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Prevalence of Intestinal Parasites and Gastrointestinal Carriage of Gram Negative Enteric Bacteria among Apparently Healthy Food Handlers of Public Hospitals, Addis Ababa, Ethiopia

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This is to certify that the thesis is prepared by Tegegn Belhu entitled **"Prevalence of Intestinal Parasites and Gastrointestinal Carriage of Pathogenic Gram negative enteric Bacteria among Apparently Healthy Food Handlers of Public Hospitals, Addis Ababa, Ethiopia"** submitted in partial fulfillment of the requirements for the Degree of Master of Science in Clinical Laboratory Science (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Contents

List of tables

List of figures

List of Abbreviations

ALERT	All African Leprosy, Tuberculosis and Rehabilitation Training Centre
ATCC	American Type Culture Collection
CDC	Centers for Disease Control & Prevention
CI	Confidence Interval
CLSI	Clinical and Laboratory Standard Institute
DCA	Deoxy Cholate Agar
DST	Drug Susceptibility Test
E.coli O_{157}:H_7	Enter hemorrhagic Escherichia coli serotype O_{157}:H_7
GIT	Gastrointestinal Tract
MDR	Multidrug resistant
MHA	Muller Hinton Agar
Ml	Milliliter
OR	Odds Ratio
µg	Micro Gram
SOPs	Standard Operating Procedures
SPSS	Statistical Package for Social Science
TVET	Technical and vocational Education and Training
TB	Tuberculosis
US	United States
WHO	World Health Organization
XLD	Xylose Lysine Dextrose

Operational definitions

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

Hygiene practice: refers to those protection measures primarily with the responsibility of the food handlers, which promote and limit the spread of infectious disease, like hand washing using soap and water, trimming nail, practicing medical checkup, etc.

Apparently healthy food handlers: are hospital food handlers that have no diarrhea and fever during data and sample collection time but they may be asymptomatic carriers of pathogenic gram negative bacteria and intestinal parasites.

Pathogenic gram negative enteric bacteria: are gram negative bacteria that can cause human gastroenteritis following consumption of contaminated food or water.

Abstract

Background: Food borne diseases are major public health problems in developing countries like Ethiopia. Food handlers with poor personal hygiene working in hospitals could be infected with different intestinal parasites and pathogenic enteric bacteria. Therefore they could pose potential risk of transmitting food borne infection to patients and the community.

Objective: To determine the prevalence of intestinal parasites and gastrointestinal carriage of pathogenic gram negative enteric bacteria among food handlers of public hospitals.

Methods: Institutional based cross sectional study was conducted from March to June, 2017. Structured questionnaires were used to collect socio demographic and other related information. About 4gram of freshly passed stool specimen was collected. Direct wet mount smear examination and formol ether concentration techniques were done for detection of parasites. Culture and series of biochemical tests were done for detection and identification of pathogenic enteric bacteria respectively. Antimicrobial sensitivity test was done by Kirby-Bauer disk diffusion method. Data was analyzed using statistical package for social science.

Results: In this study, 368 food handlers were participated and 81% were females. Out of 368 stool specimens 119(32.34%) were positive for at least one intestinal parasite. The most prevalent parasite was *Entamoeba histolytica/dispar 4*8(13%), followed by *Giardia lamblia* 36(9.78%), *Taenia Species* 21(5.7%), *Ascaris lumbricoide* 8(2.2%), *Trichuris trichiura* 5(1.4%) and *Hook worm* 1(0.3%). Regarding enteric bacteria 17(4.6%) food handlers were positive for *Salmonella* 14(3.8%) and *Shigella flexneri* 3(0.8%). No *E.coli* O_{157}:H_7 was isolated. All, (n=14), *Salmonella* isolates were resistant to ampicillin (10µg) and erythromycin (15µg) and sensitive to Ciprofloxacin(5µg), Gentamycin(10µg) and Doxycycllin(30µg). Similarly all (n=3) *Shigella flexneri* isolates were resistant to ampicillin (10µg) & tetracycline (30µg) and sensitive to Cefotaxime(5µg), Ciprofloxacin(5µg), Gentamycin(10µg), Chloroamphenicol(30µg). All *Salmonella* and *Shigella flexneri* isolates were multidrug resistant.

Conclusion: Strong system to provide health education and training, frequent, regular and comprehensive medical screening of food handlers could be important intervention to break the chain of infection that may arise from them.

Key words*: Addis Ababa, Food handlers, Shigella, Salmonella, Intestinal parasite.*

1. Introduction

1.1. Back ground information

Food contamination may occur at any point during its journey through production, processing, distribution, and preparation. Bacteria and parasites are among the pathogens that may cause food borne disease. About sixty six percents of food borne disease are caused by contamination of food or water with bacteria. These bacteria include *Salmonella*, *Shigella* and *Escherichia coli* O157:H7. The common way of transmission of these pathogens is through contaminated objects with faeces. But food can be contaminated in different ways. The risk of food getting contaminated depends largely on the health status of the food handlers, their personal hygiene, knowledge and practice of food hygiene. Therefore food-handlers that are infected with intestinal parasites and enteric bacteria with poor personal hygiene working in food-serving establishments could also be potential sources of infections. They can harbor and excrete intestinal parasites and contaminate foods from their faeces via their fingers, then to food processing, and finally to healthy individuals and patients [1-5].

Some group of peoples such as patients on chronic steroid treatment, elders and pregnant women are more vulnerable to food borne disease due to the reason that only little number of pathogen are enough to cause disease and have incompetent immune system. In the United Kingdom, people aged over 65 comprise 16% of the population. Similarly in US, vulnerable peoples were estimated to represent almost 20% of the population. Therefore, the supply of contaminated foods to these vulnerable peoples poses a great danger of food borne infection. As an example, *Salmonella* can cause nosocomial bacteraemia among 20% to 30% of transplant cases [6-11]. The World Health Organization global report on surveillance of antimicrobial resistance in 2014 report makes a clear case that resistance to common bacteria has reached alarming levels in many parts of the world [4]. The increased vulnerability of peoples to food borne illness with the rapid emergence of drug resistant pathogens indicates that special attention should be given for food establishment system and food handlers' health.

1.2. Statement of the problem

In developed countries up to 10% the population might suffer from food borne disease. Intestinal infection which is mostly caused by contaminated food is still a widely prevalent public health problem in developing countries like Ethiopia. The main causes of food borne illness are bacteria which accounts for 66% of food borne disease. Four percent of food borne disease is caused by intestinal parasites [2]. In developed countries, up to 30% of the population suffers from food borne diseases each year [12]. Foods borne diarrheal disease are responsible for the death of 1.9 million peoples annually across the world whereas up to 2 million deaths are estimated per year in developing countries [13]. According to CDC, food borne diseases cause an estimated 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the US each year. The cost of the most common food borne illnesses in the United States is estimated at $6.5–$34.9 billion annually [2].

Food can be contaminated by enteric pathogens in different ways. Food handlers may be infected by a wide range of enteric pathogens and have been implicated in the transmission of many infections to the public in the community and to patients in the hospital setting. The American cook "Typhoid Mary" is one of the historically notorious examples of food- borne outbreaks which was responsible for 7 epidemics of typhoid affecting more than 200 persons [14]. A study conducted in Malaysia showed that approximately 10-20% food-borne outbreaks in the community are due to food handlers [15]. During the year 2001, there was an outbreak of *Entero Pathogenic Escherichia Coli* in the neonatal intensive care unit advanced pediatric Centre, in India that was traced back to a carrier food handler involved in the preparation of milk feeds. Shiga toxin producing *E.coli* is a bacterium that can cause severe food borne disease. *Escherichia coli* 0_{157}:H_7 is the most important related to public health [16]. Therefore, food handlers infected by enteric pathogens with poor hygiene can be the main source of food contamination because they come in contact with it from the time of preparation to the time of serving.

Drug resistance is a growing threat to treatment of disease caused by bacteria. Hospital environment can be colonized by many drug resistant bacteria. Peoples who are working in this environment such as food handlers may have a greater risk of being colonized by drug

resistant bacteria than peoples working in the community setting and can serve as a reservoir of these drug resistant bacteria [6]. The prevalence of intestinal parasitic infection and intestinal carriage of pathogenic enteric bacteria with their associated risk factors among food handlers who are working at hospital setting in Addis Ababa is not well studied. Therefore the present study is designed to detect hospital food handlers, who are infected with different intestinal parasites, intestinal carriage of *Salmonella*, *Shigella* and *Escherichia coli* 0_{157}:H_7.

1.3. Significance of the study

Even though many studies have been conducted in Ethiopia, particularly in Addis Ababa, to determine prevalence of intestinal parasites and pathogenic enteric bacteria among different groups of food handlers, there is no information found among food handlers who are working at hospital settings. This indicates that the possible risk of infection posed by these food handlers to peoples served by them had denied enough attention from researchers. Therefore this study will be used as an eye opener.

Asymptomatic, infected and carrier food handlers pose greater danger to the patients and the public because they keep on working unmindful of transmitting pathogenic bacteria and parasite. Therefore, the result of this study may indicate the danger posed by asymptomatic food handlers on patients.

The information generated from this study and the suggested recommendation can also be used as an input for policy makers and hospital administrators to take measures that mitigate the transmission of pathogenic gram negative enteric bacteria and intestinal parasites from food handlers to patients and community. These will help them to improve quality of hospital care to their patients.

Furthermore, information generated from this study can be used as reference for further similar studies in Ethiopia as well as in the world.

2. Literature review

Food borne disease outbreak can be occurred at any time in a community. And a study by Selman CA *et al*. showed that it is difficult to investigate and identify contributing factors to the outbreak. Since transmission of intestinal parasites and enteric bacteria is effected through objects contaminated by faeces, food handlers may carry and convey enteric bacteria and parasites that can be associated with food borne disease outbreak. A study by Francis *et al.* showed that 65.7% and 79% finger and nail carriage of *Salmonella* among food handlers respectively [17]. Another study at children hospital, in Ahvaz, Iran reported 4% of *Shigella* among children suffering from dysentery. According to this study *Shigella flexneri* was predominant and showed higher resistance rate, 85% to trimethoprim-sulfamethoxazole and 87.5% to ampicillin was recorded. Ten percent isolates of *Shigella flexneri* showed resistance to chloroamphenicol and Nalidixic acid. Marked resistance rate, 18.2% was seen to gentamycin [18]. For this reason knowing the health status of food handlers is crucial to mitigate food borne disease outbreak.

Several studies have been conducted in Iran on prevalence of intestinal parasitic infection among food handlers of different groups at different parts of the country and revealed prevalence between 2% and 61% [19-21]. The prevalence level of parasitic disease in these studies was as follows: Mazandaran (21%) [22], Ardabil (27.7%) [23] and Khuzestan (8.8%) [24].

Studies in different parts of India revealed that there is infection of intestinal parasites and enteric bacteria among food handlers. Among these studies a study which was conducted for six consecutive years on food handlers of tertiary care hospital have been showed a prevalence of 1.4 to 16% of intestinal parasitic infection and 0 to 13.3% of enteric bacteria from 2001-2006. In this study the most common intestinal parasite was *Giardia lamblia* with 0 to 4.05% prevalence followed by *Entamoeba histolytica*, 0 to 2.6% and *Hook worm*, 0 to 1.4%. In this study *Shigella* was the most prevalent enteric bacteria ranging from 0% to 13.3% followed by *Salmonella*, 0 to 9.3% within six years. And no *Escherichia coli* O157:H7 was detected [25].

Another study in northwest Iran revealed 3.75% prevalence of intestinal parasitic infection.

In this study there was no statistically significant difference between risk of intestinal parasitic infection between age and gender (p=0.094) [26]. The prevalence of intestinal parasitic infection in Iran, according to another study was 10.4%. In this study statistically significant difference in the prevalence of parasite infection was reported between males and females (p=0.024), while there was no difference among different educational groups [27]. A study in Sari, Northern Iran reported 15.5% food handlers were positive for at least one type of parasite. In this study education level, sex and hand washing after toilet use were significantly associated with risk of intestinal parasitic infection [28]. Kheiradish *et al*. revealed 19% intestinal parasite prevalence; education level and gender were significantly associated with intestinal parasitic infection in this study model [29].

A study conducted in Dubai which is the second largest city of United Arab Emirates showed that, 2% over all prevalence of intestinal parasites among food handlers belonging to different nationalities. The prevalence of *Giardia lamblia* and *Ascaris lumbricoide* in this study was 1.6% and 0.2% respectively [30].

A study conducted at Zuila state Venezuela about the prevalence of *Cryptosporidium* and other intestinal parasites among food handlers showed that 48.7% general prevalence of intestinal parasite. The most prevalent parasite was *Giardia lamblia* (13.4%) followed by *Entamoeba histolytica/dispar* (9.2%) [31].

Another study conducted in the city of Uberlandia, Minas Gerais, Brazil revealed that 47.1% of school food handlers were positive for different intestinal parasites. The most prevalent parasite isolated in this study was *Giardia lamblia* (21.1%). Prevalence of *Hook worm* and *Ascaris lumbricoide* were 9.6% and 5.8% respectively [32].

The prevalence of intestinal parasites among food handlers in Anatolia was about 52.2%, according to a study done by Simsek Z *et al*. No *Salmonella* and *Shigella* were isolated [33]. Comparable research, done by Kusolusk *et al.* in Kanchanaburi province, Thailand revealed, 10.3% of prevalence of intestinal parasites. Similarly no *Salmonella* and *Shigella* was detected in this study [34].

A study conducted within one year period to determine prevalence of *Salmonella, Shigella* and intestinal parasites among food handlers in Ibrid, Jordan revealed, 6%, 1.4% and 18.1% prevalence respectively. In this study MDR was reported among both *Salmonella* and *Shigella* isolates [35].

Further studies conducted in different parts of the world showed different prevalence level of intestinal parasitic infection: in Gaza strip Palestine (24.3%) [36], in Holly city of Makkah (31.94%) [37].The prevalence of intestinal parasitic infection among food handlers in Jenin Governorate, North Palestine, was 32% to 41.5% in 10 years [38]. Intestinal parasite among street food handlers in Abeokuta, Nigeria was also 97% [39].

In Khartoum, Sudan 29.4% food handlers attending public health laboratory for medical checkup were harboring intestinal protozoa and Helminthes. *Giardia lamblia, Entamoeba histolytica* and *Taenia species* were 9.7%, 4.3% and 0.3% prevalent respectively [40]. Referring, another study conducted in Ombdurman, Sudan, *Salmonella* and *Shigella* was isolated from 3.8% and 1.3% of food handlers respectively. The overall prevalence of intestinal parasitic infection according to Seada AH *et al* was 6.9% [41]. The carrier rate of *Salmonella* among food handlers in Kumasi, Ghana was 2.3% [42]. In Kenya, according to Kumatu *et al*, prevalence of intestinal parasite among food handlers in Nairobi, who had valid medical certificate, was 15.7% [43].

Parasitic infection among food handlers working in Gondar University student's cafeteria was 25%. In the later study statistical significance test was done, and have not been shown significant association between intestinal parasitic infection and socio demographic variables and certification, but, with hand washing practice with water and soap after toilet use of food handlers [11].

*Acc*ording to Kifelew G *et al*. *Salmonella*, among food handlers in Gondar Town was 3% prevalent. According to this study 69.2 and 61.5% of isolates were resistant to amoxicillin and ampicillin respectively. While 46.2% were resistant to tetracycline. Forty six percent of the

isolates were MDR [44].

A cross sectional study conducted in southern Ethiopia showed 36% of food handlers were positive for different intestinal parasites. Based on this study intestinal parasitic infection prevalence was not significantly associated with age and gender (p=0.053), with years of service (p=0.086). But was associated with hand washing after toilet use (p = 0.029), practice of using common knife for cutting raw flesh food (p = 0.046), and preparing food when suffering from disease like diarrhea, (p = 0.023). Food handlers who were using water only to wash their hands after toilet use [OR: 1.71, 95% CI; (1.057-2.765)] and use common knife for cutting raw flesh food and other food [AOR: 1.72, 95 % CI: (1.01–2.92)] had a more likely risk of intestinal parasitic infection (with 71% and 72% respectively) than that use water and soap and do not share knife [45].

About forty nine percent (49.4%) food handlers working at Mekelle university student's cafeteria were positive for intestinal parasitic infection. Food handlers who use soap for hand washing after toilet [AOR: 0.06, 95% CI (0.02- 0.14)] and practice of medical checkup [AOR: 0.47, 95% CI (0.22-0.97)], were determinants for intestinal parasitic infection. These factors were preventive for intestinal parasitic infection [46]. The prevalence of intestinal parasitic infection among food handlers in Yebu Town, Southwest Ethiopia was 44.1%. Age of participants greater than 35 [AOR: 4.8, 95% CI (1.1-21.8)] and untrimmed finger nail [AOR: 14.7, 95%CI (2.8-75.4)] were among the indicated intestinal parasitic infection predictors. There was no significant difference in gender [47].

A study conducted in Ethiopia, by Assefa *et al*. among Jimma University student's cafeteria food handlers, culture of hand rinse samples revealed many enteric bacteria. This includes *Escherichia coli* (10.9%), *Salmonella* species (0.9%) and no *Shigella species*. According this study bacterial contamination rate was significantly associated with service year with p=0.006 and carriage of *Salmonella* higher among food handlers who have worked for longer years [48].

Zero percent and 3.5% *Shigella* and *Salmonella* respectively were isolated from stool culture

of food handlers working in Addis Ababa University student's cafeteria in Addis Ababa in study done by Aklilu et.al. According to this study, 100%, *Salmonella* isolates were resistant to ampicillin, amoxicillin and erythromycin. *Salmonella* isolates that showed resistance to Trimethoprime/sulfamethoxazole and cefotaxime were 16.7%. All, 100% of *Salmonella* isolates were MDR [49]. According to Teklemariam *et al*. prevalence intestinal parasitic infection, among food handlers in catering establishments of Awassa Town was 52.2%. While the prevalence of intestinal parasitic infection was higher no *Salmonella* or *Shigella* was isolated [50].

3. Objectives

3.1. General objective

The study was aimed to determine the prevalence of intestinal parasites and pathogenic gram negative enteric bacteria among apparently healthy food handlers of public hospitals, Addis Ababa, Ethiopia from March to June, 2017.

3.2. Specific objectives

The main specific objectives of this study were:

- To determine the prevalence of intestinal parasites
- To determine the gastrointestinal carriage of *Salmonella*, *Shigella* and *Escherichia coli* O_{157}:H_7.
- To describe the drug susceptibility pattern of each bacterial isolates.
- To assess associated risk factors for intestinal parasitic infection and carriage of *Salmonella*, *Shigella* and *Escherichia coli* O_{157}:H_7.

4. Hypothesis

- No significant prevalence of intestinal parasite infection among food handlers of public hospitals compared to previous reports.
- Socio demographic characteristics and hygiene practice of food handlers has no association with the risk of intestinal parasitic infection and carriage rate of pathogenic Gram Negative enteric bacteria.

5. Materials and Methods

5.1. Study area

The study was conducted in Addis Ababa which is the capital city of Ethiopia and Africa which has an area of 527 square kilometers. It has ten sub cities and one hundred sixteen weredas. The total population of the city was about 3,384,569 and all are urban inhabitants based on a national census conducted in 2007. Addis Ababa has a subtropical highland climate. The city has a complex mix of highland climate zones, with temperature differences of up to ten degree Celsius, depending on elevation and prevailing wind patterns. The high elevation moderates temperatures year-round, and the city's position near the equator means that temperatures are very constant from month to month [51].

According to the Federal ministry of health of Ethiopia, there were about eleven public hospitals in Addis Ababa city in 2017. Based on the information collected from human resource management about four hundred twenty food handlers were working within these hospitals. The average number of food handlers in each hospital was about thirty nine.

5.2. Study design and period

Institutional based cross- sectional study was conducted to determine the prevalence of intestinal parasites and pathogenic gram negative enteric bacteria among apparently healthy food handlers of public hospitals, Addis Ababa, Ethiopia from March to June, 2017.

5.3. Source population

All food handlers who have direct contact in food preparation and handling regardless of their employment status either permanent or contract employee during the study period within the selected eleven public hospitals in Addis Ababa, Ethiopia was taken as a source population.

5.4. Study population

All volunteering food handlers who have direct contact in food preparation and handling regardless of their employment status either permanent or contract employee within the selected eleven public hospitals in Addis Ababa, Ethiopia that fulfill the inclusion(eligibility) criteria were participated in the study.

5.5. Inclusion criteria

Food handlers working in the selected public hospitals and given informed consent were included in the study.

5.6. Exclusion criteria

Food handlers, who had diarrhea and fever, that were received antibiotic or anti-parasitic treatment within the past two to three weeks prior to the study or who were taking antibiotics and or anti-parasitic for GIT related cases during data collection were excluded from the study.

5.7. Study variables

5.7.1. Dependent variables

Prevalence of intestinal parasites and carriage of pathogenic gram negative enteric bacteria and drug susceptibility pattern of culture isolates were dependent variables.

5.7.2. Independent variables

The independent variables in this study were age, sex, food preparation and handling training, hand washing habit after toilet, educational status, medical checkup, swimming habit, total year working at hospital, nail trimming habit, sharing knife and other equipments used for food preparation, habit of eating raw or undercooked food, preparing food when suffering from diarrhea and wash hand before touching food.

5.8. Sampling methods and Sample size

The study was conducted at Tikur Anbesa Specialized Hospital, ALERT Hospital, Saint Paul Specialized Hospital, Saint Amanuel Mental Hospital, Saint Peter's TB Specialized Hospital, Yekatit 12 Medical College Hospital, Zewditu Memorial Hospital, Ghandi Memorial Hospital, Minilik II Memorial Hospital, Ras Desta Damtew Memorial Hospital and Tirunesh Bejing Hospital. Each hospital was chosen by purposive sampling technique. Then the total number

of food handlers working at these eleven public hospitals was collected from human resource management of each hospitals and it was about three hundred ninety which was taken to be a sample size. Convenience sampling technique was used to enroll each study participants.

5.9. Data and specimen collection

Face to face interviewing method using structured questionnaires and observation were used to collect socio demographic and food handlers hygienic practice related information. Then about 4gram (about 4 times size of pea) freshly passed stool specimens were collected with a uniquely labeled sterile container from March to Jun, 2017. Direct saline, Iodine mount and formalin ether concentration techniques were done at each selected hospitals or nearby volunteer laboratories. Then the remaining specimen was transferred using the scoop into Carry-Blair medium until the level of liquid reaches the fill line. The specimen was mixed into the medium using the scoop or sterile wooden applicator stick. Then it was transported to Addis Ababa Public Health and Emergency Management core process laboratory with the remaining sample in the stool cup using triple packaging at room temperature within 2 hours of collection for parasitological tests. Specimens with Cary Blair transport medium were transported with cold chain.

5.10. Culture and identification

As soon as arrived in the laboratory, specimens were inoculated primarily on selenite F broth, XLD and sorbitol MacConkey followed by incubation at 37°C for 24 hours . Then growth on selenite F broth was sub cultured on to XLD. SMAC and XLD were examined for colorless and red or reddish with black center colonies respectively. Biochemical tests were done for all colorless colonies on SMAC to confirm that, it is *Escherichia coli*. None lactose fermenting colonies on XLD were sub cultured onto Sheep Blood Agar and biochemical tests were performed for final identification of the isolates. Enteric bacteria were identified by performing a series of biochemical tests namely; Triple Sugar Iron Agar, Indole, Simon's Citrate, Ly-sine Iron Agar, Motility, Mannitol, Oxidase and Urea hydrolysis for growths on the primary isolation media. Then slide agglutination test was done to identify *Shigella species* using polyvalent and monovalent anti-sera.

5.11. Antimicrobial Susceptibility Testing

Antimicrobial sensitivity of the bacterial isolates was done by the Kirby-Bauer disc diffusion method. Fresh sub-cultures of bacterial isolates were used after overnight growth on Muller Hinton Agar. The inoculums were prepared by suspending several of the colonies in sterile phosphate buffered saline (pH 7.2) to achieve a turbidity of 0.5 McFarland standards. A sterile cotton swab was dipped into the bacterial suspension, elevated above the liquid and was rotated several times against the inside wall of the tube to remove excess of the inoculums. The swab then was streaked evenly in three different directions onto the Muller Hinton Agar, and then appropriate antibiotic disks were dispensed aseptically and incubated at 37 degree Celsius for 24 hours.

Organisms' sensitive to the antibiotic were inhibited from growing in a circular zone around the antibiotic impregnated paper disk. Diameters of the zone of inhibition around the discs were measured to the nearest millimeter using a ruler and classified as sensitive, intermediate, and resistant according to the standardized table supplied by CLSI (see annex 11). All intermediate results were included into resistant category. The following were antibiotic disks with their concentration; trimethoprime /sulfamethoxazole (1.25/23.75µg), ampicillin (10µg), erythromycin (15µg), azithromycin (15µg), gentamycin (10µg),Doxycyclin (30µg), Ciprofloxacin (5µg), naldixic acid (30µg), cefotaxime (30µg), Chloroamphenicol (30µg) and Tetracycline (30µg) (see Annex11). These drugs were selected based on second edition Ethiopian medicine formulary 2013. An isolate was considered as MDR if resistant to two or more drugs belonging to different classes and for one or two antibiotics from each class [52].

5.12. Parasitological examinations

About 2mg of stool was picked with applicator stick and emulsified in a drop of normal saline (0.85% NaCl) at the one end of a clean, non scratched glass slide, and the same size of stool in Lugol's iodine at the opposite end of the same glass slide. Then cover slip was placed on both preparations and scanned under 10× and 40× objective lenses of a light microscope for detection of intestinal protozoan trophozoite, cysts and ova of nematodes. Briefly, formol ether concentration technique was done as follows: about 1gm (pea size) of fresh stool specimens were emulsified in 8ml of formol water. The resulting suspension was filtered through three layers of wet cotton gauze in a funnel into a centrifuge tube and about 4ml of diethyl acetate was added. The centrifuge tube was shaken vigorously and centrifuged at 750-100g for 1 minute. After the supernatant was poured of the sediment was slightly shaken and one drop was added at the centre of clean, scratch free glass slide. Cover slip was applied on the preparation and was examined in the same way as explained earlier. Small drop of Lugol's iodine was run under the cover slip to observe the characteristic features of cyst. The different intestinal parasites identified were recorded on laboratory investigation result recording form [53-54].

5.13. Data recording and analysis

The data was entered into Microsoft excel 2007, edited and cleaned before exported to statistical package for social science version 20, which was used for data management and analysis. Univariate and bivariate analysis were done. Then crude and adjusted odds ratios with 95%CI were calculated to test statistical significance. Variables with p value < 0.3 in a bivariate analysis were considered to multivariate analysis to look their relative effect on the outcome (intestinal parasitic infection) and carriage of *Salmonella* and *Shigella* by controlling other possible confounding factors.

5.14. Quality assurance

To assure quality of data questionnaires were translated to Amharic and pretested, data collectors were trained a day before and after a pretest, continuous supervision was done by the principal investigator, standard operating procedures were followed, and laboratory investigations were done by competent laboratory professionals.

Routine quality controls were performed for all culture media using ATCC strains recommended by manufacturers. Growth and recovery of *Salmonella typhimurium* ATCC 14028, *Shigella flexneri* ATCC 12022 were used as a quality control for Carry-Blair medium. *E.coli O157:H7* ATCC -35150 and *E.coli* ATCC- 25922 were used as a positive and negative control for SMAC respectively. For XLD *Salmonella enterica* ATCC- 14028 and *Shigella flexneri* ATCC-12022 were used as positive control, while *E.coli* ATCC-25922 was used as a negative control. *Salmonella typhimurium* ATCC-14028 and *E.coli* ATCC-25922 were used as a positive and negative control for Selenite-F broth respectively. Growth of *E.coli* ATCC-25922 was also used as a quality control for MHA. American Type Culture Collection stains *Salmonella enterica* 14028, *Escherichia coli* 25922, *Pseudomonas aeruginosa* 27853 and *Shigella sonnei* 19290 were used as quality control for Triple Sugar Iron Agar medium. For Antibiotic Sensitivity Testing *E.coli* ATCC- 25922 was used as a control based on CLSI guideline (see Annex 12) [57]. Media sterility check was done by overnight incubation at 37 degree Celsius. We examined the normal saline for turbidity microscopically daily. Data was entered on excel and reviewed before it was exported to SPSS.

5.15. Ethical considerations

Ethical approval letter was obtained from the Departmental Research and Ethics Review Committee of Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Laboratory Sciences, and Addis Ababa City administration Health Bureau. Written informed consent was also obtained from study participants. Strict confidentiality was maintained during the interview process as well as anonymity was kept during data processing and report writing. Food handlers who were found to be positive for enteric pathogens were referred to their respective staff medical center for appropriate antimicrobial treatments.

6. Results

6.1. Socio demographic characteristics of study participants

A total of 368 food handlers were participated in this study with none response rate of 6%. Two hundred ninety eight (81%) of the participants were females. The mean age was 34.07±9 years and ranging from 18–60 years. The majority of food handlers, 210 (57.1%) were in the age group of 18–35 years. About 36% of the study participants were working at a position of cooking and the rest 24%, 16% and 24% were working at hosting, cleaning utensils and other job positions in dining rooms respectively. All food handlers reported that they have shoe wearing habit and use pipe water source. One hundred forty nine (40.5%) participants had completed primary school. All, (100%) of food handlers who were participated in this study were urban residents. Two hundred sixty eight (72.8%) were worked for greater than 2 years in dining rooms. Only 77(20.9%) and 187(50.8%) of the food handlers participated in this study had formal food preparation and handling training and took medical checkup at least within the last six months respectively (Table 1).

Table 1: Socio demographic characteristics of food handlers in public hospitals, Addis Ababa, Ethiopia March to June, 2017(n=368).

Socio demographic data	Frequency	Percent
Sex		
Male	70	19
Female	298	81
Age		
18-35	210	57.1
36- 53	151	41.0
>53	7	1.9
Educational status		
Illiterate	48	13.0
Primary school	149	40.5
Secondary school	133	36.1
Technical & vocational	17	4.6
Collage/University	21	5.7

n = sample size

6.2. Prevalence of intestinal parasites and carriage rate of enteric bacteria

Among the 368 food handlers, 119 (32.34%) were found to be positive for six different intestinal parasites. No multiple parasitic infections were detected. The most prevalent parasite among food handlers was *Entamoeba histolytica/dispar* 48(13%) followed by *Giardia lamblia* 36(9.78%), *Taenia Species* 21(5.7%), *Ascaris lumbricoide* 8(2.2%), *Trichuris trichiura* 5(1.4%) and *Hook worm* 1(0.3%). Among detected intestinal parasites *Entamoeba histolytica/dispar* was predominant (Fig. 1).

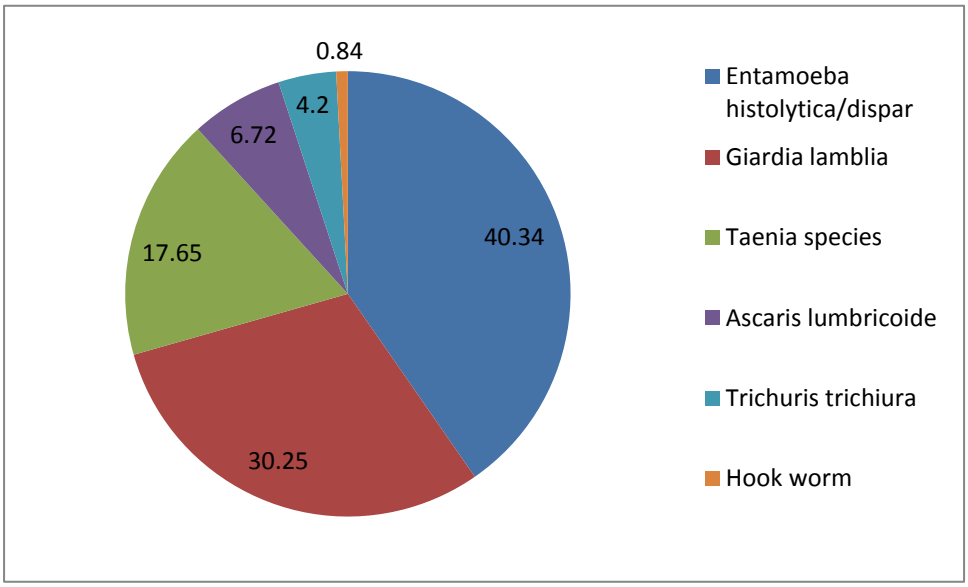

Fig 1: Type and percentage of intestinal parasites isolated from stool specimens of food handlers in public hospitals, Addis Ababa, Ethiopia. March to June, 2017 (n=119).

Regarding the carrier state of enteric bacteria, among 368 study participants, 17(4.6%) of them were found to be positive for *Salmonella species* and *Shigella flexneri*. The most prevalent bacterial isolate in this study was *Salmonella* 14(3.8%) followed by *Shigella flexneri* 3(0.8%). The prevalence of enteric bacteria was 17(5.7%) and 0(0%) among female and male respondents respectively. Food handlers that washed their hands after toilet use with water and detergent were 218(59.2%) and about 9(4.1%) were positive for enteric bacteria (Table 2).

Table 2: Prevalence of intestinal parasites and carriage of gram negative enteric bacteria among food handlers in public hospitals, Addis Ababa, Ethiopia, March to June, 2017(n=368).

Variables		Total tested No.(%)	Positive intestinal parasite No.(%)	Positive Enteric bacteria No.(%)
Sex	Male	70(19)	20(28.6)	0(0)
	Female	298(81)	99(33.2)	17(5.7)
Age group	18-35	211(57.3)	69(32.7)	11(5.2)
	36-53	150(40.8)	46(30.7)	5(3.3)
	>53	7(1.9)	4(57.1)	1(14.3)
Educational status	Illiterate	48(13.1)	8(16.7)	3(6.25)
	Primary school	149(40.5)	60(40.3)	7(4.7)
	Secondary school	133(36.1)	45(33.8)	6(4.5)
	TVET	17(4.6)	3(17.6)	1(5.9)
	College/University	21(5.7)	3(14.3)	0(0)
Years working at hospital	< 2 years	100(27.2)	33(33)	3(3)
	2-10 years	167(45.4)	51(30.5)	8(4.8)
	11-20 years	68(18.5)	25(36.8)	5(7.4)
	≥ 21 years	33(8.9)	10(30.3)	1(3)
Food preparation and handling training	Yes	77(20.9)	13(16.9)	0(0)
	No	291(79.1)	106(36.4)	17(5.84)
Hand washing habit after toilet	With water only	150(40.8)	68(45.3)	8(5.3)
	With water & detergent	218(59.2)	51(23.4)	9(4.1)
Eat raw/undercooked food	Yes	217(59)	88(40.6)	10(4.6)
	No	151(41)	31(20.5)	7(4.6)
Wash hand before touching food.	Yes	337(99.6)	110(32.6)	14(4.2)
	No	31(8.4)	9(29)	3(9.7)
Prepare food when suffering from diarrhea	Yes	50(13.6)	19(38)	2(4)
	No	318(86.4)	100(31.4)	15(4.7)
Nail trimming habit	Yes	322(87.5)	102(31.7)	14(4.3)
	No	46(12.5)	17(37)	3(6.5)
Swimming habit	Yes	53(14.4)	13(24.5)	1(2)
	No	315(85.6)	106(33.7)	16(5.1)
Sharing knife and other equipments	Yes	271(73.6)	102(37.6)	12(4.4)
	No	97(26.4)	17(17.5)	5(5.2)
Medical checkup within the past 6 months	Yes	187(50.8)	37(19.8)	8(4.3)
	No	181(49.2)	82(45.3)	9(4.9)

6.3. Associated risk factors of intestinal parasitic infection and enteric bacteria

About 99(33.2%) of female and 20(28.6%) of male participants were positive for intestinal parasitic infection. Although about 218(59.2%) of respondents, reported that they had a habit of washing hand with water and detergent after toilet use 51(23.4%) of them were positive for intestinal parasitic infection. Only half of the respondents, 187(50.8%) did medical checkup within the past six months. Majority, 322(87.5%) participants stated that they had a regular nail trimming habit. But 102(31.7%) of them were positive for intestinal parasitic infection. (Table 2).

No statistically significant differences were found between female and male food handlers with different age group, p= 0.482 and 0.91 respectively. In addition no statistical association was found between food handlers who had; different educational status(p=0.42), more than two years work experience in the hospital(p=0.9), swimming habit(p=0.37), prepared food when suffering from diarrhea(p=0.47), do not washed their hand before touching food(p=0.8), and had no regular nail trimming habit(p=0.5) related to risk of parasitic infection.

Similarly no statistically significant associations were found between all tested variables and risk of gastrointestinal carriage of *Salmonella* and *Shigella flexneri* such as gender (p=0.99), age (p=0.43), between food handlers who had; different educational status (p=0.9), different marital status (p=0.43), more than two years work experience in the hospital (p=0.67), swimming habit (p=0.63), prepared food when suffering from diarrhea (p=0.66), do not washed their hand before touching food (p=0.31), and had no regular nail trimming habit (p=0.43), food preparation and handling training (p=0.89), washed their hands after toilet use with water and detergent (p=0.30), habit of eating raw or undercooked food (p=0.43), medical checkup within the past six months (p=0.43) and share knife and other equipments used for food preparation (p=0.80).

The logistic regression analysis result showed that food handlers who had food preparation and handling training had a more likely protective effect (63%) from getting intestinal parasitic infection [OR:0.37,95%CI(0.19-0.75)] than food handlers who did not trained.

Intestinal parasitic infection was less likely to occur (62% protective effect), among food handlers who washed their hands after toilet use with water and detergent [OR: 0.38, 95%CI (0.23-0.62)] than those who did wash with water only after toilet use.

The extent of intestinal parasitic infection was also less likely to occur with a protective effect of 64% , among food handlers who had no habit of eating raw or undercooked food [OR:0.36, 95%CI (0.22-0.61)] compared to those who had habit of eating raw or undercooked food. Food handlers who had practiced medical checkup within the past six months had a less likely risk of being infected with intestinal parasites [OR: 0.35, 95%CI (0.22-0.58)] as compared to those food handlers who did not practiced the checkup. The risk of getting intestinal parasitic infection is less likely among food handlers who did not share knife and other equipments used for food preparation [OR: 0.23, 95%CI (0.23-0.8)] compared to those who share knife and other equipments (Table 3).

Table 3: Bivariate and Multivariate logistic regression analysis: Risk factors for intestinal parasitic infection among food handlers working in public hospitals, Addis Ababa, Ethiopia, March to June, 2017 (n=368).

Predictors		Positive No.(%)	Negative No.(%)	Crude OR 95% CI	Adjusted OR 95%CI
Formal food preparation training	Yes	13(16.9)	64(83.1)	0.36(0.19-0.67)	0.37(0.19-0.75)
	No	106(36.4)	185(63.6)	1.00	1.00
Wash hand with water and detergent after toilet use	Yes	51(23.4)	167(76.6)	0.37(0.24-0.58)	0.38(0.23-0.62)
	No	68(45)	82(55)	1.00	1.00
Eat raw/ undercooked food	No	31(20.5)	120(79.5)	0.38(0.24-0.61)	0.36(0.22-0.61)
	Yes	88(41)	129(59)	1.00	1.00
Regular medical checkup within the past 6 months	Yes	37(19.8)	150(80.2)	0.3(0.19-0.47)	0.35(0.22-0.58)
	No	82(45)	99(55)	1.00	1.00
Share knife	No	17(17.5)	80(82.5)	0.35(0.2-0.63)	0.23(0.23-0.8)
	Yes	102(38)	169(62)	1.00	1.00

n= Sample size

6.4. Antimicrobial susceptibility pattern of bacterial isolates

All, 100% (n=14) *Salmonella* isolates were found to be resistant to ampicillin and erythromycin (Table 4). Two (14.3%) isolates were found to be resistant to three drugs of different groups (ampicillin, erythromycin and Trimethoprime/sulfamethoxazole). Therefore, all *Salmonella* isolates were MDR (Table 6). All, 100% (n=3) *Shigella flexneri* isolates were found to be resistant to ampicillin and tetracycline (Table 5). All (100%) *Shigella flexneri* isolates were found to be MDR (Table 6).

Table 4: Antibiotic sensitivity pattern of *Salmonella* isolated from stool specimens of food handlers in public hospitals, Addis Ababa, Ethiopia, March to June, 2017(n=14).

Antibiotics	**AST**			
	R	%	S	%
Ampicillin(10µg)	14	100	0	0
Cefotaxime(5µg)	1	7.1	13	92.9
Erythromycin(15µg)	14	100	0	0
Ciprofloxacin(5µg)	0	0	14	100
Gentamycin(10µg)	0	0	14	100
Doxycycllin(30µg)	0	0	14	100
Trimethoprime/sulfamethoxazole (1.25/23.75µg)	2	14.3	12	85.7

R= resistant, S= sensitive

Table 5: Antibiotic sensitivity pattern of *Shigella flexneri* isolated from stool specimens of food handlers in public hospitals, Addis Ababa, Ethiopia, March to June, 2017(n=4).

Antibiotics	AST			
	R	%	S	%
Ampicillin(10µg)	3	100	0	0
Cefotaxime(5µg)	0	0	3	100
Azithromycin(15µg)	1	33.3	2	66.7
Naldixic acid(30µg)	1	33.3	2	66.7
Ciprofloxacin(5µg)	1	33.3	2	66.7
Gentamycin(10µg)	0	0	3	100
Chloroamphenicol(30µg)	0	0	3	100
Tetracycline(30 µg)	3	100	0	0
Trimethoprime/sulfamethoxazole(1.25/23.75µg)	1	33.3	2	66.7

R= resistant**,** S= sensitive

Table 6: MDR pattern of *Salmonella* and *Shigella flexneri isolates* from stool specimen of food handlers in public hospitals. Addis Ababa, Ethiopia, March to June, 2017.

Bacterial isolates	R_0%	R_1%	R_2%	R_3%	R_4%	R_5%	R_6%	R_7%	R_8%
***Salmonella species*(n=14)**	0(0)	0(0)	12(85.71)	2(14.29)	0(0)	0(0)	0(0)	0(0)	NA
***Shigella flexneri*(n=3)**	0(0)	0(0)	2(66.7)	1(33.3)	0(0)	0(0)	0(0)	0(0)	0(0)

R_0- sensitive to all tested antibiotics, R_1- resistance to only one antibiotic etc. NA- not applicable

7. Discussion

Gastroenteritis caused by different intestinal protozoa, helminthes and enteric bacteria is still a widely prevalent public health problem in developing counties. Healthy peoples, patients visiting hospitals and the community at large are at risk of food and water borne gastroenteritis. Beside other factors, food handlers may act as an important vector to harbor intestinal parasites and pathogenic enteric bacteria that may be transmitted to people served by them. Therefore this study was conducted to determine the prevalence of intestinal parasite infections and carriage rate of pathogenic gram negative enteric bacteria among food handlers of public hospitals in Addis Ababa, Ethiopia. In Ethiopia the impact of food borne disease that have been arise from asymptomatic and carrier hospital food handlers on patient care is not well studied yet.

The prevalence of intestinal parasites among food handlers from different studies was reported as follows: by Assefa T *et al*. at southern part of Ethiopia 36% [48], Andargie G *et al*. in Gondar Town, Northwest Ethiopia 29.1% [55], Bakier MA *et al*. in Khartoum, Sudan 29.4% [40], Seada AH *et al*. in Omdurman, Sudan 30.1% [41], and by Wakid MH *et al*. in holly city of Makah was 31.94% [37]. All these findings are consistent with the current finding (32.4%).

Higher prevalence of intestinal parasite among food handlers has been reported from a studies conducted at different parts of the world such as: Akililu A *et al*. in Addis Ababa, Ethiopia 45% [49], Teklemariam S *et al*. in Hawassa, Ethiopia 63% [50], Nigussie D *et al*. in Mekelle, Ethiopia 49.4% [46], Abera B *et al*. in Bahirdar Town, Northwest Ethiopia 41.1% [56], Idowu OA *et al*. in Abukota, Nigeria 97% [39], Al-lehham AB *et al*. in Ibrid, Jordan 48% [35], Sinesk Z *et al*. in Sanliurfa, South eastern Anatolia 52.2% [33], Al-suwaidi AHE *et al*. in Minas, Brazil 47.1% [32] and Ferites A *et al*. in Zulia state, Venezuela 48.7% [31].

Lower prevalence of intestinal parasite were reported from a studies that had been conducted among food handlers by Modrek MJ *et al* in Northwest Iran 3.73% [26], Motazedian MH *et al*. in Shiraz, Iran 10.4% [27], Al-hindi A *et al*. in Gaza strip, Palestine

24.3% [36], Al-lehham AB *et al*. in Ibrid, Jordan 15.1% [35], Kusolsuk T *et al*. among food handlers in tourist area restaurants & educational- institutions cafeteria, in Thailand 10.3% [34] and by Khurana S *et al*. among tertiary care hospitals North India 1.3 to 7% [25]. This discrepancy may be due to much difference in sample size and geographical location. Differences in education status, personal hygiene, dissimilar socio demography and environmental conditions of these diverse populations may also contribute for the discrepancy.

According to Mulat *et al*. the common causes of protozoan intestinal parasitic infection was Giardiasis 11% [11]. In the current study among total number of intestinal parasites detected in the laboratory (119), *Entamoeba histolytica/dispar* was predominant, 40.3% followed by *Giardia lamblia* 30.25%. The higher frequency of *Entamoeba histolytica/dispar* than *Giardia lamblia* may be due to the reason that cyst stage of *Entamoeba histolytica* and *dispar* were not differentiated and most food handlers had habit of eating raw/uncooked food in this study. Since all food handlers participated in this study reported that they used pipe water the high prevalence of *Giardia lamblia* may be due to resistance to chlorination or under chlorination of the water. About 59% of food handlers participated in this study had habit of eating raw or undercooked foods. This can be attributed to higher frequency of *Taenia species* in this study.

In the current study 3.8% and 0.81% of food handlers were positive for *Salmonella* and *Shigella flexneri* respectively. The carriage *Salmonella* was in agreement with similar studies conducted on food handlers of different groups: by Akililu A *et al*. in Addis Ababa, Ethiopia 3.5% [49], Andargie G *et al*. in Gondar Town, Northwest Ethiopia 3.1% [55], Kifelew G *et al*. in Gondar, 3% [44], Seada AH *et al*. in Ombdurman, Sudan 3.8% [39], Khurana S *et al*. in tertiary care hospital, North India 5% [25]. Higher carriage rate of *Salmonella* has been reported from a study by Al-lehham AB *et al*. in Ibrid, Jordan 6% [35]. Lower prevalence of *Salmonella* was found in a studies conducted by Assefa T *et al* in Jimma from hand rinse samples 0.9% [48], Khurana S *et al*. on food handlers of tertiary care hospital in India 0-2.5% [25], Francis SP *et al*. in Kumasi, Ghana 2.3% [17], zero percent by Sinask Z *et al*. in Anatolia [33], Kusolusk T *et al* in Thiland [34], Andargie G *et al*. in Gondar town [55] and Teklemariam S *et al*. in Hawassa, Ethiopia [50].

The prevalence of *Shigella* in this study is in line with reports among food handlers from previous studies by Khurana S *et al*. in India 1.28%, and 1.23% in two years [25], Al-lehham AB *et al*. in Ibrid Jordan 1.4% [35], 1.3% by Seada AH *et al*. in Omdurman, Sudan [39]. Higher results have been reported by Andargie G *et al*. 3.1% at Gondar town, Northwest Ethiopia [55], Khurana S *et al*. in India, 9.3% [25]. The higher prevalence of *Shigella* from a study conducted in India might be food handlers who were suffering from dysenteries were included. Lower carriage, 0% had reported by Assefa T *et al*. from hand rinse sample in Jimma, Ethiopia 0% [48] and by Akililu A *et al*. in Addis Ababa [49]. The overall discrepancy between findings of these studies and the current study may be due to difference in culture of consuming uncooked or raw vegetables, fruits, meat, and unpasteurized milk. It may also be due to difference in contact with domestic or wild animals which can act as a reservoir for *Salmonella* and *Shigella*, hand washing habit and access to clean water among food handlers.

Regarding the drug susceptibility pattern of *Salmonella* isolates all 100% (n=14) were found to be resistant to ampicillin and erythromycin. This is in agreement with the result of the study conducted among food handlers, by Akililu A *et al*. in Addis Ababa university student's cafeteria, Ethiopia [49] by which all isolates of *Salmonella* were resistant. The result was consistent with the result of Abera B *et al*. in Bahirdar Town, Northwest Ethiopia [56], by which all, 100% isolates were resistant to ampicillin. However slightly lower resistance rate, 61.5% to ampicillin was recorded in a study conducted in Gondar, Northwest Ethiopia by Kifelew G *et al*. [44].

All, 100% (n=14) *Salmonella* were sensitive to ciprofloxacillin, doxycyclin and gentamycin and the result was in line with the result of a study in Addis Ababa, Ethiopia [49] by which 100% of the isolates were sensitive. But Abera B *et al*. in Northwest Ethiopia [56] showed 16.6% and 66.7% resistance to ciprofloxacillin and doxycyclin respectively. All *Salmonella* isolates were found to be MDR, but in a study conducted at Gondar university [44] 46.2% isolates were MDR.

All isolates of *Shigella flexneri* were found to be resistant to ampicillin and tetracycline in the present study. The result is comparable with the result of Khaghani S *et al*. in

southwest Iran that 87% of *Shigella flexneri* isolates were resistant to ampicillin [18]. All of *Shigella flexneri* were found to be MDR. Higher rate of resistance by *Salmonella* and *Shigella* from the present study may be due to the reason that these hospital food handlers may acquire these bacteria from hospital environment where, indiscriminate use of antibacterial for treatment and nosocomial infection is common.

In the present study no statistically significant difference was found in magnitude of intestinal parasite infection between gender, age, educational status, and working in hospital for more than two years. The result is in agreement with a study conducted in Northwest Ethiopia, Gondar University [55] showed that there was no statistically significant difference for all tested socio demographic variables. Similarly no statistically significant difference for gender in a study conducted at northwest Iran [26], southern Ethiopia [45], educational status and service year in a study conducted at Shiraz, Iran [27].

In this study food preparation and handling training [OR:0.37 (0.19-0.75)], washing hands after toilet use with water and detergent [OR: 0.38 (0.23-0.62)], avoiding habit of eating raw or undercooked food [OR:0.36 (0.22-0.61)], practicing medical checkup [OR: 0.35 (0.22-0.58)], did not share knife and other equipments used for food preparation [OR: 0.23 (0.23-0.8)] were protective from intestinal parasite infection. But, according to Mama M *et al*. sharing knife to cut vegetables and fruits [AOR: 1.72: (1.01–2.92)], not washing hand with water and soap after toilet use [AOR=1.69 (1.04–2.75)] were risk factors for intestinal parasitic infection [45]. Nigusse D *et al*. reported washing hand with water and soap after toilet use [AOR: 0.47 (0.22-0.97)] and practicing regular medical checkup [AOR: 0.06 (0.02- 0.14)] [46] were protective measures to intestinal parasitic infections. These findings are consistent with the results of current study.

8. Limitation and Strength of the study

8.1. Limitation

- Species identification for *Salmonella* isolates was not done.
- And the laboratory method was not designed in such a way to detect other bacteria that are the probable causes of gastroenteritis such as *Yersinia enterocolitca* and *Campylobacter jejuni* because of unavailability of special media required for isolation.
- *Entamoeba histolytica* and *dispar* cysts were not differentiated.

8.2. Strength

- It is the first, that have attempted to determine to what extent food handlers working in public hospitals are infected with intestinal parasites, and carriage of *Salmonella, Shigella* and *Escherichia coli* O_{157}:H_7 and to assess risk factors for intestinal parasitic infection and carriage these enteric bacteria with their drug susceptibility pattern particularly in Public Hospitals, at Addis Ababa.
- Data quality assurance systems were strongly implemented.

9. Conclusion and recommendation

9.1. Conclusion

The present study revealed that about 32.34% of the food handlers working at public hospitals in Addis Ababa, Ethiopia were positive for different intestinal parasites. The most prevalent parasite was *Entamoeba histolytica/dispar* followed by *Giardia lamblia*. The prevalence of parasites is significantly high.

Socio demographic characteristics of the food handlers were not significantly associated with risk of intestinal parasitic infection. But taking food preparation and handling training, hand washing with water and detergent habit after toilet use, practicing regular medical checkup, not sharing knife and other equipments between food handlers used for food preparation and no habit of eating raw or undercooked food were found to be associated with risk of infection with intestinal parasites and they were preventive. All tested variables were not significantly associated with risk of getting *Salmonella* and *Shigella flexneri*.

The cumulative carriage rate of *Salmonella* and *Shigella flexneri* among food handlers was significant. No *Escherichia coli* $O_{157}:H_7$ was isolated. All *Salmonella* and *Shigella flexneri isolates* were found to be multidrug resistant.

9.2. Recommendation

The findings of this study indicated that the food handlers may pose a risk of food borne illness to patient's population being served. So it is recommended that hospital administration should provide food preparation and handling training, continuous medical checkup, give health education about personal hygiene for food handlers and food hygiene to minimize the risk of infection with intestinal parasites as well as transmission to patients and other group of peoples. The food handlers, participated in this study were also recommended to improve their hygiene practice.

Since the present study had also indicated that food handlers working at public hospitals in Addis Ababa, Ethiopia were asymptomatic carriers for *Salmonella* and *Shigella flexneri* with high rate of drug resistance to commonly used antibiotics, which necessitate screening of food handlers.

Further studies should be conducted to indicate the impact of food borne disease arise from carrier food handlers on patient care.

10. References

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Annex

Annex-1: Consent form information sheet for study participants

Principal Investigator: Tegegn Belhu

Institution: Addis Ababa University College of Health Sciences

Purpose: The purpose of this study is to investigate the magnitude of gram negative entero-pathogenic bacteria with their drug susceptibility pattern and intestinal parasite among apparently healthy hospital food handlers. If you are willing to participate in this study you are expected to answer some questions and give two stool specimens.

Risks associated with the study: There is no risk associated with participating in this study. You never waste time except the time required to give some information and specimen.

Benefits of the study: There will be no financial benefit to you. But the result of the study will be used for your hospital to take care of you and the patients you are serving. If bacteria and or parasite that will be harmful to your health are detected we will facilitate to take treatment,

Confidentiality of your information: The results of the laboratory findings will be kept confidential and could only be accessed by the researcher and responsible body. There will be no personal information to be attached to your data.

Termination of the study: We will respect your decision if you later changed your mind.

Based on the above information I agree to participate in the research

Signature: _____ Date: _____

Name of Data collector _____ Signature _____

Remember; if you have any question you can ask the principal investigator

Tegegn Belhu

Addis Ababa University College of Health Sciences,

Department of Medical Laboratory Sciences

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E-mail:- tegegnbelhu@gmail.com

Annex-2: Consent form information sheet for study participants (Amharic version)

ጥናቱን የሚያጠናው፤ ተገኝ በልሁ፤ በአሁኑ ሰዓት በአዲስ አባባ ዩኒቨርስቲ በክሊኒካል ላቦራቶሪ ሳይንስ የሁለተኛ ዲግሪ ፕሮግራም በዲያግኖስቲክ እና ፐብሊክ ሄልዝ ማይክሮባዮሎጂ ትምህርት ክፍል እየተከታተልኩ እገኛለሁ፡፡

የጥናቱ አላማ፡ የምግብ አብሳዮች እና አቅራቢዎች አንጀት ውስጥ ህመም ሳያስከትሉ በመየት በየትኛውም ጊዜ በምግብ አብሳዮች እና አቅራቢዎች ላይ በሽታ ሊያመጡ የሚችሉ ፡እንዲሁም በምግብ ብክለት ወደ ተጠቃሚዎች ሊተላለፉ የሚችሉ ባክቴሪያዎችን እና ጥገኛ ተህዋሲያን በላቦራቶሪ ምርመራ ማግኘት እና በእነዚህ ባክቴሪያዎች የሚመጡ ህመሞችን ለማከም ለሚያገለግሉ መዲሀኒቶች ያላቸውን ተጋላጭነት ማጥናት ነዉ፡፡በጥናቱ ወቅት ከ እርስዎ የሚጠበቀው በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ ናሙና የሚሰበስቡት ባለሙያወች ለሚጠይቆወት የተወሰኑ ጥያቄወች መልስ መስጠት እና የሰገራ ናሙና መስጠት ነው፡፡

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት: በጥናቱ መጀመሪያም ይሁን መጨረሻ በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም፡፡ መረጃ እና ናሙና ለመስጠት ከሚያስፈልገው ጊዜ ውጭ የሚያባክኑት ምንም ጊዜ አይኖርም፡፡

ለጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም: በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም፡፡ ነገር ግን ከጥናቱ የሚገኘው ውጤት እርስዎ ህክምና እንዲያገኙ እና ከእርሰዎ ወደ ታካሚዎች እንዲሁም ወደ ህብረተሰቡ ሊተላለፉ የሚችሉ በሽታወችን ለመከላከል ይጠቅማል፡፡

የመረጃ ሚስጥራዊ አጠባበቅ: የሚሰጡትመረጃ በጥናቱ ወቅትም ሆነ ከዚያ በኋላ ባሉት ጊዜያት ሙሉ በሙሉሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡ በጥናቱ ላይ ያለመሳተፍ መብት አለዎት፡፡ይህ መረጃ በጥንቃቄ የሚያዝ ይሆናል፡፡ በመጨረሻም የጥናቱ ውጤት ለሚመለከተዉ አካል ብቻ የሚገለፅ ይሆናል፡፡

ከጥናቱ አቋርጦ ስለመውጣት፡ በማንኛውም ጊዜ ለማቋረጥ ከወሰኑ ጥያቄዎተን እንቀበላለን፡፡

እኔም የጥናቱ ተሳታፊ ይህንን በመገንዘብ ጥናቱ ላይ ለመሳተፍ ተስማምቼያለሁ፡፡

ፊርማ ----------

መረጃውን የሰበሰበው ግለሰብ ስም----------

ፊርማ ----------

ያስታውሱ፤ ስለዚህ ጥናት ማንኛውም ጥያቄ ካለዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ፡፡

የዋና ተመራማሪው አድራሻ፤

ተገኝ በልሁ፤ የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት፣ የጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ- አዲስ አበባ፣ ኢትዮጵያ , ኢ-ሜይል፣ tegegnbelhu@gmail. com ስልክ ፣ +251936661909

Annex-3: Questionnaire (English version)

Interviewers name……..

Date of the interview……..

Participant code_____

Part I: Socio demographic characteristics of participants

1. Age_____
2. Sex_____
3. Educational Status: Illiterate □ primary school (1-8) □ Secondary school (9-12) □ technical and vocational school □ Collage/University □
4. For how many days/months or years you have worked as food handler in this hospital? Less than 2 year □ 2-10 years □ 11-20 years □ >21 years □
5. Address you are living now: Urban □ Rural □
6. Job position in the food handling job: Cook /chef, cleaning utensils and waiter others/specify_____
7. Presence of latrine at hospital: 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. Swimming habit: Yes □ No □
11. Habit of eating uncooked raw foods (meat, vegetables…) Yes □ No □
12. Have you taken anti-helminthes/protozoa within the past six months? Yes □ No □
13. Source of water pipe water □ other source □
14. Do you wash your hands after toilet use? Yes □ No □
15. If yes? with water only □ with water and detergent □
16. Certified in food preparation and handling? Yes □ No □
17. Do you take regular medical checkup? Yes □ No □
18. Do you wash your hands before preparing and serving any food? Yes □ No □
19. Do you prepare food when suffering from disease like diarrhea? Yes □ No □
20. The practice of using common knife for cutting raw flesh food and other food.

Yes □ No □

21. Do you trim your finger nail regularly? Yes □ No □

Laboratory investigation result recording form

Parasitology result

Code	Intestinal parasite	Remark

Culture and AST result

Code	Growth		Entero-pathogenic bacteria	AST result	Remark
	Yes	No			

Annex-4: መጠይቅ

የጠያቂው ስም _____

ቀን _____

የጥናቱ ተሳታፊ የሚስጥር ቁጥር _____

ክፍል፡1 የተሳታፊው socio demographic ሁኔታ

1. ዕድሜ_____
2. ፆታ_____
3. የትምህርት ደረጃ፡ አልተማረኩም □ የመጀመሪያ ደረጃ □ ሁለተኛ ደረጃ □ የሙያ ማሰልጠኛ ተቋም □ ከሌጅ/ዩኒቨረሲቲ □
4. በዚህ ሆስፒታል ውስጥ ለምን ያህል ጊዜ አገልግለዋል? ከ 2 አመት በታች □ 2-10 አመት □ 11-20 አመት □ ከ 21 አመት በላይ □
5. አድራሻ፡ ከተማ □ ከ ከተማ ክልል ውጭ □
6. በምግብ ስራ ውስጥ ያለህ/ሽ የስራ ድርሻ፡ አብሳይ /አጣቢ/አስተናጋጅ ሌላ ይጠቀስ ----------
7. ሽንት ቤት አለ? አለ □ የለም □
8. ሽንት ቤቱ ከካባቢው እጅ መታጠቢያ አካባቢ ሳሙናን የመሳሰሉ ቁሳቁሶች አለ?አለ □ የለም □

ክፍል 2- የተሳታፊወች የንፅህና አጠባበቀቅ ተግባር

9. ጫማ የማረግ ልምድ፡ አለኝ □ የለኝም □
10. የዋና ልምድ፡ አለኝ □ የለኝም □
11. ያልበሰሉ ነገሮችን ይመገባሉ (ስጋ፣ አትክልት …) አዎ □ አልበላም □
12. የሆድ ውስጥ ትላትል ለማጥፋት የሚጠቅሙ መድሃኒቶቸን ባለፉት ስድስት ወራት ውስጥ ወስደዋል? አዎ □ አልወሰድኩም □
13. ውሃ ከየት ይጠቀማሉ? ከቧንቧ □ ከሌላ ምንጭ □
14. ሽንት ቤት ከተጠቀሙ በኋላ እጂወትን የመታጠብ ልምድ አለወት? አዎ □ የለኝም □
15. ከታጠቡ? በውሃ ብቻ □ በውሃ እና ሳሙና □
16. የምግብ ዝግጅት ስልጠና ወስደዋል? አዎ □ አልወሰድኩም □
17. መደበኛ የህክምና ምርመራ ያደርጋሉ? አዎ □ አላደርግም □
18. ምንም አይነት ምግብ ከማብሰልዎ እና ማቅረበወ በፊት እጅወትን ይታጠባሉ? አዎ □ አልታጠብም
19. ተቅማጥ ያለው ህመም እየተሰማወት ስራ ይሰራሉ? አዎ □ አልሰራም □
20. ቢላዋ በጋራ ይጠቀማሉ? አዎ □ አልጠቀምም □

21. ጥፍረወን የመቁረጥ ልምድ አለወት? አዎ □ የለኝም □

Laboratory investigation result recording form

Parasitology result

Code	Intestinal parasite	Remark

Culture and AST result

Code	Growth		Entero-pathogenic bacteria	AST result	Remark
	Yes	No			

Annex-5: Procedure for Indole test

Purpose: This procedure provides instructions to detect the production of indole by bacteria growing on media containing tryptophan.

Principle: The indole test determines the ability of an organism to produce indole from the degradation of the amino acid tryptophan. Tryptophan is hydrolyzed by tryptophanase to produce three possible end products –one of which is indole. A colored product is produced when the indole is combined with certain aldehydes (kovacs Reagent)

Reagents:

- Nutrient Broth

Supplies:

- Pasteur pipette
- Rubber tit
- Wire loop

Equipment:

- Incubator 37ºC
- Bunsen burner

Sample type: The colony which is suspended in nutrient broth

Limitations: Mixed colony

Sample retention**:** Samples are discarded after 24 hrs

Quality control: ATCC strain

Control preparation:

- Reconstitute the lyophilized sample by TSY broth or Normal saline
- Open the seal and aseptically add 1ml of broth or Saline
- Inoculate on medias (Blood or MaCconkey Agar Plate)
- Incubate at 37ºC incubaton
- After overnight incubation (18-24hr) observe the colony and perform biochemical tests

Escherichia coli ATCC 25922 -dark pink color develops

Enterobacter aerogenes ATCC13048 -fair to good growth blue

Note: If the colony is not pure re-culture from the stock

Procedure:

1. Take nutrient Broth tube
2. Label the tube
3. Take pure colony on BAP near Bunsen burner
4. Suspend in Nutrient Broth
5. Vortex the suspension
6. Incubate at 37ºC incubator
7. When the suspension become turbid take drop of suspension aseptically and add drops into broth
8. Incubate the inoculated media at 37ºC incubator for overnight
9. Add drops of kovacs reagent
10. Observe the production of red Ring or not

Result interpretation: Indole positive bacteria such as *Escherichia coli* produce tryptophanase, an enzyme that cleaves tryptophan, producing indole and other products. When Kovac's reagent (p-dimethylaminobenzaldehyde) is added to a broth with indole in it, a dark pink color develops.

Limitations: The indole test must be read by 48 hours of incubation because the indole can be further degraded if prolonged incubation occurs. The acidic pH produced by *Escherichia coli* limits its growth.

Clinical utility: A test used to identify members of the Enterobacteriaceae family and other Gram-negative bacilli, based on the ability of the organisms to produce indole from tryptophan

References :

1. WHO: Basic laboratory Procedures in Clinical Bacteriology. 2nd edition. Geneva: WHO; 2003. P.37-50.
2. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Manual of clinical microbiology. 6th ed. Washington DC: ASM Press; 1995.
3. Finegold SM, Martin WJ, Scott EG. Bailey and Scott’s diagnostic microbiology. 5th ed. Saint Louis: CV Mosby Company; 1978.

Annex-6: Procedure for Motility Test

Purpose: This procedure provides instructions for performing the detection of motility of gram-negative enteric bacilli.

Principle: Bacterial motility can be observed directly from examination of the following incubation. Growth spreads out from the line of inoculation if the organism is motile. Highly motile organisms provide growth throughout the tube. Growth of non motile organisms only occurs along the stab line

Reagents:

- Nutrient Broth

Supplies:

- Semi solid agar
- Pasteur pipette
- Rubber tit
- Wire loop

Equipments:

- Incubator 37ºC
- Bunsen burner

Sample: colony from MacConkey agar

Sample retention: Samples are discarded after 24 hrs

Quality control: ATCC strain

Control preparation: the same as indole test

Shigella strain (ATCC) = non motile control

Note: If the colony is not pure re-culture from the stock

Procedure:

1. Take nutrient Broth tube
2. Label the tube
3. Take pure colony on Blood Agar Plate near Bunsen burner
4. Suspend in Nutrient Broth
5. Vortex the suspension

6. Incubate at 37ºC incubator
7. When the suspension become turbid take drop of suspension aseptically and stab the semi solid medium not drop the broth
8. Incubate the inoculated media at 37ºC incubator for overnight
9. Observe change of color (diffusion of bacteria) on the media

Result interpretation: Motility is observed visually by diffuse growth spreading from the line of inoculation. Certain strains of motile bacteria will show diffuse growth throughout the entire medium, while others may show diffusion from one or two points only, appearing as nodular growths along the stab line. Non-motile organisms grow only along the line of inoculation.

Limitation:

1. Many organisms fail to grow deep in semisolid media; inoculating pour plates may be advantageous.
2. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Clinical utility: This test is used to determine if an organism is motile or non-motile. Motile organisms are generally bacilli although a few motile cocci do exist.

References :

1. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Manual of clinical microbiology. 6th ed. Washington DC: ASM Press; 1995.

Annex-7: Procedure for Urea hydrolysis test

Purpose: This procedure provides instructions for the differentiation of bacteria on the basis of urea hydrolysis.

Principle: The urea medium of Rustigian and Stuart3 is particularly suited for the differentiation of *Proteus species* from other gram negative enteric bacilli capable of utilizing urea. The complete Urea Agar contains 15.0 g/L of agar in addition to the ingredients in the base medium. When organisms utilize urea, ammonia is formed during incubation which makes the reaction of these media alkaline, producing a red-pink color. Consequently, urease production may be detected by the change in the phenol red indicator.

Reagents:

- Nutrient Broth
- 40% urea solution

Supplies:

- Urea Agar
- Pasteur pipette
- Rubber tit
- Wire loop

Equipments:

- Incubator 37ºC
- Bunsen burner

Sample type and retention: the same as indole test

Quality control: ATCC strain

Control preparation: Refer procedure for indole test (Similar)

Escherichia coli ATCC 25922 yellow slant

Proteus mirabilis ATCC 49565 pink red slant and butt

Note: If the colony is not pure re-culture from the stock

Procedure:

1. Take nutrient Broth tube
2. Label the tube
3. Sterilize wire loop using the Bunsen burner

4. Using wire loop take a heavy inoculums of growth from an 18-24 hour pure culture
5. Suspend in Nutrient Broth
6. Vortex the suspension
7. Incubate at 37ºC incubator
8. When the suspension become turbid takes drop of suspension aseptically and add drops into urea slant
9. Incubate the inoculated media at 37ºC incubator for overnight
10. Observe change of color on the media

Interpretation of results: The production of urease is indicated by an intense pink-red (red-violet) color on the slant. The color may penetrate into the agar (butt); the extent of the color indicates the rate of urea hydrolysis. A negative reaction is no color change. The agar medium remains pale yellow.

Limitations:

1. *Pseudomonas aeruginosa*, for example) or other proteins which raise the pH due to protein hydrolysis and the release of excessive amino acid residues. To eliminate possible protein hydrolysis, perform a control test with the same test medium without urea.
2. Do not heat or reheat the medium because urea decomposes very easily.
3. Urea Agar detects rapid urease activity of only the urease positive
4. *Proteus species*. For results to be valid for the detection of *Proteus*, the results must be read within the first 2-6 hours after incubation. Urease-positive *Enterobacter*, *Citrobacter* or *Klebsiella*, in contrast, hydrolyze urea much more slowly, showing only slight penetration of the alkaline reaction into the butt of the medium in 6 hours and requiring 3-5 days to change the reaction of the entire butt.

Clinical utility: Urea Agar and Urease Test Broth are used for the differentiation of organisms, especially the Enterobacteriaceae, on the basis of Urease production.

Annex- 8: Procedure for Citrate test.

Purpose: This procedure provides instruction to perform citrate utilization test for enteric bacteria.

Principle: citrate agar is used to test an organism's ability to utilize citrate as a source of energy. The medium contains citrate as a sole carbon source and inorganic ammonium salts as source of nitrogen. Bacteria that can grow on this medium produce an enzyme citrate permease, capable of converting citrate to pyruvate. Pyruvate can then enter the organism's metabolic cycle for production of energy. Growth is an indicative utilization of citrate. During citrate utilization ammonium salts are broken down to ammonia which increases PH of medium that leads to change in the color of bromthymol from green to blue.

Quality control: Citrate positive- *Klebsiella pneumoniae* ATCC 13883

Citrate negative- *Escherichia coli* ATCC 25922

Procedure:

1. Streak the slant back and forth with a light inoculums picked from the centre of a well isolated colony
2. Inoculate at 37 degree Celsius for up to 4-7 days.
3. Observe a color change from green to blue through the slant.

Interpretation of the result:

Positive if color change from green to blue through slant. Negative result show no color change

Limitation: some organisms capable of to grow on citrate but no color change and the test is not sufficient to identify to species level.

Anne-9: Procedure for TSIA Test

Purpose: This procedure provides instruction to determine whether gram negative bacteria utilize glucose and lactose or sucrose through fermentation and produce H_2S.

Principle: The medium contains glucose, lactose, sucrose, phenol red, peptone, ferrous sulphate. Fermentation sugars resulted in production of acid that cause the change in the PH of the medium. The change in Ph leads to change in the color of indicators.

Quality control: American Type Culture Collection stains *Salmonella enterica* 14028, *Escherichia coli* 25922, *Pseudomonas aeruginosa* 27853 and *Shigella sonnei* 19290 can be used as quality control.

Procedure:

1. Touch a well isolated colony with a sterile straight wire.
2. Inoculate TSI by first stabbing through the center of the bottom of the tube and then streak the surface of the butt
3. Leave the cap loose and incubate at 37 degree Celsius for 24 hours aerobically
4. Observe the reaction.

Interpretation of the result: initially the color of TSI is red. If no color changes (red slant/red butt) implies no fermentation of glucose, lactose and sucrose. Yellow slant/yellow butt at 8 hours of incubation and Red slant/yellow but at 24 hours of incubation indicate only glucose fermentation. If Yellow slant/yellow but persists over 24 hours, glucose, lactose and/or sucrose are fermented. Black precipitated indicates H_2S production. If there is crack or bubbles produced CO_2 or H_2 is produced.

Annex-10: Antibiotic sensitivity testing procedure

Purpose: This procedure provides instructions to determine the drug sensitivity pattern of bacteria using Kirby Bauer disk diffusion method.

Principle: The antibiotic will diffuse in a radial manner from the disc and will inhibit bacterial growth around it.

Abbreviation:

ATCC= American Type Culture Collection

CLSI= Clinical and laboratory standards institute

Reagents: 0.5 McFarland standards

Reagent preparation:

Turbidity standard number	Barium chloride dihydrate (1.175%)	Sulfuric acid (1%)	Corresponding approximate density of bacteria
0.5	0.5ml	99.5ml	1x108

Reagent stability: for six month at +2:+8 oc

Supplies:

- Muller hinton agar
- Mulleur Hinton agar with 5% sheep blood
- Normal saline
- Test tube
- Wooden applicator sticks with cotton
- Antimicrobial disks

Equipments:

- Safety cabinet
- Bunsen burner
- Incubator
- Measuring caliper
- Vortex
- Candle jar

Sample

Sample type	Amount required	Transport and Storage	Stability
Pure colony equivalent to 0.5 McFarland	2 ml	The test should be done immediately after the suspension have been made.	Stable for 24 hours

Limitations: Comparing the inoculums turbidity with the standard McFarland is subjective.

Sample retention: Samples are discarded after the test has been done. .

Quality Control

Control	Stability	Frequency	Preparation (Y/N)
ATCC strains	6 weeks at room temperature	Weekly subculture	Y

Y: yes N: no

Control preparation:

Reconstitute the lyophilized standard strain in to 1ml TSY broth or Normal Saline.

Inoculate in to BAP & MAP

Incubate for 16 – 24 hrs at 35 – 37 oC

Perform sensitivity test

Compare the sensitivity result with CLSI guideline.

Note: If the results are out of expected value, repeat the test and take corrective action.

Procedure:

1. Prepare pure colony suspension in to normal saline equivalent to 0.5 Mcfarland standards.
2. Streak on appropriate media the entire surface.
3. Select antimicrobial agents according to the CLSI guideline & Put the disc on the plate aseptically
4. Incubate for 16 – 24 hrs at 35 +/- 2^{o}C

5. Measure zone of inhibition and interpret the result based on CLSI break point.

Result interpretation:

1. Susceptible (S):

The 'susceptible' category implies that isolates are inhibited by the usual achievable concentration of antimicrobial agent when the recommended dosage is used for the site of infection.

2. Intermediate (I):

The 'intermediate' category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower that for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated or when a higher than normal dosage of a drug can be used. This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretation, especially for drugs with narrow pharmacotoxicity margin.

3. Resistant **(R):**

The 'resistant' category implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules, and/or that demonstrate MICs or zone diameters that fall in the range where specific microbial resistant mechanisms are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies.

Limitation: The response to antimicrobial therapy in vivo may not always reflect results in vitro.

Clinical utility: To detect in vitro the relationship between an organism and an antibiotic to predict the failure or success of therapy in vivo (in patient).

References :

1. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. USA: CLSI: M100-S25; 2015.

Annex-11: List of antibiotic disks with their concentration and zone of inhibition

Drug	Conc. (µg)	Sensitive	Intermediate	Resistant
Ampicillin	10	≥ 17	14-16	≤ 13
Gentamicin	10	≥ 15	13-14	≤ 12
Cefotaxime	30	≥ 26	23-25	≤ 22
Ciprofloxacin	5	≥ 21	16-20	≤ 15
Tetracycline	30	≥ 15	12-14	≤ 11
Trimethoprime/sulfamethoxazole	1.25/23.75	≥ 16	11-15	≤ 14
Chloramphenicol	30	≥ 18	13-17	≤ 12
Erythromycin	15	≥23	14-22	≤13
Doxycyclin	30	≥ 14	11-13	≤10
Azithromycin	15	≥13	-	≤12
Naldixic acid	30	≥19	12-14	≤11

Source: CLSI guideline

Annex -12: Antibiotic sensitivity discs zone size interpretive chart (based on results obtained using Muller Hinton Agar) as per CLSI

S. No	Discs(Concentration)	Control ATCC	Acceptable zone size
1	Ampicillin(10µg)	*E.coli* 25922	15-22
2	Cefotaxime(5µg)	*E.coli* 25922	29-35
3	Erythromycin(15µg)	-	-
4	Ciprofloxacin(5µg)	*E.coli* 25922	30-40
5	Gentamycin(10µg)	*E.coli* 25922	19-26
6	Doxycycllin(30µg)	*E.coli* 25922	18-24
7	Trimethoprime/sulfamethoxazole (1.25/23.75µg)	*E.coli* 25922	23-29
8	Azithromycin(15µg)	*E.coli* 25922	28-36
9	Naldixic acid(30µg)	*E.coli* 25922	22-28
10	Chloroamphenicol(30µg)	*E.coli* 25922	21-27
11	Tetracycline(30 µg)	*E.coli* 25922	18-25

Source: CLSI, 2015

Annex-13: Microscopic Examination of stool Specimens

Procedure for Direct Wet Mount preparation

1. Place a drop of fresh physiological saline on one end of a slide and a drop of iodine on the other end.
2. To avoid contaminating the fingers and stage of the microscope, do not use too large a drop of saline or iodine.
3. Using a wire loop or piece of stick mix a small amount of specimen, about 2mg, (matchstick head amount) with the saline and a similar amount with the iodine. Make smooth thin preparations. Cover each preparation with a cover glass.

   Note: Sample from different areas in and on the specimen or preferably mix the faeces before sampling to distribute evenly any parasites in the specimen. Do not use too much specimen otherwise the preparations will be too thick, making it difficult to detect and identify parasites.
4. Examine systematically the entire saline preparation for larvae, ciliates, helminthes eggs, cysts, and oocysts. Use the 10x objective with the condenser iris closed sufficiently to give good contrast. Use the 40x objective to assist in the detection and identification of eggs, cysts, and oocysts.
5. Always examine several microscope fields with this objective before reporting 'No parasites found'.
6. Use the iodine preparation to assist in the identification of cysts
7. Report by specifying the parasite name and the stage of development (egg, larvae, trophozoite and cyst in the entire saline preparation.

Formol Ether Concentration Technique

Required

– Formol water, 10% v/v.

Prepare by mixing 50 ml of strong formaldehyde solution with 450 ml of distilled or filtered rain water.

– Diethyl ether or ethyl acetate.

– Sieve (strainer) with small holes, preferably 400–450_m in size.

The small inexpensive nylon tea or coffee strainer available in most countries is suitable (can be used many times and does not corrode like metal sieves).

Procedure for formal ether concentration technique:

1. Label screw caped test tubes with unique specimen number.
2. Add about 4ml of formol water solution (10% v/v) into each labeled test tubes.
3. Using applicator stick, emulsify an estimated 1g (pea-size) of faeces into each tube.
4. Note: Include in the sample, faeces from the surface and several places in the specimen.
5. Add a further 3–4 ml of 10% v/v formol water and cap the bottle.
6. Mix well by shaking.
7. Sieve the emulsified faeces and collect the sieved suspension in a beaker.
8. Transfer the suspension to a conical (centrifuge) tube.
9. Add 3–4 ml of diethyl ether or ethyl acetate.

Caution: follow universal safety precautions

10. Stopper the tube and mix for 1 minute.
11. Centrifuge immediately at 750-1000g for 1 minute.
12. After centrifuging, the parasites will have sediment to the bottom of the tube and the fecal debris will have collected in a layer between the ether and formol water (look figure)

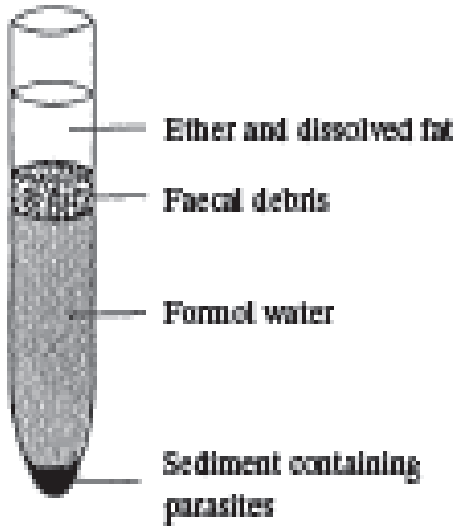

Fig : Formol ether sedimentation concentration technique, after centrifugation.

13. Using a stick or the stem of a plastic bulb pipette loosen the layer of fecal debris from the side of the tube and invert the tube to discard the ether, fecal debris, and formol l water. The sediment will remain.
14. Return the tube to its upright position and allow the fluid from the side of the tube to drain to the bottom.
15. Tap the bottom of the tube to re-suspend and mix the sediment.
16. Transfer the sediment to a slide, and cover with a cover glass.
17. Examine the preparation microscopically using the 10x objective with the condenser iris closed sufficiently to give good contrast.
18. Use the 40x objective to examine small cysts and eggs.
19. To assist in the identification of cysts, run a small drop of iodine under the cover glass.

References:

1. Cheesbrough M. District laboratory Practice in Tropical Countries. *Cambridge University Press*; 2004; 192-236
2. Suwansaksri J, Nithiuthai S, Wiwanitkit V, Soogarun S, Palatho P. The formol-ether concentration technique for intestinal parasites: Comparing 0.1N sodium hydroxide with normal saline preparations. *Southeast Asian J. Trop. Med. Public Health*. 2002: 33(3): 97-98.

Annex-14: Declaration

I, the undersigned person, declare that this thesis is my original work, has not been worked and presented either in Addis Ababa University or any other Universities. I also declare that all sources of references used have been duly acknowledged.

Name of the candidate: Tegegn Belhu Abebe(BSc)

Signature_____

Place: Addis Ababa University, School of Medical Laboratory Sciences, Ethiopia

Date of submission_____/_____/_____

This thesis has been submitted with my approval as an advisor.

Name of advisor: Mr Kassu Desta (Asst professor)

Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia

Date of submission_____/_____/_____

Name of advisor: Dr Abreham Tesfaye

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Place: Addis Ababa city administration health bureau

Date of submission_____/_____/_____

Name of advisor: Dr Yohannes W/kidan

Signature _____

Place: Addis Ababa city administration health bureau

Date of submission_____/_____/_____