.74%) of the participants had intestinal parasites. *Entamoebahistolytica/dipar* was the most widespread parasite (5.7%) of those who provided stool samples. In addition, 1.6% (4/247) of the samples was positive for *Salmonella*. All of the *Salmonella* isolates were sensitive for gentamicin, ciprofloxacin, ceftriaxone, tetracycline, chloramphenicol, amoxicillin / clavulanic acid, ampicillin-sulbactam, Nacidixcacid, cotrimoxazole, imipenem, and marocain and all isolates were resistant to antibiotics like Ampicillin, Ceftazidime, Cefotaxime, and Cefuroxime.

Conclusions:

We conclude that the health problems in the study area areintestinal parasite and *Salmonella*. To address foodborne illness in Addis Ababa Police Commission Camps regular health education and training programs among food handlers had requested to address.

Keywords: Intestinal parasite, *Salmonella*, *Shigell*a, food handler

1. Introduction

1.1 Background

Foodborne illnesses are a global concern, as they can be responsible for illness, death, and economic hardship(1)(2). Many different diseases, including those caused by bacteria, viruses, and parasites, can be transmitted to humans through contaminated food (3). Outbreaks appear to be exacerbated during foodborne illness. In recent decades, food globalization has also led to the rapid movement of food-borne pathogens across international borders(4). The foodborne epidemic has had a negative impact on trade and food security (5)(6). In response to foodborne illnesses, national governments and international bodies have established sophisticated systems to monitor and improve food security(7).

The Centers for Disease Control and Prevention found that third most reported food preparation practice in foodborne illnesses were personal hygiene(8). *Salmonella* is transmitted from infected people, from regular asymptomatic carriers to food or water (9). *S. paratyphi A*, *B*, and C are often serious and occasionally life-threatening bacteria.In the United States, an estimated 80 cases of paratyphifever are reported each year, with 90% occurring during international travel. Paratyphifever cases of serotypes *S. paratyphoid*B and C have been reported more frequently. Ongoing *S. Paratyphi*infections are important to identify and control outbreaks, determine public health priorities, monitor disease trends, and evaluate the effectiveness of public health interventions (10).Moreover, 79.2% and 44.1% were resistant to chloramphenicol and gentamicin, respectively for*Salmonella*reported that clinical specimens at the University of Gondar Hospital (11).

In the world, especially in developing countries, parasites aremajor health problems(12). According to the World Health Organization (WHO), about two-thirds of the world's population is infected with the same type of intestinal parasite, and *Ascariasis* and Giardia infections are the most common of all (13). In some parts of the country, parasites are more prevalent due to the region's climate, local customs, and the use of human and animal waste as fertilizers(14). Lack of clean and safe water, overcrowding, lack of proper waste disposal, lack of respect for health (social and individual), inadequate vegetable washing, and lack of well-cooked meat increase the risk of intestinal parasites. (17,18).A study of Addis Ababa University students' cafeteria, found that 3.4%*Salmonella* strains from food handlers were tested (19). In a study conducted in Tigray

Prison Centers in Ethiopia, majority of participants had one or more intestinal parasites. In the 23.7% of the study participants protozoan*Entamoebahistolytica/dispar*was detected. In addition, 60.6% of the participants had a good level of knowledge and 51.5% had a good level of practice in foodborne illness and food safety (20).The study was designed to detectintestinal parasites, *Salmonella* and *Shigella*, Associated Risk Factors and Antibiotics Susceptibility Pattern of isolates among food handlers in Addis Ababa police commission camps, Addis Ababa, Ethiopia.

1.2 Statement of the Problem

The burden of foodborne illness is high - an estimated 420,000 people died worldwide, and 1 in 10 people get sick each year, and 33 million healthy lives are lost. Foodborne illness can be especially serious for young children. Diarrhea is the most common foodborne illness, affecting 550 million people each year, including 220 million children under the age of five. Malnutrition is one of the four major causes of diarrhea in Africa, with 137,000 deaths each year and 91 million acute illnesses. (21). Food borne trematodes loses 2 million lives a year worldwide due to disability and death (21).

According to the Centers for Disease Control (CDC), food-related illnesses kill an estimated 76 million people each year, with 325,000 hospitalizations, and 5,000 deaths in the USA (22).The survey in Bahir Dar, northwestern Ethiopia, found that 384 food handlers had no previous medical examinations 54 (14%) were food handlers.The food handlers had intestinal parasites, *S. typhi*, and diarrheawere 41.1%, 1.6%, and 6.5%, respectively. *E. histolytic / dispa*r 12.76% and *G.lamblia* 7.0% and *A. lumbricoides*, 11.7%, *Hookworm*, 8.1%, *S. stercoralis*, 2.86%, *S. manson*, 1.8%, *Tania species*, 1.3%, *H. nana*, 0.5% and *T.trichiria*, 0.5%(23).

According to a study conducted by Addis Ababa University student cafeteria, some of food handlers were positive for various intestinal parasites and fecal cultures. (24). Therefore, hygienic pathogens can be a major source of food contamination, as infected food handlers come in handy from the time of preparation to serving. A community-based cross-sectional study done in Ethiopia by working in food service establishments. For food handlers, 41% had one or more intestinal parasites and 2.5% exclusion was *Salmonella typhi*. Eating raw meat, washing hands after using the toilet and touching contaminated materials have been linked to intestinal parasite. Regular check-ups are essential to prevent the transmission of intestinal parasites and *salmonellosis*(25).It was reported that*S. typhi* 79.2% and 44.1% of the clinical samples at Gondar University Hospital were respectively resistant to chlorexidine and gentamicin(12).The spread of intestinal parasites,*Salmonella, andShigella*among food handlers in the Addis Ababa Police Commission camps are not known. Therefore, this study is designed to identify food handlers who are affected by the intestinal parasites, *Salmonella,* and *Shigella* among food handlers in the Addis Ababa Police Commission camps.

1.3 Significance of the Study

This study was providing information about intestinal parasites,*Salmonella* and *Shigella* toward food handlers. This study provides concrete evidence of *Salmonella* and *Shigella*helps to create awareness among related health problems by developing strategies to prevent and control the spread of intestinal parasites among food and micro-organisms in the study area. It serves as a starting point for further research.In addition, information from this study can be used as a reference for further similar studies in Ethiopia and around the world.

2. Literature review

It kills an estimated 420,000 people worldwide, 1 in 10 gets sick each year, and 33 million healthy lives are lost. Foodborne illness can be especially serious for young children. Diarrhea is the most common foodborne illness, affecting 550 million people each year, including 220 million children under the age of five. In Africa, food poisoning is responsible for 137,000 deaths each year and 91 million acute illnesses, particularly affecting children under the age of five(21).

The spread of intestinal parasites in Bandar Abbasfood monitors in southern Iran was 34.9% positive for fecal parasites. The most affected individuals are 54.3% of bakery workers, 41.1% of fast food factories, 34.7% of supermarkets, 34.7% of supermarkets, 33.9% of restaurants, 29.8% of offices, 27.3% of butchers and coffee shops, 26.7% ($P < 0.05$), respectively. Intestinal parasites Blastocystishominis were 24.3%, *Entamoeba Coli* 8%, *Giardia lamblia*6.8% and *Dientamoeba fragilis*4.3%. In this study, only two infections were found in *Hymenolepis nana*(0.3%) and *Enterobiusvermicularis*(0.1%). Workplace contact and direct contact with raw food influenced the spread of intestinal parasites ($P < 0.05$) (26).

In a study of 984 participants in the northern province of Mazandaran, the IPI's overall prevalence was 12.1%. The prevalence ofparasite was13.2%. The most common protozoa IPs are included *Giardia lamblia* (3.3%), *Entamoeba coli* (2%), *Blastocystishominis* (1.3%), *Entamoebahistolytica/dispar* (1.1%), *Entamoebahartmanni* (1.1%), and *Cryptosporidium spp*. (0.6%). Also, major helminthic infections were including *Trichostrongylus spp*. (2.6%), *Hookworms* (1.7%), *Strongyloidesstercoralis*(1.1%), *Hymenolepis nana* (0.6%) and *Dicrocoeliumdendriticum* (0.6%)(27).

In a study conducted in Lagos, Southwest, Nigeria, 31.5% of the population surveyed reported IgG and IgM anti-salmonella immunoglobulin in the past or present, and 68.5% had no recent or previous infection. Ninety-three respondents are male and one hundred and forty-two are active working-age braces between 11-60 years. Of the 93 men tested, 28.0% were infected, 72.0% were uninfected, and 33.8% of the 142 women screened for Salmonella *enteric serovarTyphi* and *Paratyphi*infections were infected (28).

In the study done southern, Ethiopia, intestinal parasites were predicted by 10.0%, and the corresponding *Ascarislumbricoides*, hookworm, and *Trichuristrichiura were* 41.7%,33.3%, and 25.0% intestinal parasite has been identified, respectively. *Salmonella typhoid*were 6.3%

distribution recorded (29), and also in the study of eastern Ethiopia, the total prevalence of intestinal parasites in this study was 61.9%. The most common parasite *A.Alumbricoides* was 45.6%. Protozoan infection was higher than helminth infection. Several intestinal infections have been identified; 34.6% of the study participants had a double infection. The most logical causes of intestinal parasites are comparative condition, habitat, and food contamination information related to intestinal parasites, water before and after contact with food only (30).

A study conducted by Hawassa University's main campus and the College of Health Sciences found that 20.6% tested for gastrointestinal pathogens were positive for various intestinal parasites. Among the parasites, *Ascarislumbricoides* were the most widespread parasites (9.5%), followed by *Strongyloidesstercoralis* (2.2%) and *Entamoebahistolytica /dispar* (2.2%). No *salmonella spp*and *Shigella spp.*was found in stool culturesbuttwenty-two (8.1%) of food handlers were positive for the Widaltest (31).

According to a study by Bahir Dar University, the prevalence of *S. typhi* and intestinal parasites among food handlers was 2.7% and 12.9%, respectively. Of the eight identified intestinal parasites, two were the most common intestinal parasites, hookworm 6.3%, and *G. Lamblia* was 3.1%. Female food handlers were more likely to be positive for typhiod and intestinal parasites than male. In addition, dieters with a history of routine medical examination were less likely to be infected with intestinal parasites(32).InYebu Town, Southwest Ethiopiaa total of 376 food inspectors were registered, 31 of whom refused to participate in feces testing. 36% of food handlers for various intestinal parasites, 14% of which is *Entamoebahistolytica/dispar*, followed by *Ascarislumbricoides*, 9.27% (33).

According to a study conducted in Dire Dawa, the overall distribution of *Salmonella* and *Shigella* was 8.7%. The most common identified were *Salmonella* (6%). Most of the isolates were resistant to amoxicillin, ampicillin, and tetracycline were 97.7%, 89.5%, and 68.4%, respectively. About half (47.4%) were resistant to *Salmonella*. Those who did not wash their hands with soap and did not cut their nails after using the toilet had a higher risk of exposure than their counterparts like *Salmonella* and *Shigella* (34).

A study of cafeteria stool samples by Addis Ababa University students revealed that 3.4% of the *Salmonella* species collected from 233 food handlers. Two of these were *S enteric serovartyphi*, one *S paratyphi* A and the other five were *Salmonella serovars*. Among the risk factors, post-toilet and soap-free hand washing practices were statistically significant for *Salmonella* carriers.

The presence of 3.4% *salmonella* species may be a source of salmonellosis unless carriers undergo routine tests and other preventive measures such as health education, food handlers training (18).

According to a study among food handlers at WolayitaSodo University student restaurants, the prevalence of intestinal parasites was 23.6% and 12.4% was due to amoeba cyst. Uncontaminated nails and regular hand washing before food handling have been found to be highly associated with intestinal infections (35).

Results of all 40 *Salmonella* and 17 *Shingles* isolates. The highest resistance was found in *Shigellaspp.* for tetracycline 14 (82.4%) and co-trimoxazole 13 (76.5%). And ampicillin 24 (60%) and tetracycline 21 (52.5%) in *salmonella spp*. On the other hand, the highest levels of exposure to ceftriaxone and ciprofloxacin were found to be 100% and 88.2%, respectively. In the majority of*Shigella*species, resistance to one or more antimicrobial agents was found and 9 (56.25%) showed multiple resistance patterns (36).

In a study conducted by the University of Adigrat, antimicrobial cracking was performed for 22 *Salmonella* and 9 antimicrobial agents, selecting 11 *Shigella*. Both *Salmonella* and *Shigella* isolates were100%resistantto amoxicillin. *Salmonella*isolates were 90.90% resistant to gentamicin (42).

According to a study conducted by food handlers in the town of Bebu in southwestern Ethiopia, the total prevalence of intestinal parasites was 44.1%. *Ascarislumbricoides* and *hookworm* contain the parasites identified in the feces of the study participants. Hand washing before meals is not a regular practice, and untested fingernails have been the most common prognosis for intestinal parasites among food handlers (36).

In a cross-sectional study conducted at Tigray Prison Centers, Ethiopia the majority of participants with one or more intestinal parasites. Protozoan *Entamoebahistolytica/dispar* was detected among 23.7% of the study participants. In addition, the participants had a good level of knowledge and a good level of practice in foodborne illness and food safety were 60.6% and51.5%, respectively (19). Among the asymptomatic food handlers working at Haromaya University cafeterias, the findings highlight the fact that food handlers are the source of foodborne infections that require proper hygiene measures in university cafeterias (37).

According to Mengistu G. Ital, 10.5% of *salmonella* species were isolated from 382 patients. *Salmonella* species were identified: 15% of group A (somatic antigen O, O: 2), each group B (O:

4), D1 (O: 9) and D2 (O: 9, 46), and 7.5% of group C (O: 7 / O8) could not be typed with 40% antisera while isolated (38).

In Nekemte, western Oromia, Ethiopia, the prevalence of intestinal parasites in food service was 52.1%. *Entamoebahistolytica/dispar* was (56.8%), *Ascarislumbricoides* (26.4%), *Taeniasaginata* (16%), and *Hookworm* (16.8%). Hygiene practices such as toilet flushing, post-toilet flushing with soap and water, nail trimming, and proper work clothes and shoes were statistically significant for intestinal infections (39).

A study of food and beverage service providers in northern Ethiopia has identified five intestinal parasites (IPs). The prevalence of at least one intestinal parasite was 14.5%. It is 12.3 times more likely to be positive for at least one intestinal parasite infection than for a chef every 9 months. The risk of developing intestinal parasites was 3 times higher among those with no formal education than those in high school and higher. Food trainers who have received food hygiene and safety training have a 66% lower risk of developing intestinal parasites compared to those who have not been trained (40).

A study of health food managers in Addis Ababa University Student Cafeteria, Addis Ababa, Ethiopia found that 45.3% of food handlers had*Entameobahistolytica/dispar*, *Giardia lamblia*, *Taenia specie,Ascarislumbricoides,hookworm*, and *Trichuristrichiura were 70.8%, 18.8%, 5.2%*,2.1%,2.1%, and 1.1%, respectively. Stool cultures *Salmonella*were 3.5% and *Shigella* species are not isolated from any fecal samples. All *Salmonella* profiles were sensitive to ciprofloxacin, amikacin, and gentamicin, but were resistant to amoxicillin, clindamycin, and erythromycin (24).

A community-based cross-sectional study of unknown food processors in the South; was done in Ethiopia by working in foodservice establishments. For food handlers, 41% had one or more intestinal parasites. Intestinal pathogens have been linked to eating raw meat, washing hands after using the toilet, and touching contaminated materials. Regular check-ups by food handlers are essential to prevent the transmission of intestinal parasites and salmonellosis (25).

In a study of food handlers at the Adigrat University student cafeteria in northern Ethiopia, 7.3% of food handlers were positive for *salmonella*. Hand washing after touching dirty items, not washing hands before handling food and undiagnosed nails have been identified (41).

3. Objectives

3.1 General Objective

To assess prevalence of Intestinal parasites, *Salmonella* and *Shigella*, Associated Risk Factors and Antibiotics Susceptibility Pattern of isolates among food handlers in Addis Ababa police commission camps, Addis Ababa, Ethiopia.

3.2 Specific Objectives

- To determine the prevalence of intestinal parasites,*Salmonella* and *Shigella*among food handlers work at Addis Ababa police commission camps, Addis Ababa,Ethiopia.
- To determine risk factors associated with intestinal parasite and *Salmonella* infection among food handlers work at Addis Ababa police commission camps, Addis Ababa, Ethiopia.
- To identify the antimicrobial profile of the bacterial isolates at Addis Ababa police commission camps, Addis Ababa, Ethiopia.

4. Materialsand Methods

4.1. Study Design and Area

From February to May 2021, a cross-section study was done from asymptomatic food handlers of the Addis Ababa.Addis Ababa is the capital of Ethiopia and the largest city in the country. Addis Ababa is located in the heart of the country with a population of 7.5 million and stands at an altitude of 7,726 feet above sea level. The city is divided into 10 sub-cities: such as Addis Ketema, AkakiKaliti, Arada, Bole, Gullele, Kirkos, KolfeKeranio, Lideta, NifasHar-Lafto, Yeka(42)

4.2. Study Population

The study population wasAddis Ababa police commission cafeterias food handlers working more than threemonths.

4.3 Eligibility

4.3.1. Inclusion criteria

Addis Ababa police commission cafeterias working food handlers greater than 3 months and give informed consent were included in the study.

4.3.2. Exclusion criteria

Food handlers who had taken antibiotics, anti-helminthic in the last three months, and incomplete questionnaires were excluded from the study.

4.4. Study Variables

4.4.1. Dependent variables

- The prevalence of intestinal parasite
- The prevalence of *Salmonella*
- The prevalence of *Shigella*
- *AST*

4.4.2. Independent variables

- Sex
- Age

- Hand washing habit
- Medical cheek up
- Educational level
- Source of drinking water
- Certified in food training
- Latrine usage
- Total year working
- Nail trimming habit
- Sharing knife and other equipment's used for food preparation
- Habit of eating raw or undercooked food
- Preparing food when suffering from diarrhea

4.5. Sample size determination and sampling technique

4.5.1. Sample size determination

The sample size was determined by using a single population proportion formula taking into account the following assumptions: formula (n=Z2/2p (1-p)/d2) that have a power of 50%; where n= minimum sample size, p = proportion in the population believed to be an intestinal parasite, Salmonella typhi is 50%, Z= a value of standard normal deviate at 95% usually set at 1.96 confidence level, d=level of precision=0.05 (sampling error of 5%). Zα/2 = 1.96 for the standard scale of 95% level of confidence, level of precision = 5%:

(n=Z2/2 p (1-p)/d2) =384

Since the total number of the source, the population is 1000 and below 10,000 a correction formula was used to adjust the sample size as follows:

n/1+ n/N= 384/1+384/700≈ 247

4.5.2. Sampling technique

For the selection of study subjects, a simple random sampling technique was used. A 700 list of food handlers was obtained from the Addis Ababa Police Human Resources Management and 247 are taken.

4.6. Data Collection Tools and Procedure

The food handlers were informed about the study, the objective, and asked for consent. After Food handlers signed informed consents Socio-demographic information and Stool sample was collected from each study subject at Addis Ababa police commission all sub city clinic.

4.6.1 Stool specimens collection

A stool sample was collected by providing stool containers, tissue paper, and a clean applicator stick, to each study subject. Then the stool samples were examined in sub-city police clinic for the intestinal parasites and the other samples were transported 1 gram swabs and placed in a container with transport medium Cary–Blair to Yekatete 12 hospital microbiology laboratory for bacterial study.

4.7. Diagnosis of intestinal parasites

4.7. 1.Direct smear examination for stool samples

On a glass slide, the stool was emulsified in a standard saline drop (0.85% NaCl) and approximately 1-2 mg. A cover sheet was placed on each slide and, as needed, the slides were removed from the optical microscope with 10x and 40x real lenses. Direct detection of intestinal protozoan trophozoites, which are preserved in direct saline smear liquid or semi-liquid samples, was mainly used(43).

4.7.2Formol-Ether Sedimentation Concentration Technique

Formol ether procedure cannot detect trophozoites; it is deemed the best concentration technique used in diagnostic for the discovery of cysts, ova, and larvae (44, 45). To kill and preserve the diagnostic stagesregularly, 10% formal saline was used. Diethyl ether collects most of the debris in a separate layer. All diagnostic stages that are applicable to the technique were collected at the bottom of the analysis centrifuge tube. However, safety precautions were taken as formalin is carcinogenic and diethyl ether is flammable and explosive. The greater the amount of stool used, the greater the chance of recovery from the diagnostic stages. The sedimentation technique was performed by emulsifying approximately 2 g of feces in 10-15 ml of 10% formaldehyde saline. The suspension was allowed to stand for 30 minutes and was then cast through two layers of cheesecloth into a 15 ml conical centrifuge tube and centrifuged at 2000 rpm for 5 minutes. When necessary, the washing step was repeated until the supernatant cleared. The pellet was

suspended with 10 ml of 10% formal saline and allowed to stand for 5-10 minutes. A total of 3 ml of diethyl ether is added and then the tube is vigorously shaken for 30 seconds and centrifuged at 2000 rpm for 5 minutes. After centrifugation, the applicable diagnostic step was the sediment at the bottom of the tube. The fecal waste will separate into a layer between the diethyl ether and the formalin-10% saline layers. A layer of fecal waste is loosened with a wooden stick and the tube is quickly inverted to discard the top three layers while the sediment remains at the bottom. Then, part of the pellet was transferred to a microscope slide, covered with a coverslip, and scanned microscopically with high and low objective lenses. (46).

4.7.3 Zinc sulfate Flotation technique

Flotation techniques (zinc sulfate) use solutions which have higher specific gravity than the organisms to be floated so that the organisms rise to the top and the debris sinks to the bottom. The main advantage of this technique is to produce a cleaner material than the sedimentation technique. The disadvantages of most flotation techniques are that the walls of eggs and cysts will often collapse, thus hindering identification. Also, some parasite eggs do not float.

4.8. Diagnosis of*Salmonella* and *Shigella*

4.8.1 Culture and Isolation of *Salmonella* and *Shigella*

Ten (10) mL of Selenite F broth, an enrichment medium that inhibits the growth of coliforms while allowing the growth of *Salmonella* and *Shigella* species was inoculated and incubated overnight at 37oC aerobically. The overnight incubates Selenite F broth will be subculture onto XLD and DCA further incubate for 18-24 hours at 37o C. Non-lactose fermenting (NLF) colonies growing on plates was identified on the biochemical tests (nutrient broth, KIA, Citrate, LIA, Urea, and Motility) and *Salmonella* agglutinating test performed(16).

4.8.2 Antimicrobial susceptibility tests for *Salmonella* and *Shigella*

Antimicrobial susceptibility test was done for identified bacteria using disk diffusion method on Muller Hinton agarplates against ampicillin (10 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg) (47).

4.9. Data Quality Control

The data collector was a trainee in the processes of the data collection procedure. The completeness and simplicity of the collected data were monitored every day. Pretests, structured

questionnaire were used for the data collection on socio-demographic characteristics and associated risk factors. The questionnaire was originally provided in English and interpreted into the local language, Amharic.

4.10. Laboratory Quality Control

To assure the quality of data questionnaires was translated to Amharic and pretested, data collectors were trained a day before and after a pretest, continuous supervision will be done by the principal investigator, standard operating procedures should follow in pre-analytical sample collection, analytical processing, post-analytical result interpretation, and record and also laboratory investigations were done by competent laboratory professionals.

The sterility of culture media was checked by incubating about 5% batch of the media at 35–37°C overnight and assess for potential contamination. Standard source strains of *S.typhimurum* (ATCC-14028) and *S. flexneri* (ATCC 12022) were used as quality control throughout the study for culture. Data quality was warranted at various activities of the study by attending the provided standard operating procedure (SOP) of the laboratory.

4.11. Data Analysis and Interpretation

Data were entered and analyzed with the Statistical Package for Social Sciences (SPSS) version25 software.Frequencies test andthe associations between proportions were tested using the Chi-square test. P values ≤ 0.05 were considered to indicate statistical significance.

4.12. Ethical Considerations

Ethical clearance was obtained from the School of Medical Laboratory Sciences, University of Addis Ababa ethical review committee, and a letter informing the Addis Ababa police commotion about the purpose of the study was written by the School of Medical Laboratory Sciences, University of Addis Ababa. Support letter was obtained from the Addis Ababa police commotion before data collection. Informed Consent was obtained from each study participant after explaining the purpose and objective of the study. The study participant was informed that all data and samples obtain from them are kept confidential by using codes instead of any personal. Participants found positive for intestinal parasites and Salmonella was treated.

4.13. Operational Definitions

Intestinal parasite infection: is a condition in which a parasite infects the gastrointestinal tract of food handlers.

***Salmonella*:** is a symptomatic infection caused by bacteria of the *Salmonellosis in food handlers.*

Shigella:species are bacteria that cause a foodborne illness called shigellosis.

Food handler: a person who is working in cafeteria and engaged in the process of food preparing, serving and cleaning.

5. Workflow

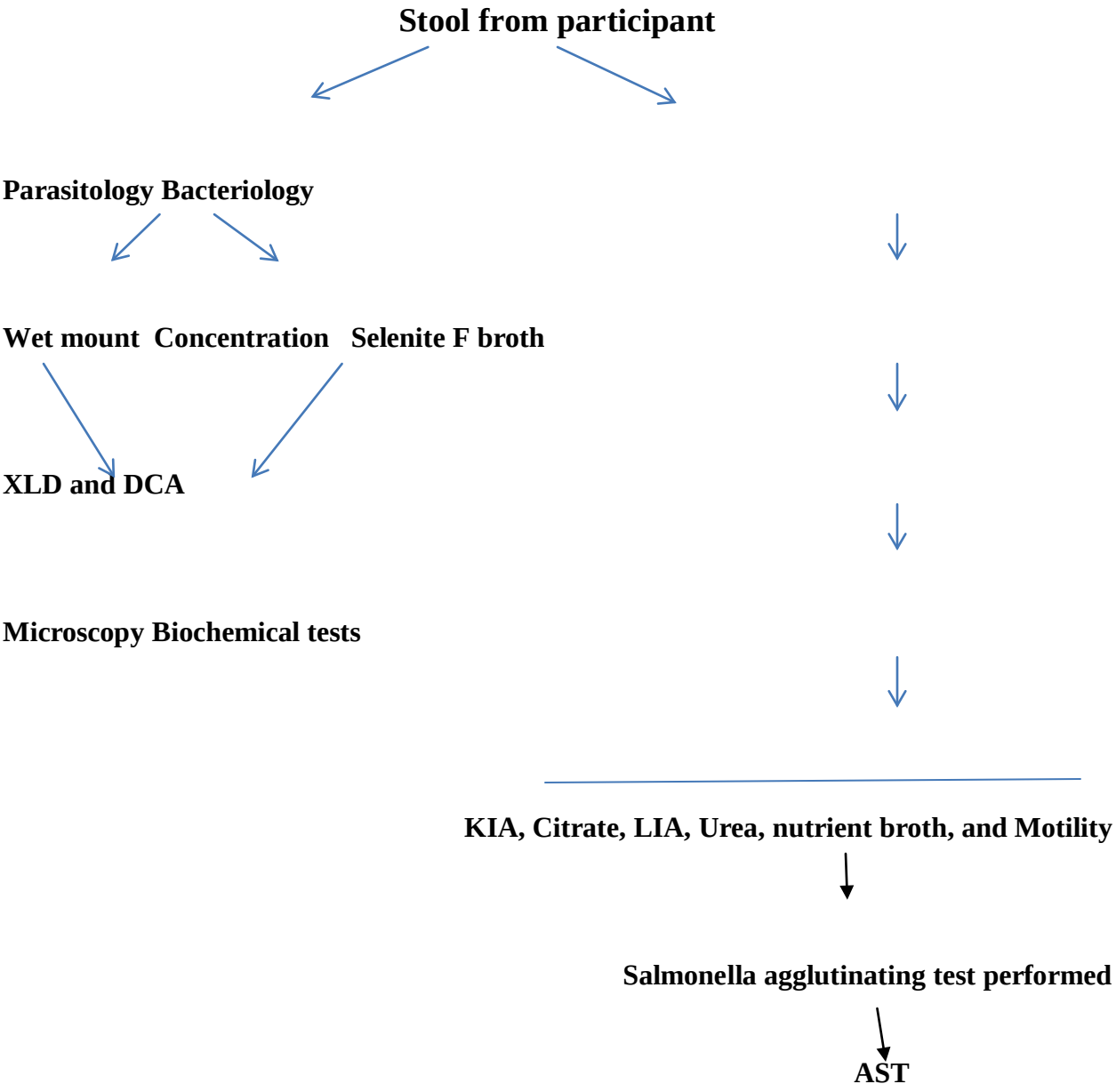

Fig. 1 Sample preparation and test workflow

6. Results

6.1 Socio-demographiccharacteristics

The majority of the food handlers (83%) were young aged 26 years and below, the mean age of the respondents 22.7, and range 38. All of the food handlers were female. One hundred forty-three (57.9%) food handlers had a primary school (grades 1-8) educational status, 74 (29.9%) food handlers was grade 9 and above, and 30 (12.2%) was illiterate. The majority 134(54.3%) of food handlers had served below 1 years, 66 (26.7%) of food handlers had served 1up to 2 years, and 47 (19%) of food handlers had served above 2 years. Majority of the study participants (59.1%) were kitchen cookers /chef(table 1).

Table 1: Socio-demographic characteristics of the food-handlers, Addis Ababa police commotion camps, Addis Ababa, Ethiopia.

Characteristics	**Frequency (%)**
Age	
26 years and below	205 (83)
Above 26 years	42 (17)
Educational Status	
Illiterate	30(12.2)
1-8 grade	143(57.9)
grade 9 and above	74 (29.9)
Service year	
Below 1 year	134(54.3)
1-2 years	66(26.7)
Above 2 years	47(19)
Job position	
Cook/chef	146(59.1)
Waiters and utensil cleaner	101(40.9)

6.2 Intestinal parasites

From total (247) stool specimens diagnosed by direct wet mounts andformol-ether concentration techniques to identify intestinal parasites,twenty-nine (11.74%) specimens were found positive for four different intestinal parasites, namely for*E. histolytica/dispar,G. lamblia,Taeniaspp andA.lumbrcoides. E. histolytica/dispar*5.7% was the most prevalent parasite, which consecutively followed by *Giardia lamblia*2.8%, Taenia *spp.*2% and *Ascarislumbrcoides*0.8% (Fig. 2.).

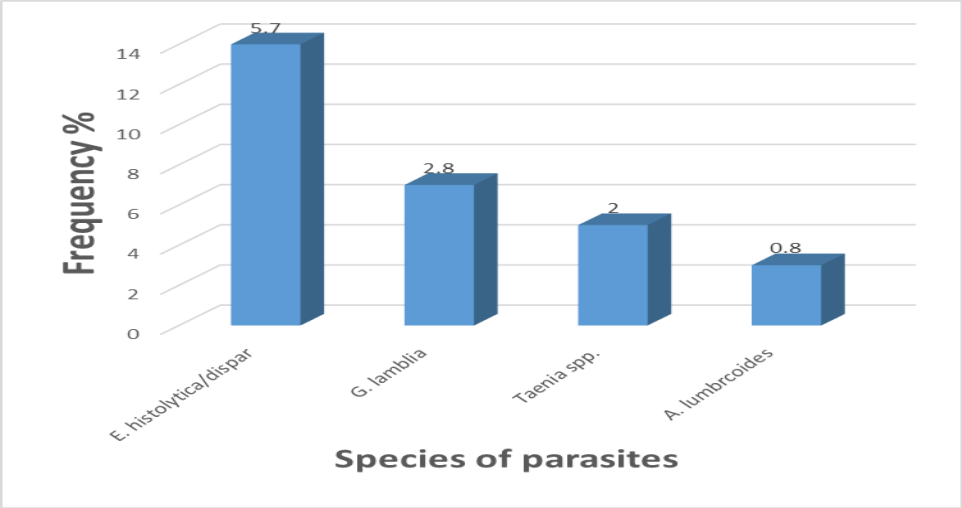

Figure 2. Prevalence of intestinal parasites among food handlers of Addis Ababa police commission camps, Addis Ababa, Ethiopia, 2021.

The majority of the food handlers infected with parasite 26(89.66%) were 26 years and below and 3 (10.34%) infected with parasite above 26 years. fourteen (48.28%) food handlers were grades 1-8 educational status, 10 (34.48%) food handlers was grade 9 and above, and 5 (17.24%) infected with parasite were illiterate. Thefood handlers infected with parasite13 (44.83%), 9 (30.03%), and 7 (24.14%)were below 1 year,above 2 years, and 1-2 years had service year, respectively. There is no statistically significance (P< 0.05) (table 2).

Table 2: Frequency of parasitic infection and demographic factors among food handlers in Addis Ababa police commission camps, Addis Ababa, Ethiopia

	Variable	Number of examined	Number of infected (%)	P-value
Age	**26 years and below**	205	26(89.7)	0.733
	Above 26 years	42	3(10.34)	
Educational Status	**Illiterate**	30	5(17.24)	0.686
	1-8 grade	143	14(48.28)	
	Grades 9 and above	74	10(34.48)	
Service year	**Below 1 year**	134	13(44.83)	0.403
	1-2 years	66	7(24.14)	
	Above 2 years	47	9(30.03)	

6.3 Potential risk factors for food borne diseases

In this study, 244(98.8%) of the participants questioned had to toilet in the working area. Only 52(21.1%) of the food handlers responded that they had taken regular medical checkups. Besides, 68(27.5%) of the respondents claimed that they always cooked food when suffering from illnesses like diarrhea. Unfortunately, 244(98.8%) of the participants claimed that they always wash their hands before preparing and serving any food. We also noted that 42(17%) of the food handlers did not cut their fingernails constantly. There is statistically significant parasite disease with approved in food preparation and handlingp value 0.008 ($P< 0.05$) (table 3).

Table 3. The association between intestinal parasite and risk factor on food handlers at Addis Ababa police commotion camps, Addis Ababa, Ethiopia (n = 247).

Potential risk factors		Number 0f tested	No parasite (%)	Adjusted OR (95% CI)	Chi2, p value
Presence of hand washing material such as soap around the latrine	No	113	13(44.8)	0.667(0.283-1.572)	0.946
	Yes	134	16(52.2)		
Shoe wearing habit	No	11	5(17.2)	0.137(0.036-0.520)	0.396
	Yes	236	24(82.8)		
Habit of eating uncooked raw foods	No	120	13(44.8)	1.013(0.432-2.373)	0.517
	Yes	127	16(55.2)		
Certified in food preparation and handling?	No	224	26(89.7)	1.568(0.376-6.542)	0.008
	Yes	23	3(10.3)		
Do you take regular medical checkup?	No	195	22(75.9)	1.790(0.627-5.107)	0.872
	Yes	52	7(24.1)		
Do you prepare food when suffering from disease like diarrhea?	No	179	24(82.8)	0.482(0.150-1.546)	0.463
	Yes	68	5(17.2)		
The practice of using common knife for cutting raw flesh food and other food	No	148	21(72.4)	0.437(0.164-1.162)	0.977
	Yes	99	8(27.6)		
Do you trim your finger nail regularly?	No	42	10(34.5)	2.402(0.891-6.475)	0.327
	Yes	205	19(65.5)		

Salmonella isolatesout of 247 participated food-handlers, four *Salmonella* species and none *Shigella*specieswere isolated. All of the *Salmonella*species isolates werewith in age 26 years and below, majority of isolate were in the educational status and service years, grade 8 and below, and less than one year, respectively. There is no statistically significant ($P< 0.05$) (table 4).

Table 4: Frequency of *salmonella* and demographic factors among food handlers in Addis Ababa police commission camps, Addis Ababa, Ethiopia.

Variable		Number. of examined	Numbersalmonella positive (%)	P-value
Age	26 years and below	205	4(100)	0.659
	Above 26 years	42	0	
Educational Status	Illiterate	30	1(25)	0.869
	1-8 grade	143	2(50)	
	Grades 9 and above	74	1(25)	
Service year	Below 1 year	134	3(75)	0.812
	From 1-2 years	66	1(25)	
	Above 2 years	47	0	

Antimicrobial susceptibility of the Salmonella isolates

All the Salmonella isolates were sensitive to Gentamicin, Ciprofloxacin, Ceftriaxone, Tetracycline, Chloramphenicol, Amoxicillin/clavulanic acid, Ampicillin sulbactan, Nacidixc acid, Imipcnene, Mcropcnen, and Azithromicne. To the contrary, all the Salmonella isolates were resistant to Ampicillin, Ceftazidine, Cefotaxine, and Cefuroxime (Table 5).

Table 5. Antimicrobial susceptibility testing of Salmonella and Shigella isolates among the food handlers in Addis Ababa police commotion camps, Addis Ababa, Ethiopia, 2021(n = 4).

	Salmonella isolates (n = 4)	
	Sensitive n (%)	Resistant n (%)
Gentamicin	4(100)	
Ciprofloxacin	4(100)	
Ceftriaxone	4(100)	
Tetracycline	4(100)	
Ampicillin		4(100)
Chloramphenicol	4(100)	
Amoxicillin/clavulanic acid	4(100)	
Ceftazidine		4(100)
Cefotaxine		4(100)
Ampicillin sulbactan	4(100)	
Cefuroxime		4(100)
Nacidixc acid	4(100)	
Imipcnene	4(100)	
Mcropcnen	4(100)	
Azithromicne	4(100)	

7. Discussion

Foodborne illness has long had an impact on human health and economic well-being. Food regulators play an important role in the spread of these diseases in different communities (1) (2). There is limited information available on the food knowledge and practice of food handlers in Addis Ababa police commission camps in Ethiopia.

Of the 247 stool specimens diagnosed by direct wet mounts and formol-ether concentration techniques to identify intestinal parasites, twenty-nine (11.74%) specimens were found positive for four different intestinal parasites, namely for E. histolytica/dispar, G. lamblia, Taenia spp and A. lumbrcoides. E. histolytica/dispar 5.7% was the most prevalent parasite, which consecutively followed by Giardia lamblia2.8%, Taenia spp.2% and Ascarislumbrcoides0.8%. In other studies in Ethiopia, this finding reached 61.9% in Gojjam (26) and Nekemte (37) at 52.1%. However, it can be associated with reports from Ethiopia (Axum city, 14%) (39)and western Iran (9%) (47). Differences in the distribution of intestinal parasites may be due to variations in local situations, such as water supply and hygiene.

The study found that 5.7% of participants who gave stool samples were the most pathogenic *E. histolytica/dispar*. Other studies have reported a high incidence of this parasite in Ethiopia (29, 48). Poor hygiene practices contribute to the transmission of the disease in areas where it is prevalent, mainly through the feco-mouth line (49).

Of the 247 feces tested in culture and biochemical experiments, 1.6% had *salmonella*. This finding is that 3.4% (18), 5.9% (50) and 5.04% (35) of these bacterial isolates are lower than in previous studies. These differences may be due to differences in the hygienic practices of food handlers, the type of test samples, and the socio-economic and educational level of the participants. Food handlers have a social responsibility to ensure food safety, as 20% of foodborne illnesses are transmitted due to improper handling of food by supervisors (51). This is common in overcrowded prisons, where health care is inadequate, and foodborne illness can occur.

Regarding drug susceptibility, *Salmonella* isolates were 100% sensitive to gentamicin, ciprofloxacin, ceftriaxone, tetracycline, chloramphenicol, amoxicillin / clavulanic acid, ampicillin-sulbactam, nalidixic acid, imipenem, mcropcynenicin Other similar studies have shown a risen susceptibility of these bacteria to these antibiotics (50, 52). *Salmonella* isolates were also 100% resistant to several of the antibiotics in the present study for ampicillin,

ceftazidime, cefotaxime, and cefuroxime. These findings advised that the problem of antimicrobial resistance has still to be solved in Ethiopia. Antibiotic resistance has become a main public health threat throughout the world, demanding the collaborative intervention(53).

In this study no Shigella species were isolated similar to Addis Ababa University Student Cafeteria, Addis Ababa, Ethiopia (24), and differ from in a study conducted by the University of Adigrat (42).

Our data suggest that 98.8% of the participants interviewed had latrine in the working area. The majority of the respondents claimed that they don't prepare food when suffering from diseases like diarrhea. And also, 98.8% of the participants claimed that they always wash their hands before preparing and serving any food. We also noted that the majority of the food handlers cut their fingernails regularly. The level of practice of the study food handlers in our study is higher than with a study carried out in Dangila town, Ethiopia; 52.5% and the food handlers in Debark town (40.1%), and similar to a study in Jordanian military hospitals (89.4%) (56). The variations in the level of applications among the different studies may be due to the differences in the method of evaluations, the level of knowledge of the food handlers, the working environments, or the socio-economic outlines of the food handlers. The WHO suggests the use of white coats during food preparation and service to ensure that food is not exposed to any clothing underneath. Food handlers are also supposed to cover white caps or aprons to defend food from hair. (57).

8. Strength and limitations

Our study filled the knowledge gap on *Salmonella* and parasite infection. *Salmonella* was identified only on the basis of the types of material deficiency. Molecular techniques were also not available to identify the*Salmonella*, and between *E. histolytica* and *E. dispar*among food handlers.

9. Conclusions

In summary, foodborne pathogens are the main health problems in the study areas. Furthermore, ongoing health education and training programs are essential to improve the level of knowledge

and practice of food handlers. The provision of essential facilities such as potable water, clean toilets and soap is also recommended to improve the personal hygiene of food facilities.

10. Recommendation

Based on the results of the study, the following recommendations are suggested: Increasing the knowledge and awareness of food handlers via providing information about food contamination related to*Salmonella*and intestinal parasitic infections. Training must be given to food handlers on personal hygienic conditions (like finger trimming and handwashing after toilet and before having contact with food with water and soap). It is better if the employees were trained and certified food handlers. Further studies should be undertaken on the prevalence of *Salmonella* and intestinal parasite infections and associated risk factors. Continuous checkup of food handlers should be mandatory to alleviate the problem by the concerned body.

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ANNEXES

Annex I. Information sheet in English Version

Title of the Research Project: To evaluate prevalence of Intestinal parasites, *Salmonella* and *Shigella*, Associated Risk Factors and Antibiotics Susceptibility Pattern of isolates among food handlers in Addis Ababa police commission camps, Addis Ababa, Ethiopia.

Principal Investigator: FelekeTalegetaEngdawork (BSc, MSc candidate)

Name of the Organization: Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University

Introduction

You are invited to participate as a study subject in a research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntarily. The research teams will include one principal investigator, two advisors; from Addis Ababa University Department of Medical Laboratory Sciences. Please take as much time as you need to read or listen in the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we will try to evaluate prevalence of Intestinal parasites, *Salmonella* and *Shigella*, Associated Risk Factors and Antibiotics Susceptibility Pattern of isolates among food handlers in Addis Ababa police commission camps, Addis Ababa, Ethiopia.

Purpose of the research:

The health laboratory plays an indispensable role in the health care system. It supports diagnosis (to rule in or rule out a diagnosis), monitoring of response to treatment, epidemiological surveillance, prevention as well as Research (to understand the pathophysiology of a particular disease process). Especially there is lack of local reference interval for indigenous population. Therefore, the purpose of this proposed study is to determine Prevalence of intestinal parasite and typhoid among food handlers in Addis Ababa police commotion camps, Addis Ababa, Ethiopia. You have been chosen for this study. Therefore, we invite you to take part in this study and contribute to the establishment of indigenous reference values. The values are needed for

providing quality laboratory service. Thus, result from this study is anticipated to improve the health status of the adult population at large in Ethiopia.

Procedures and the expected participation

If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also specimen collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staffs as it is needed. The required clinical sample will be collected and provide to residents of laboratory department. Then, you are requested to give your consent to the sample collector. After consent, a sample will be asked to provide fresh stool. Moreover, there will be a face-to-face interview for additional questions.

Procedures: After agreeing that you can take part, one or more of our research staff will ask you some questions which will take up to 15 minutes. You will be asked to provide fresh stool on a particular container we provide. We will conduct laboratory examination to determine different bacteriological and parasitological values.

Potential risks and Discomforts

There will be minimal discomfort in giving stool samples. However, we will try to minimize the discomfort as much as possible.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study. There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Potential benefits to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. However, based on the diagnosis result you will be treated in view of that. In addition, the result of the study will be beneficial for the detection and managing of intestinal parasite and typhoid. Hence, you are indirectly benefiting other patients and the society in this respect.

Participation and Withdrawal from the Study

The participation is voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result free.

Contact information

If you have any questions about this study you can contact the following principal investigators and advisors for further information.

	Name	Phone:	E-mail:
IP	FelekeTalegeta	+251 910501726	talegetafeleke@gmail.com
Advisors	Dr. MistireWolde	+251 911699710	

Annex II. Information sheet in Amharic Version

የተሳታፊዎችፈቃድናመተማመኛቅፅ

በአዲስአበባዩኒቨርሲቲጤናሳይንስኮሌጅየሕክምናላቦራቶሪሳይንስት/ክፍልበማስተርስድግሪተማሪየመመረቂያጥናትላይእዲሳተፉተጋብዘዋል።እባክዎበዚህጥናትለመሳተፍከመስማማትዎበፊትከዚህቀጥሎየሚገኘዉንምንባብበጥሞናያንብቡናግልጽያልሆነልዎትንማንኛዉምሃሳብይጠይቁ።

መግቢያ

የጥናቱርዕስ “TO EVALUATE PREVALENCE OF INTESTINAL PARASITES, *SALMONELLA* AND *SHIGELLA*, ASSOCIATED RISK FACTORS AND ANTIBIOTICS SUSCEPTIBILITY PATTERN OF ISOLATES AMONG FOOD HANDLERS IN ADDIS ABABA POLICE COMMISSION CAMPS, ADDIS ABABA, ETHIOPIA”.

የእርስዎበዚህጥናትላይየሚኖርዎትተሳትፎሙሉበሙሉበበጎፈቃደኝነትላይየተመሰረተነዉ።በዚህጥናትዉስጥላለመሳተፍወይምለመሳተፍከወሰኑበኃላለማቋረጥየሚወስኑቢሆንምእንኩዋበዚህክሊኒክየሚሰጠዉማንኛዉምአገልግሎትአይቋረጥም፡በጥናቱለመሳተፍየሚስማሙሆነስምምነትቅጹላይበጹሁፍወይምበጣትፊርማማስቀመጥይጠበቅዎታል።

የጥናቱተሳታፊለመሆንየሚጠበቅበዎትምንድንነው?

በዚህጥናትለመሳተፍየሚስማሙከሆነናሙናዎለጥናቱእንዲሚዉልመስማማትይጠበቅብዎታል።ከተወሰደዉናሙናላይየሚገኙመረጃዎችከዚህክሊኒክዉጭለሚገኙናለስራዉአግባብነትላላቸዉሰዎችቢነገርየማይቃወሙመሆኑንመስማማትይጠበቅብዎታል።ይሁንእንጅይህአይነቱመረጃየርስዎንማንነትየሚገልጡመረጃዎችንማለትምስም፤አድራሻናየስልክቁጥርየመሳሰሉትንመረጃዎቸንአይጨምርም።ይልቁንምለዚህአገልግሎትብቻየሚዉልእርስዎንለማወቅየሚያስችልመለያቁጥርጥቅምላይእንዲዉልይደረጋል።በተጨማሪምስለርስዎአጠቃላይየጤናሁኔታለሚቀርቡአንዳንድተጨማሪጥያቄዎችመልስመስጠትይኖርብዎትአል።

በዚህጥናትመሳተፍየሚያስከትላቸዉችግሮችምንድንናቸዉ?

ናሙናበሚሰበሰብበትወቅትምንምአይነትየከፋችግርአያጋጥምዎትም።ሆኖምግንናሙናዉንለመሰብሰብምድያለዉባለሙያስለሚመደብናአስፈላጊዉየጥንቃቄእርምጃስለሚወሰድችግርአይኖርም።

የህክምናመረጃበሚስጥርተጠብቆመቆየትየሚችለዉእንዴትነዉ?

ስለራስዎየሰጡትማንኛዉምመረጃናከተወሰደዉናሙናላይየተገኘዉየላቦራቶሪዉጤትየሚዉለዉለጥናቱአ
ላማብቻነዉ።ይህንማህደርሊያገኙየሚችሉትየተወሰኑየጥናቱተባባሪሰዎችብቻናቸው።ከዚያምበላይስለእር
ስዎያለውንማንኛውንምመረጃየተለየየይለፍቃልባለውየኮምፒውተርየመረጃማህደርውስጥእንዲቀመጥይ
ደረጋል።

በዚህጥናትመሳተፍየሚያስገኛቸውጥቅሞችምንድንናቸው ?

ይህጥናትየማስተርስዲግሪመመረቂያእንደመሆኑመጠንበዚህጥናትበመካፈልዎበገንዘብየሚያገኙትጥቅም
ባይኖርምከጥናቱበሚገኝውውጤትግንተጠቃሚነዎት።የእርሶዎተሳትፎየእርስዎንናየወገንዎትንለማከታተ
ልከፍተኛጥቅምይኖረዋል።

በዚህጥናትተሳታፊየመሆንዎመብቶችምንድንናቸው ?

በዚህጥናትመሳተፍሙሉበሙሉበእርስዎፈቃደኝነትየተመሰረተበመሆኑበማንኛውምሰዓትናቦታየማቋረጥ
ሙሉመብትየተጠበቀከመሆኑምበላይእራስዎንከጥናቱበማግለልዎምክንያትየሚቀርብዎትምንምአይነትክ
ሊኪክልአገልግሎትአይኖርም።ከዚህምበተጨማሪጥናቱንበተመለከተማንኛውንምአይነትጥያቄየመጠየቅና
ገለጻየማግኘትመብትአለብዎት።የላብራቶሪምርመራውጤቱንምበነጻማግኘትይችላሉ።ነገርግንእርስዎበሚ
ሰጡንመረጃየችግሩንስፋትለመከላከልእናለመቆጣጠርጠቃሚስለሆነለሚቀርብልዎትጥያቄቀጥተኛመልስይ
ሰጡንዘንድበታላቅአክብሮትእንጠይቃለን።

ጥያቄካለኝወይምችግርቢያጋጥመኝምንማድረግይገባል?

ይህንንጥናትበተመለከተወይከዚህጥናትጋርበተዛመደመልኩስለሚያጋጥሙድንገተኛአደጋዎችወይምጥያቄ
ካለዎትበሚመለከተውአድራሻይጠቀሙ።

ሥምስልክ: E-MAIL:

ፍለቀታለጌታ+251 910501726 TALEGETAFELEKE@GMAIL.COM

ADVISORS DR. ሚስጥረወልዴ +251 911699710

Annex III. Informed consent form in English version

Card no…..……

I had been informed that the objective of this study is to assess to evaluate prevalence of Intestinal parasites, *Salmonella* and *Shigella*. The results of this study have an importance to treat me and other patients, and to be used as an input for the future development of strategies or guidelines for diagnosing of intestinal parasite and thypoid in Ethiopia. I had been also informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data that include fresh stool sample, and filling questionnaire. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent for providing the requested information and specimens as the doctors find best for me.

Signature: _____ Date_____

Annex IV. Questionnaire in English version

Part I: Socio demographic characteristics of participants

1. Age_____

2. Sex_____

3. Educational Status: Illiterate □ primary school (1-8) □ above grade 9 □

4. For how many days/months or years you have worked as food handler in this cafteria?

Less than 1 year □ 1-2 years □ above 2 years □

5. Address you are living now: _____

6. Job position in the food handling job: Cook /chef, cleaning utensils and waiter others/specify_____

7. Presence of latrine at working area: Yes □ NO □

8. Presence of hand washing material such as soap around the latrine: Yes □ No □ Part II- Hygienic practice of food handlers

9. Shoe wearing habit: Yes □ No □

10. Habit of eating uncooked raw foods (meat, vegetables…) Yes □ No □

11. Have you taken anti-helminthes/protozoa within the past three months? Yes □ No □

12. Source of water pipe water □ other source □

13. Do you wash your hands after toilet use? Yes □ No □

14. If yes? with water only □ with water and detergent □

15. Certified in food preparation and handling? Yes □ No □

16. Do you take regular medical checkup? Yes □ No □

17. Do you wash your hands before preparing and serving any food? Yes□ No □

18. Do you prepare food when suffering from disease like diarrhea? Yes □ No □

19. The practice of using common knife for cutting raw flesh food and other food. Yes □ No □

20. Do you trim your finger nail regularly? Yes □ No □ Laboratory investigation result recording form Parasitology result Code Intestinal parasite Remark

Annex V. Informed consent form in Amharic version

የተሳታፊዎችስምምነትማረጋገጫ

የሚስጥርቁጥር ----------

የተሳታፊዉስም ----------

እኔስሜከላይየተጠቀሰውተሳታፊ “To evaluate prevalence of Intestinal parasites, *Salmonella* and *Shigella*, Associated Risk Factors and Antibiotics Susceptibility Pattern of isolates among food handlers in Addis Ababa police commission camps, Addis Ababa, Ethiopia.” ጥናትላይበቂገለጻተደርጎልኛል።ለጥናቱምየሰገራናሙናእንደሚያስፈልግተገልጾልኛል።የጥናቱንምአላማዎች ምተረድቻለሁ።

በቃለመጠይቁላይየገለጽኪቸውመረጃዎችበሙሉበሚስጥርየተጠበቁእንደሚሆኑተነግሮኛል።በጥናቱላይያለ መሳተፍናማንኛውንምመረጃያለመስጠትእንዲሁምበማንኛውምጊዜከጥናቱራሴንየማግለልመብቴየተጠበ ቀእንደሆነተገልጾልኛል።

ስለዚህለዚህጥናትመረጃናየስምምነትቃሌንየሰጠሁትበአጠቃላይሁኔታውንበመረዳትናበፍጹምፍቃደኝነትነ ው፡በተጨማሪምጥያቄለመጠየቅተፈቅዶልኝለማወቅየፈለኩትንያህልማብራሪአግኝቻለሁ።የዚህጥናትተሳ ታፊበመሆኔየማገኘውጥቅምየሁሉንምምርመራውጤትበነጻማግኘትእንደሆነተረድቻለሁ።

በአጠቃላይእኔከላይበመተማመኛቅፅየተጠቀሱትንሁሉበሚገባናበተረጋጋመንፈስአንብቤዋለሁኝ።ስለዚህበ ዚህጥናትለመሳተፍፈቃደኛመሆኔንበፊርማዬአረጋግጣለሁ።

ፊርማ---------- ቀን ----/---/----------

(የስምምነትቅጹንማንበብለማይችሉተሳታፊዎች)

የአማካሪስም ---------- ፊርማ ----------

ቀን----------

Annex VI. Questionnaire in Amharic version

ክፍሌ፡1 የተሳታፉው socio demographic ሁኔታ

1. ዕድሜ_____

2. ፆታ_____

3.የትምህርትደረጃ፡አልተማረኩም□የመጀመሪያደረጃ(1-8) □9 በላይ□

4. በዚህየስራቦታውስጥምንያህልጊዜአገልግለዋል?

ከ1አመትበታች□ 1-2አመት□ከ 2አመትበላይ□

5. አድራሻ፡ክ/ከተማ_____

6. በምግብስራውስጥያለህ/ሽየስራድርሻ፡አብሳይ /አጣቢ/አስተናጋጅሌላይጠቀስ ----------

7. ሽንትቤትአለ? አለ□የለም□

8. ሽንትቤቱከካባቢውእጅመታጠቢያሳሙናንየመሳሰለቁሳቁሶችአሉ? አለ□የለም□

ክፍል 2- የተሳታፉዎችየንፅህናአጠባበቀቅተግባር

9. ጫማየማረግልምድ፡አለኝ□የለኝም□

10. ያልበሰለነገሮችንይመገባሉ (ስጋ፣አትክሌት …) አዎ□አልመገብም□

11.የሆድውስጥትላትልማጥፊትየሚጠቅሙመድሃኒቶቸንባለፈትሶስትወራትውስጥወስደዋል?

አዎ□አልወሰድኩም□

12. ውሃከየትይጠቀማሉ? ከቧንቧ□ከሌላምንጭ□

13. ሽንትቤትከተጠቀሙበኋላእጂወትንየመታጠብልምድአለወት? አዎ□የለኝም□

14. ከታጠቡ? በውሃብቻ□በውሃእናሣሙና□

15. የምግብዝግጅትስልጠናወስደዋል? አዎ□አልወሰድኩም□

16. መደበኛየህክምናምርመራያደርጋሉ? አዎ□አላደርግም□

17. ምንምአይነትምግብከማብሰልዎእናማቅረበዎበፉትእጅ

18. ተቅማጥያለውህመምእየተሰማወትስራይሰራሉ? አዎ□አልሰራም□

19. ቢሊዋበጋራይጠቀማሉ? አዎ□አልጠቀምም□

20. ጥፍረዎንየሞቁረጥልምድአለዎት? አዎ □ የለኝም □

AnnexVII. Procedures

Examination of faces (wet mount)

1. Place a drop of fresh physiological saline to avoid contaminating the fingers and stage of the microscope, do not use too large a drop of saline.

2. using a piece of stick mix a small amount of specimen, about 2 mg (matchstick head amount) with the saline. Make smooth thin preparation. Cover each preparation with a cover glass. Important: sample from different areas in and on the specimen preferably mix the faces before sampling to distribute evenly any parasites in the specimen. Do not use too much specimen otherwise, the preparations will be too, making it difficult to detect and identify parasite

3. Examine systematically the entire saline preparation for larvae, ciliates,helmintheseggs,cysts,andoocysts. Use the10 objective with the condenser iris closed sufficiently to give a good contrast. Use the 40 objectives to assist in the detection and identification of eggs, cysts, and oocysts. Always examine several microscope fields with this objective parasite found

4. Use the iodine preparation to assist in the identification of cysts.

5. Report the result.

Stool examination (concentration technique)

Principle of Formal ether (Formalin-Ethyl Aceatate) sedimentation technique

Sedimentation techniques use solutions of lower specific gravity than the parasitic organisms, thus concentrating the latter in the sediment. It takes advantage of the high specific gravity of protozoan cysts and helminth eggs compared to water. Their natural tendency to settle out in aqueous solutions can be accelerated by light centrifugation. Formalin fixes the eggs, larvae, oocysts, and spores, so that they are no longer infectious, as well as preserves their morphology. Fecal debris is extracted into the ethyl acetate phase of the solution. Parasitic elements are sedimentedat the bottom.

Materials required

- Glass container
- Guaze
- Funnel
- Centrifuge tube (15ml capacity)
- Centrifuge
- Physiological saline (0.85% w/v Nacl)
- 10% buffered formalin
- Ether (ethyl acetate)
- Test tubes with stopper
- Glass rod
- Microscope

Procedure for Formal Ether Sedimentation Technique

1. Wear gloves when handling stool specimens.
2. In a suitable container, thoroughly mix a portion of stool specimen about the size of a walnut into 10mL of saline solution. Mix thoroughly.
3. Filter the emulsion through fine mesh gauze into a conical centrifuge tube.
4. Centrifuge the suspension at relative centrifugal force (RCF) of 600 g (about 2000 rpm) for no less than 10 minutes. The suspension should yield about 0.75mL of sediment for fresh specimens and 0.5 mL for formalinized feces.
5. Decant the supernatant and wash the sediment with 10 mL of saline solution. Centrifuge again and repeat washing until supernatant is clear.
6. After the last wash, decant the supernatant and add 10 mL of 10% formalin to the sediment. Mix and let stand for 5 minutes to effect fixation.
7. Add 1 to 2 mL of ethyl acetate, Stopper the tube and shake vigorously.
8. Centrifuge at 450 g RCF (about 1500 rpm) for 10 minutes. Four layers should result as follows

- a top layer of ethyl acetate;
- plug of debris;
- layer of formalin; and
- sediment

9. Free the plug of debris from the side of the tube by ringing with an applicator stick. Carefully decant the top three layers.
10. With a pipette, mix the remaining sediment with the small amount or remaining fluid and transfer one drop each to a drop of saline on a glass slide. Cover with a coverslip and examine microscopically for the presence of parasitic forms.
11.

Quality Control

1. Check solutions with each use to be sure they are clear and free of any bacterial contamination.
2. Run known positive specimens through the procedure to verify organism recovery. This should be done at least two times per year.

Observation and Results

Systematically examine the entire surface of each coverslip with the 10x objective or, if needed for identification, higher power objectives of the microscope in a systematic manner so that the entire coverslip area is observed. When organisms or suspicious objects are seen, switch to higher magnification (40X) to see more detailed morphology of the object in question.

Procedure Notes-sedimentation technique

1. The sedimentation procedure can also be used to process polyvinyl alcohol (PVA) fixed specimens. After the procedure by filing one half of a tube with the stool-PVA mixture and add 0.85% NaCl almost to the top of the tube. Then filter the mixture through wet gauze into a 15 mL centrifuge tube and follow the remaining standard procedure.
2. If ethyl acetate is used, swab the insides of the tube with a cotton-tipped applicator stick after the plug of debris is rimmed and the excess fluid is decanted. Excess ethyl acetate in the sediment at the time smears are prepared will lead to bubbles that may obscure parasitic forms one is attempting to observe.
3. Errors in interpretation may occur if too much or too little feces is used in the sedimentation procedure. Adhere to the recommended formula of 0.75 mL of sediment for fresh specimens and 0.5 mL for formalinized feces.
4. Allow the centrifuge to reach maximum speed before the time is monitored. If the centrifugation time is too little, certain smaller parasitic forms, such as the oocysts of Cryptosporidium species, may not reach the sediment.

Limitation of Sedimentation Technique

1. Certain parasites, such as Giardia lamblia, hookworm eggs, and Trichuris egg may not concentrate well from PVA-preserved specimens. The oocysts of Isospora belli do not routinely appear in concentrates. Therefore, examination of permanently stained smear is highly recommended.

2. With both the sedimentation and the flotation techniques, species identification may not be possible in all cases, depending on the clarity of the forms observed. Permanently stained smears are usually required to make the final identification, particularly when attempting to confirm the identity of the Entamoebahistolytica.

Principle of zinc sulfate Flotation technique

Flotation techniques (most frequently used: zinc sulfate or Sheather's sugar) use solutions which have higher specific gravity than the organisms to be floated so that the organisms rise to the top and the debris sinks to the bottom. The main advantage of this technique is to produce a cleaner material than the sedimentation technique. The disadvantages of most flotation techniques are that the walls of eggs and cysts will often collapse, thus hindering identification. Also, some parasite eggs do not float.

Preparation of culture media.

The major process during the preparation of culture media

- Weighing and dissolving of culture media ingredients
- Sterilization and sterility testing
- Addition of heat-sensitive ingredients
- Dispensing of culture
- PH testing of culture media
- Quality assurance of culture media
- Storage of culture media

1. Weighing and dissolving of culture media ingredients

Apply the following while weighing and dissolving culture media ingredients.

- Use ingredients suitable for microbiological use.
- Use clean glassware, plastic, or stainless equipment.
- Use distilled water from a glass still.

- Do not open new containers of media before finishing the previous nose
- Weigh in a cool, clean, dry, and draught-free atmosphere.
- Weigh accurately using a balance.
- Wear a facemask and glove while weighing and dissolving toxic chemistry.
- Do not delay in making up the medium after weighing. Add powdered ingredients to distilled water and mix by rotating or stirring the flask.
- Stir while heating is required to dissolve the medium.
- Autoclave the medium when the ingredient is dissolved

2. sterilization and sterility testing

Always sterilize a media at the correct temperature and for the correct length of time as instructed in the method of preparation methods used to sterilized culture media

A) Autoclaving

B) Steaming at 100 OC

C) Filtration

A) Autoclaving

Autoclaving is used to sterilize most agar and fluid agar media

B) Steaming at 100 OC

It used to sterilize media containing ingredients that would be inactivated at room temperature over 100 OC and re-melt previously bottled sterile agar media

C) Filtration

It is used to sterilize additives that are heat -sensitive and cannot be autoclaved.

Sterility testing

The simplest way to test for contamination is to incubate the prepared sample media At 35-37OCfor 24 hours. Turbidity in fluid media and microbial growth in solid media confirm contamination.

3. Addition of heat-sensitive ingredients

Refrigerated-heat sensitive ingredients should be warmed at room temperature before added to a molten agar media.

Using an aseptic technique, the ingredients should be added when the medium has, cooled to 50OC and should be distributed.

4. PH testing

The pH of most culture media is near neutral and can be tested using pH papers or pH meter.

5. Dispensing of culture media.

Media should be dispensed in a clean draught-free room using technique and sterile container.

Dispensing agar media in Petri-dish

- Layout the sterile Petri-dishes on a level surface.
- Mix the medium gently by rotating the flask or bottle.
- Flame sterilizes the neck of the flask or bottle.
- Pour 15 ml of medium in each petri-dish.
- Stack the plates after the medium has gelled or cooled.
- Store the plate in the refrigerator.

NB; Agar plate should be of an even depth and a firm gel. The surface of the medium should be smooth and free from bubbles.

6. Quality control

- Inoculate quarter plates of the medium with a five-hour broth culture for each control organism.
- Use the straight wire to inoculate and wire loop to spare the inoculums.
- Depending on the species, incubate aerobically, CO_2-enrich atmosphere, and anaerobically at 35-37^{0}C for 24 hours.
- Examine the degree of growth, morphology, and other characteristics of microbial colonies.
- Record the results of each control species and camper to your standard reading. Storage of culture media.
- Dehydrate culture media and dry ingredients should be stored at even temperature in a cool dry place away from direct light.
- Plates of culture media and additives like serum and blood require storage at 2-8^{O}C.
- All culture media and additives should be labeled with the name and date of preparation.

Inoculation of culture media

When inoculating culture media, an aseptic technique must be used to prevent contamination of specimen and culture media, and laboratory workers, and the environment.

Aseptic technique during inoculation of culture media

- Decontaminate the workbench before and after the work of the day.
- Use facemask and gloves during handling the highly infectious specimen.
- Flame sterilizes wire loop, straight wire, and metal forceps before and after use.
- Flame the neck of specimen and culture bottle, and tubes after removing and before replacing caps and plugs.

Procedure for culturing

1. Inoculate the sample into the selenite F broth,XLD, and DCA

2.Incubate the plate aerobically for 18-24 hours at 35 ± 2^{o}c.

3.Isolate the common pathogens. (*Salmonella and Shigella*)

4.Perform biochemical test

5. *Salmonella* agglutinating test performed

6. AST

7. Report the result.

AnnexVIII. Declaration

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

M.Sc. candidate: FelekeTalegetaEngdawork (B.Sc.MSccandidate)

Signature: _____

Date of submission: _____

This proposal has been submitted with our approval as advisors.

Advisor: Dr. MistireWolde (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: BirukZerfu (MSc, PhD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.