PREVALENCE OF *SALMONELLA*, *SHIGELLA* AND INTESTINAL PARASITES INFECTION AMONG FOOD HANDLERS WORKING IN SELECTED GOVERNMENTAL HOSPITALS OF JIMMA ZONE, SOUTHWEST ETHIOPIA

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Abstract

Introduction*:* Humans face a global public health and economic concern as a result of unsafe food. 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 handlers. Food handlers have been caught by various pathogens and are being transmitted to the community. Hence, this study is aimed at assessing the presence of intestinal parasites, *Salmonella* and *Shigella*, associated risk factors and antibiotics susceptibility pattern of isolates among food handlers in selected governmental hospitals of Jimma zone, Southwest Ethiopia.

Methods: An institutional-based cross-sectional study was conducted among 118 food handlers from June 15 to August 14, 2022. Four governmental hospitals which give food service to inpatients were sampled. Direct wet mount and formol-ether concentration techniques were applied for microscopy identification of intestinal parasites from feces samples. Culturing technique and biochemical tests were used to isolate Salmonella and Shigella species. Additionally, antimicrobial susceptibility test to selected antibiotics was performed using Kirby-Baur disk diffusion method on Muller-Hinton Agar. Data was entered and analyzed using SPSS software version 25

Results: In this study, 118 food handlers were participated and 87.3% were females. Out of 118 stool specimens 36(30.5%) were positive for intestinal parasites. The most prevalent parasite was Ascaris lumbricoide 17(14.4%) and followed by Giardia lamblia 9(7.6%). Regarding enteric bacteria 13(11%) food handlers were positive for Salmonella and no Shigella species was isolated. Salmonella isolates were highest resistance to Ampicillin 13(100%). However, among all Chloramphenicol 2(15%) shows the highest intermediate. Whereas Ceftriaxone 13(100%), Amoxicillin/clavulanic acid Ciprofloxacin 11(85%), and Chloramphenicol 10(77%), were detected susceptible respectively. Habit of hand washing after visiting toilet, regular medical checkup last 6 month, deworming and untrimmed nail status were significantly associated with Intestinal Parasites and salmonella species infection.

Conclusion: The prevalence of intestinal parasitic infections and *Salmonella* indicates the importance of food handlers as probable sources of enteropathogenic infections. In this study, 30.5% of stool specimens were positive for different intestinal parasites. Salmonella isolation rate was 11%. Therefore, constant surveillance, improvement of personal hygiene, and taking anti helminthic/protozoa drug and periodic medical check-up are recommended to control pathogens infection in food handlers.

Key words: *Salmonella, Shigella*, Intestinal Parasite, Antimicrobial susceptibility test, Multidrug resistance.

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List of Acronyms and Abbreviations

AGH	Agaro General Hospital
AML:	Amoxicillin
AMP:	Ampicillin
ART:	Antiretroviral therapy
AST:	Antimicrobial Susceptibility Testing
CAF:	Chloramphenicol
CIP:	Ciprofloxacin
CLSI:	Clinical Laboratory Standards Institute
CSAE:	Central Statistical Agency of Ethiopia
JMC	Jimma Medical Center
KIA:	Klingler Iron Agar
MDR:	Multi- drug resistance
NTS:	Non typhoid salmonella
OPD:	Outpatients Department
SCPH	Seka Chekorsa Primary Hospital
SGGH	Shenen Gibe General Hospital
SPI:	Salmonella Pathogenicity Island
SPSS:	Statistical package for social science
UNICEF:	United Nations International Children Emergency Fund
USA:	United States of America
XLD:	Xylose Lysine Deoxycholate agar

Chapter One

1. Introduction

1.1. Background

Foodborne disease is a public health issue of global concern, causing a heavy burden of disease to society. 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. Foodborne disease is any illness that results from the consumption of contaminated food, most foodborne diseases are infections caused by a variety of bacteria, viruses, and parasites. They are an important cause of morbidity and mortality worldwide(1).

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 (2).

Food handlers may be infected by a wide range of enteropathogens and have been implicated in the transmission of many infections to the public in the community and to patients in hospitals. One human carrier of disease, preparing food at home was jeopardize the heath of only a small number of persons, mainly members of the family. When such person works in the kitchen of a restaurant, hospital, factory, canteen, school, or other places where meals are prepared and supplied to many people, the number of potential victims was correspondingly be greater so that the need for a high standard of food hygiene and adequate control measures is particularly important in catering establishments of all kinds(3).

Salmonella is a leading cause of foodborne illness worldwide and can cause enterocolitis (salmonellosis), enteric fever (typhoid fever), and septicemia with general symptoms of fever, diarrhea, abdominal cramps, nausea, vomiting, chills, and prostration. Usually, the disease lasts a few days and is self-limited although occasionally the infection can be more serious, with loss of fluid and electrolytes, and can be fatal, especially to the sick, infants, and the elderly(4).

Shigellosis is caused by *Shigella spp*. and it is a worldwide problem although more prevalent in developing countries(5). *Shigella species* are limited to the intestinal tract of humans and cause bacillary dysentery leading to watery or bloody diarrhea. Humans appear to be the only normal host reservoir for *Shigella* and they become infected by ingestion of contaminated food and water (5). It is a highly infectious disease worldwide and its prevalence is the highest in tropical and subtropical regions of the world where living standard is very low and access to safe and adequate drinking water supply and proper excreta disposal system are often very limited or even absent(6).

In Ethiopia, as in many other underdeveloped nations, intestinal parasitosis is common. One third of Ethiopians are thought to be infected with *Ascaris lumbricoides,* one quarter with *Trichuris trichiura*, and one in eight with *Hookworm.* As a result, Ethiopia has the second greatest burden of ascariasis in Sub-Saharan Africa, the third highest burden of *Hookworm*, and the fourth highest burden of *trichuriasis* (7).

In developed country, intestinal parasitic infections are mainly associated with factors like poor socioeconomic conditions, poor hygiene and sanitation practices, unsafe and inadequate water supply, and environmental change (8). The most common etiologic agents of intestinal parasitic infections in Ethiopia are *Ascaris lumbricoides, Entamoeba histolytica, Giardia lamblia*, *Trichuris trichiura*, and *Hookworm* (9,10)

1.2. Statement of the Problem

Foodborne diseases are among the major public health problems worldwide. Food born disease continue to pose a significant public health, economic, and social burden around the world. According to the findings, one out of every ten individuals becomes ill each year as a result of food contaminated with microbiological or chemical agents, resulting in 600 million illnesses, 420 000 deaths, and the loss of 33 million healthy years of life worldwide.(11). The problem is more severe in developing countries because of a lack of resources for environmental sanitation and personal hygienic practices. Food handlers with poor personal hygiene working in food-serving establishments could be potential sources of foodborne infections (12). In developing countries, 70% of cases of diarrhea are associated with the consumption of contaminated food (13).

Salmonella and *Shigella* are one of major pathogens affecting nutrients contents and causes disease to human by various microorganisms worldwide caused by consumption of various food stuff contaminated with vegetative form of the pathogen and their toxins and are one of Gram-negative rods bacteria causative

agents of two third of human borne diseases worldwide with high burden in developing countries. As a result, most of foods the ability to cause diseases by various bacteria causing agents that play a significant role on both public health and economic sectors (14).

Globally, *Salmonella* remains a major cause of foodborne infection in humans, which leads to approximately 93 million infections every year. The World Health Organization (WHO) estimates that there are around 16 million new cases and 600,000 deaths due to typhoid fever each year worldwide. It causes bacterial bloodstream infections with a fatality rate of 20–25% (15,16).*Shigella* continues to play a major role in the etiology of inflammatory diarrhea and dysentery in food handlers. The annual incidence of *Shigella* is estimated to be 164.7 million people, with 69% of all deaths attributable to Shigellosis worldwide (17,18).

Intestinal parasites still remain as major causes of considerable public health problems in low-income countries where poor food hygiene practice is common. People involved in preparing and serving food, working with poor personal hygiene could pose a potential threat of spreading intestinal parasites to the public in a community(3).

In Ethiopia the cases of food-borne illnesses are not sufficiently investigated but few reports even if diagnosed in the form of outbreak or illness shows that highly linked to Intestinal parasite, *Salmonella* and *Shigella*. Center for disease control and prevention (CDC) are attributed that Bacteria and intestinal parasites are commonly found in soil, water, plants and animals including humans. People can also be exposed to some bacteria through inhalation, contaminated drinking water and fruit juices (19).

Species of the genus *Shigella* are among the bacterial pathogens most frequently isolated from patients with diarrhea. About 5 to 15% of all diarrheal episodes worldwide can be attributed to an infection with *Shigella*, including 1.1 million fatal cases(20). A total of 69% of all episodes and 61% of all deaths attributable to shigellosis involved children less than 5 years of age (21).

Salmonella is one of the most frequently isolated foodborne pathogens. It is a major worldwide public health concern, accounting for 93.8 million foodborne illnesses and 155,000 deaths per year caused by NTS (22), Enteric fever is an invasive, life-threatening, systemic disease with an estimated over 27 million cases, resulting in more than 200,000 deaths (23). Case-fatality estimates for invasive NTS disease among hospitalized patients in Africa have been in the range 4.4% to 27% for children and 22% to 47% for adults(24).

Studies conducted in different parts of Ethiopia showed different prevalence rates of *Salmonella* and *Shigella* species. For example studies from Jimma reported (10.8%, 1.1%), Hawassa (2.5%, 7.0%), Harar (11.5%, 6.7%), Gondar (1.08%, 4.57%), Bugarija (10.5%, 4.5%) for *salmonella* and *Shigella*, respectively(25–29). Other studies have also reported on HIV infected patients in Ethiopia; the prevalence of *Shigella* 3.5% in Gondar and in Jimma 4.0% *Shigella* and 8.1% *Salmonella* was reported in HIV infected individuals (30,31). The findings mentioned above emphasize food-handlers serves as potential sources for food borne pathogens and suggest health institutions for appropriate hygienic and sanitary control measures.

Extensive and uncontrolled use of antibiotics results in emerging of multi drug resistant strains of *Salmonella* and *Shigella* species. This emergence of MDR strains are challenges in the selecting of appropriate drugs and in the effective treatment of salmonellosis and shigellosis(32).

1.3. Significance of the Study

- This study provides recent information on the magnitude of *Salmonella, Shigella* and Intestinal parasites among food handlers at the study sites
- It serves as a base line for further studies of other enteric pathogens in the study area.
- The information generated from this study and the suggested recommendation can also be used as an input for hospital administrators to take measures that mitigate the transmission of pathogenic bacteria and intestinal parasites from food handlers to patients and community. These will help them to improve quality of hospital care to their patients.
- In addition, information from this study can be used as a reference for further similar studies in Ethiopia and around the world.

Chapter Two

2. Literature review

*2.1.*General characteristic of *Salmonella, Shigella* and *Intestinal Parasites*

Globally several studies indicated that Food handlers (makers) playing a significant role in transmission of food borne infections mainly intestinal parasites and enteric pathogens like *Salmonella* and *Shigella*. In Ethiopia recent studies indicated that the occurrence of those enteric pathogens associated with food are recently increased. This may be due to water quality used, environmental condition, personal hygiene status of food handlers, the sanitary conditions of service houses and equipment's have been mentioned to favor the disease. Different studies found that asymptomatic carriers are recently linked with those enteric pathogens (33–35)

Salmonella is a non-lactose fermenter, motile, and gas producer gram negative rod belonging to the *enterobactericeae* family(36). *Salmonella* are facultative anaerobic bacteria with three primary antigens: H (flagella antigen), O (soma antigen), and VI (capsular antigen) (possessing only few serovars). Their ability to metabolize citrate as a solitary carbon source and lysine as a nitrogen source, as well as create hydrogen sulfide, originally defined them (37,38).

Shigella dysenteriae (also known as Group A*), Shigella flexneri* (Group B), *Shigella boydii* (Group C), and *Shigella sonnei* are the four species that make up the *Shigella* genus (Group D). On the basis of reactivity with highly immune serum, each species can be further separated into serotypes*: S. dysenteriae* (15 serotypes), *S. flexneri* (6 serotypes and 2 variations), and *S. boydii* (20serotypes) *Shigella* is a Gram-negative rod that is non-motile and ferments glucose. It belongs to the *Enterobacteriaceae* family (37). It is a bacterium that can only be found in the human intestine. The incubation period is one to seven days, and the infectious dose is between 10 and 100 organisms (39)

Both developed and underdeveloped countries are affected by intestinal parasitic infections throughout the world. Nearly one-third of the populations in developed countries are suffering from intestinal parasitic infection. This is around five times higher in developing countries(40) .

2.2. Prevalence of intestinal parasites among food handlers

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% (41). The prevalence level of parasitic disease in these studies was as follows: Mazandaran (21%) (42), Ardabil (27.7%) (43) and Khuzestan (8.8%) (44).

Food handlers have been shown to be infected with intestinal parasites and enteric bacteria in various locations of India, according to studies. From 2001 to 2006, a survey of food handlers at tertiary care hospitals revealed a prevalence of 1.4 to 16 percent intestinal parasite infection and 0 to 13.3 percent enteric bacteria. The most prevalent intestinal parasite in this study was *Giardia lamblia*, which had a prevalence of 0 to 4.05 percent, followed by *Entamoeba histolytica*, which had a frequency of 0 to 2.6 percent, and *Hook worm*, which had a prevalence of 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(45).

According to a study conducted in Sari, Northern Iran, 15.5 percent of food workers tested positive for at least one parasite. Education level, sex, and hand washing after toilet use were all found to be significantly related to the risk of intestinal parasite infection in this study(46). In this study model, Kheiradish et al. discovered a 19% prevalence of intestinal parasites; education level and gender were substantially linked with intestinal parasitic infection (47).

A study conducted in the Venezuelan state of Zuila on the frequency of Cryptosporidium and other intestinal parasites among food handlers revealed an overall incidence of 48.7%. *Giardia lamblia* was the most common parasite (13.4 percent), followed by *Entamoeba histolytica* (12.4%). (9.2%) (48).

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 (49).

According to a study by Simsek Z et al (2009)., the frequency of intestinal parasites among food handlers in Anatolia was around 52.2 percent. There were no *Salmonella* or *Shigella* bacteria found (50). In a similar study, Kusolusk et al (2011). discovered a 10.3 percent prevalence of intestinal parasites in Kanchanaburi province, Thailand. Similarly, no *Salmonella* or *Shigella* were found in this research (51).

In Khartoum, Sudan, 29.4% of food handlers who went to a public health lab for a medical checkup had intestinal protozoa and Helminthes. The prevalence of *Giardia lamblia*, *Entamoeba histolytica*, and *Taenia species* was 9.7%, 4.3 percent, and 0.3 percent, respectively(52). In Kenya, according to Kumatu et al, prevalence of intestinal parasite among food handlers in Nairobi, who had valid medical certificate, was 15.7% (53).

In cross-sectional research in Mekelle University, out of the 307 food handlers enrolled in the study, one hundred sixty-one (52.4%) stool specimens were positive for different intestinal parasites. *Entamoeba histolytica/dispar* was the most prevalent parasite (32.3%), followed by *Giardia lamblia* (4.9%) and *Schistosoma mansoni* (2.6%). In this investigation, the prevalence of intestinal parasite infection was not significantly associated with age or gender (p=0.053), with years of service (p=0.086) service However, it was linked to hand washing after using the Toilet (p = 0.029), a common practice of cutting raw meat food with a normal knife (p = 0.046)(54).

Parasitic infection among food handlers working in Gondar University student's cafeteria was 25% and there is no evidence of a link between intestinal parasite infection and socio-demographic characteristics or certification, however food handlers who wash their hands with water and soap after using the restroom are more likely to be certified(55). In Yebu Town, Southwest Ethiopia, the prevalence of intestinal parasite infection among food workers was 44.1%. Untrimmed finger nails [AOR: 14.7, 95 percent CI (2.8-75.4)] and participants' age more than 35 [AOR: 4.8, 95 percent CI (1.1-21.8)] were two of the identified intestinal parasite infection predictors(56).

Study conducted in Addis Ababa students cafeteria About 78 (45.3%) of food handlers were found to be positive for different intestinal parasites with the most abundant parasite of *Entamoeba histolytica*/dispar 68 (70.8%) followed by *Giardia lamblia* 18 (18.8%), *Taenia species* 5 (5.2%), *Ascaris lumbricoides* 2 (2.1%), *Hookworm* 2 (2.1%) and *Trichuris trichiura* 1 (1.1%)(2).

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

Salmonella and *Shigella* infections are major global public health issues that cause mild to severe intestinal tract infections (27) and are a common source of foodborne diseases that cause morbidity and mortality around the world (57). Many Asian countries, including China, India, Vietnam, Pakistan, and Indonesia, have high rates of enteric fever, with yearly incidence rates exceeding 100 cases per 100,000 people. Pakistan and India, with 451.7 and 214.2 cases per 100,000 people, respectively, have the highest

incidence rates. Due to a lack of diagnostic resources and effective surveillance technologies in many developing countries, particularly in Sub-Saharan Africa, the burden of enteric fever is poorly characterized (22).

Salmonella species can be found in humans, domestic and wild animals, as well as reptiles, birds, and insects, all over the world (16). Enteric fever is widespread in impoverished countries where there is a shortage of clean water and sanitation. *Salmonella* strains that are not typhiod are found all over the world (58). *Salmonella* infection in humans is costly; for example, in the United States, it affects around 1.2 million patients annually, costing $365 million in medical costs (59). Salmonellosis is the second most often reported gastrointestinal infection in the European Union (EU), with a confirmed incidence rate of 20.4 cases per 100,000 people in 2011 (60). *Salmonella* is thought to be responsible for 22.2 percent of foodborne illnesses in China, and salmonellosis is the fourth most common microbial-caused foodborne illness (57).

Shigella infections are expected to affect 80–165 million people worldwide each year. The developing countries, children under the age of five, and people with impaired immune systems account for nearly all of the cases (61). Shigellosis is a global disease, with *Shigella sonnei* predominant in Europe and the United States and *Shigella flexneri* dominating in Asia and Africa. (28).

The prevalence and virulence genes of *Shigella species* isolated from diarrhea patients were studied in a study conducted in Rosario, Argentina in 2016. The prevalence of *Shigella* was 100 (9.8%) in 1022 diarrheic patients, with isolation frequency of 74 percent for *S. flexneri*, the most common species, and 26 percent for *S. sonnei*. (62).

According to Ethiopia's National Hygiene and Sanitation Strategy program, poor hygiene and sanitation are responsible for around 60% of the disease burden. The annual incidence of food-borne illnesses in Ethiopia ranged from 3.4 to 9.3 percent, with the median being 5.8 percent, according to patient morbidity statistics (Hospitals and Health centers) of selected food-borne and food-related cases (63).

The prevalence of *Salmonella* among food handlers in different parts of Ethiopia, Arba Minch, Southern Ethiopia (6.9%) (64), Haramaya, Eastern Ethiopia (3.6%) (65). Hawassa, Ethiopia 2.12% (3), Adigrat, Ethiopia 7.3%(66). However, The prevalence of *Shigella* among food handlers in different part of Ethiopia, 0.4%, 3.1%, 1.4% and 3.7% prevalence of *Shigella* species were reported from Hawassa, Ethiopia (3), Gondar, Ethiopia (67), Haramaya, Eastern Ethiopia (65) and Adigrat respectively(66).

2.4. Food Hygien Knowledge, attitudes and practices of food handlers

Foods are affected by work experience, training, education level, medical checkup, personal hygiene, cross contamination, source of foods and water, storage temperature are found the main factor that foods spoiled due to lack of knowledge, attitude, practice of handlers because human bodies are the most inhabitants for microorganisms growth and multiplication.in Uganda and Rubaga in particular being a highly populated area, diseases related to food contamination are recognized an important health problems they create big social and economic burden to the communities and to the general health system.in Ethiopia the prevalence of intestinal and bacterial parasites among university and public cafeterias indicates contamination rate is presented everywhere through feco-oral transmission of food handlers led to outbreaks of food borne illnesses(68,69)

Food handlers with poor personal hygiene and inadequate knowledge on food safety could be the source of food borne pathogens (70,71). The consequence of food contamination varies among countries and regions of the world depending on climate, geography and degree of social and economic development (71).

The study conducted in Debre Markos town, the level of food safety knowledge and handling practices were relatively low in this study, only 34.1% of food handlers had good food safety knowledge and nearly 54% of food handlers had good food handling practice. Level of, training on food safety, and favorable attitudes towards food safety were the factors associated with knowledge of food safety. Similarly, training ,a good level of knowledge and work experience were positively associated with good food handling practice(72).

2.5. Risk factors associated with intestinal parasites, *Shigella* and *Salmonella* species infections among food handlers

About forty nine percent (49.4%) food handlers working at Mekelle university student‟s cafeteria was 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(54). The prevalence of intestinal parasiticinfection 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(56).

People in developing countries are at greater risk for these infections due to poor hygiene and a lack of sanitation facilities. The risk of intestinal parasites is significantly higher among residents in developing-country cities, particularly in shanties and slums with poor trash disposal, health-care systems, and overcrowding. Vegetables, fruits, fingers, cutlery, door knobs, and money have all been shown to have intestinal parasites. Furthermore, they can be spread by flies and contaminated fingernails(73)

Shigella is highly infectious disease worldwide and its prevalence is the highest in tropical and subtropical regions of the world. Man is the sole reservoir of the disease. *Shigella* more affects people with very low living standard and with poor access to safe and adequate drinking water supply. In such conditions, proper excreta disposal system is often very limited or even absent. It is transmitted by the fecal-oral route and enter the human body via the ingestion of contaminated food or water (25).

The main reservoirs of non-typhoid *Salmonella* are humans, domestic and wild animals, while in the case of *Salmonella typhoid*, man is the only recognized reservoir. Poultry, eggs and dairy products are the most common source of foodborne *Salmonella* outbreaks. A wide variety of food products of animal and plant origin are the transition vehicles or sources of infections. Transmission of these organisms is from person to person via fecally contaminated food, water or through direct fecal oral route (22).

In Ethiopia, *Salmonella* outbreaks are related to unhygienic food preparation, cooking, reheating and storage practices that are contaminated with the pathogen. In addition, poor access to good latrine, poor sanitation and hygienic status, hand washing habit before and after meal and / or latrine, absence of proper sewage disposal system was responsible for typhoidal type of *Salmonella* infections and *Shigellosis* is common in areas where living standards are very low and access to safe and adequate drinking water and proper waste disposal systems are often very limited, or even absent(74).

2.6. Antimicrobial Resistance of *Salmonella* and *Shigella Species* Isolated from food handlers

Determining the prevalence and antimicrobial susceptibility pattern of *Salmonella* and *Shigella* is very important for the proper selection of antimicrobial agents to control the spread of infection. Antimicrobial resistance towards one or more drugs frequency against isolates of *Salmonella* strains has increased in many countries, including the USA, the UK and Saudi Arabia (22).

The increasing antimicrobial resistance of *Shigella* species is a major problem in treating shigellosis. The major route for dissemination of multiple resistances is by horizontal transfer of plasmids carrying antibiotic resistance. A commonly isolated plasmid carries resistance against ampicillin, chloramphenicol, tetracycline, sulfonamides, streptomycin and trimethoprim (75).

In Ethiopia in the past three decades, studies indicated that *Salmonella* and *Shigella* have developed varied rate of resistance against the first line antibiotics such as ampicillin, tetracycline, co-trimoxazole, chloramphenicol (76), second generation fluoroquinolones such as norfloxacin and/or ciprofloxacin (77), the third generation cephalosporins (ceftriaxone) (78).

Study conducted in Gondar, Ethiopia 2004, isolated Sixty-five Shigella species and four *Salmonella species* from the stool samples which makes their isolation rate16.9% and 1.04%, respectively. Among 65 *Shigella* isolates, resistance to TTC, AMP, SXT, CAF, GEN and CIP, respectively, was observed in 57(87.7%), 53(81.5%), 49(75.4%), 33(50.8%), 7(10.7%) and 6(9.2%). The four isolates of *Salmonella* were susceptible to all antibiotics tested. The resistance patterns were observed eleven *Shigella* species against the six antibiotics tested. Resistance to AMP, SXT, TTC and CAF was observed in 37.3% of the isolates which was followed by AMP, SXT and TTC (35.6%). About six percent of the *Shigella* isolates were found to be resistant to all the antibiotics tested. Resistance to one or more antibiotics was found in 90.8% of the Shigella isolates (75).

Similar study conducted in Harar Ethiopia, on *Salmonella* and *Shigella* showed that 28 (11.5%) *Salmonella* and 17 (6.7%) *Shigella* organisms were isolated from 244 stool samples. Sensitivity of the *Salmonella* isolates were 0.0% to ampicillin; 0.0% to amoxicillin; 14.2% to tetracycline; 28.6% to chloramphenicol; 89.3% to norfloxacin; and 92.8% to gentamicin. *Shigella* had sensitivities of 0.0% to ampicillin; 0.0% to amoxicillin; 11.8% to tetracycline; 41.2% to chloramphenicol; 88.2% to norfloxacin; and 94.1% to gentamicin. A high level of antimicrobial resistance was detected in both *Salmonella* and *Shigella* isolates (27).

Another study conducted in Jimma Ethiopia, indicated that prevalence of *Salmonella* and *Shigella* were 19(10.8%) and 2(1.1%), respectively. All the 19 isolates of *Salmonella species* were susceptible to ciprofloxacin and norfloxacin followed by gentamycin (94.7%), chloramphenicol (94.7%) and amikacin (89.5%). However, the highest frequency of resistance was observed for ampicillin of two (100%) followed by tetracycline (47.4%) and nalidixic acid (26.3%). The MDR profile of *Salmonella* species

indicated that, 42.1% of the isolates were resistant to two antibiotics followed by three (26.3%) and four antibiotics (21.0%) The maximum number of antibiotics resisted by *Salmonella* species, was four although the highest MDR (26.3%) was observed for combinations of two antibiotics: TE/AMP (resistance to tetracycline and ampicillin) (25)

Chapter Three

3. Objectives

3.1. General Objective

- To determine the prevalence of intestinal parasites, *Salmonella* and *Shigella*, associated risk factors and test the antimicrobial drug sensitivity pattern of the *Salmonella* and *Shigella* isolates among food handlers working in selected governmental hospitals of Jimma zone, southwest Ethiopia during the study period from June 15 to August 14, 2022.

3.2. Specific Objectives

- To isolate and identify *Salmonella* and *Shigella* from stool samples of apparently health food handlers in selected hospitals.
- To determine the prevalence of *Intestinal parasites* infections among the study subjects during the study period
- To assess the knowledge, attitude and practice of food handlers towards food hygiene.
- To evaluate the antimicrobial susceptibility pattern of *Salmonella* and *Shigella* isolates
- To assess associated risk factors with the bacterial and *Intestinal parasites* infections among food handlers.

Chapter Four

4. Materials and Method

4.1.Study Area and Period

The s*tudy was c*onduc*ted in J*imma Zone which is located in Oromia regional state, Southwest Ethiopia. It is bordered on the south by the Southwest people's region, on northwest by Buno Bedele zone, on the north by East Welega zone, and on the northeast by West Shewa zone. Jimma town is the capital and administrative center of the Zone and is located at a distance of 356 km away from the capital of Ethiopia-Addis Ababa with astronomical location of 7º 4' North Latitude and 36º 5' East Longitude.

According to the 2007 Ethiopia census reports, a projected total population of Jimma zone was 3,425,206. According to Jimma zonal health office report of 2021/22, the zone has 1 tertiary hospital, 3 general hospitals, 4 primary hospitals, 122 health centers and 512 health posts.

The study was conducted from June 15 to August 14, 2022 in selected govermental hospitals of Jimma zone. Those are, Jimma Medical Center, Shenen Gibe General Hospital, Seka Chekorsa primary hospital and Agaro General Hospital.

4.2. Study Design

- Institutional based Cross-Sectional Study was conducted

4.3. Source Population

- All food handlers working at governmental Hospital of Jimma zone

4.4. Study Population

- All individuals engaged in food handling in the selected study Hospitals during the study period.

4.5. Inclusion and Exclusion Criteria

4.5.1. Inclusion Criteria

- All food handlers working at the selected hospitals of Jimma zone who are volunteer and consent to participate in the study

4.5.2. Exclusion Criteria

- Food handlers who taking antibiotics treatments within the last 14 days at the start of the study and taking anthelminthic and anti-protozoa drugs

4.6. Study Variables

4.6.1. Dependent Variables

- *Salmonella* and *Shigella* isolates
- intestinal parasites
- Antimicrobial drugs susceptibility pattern

4.6.2. Independent Variables

- Sex
- Age
- Personal hygiene
- Environmental hygiene
- Source of water
- Deworming
- Educational level of food handler, and Health status (medical checkup)
- Training on food handling
- Knowledge, Attitude, Practice of food handlers towards food hygien

4.7. Sample Size and Sampling Techniques

- There is list of 8 governmental Hospitals providing food service for inpatient, and from eight of them four (4) hospitals were selected by simple random sampling technique. These are Jimma Medical Center, Shenen Gibe General Hospital, Seka Chekorsa primary hospital and Agaro General Hospital.
- Total population sampling technique is used to enroll the study participants because the number of food handlers in the study areas is very small, so all of them were enrolled in the study. Therefore, the total sample size for this study is 118 food handlers (95 from JMC, 8 from Shene

Gibe General Hospital, 5 from Seka Chekorsa primary Hospital and 10 from Agaro General Hospital).

4.8. Data and specimen collection

4.8.1. Data Collection Tools

Face to face interviewing method using pre tested structured questionnaires was used to collect socio demographic and food handlers' knowledge, attitude and practice related information

4.8.2. Sample Collection, Handling and Transport

The study participants were instructed to bring about 4 grams (thumb size) of stool specimen with clean dry container which is pre-labelled (with date, time, identification code, age), leak proof, wide mouth and screw-capped. Fresh stool specimen from selected Hospital was collected and then immediately direct wet mount method was performed and then transported to JMC laboratory by transport medium Cary–Blair for further processing.

4.8.3. Direct smear examination for stool samples

On a microscope slide, about 1–2 mg of stool was emulsified in a drop of normal saline (0.85% NaCl) on the left-hand side of the slide, and in Lugol's iodine on the right side of the slide. A cover-slip was then placed on each side, and the slides were scanned under 10× and 40× objective lenses of a light microscope, as required. Saline direct smear is used mainly for detection of motility of intestinal protozoan trophozoites, which are seen in liquid or semi-liquid specimens. Iodine direct smear shows the characteristic features of the diagnostic stages in more details.

4.8.4. Formol-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 (37). Formol-ether concentration technique was performed from each stool specimens collected from the study participants. Using an applicator stick, an estimated 1 g (pea-size) of representative feces was emulsified in about 4 ml of 10% formol water contain in a screw-cap tube. Then further 4 ml of 10% v/v formol water was added and mix well by shaking. The emulsified feces were sieved and transferred to 15ml centrifuge tube. Then, 4 ml of diethyl ether was added and the tube was mixed for 1 minute and immediately centrifuged at 3000 revolutions per minute (rpm) for 1 min. After centrifugation, the supernatant was poured and the sediment at the bottom of the

tube was transferred to a slide and covered with a cover glass. Then the preparation was examined microscopically using the 10× and 40× objective lenses (37).

4.8.5. Auramine-O staining Method

This method was also performed on fecal smears to detect oocysts of intestinal coccidian parasites. Thin smear was prepared directly from sediment of concentrated diarrheic stool, allowed to air dry and fix in methanol for 1min. Then, flood the slide with Auramine-phenol solution and left for 15 minutes. After rinsing with tap water, decolorize with 0.5% acid alcohol for 2 minutes. Then, the slide was washed and counter stained with 0.1% potassium permanganate for 2 minutes. Finally, the slide was washed in tap water, air-dried and observed under fluorescent microscope.

4.8.6. Bacterial Culture and Identification

The isolation and characterization of *Salmonella* and *Shigella* species were performed based on the standard procedure (79). Briefly, a mixture of a stool sample (1 mL) was transferred from the Cary Blair medium into a tube containing 9 mL of Selenite F broth (Oxoid, Ltd. UK) and incubated at 37°C for 24 hours to enrich the bacteria. An inoculum from Selenite F broth was sub-cultured on Salmonella and Shigella (SS) agar (OXOID company England) and xylose lysine deoxycholate (XLD) agar (Oxoid, Ltd UK). After overnight incubation at 37°C the growth of Salmonella and Shigella was differentiated by their colony characteristic appearance on XLD agar (Shigella: red colonies, Salmonella red with a black center) and Shigella (SS) agar (Shigella: colorless, Salmonella black center colorless colonies). Pure colony with or without black centered on SSA or XLD were picked and suspended in sterile normal saline (0.85% NaCl) (80). Five series of biochemical tests such as Klinger iron agar (KIA), Simmons citrate agar, sulfide indole motility test (motility, H2S production, indole), lysine iron agar (LIA), and urease test were used for final identification of bacterial isolate(37).

4.8.7. Antimicrobial Susceptibility Testing

The antimicrobial susceptibilities of all identified bacterial isolates was performed according to the criteria of Clinical and Laboratory Standards Institute (CLSI) (81) using the Kirby-Bauer disc diffusion method on Muller-Hinton Agar. A loop full of bacteria was taken from a pure culture colony and transferred to a tube containing 5ml of normal saline and mixed gently until it forms a homogenous suspension. The turbidity of the suspension was adjusted to the turbidity of McFarland 0.5 in order to standardize the inoculum size and swabbed on Muller Hinton medium using a sterile cotton swab. Antibiotic discs were dispensed onto the inoculated medium after drying the plate for 3-5 minutes and incubated at 37oC for 24

hours. Diameters of the zone of inhibition around the discs were measured using a digital meter caliper. The following antimicrobials were tested with their respective concentration: Ampicillin (AMP, 10µg), Amoxicillin-clavulanate (30 µg), Tetracycline (TTC 30-µg), Chloramphenicol (CAF, 30-µg), Ciprofloxacin (CIP,5-µg) and Ceftriaxone (CRO, 30µg). These antimicrobial drug disks are selected based on Clinical and Laboratory Standards Institute (CLSI) and also by considering the availability and frequent prescriptions of these drugs for the treatment of Salmonella and Shigella infection in the study area. The results were interpreted according to CLSI guidelines antimicrobial susceptibility breaking points recorded as sensitive (s), intermediate (I) or resistance(R) according to CLSI 2018 (81).

4.9. Data quality assurance

To generate quality and reliable data, all quality control checks were done before, during and after data collection. All the questions in structured questionnaire were be prepared in a clear and precise way and translated into local language (Amharic and Oromifa). Data collectors were trained how to collect; the entire questionnaire was checked for completeness, during and after data collection by the principal investigator. Moreover, all laboratory assays were due by maintaining the quality control procedures. The raw data (the laboratory, clinical and demographic data) was checked for completeness and representativeness prior entry to the database.

The participants were oriented on proper sample collection. Besides, the media or reagent was checked for the expiry date of the reagents before use. The quality of the culture media was checked by inoculating known strains of *Salmonella* (ATCC14028) and *Shigella* (ATCC23354) species. The temperatures of the incubator and the refrigerator were regularly being monitored. All the laboratory procedures were conducted as per the standard operating procedures.

4.10. Data analyses

Data was entered and analyzed using SPSS version 25. Results were analyzed carefully to keep their accuracy, reliability and validity using descriptive statistics, Frequency distributions and percentages were computed for categorical variables. Descriptive statistics followed by bivariate logistic regression analyses was computed. Factors associated with dependent variables were selected in bivariate analysis with a p-value of < 0.25 for further analysis in multivariate regression analysis to adjust the effects of cofounders on the outcome variable. Statistical significance was declared at $p < 0.05$ and Odds ratio with 95%

confidence interval was computed to identify the presence and strength of associations. Results of the analysis were displayed by using tables, and graphs. A p-value ≤ 0.05 at 95% confidence level was considered as statistically significant association.

4.11. Ethical considerations

Ethical approval was obtained from Institutional Research Ethics Review Committee of, Institute of Health, Jimma University. Official cooperation letters were obtained from Jimma University to selected hospitals. Moreover, prior to commencing the study, a written informed consent was obtained from each participant after explaining about the study including their right to withdraw at any step. Participants' confidentiality and any special data security requirements was maintained and assured through password protection of electronic files and locking of hard copies. Results of the laboratory examinations that have a direct benefit in the health of the study participants was informed to physicians and the participants get their results and treatment duly as required.

4.12. Dissemination plan for results

The result of this study will be disseminated to concerned bodies including the hospitals. The results will submit to the Department of Medical Laboratory Sciences and presented at public defense. The results of the study will also be presented in national and international conferences and manuscript will be prepared and submitted for publication in peer-reviewed journals

Chapter Five

5. Results

5.1 Socio-demographic characteristics

A total of 118 food handlers were enrolled in this study, of whom 103 (87.3%) were female. The median age was 27.5 with an age range of 18 to 50 years. Sixty-seven (56.8%) of the food handlers were age above 26 years. Majority (63.6%) of the food handlers were not medically certified. About 96(81.4%) of the food handlers were above high school level or grade 9 and above.

Table 1: Socio-demographic characteristics and occupational status of the food-handlers in Selected Governmental Hospitals of Jimma Zone, Southwest, Ethiopia 2022

Characteristics	Characteristics	Frequency	Percent (%)
Sex	Male	15	13
	Female	103	87
Age	below 26 years	51	43
	Above 26 years	67	57
Educational Status	Illiterate(unable to read and write)	9	8
	1-8 grade	18	15
	grade 9 and above	91	77
Service year	Below 1 year	12	10
	1-2 years	59	50
	Above 2 years	47	40
Job division	Cleaning utilities	16	14
	food handling	79	67
	Cooking	23	19
Area of work	JUMC	95	81
	SGGH	8	7
	SCPH	5	4
	AGH	10	8

5.2. Prevalence and Types of Intestinal Parasites

From total 118 stool specimens diagnosed by direct wet mounts and formol-ether concentration techniques to identify intestinal parasites; 36(30.5%) specimens were found positive for five different intestinal parasites. *Ascaris lumbricoides* was the most prevalent parasite isolated 17(14.4%), followed by *Giardia lamblia* 9*(*7.6%), and Taenia *spp*. 4 (3.4%). the least parasites isolated were *E. histolytica/dispar* 4(3.4%) and *Schistosoma mansoni* 2(1.7%). No multiple parasites infection were isolated from the study participants.

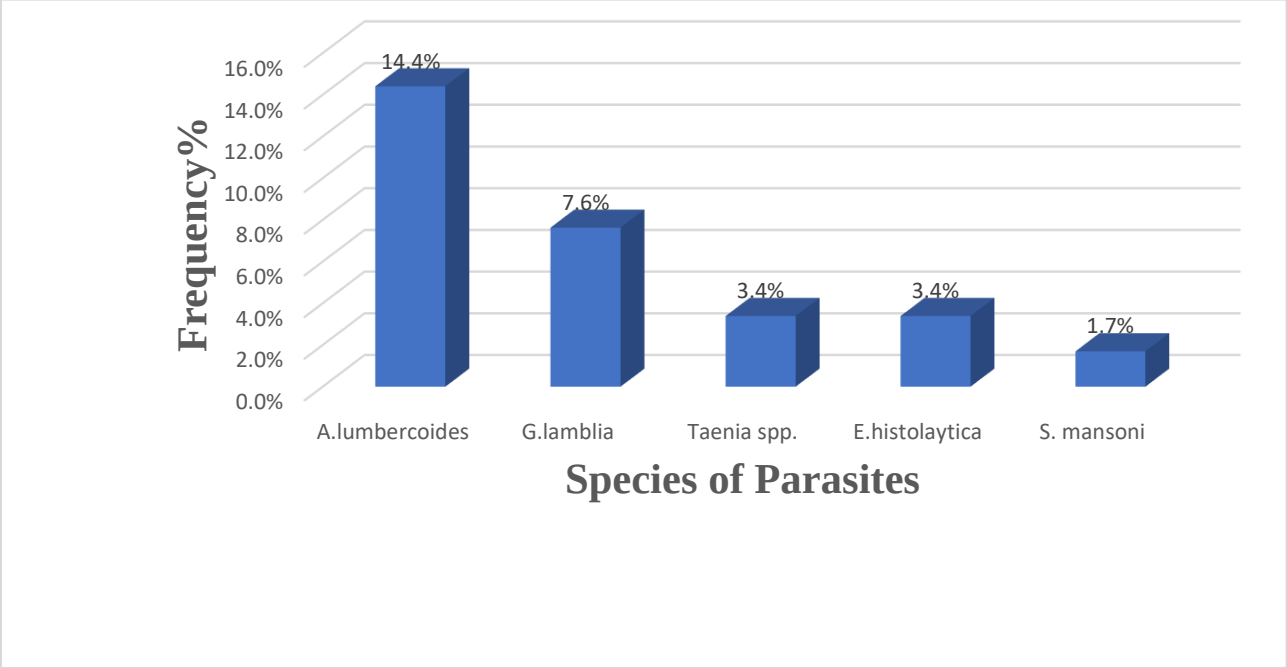

Figure 1: Bar chart showing the proportion of different intestinal parasite species among positives in Selected Governmental Hospitals of Jimma Zone, Southwest, Ethiopia 2022

The majority of the food handlers infected with parasite 23(63.8%) were above 26 years and 17(47.2%) food handlers were service years of above 2 years, 26 (72.2%) food handlers was grade 9 and above, and 6 (16.6%) infected with parasite were unable to read and write. The food handlers infected with parasite 3(8.3%), 16 (44.44%), and 17 (47.22%) were below 1 year, 1-2 years and above 2 years had service year, respectively.

Table 2: Frequency of parasitic infection and demographic factors among food handlers in Selected Governmental Hospitals of Jimma Zone, Southwest, Ethiopia 2022

Characteristics	Characteristics	Intestinal parasite	
		Positive	Negative
Sex	Male	6(40.0)	9(60.0)
	Female	30(29.1)	73(70.9)
Age	Below 26 years	13(25.5%)	38(74.5%)
	Above 26 years	23(34.3%)	44(65.7%)
Educational Status	Unable to read and write	6(66.7%)	3(33.3)
	1-8 grade	4(22.2%)	14(77.8%)
	grade 9 and above	26(28.6%)	65(71.4%)
Service year	Below 1 year	3(25%)	9(75%)
	1-2 years	16(27.1%)	43(72.9%)
	Above 2 years	17(36.2%)	30(63.8%)
Area of work	JUMC	28(29.5%)	67(70.5%)
	SGGH	4(50%)	4(50%)
	SCPH	2(40%)	3(60%)
	AGH	2(20%)	8(80%)

5.3. Factors associated with the occurrence of intestinal parasites

The findings from the bivariate analysis showed that 5 variables meet the criteria of p-value < 0.2 to be included for multivariate analysis (Table 3). From the total of 5 variables that met the criteria only 3 variables were significantly and independently associated with the occurrence of intestinal parasites at a $p < 0.05$ and the 95% confidence interval. The analysis from the multivariate logistic regression showed that food handlers who don't have Medical Checkup last 6 month were 3.7 times more likely to be positive for intestinal parasites compared to their counterparts [AOR = 3.71, 95% CI = 1.149-11.989]. Additionally, food handlers who washed their hands sometimes by soap and water after visiting toilet were 15.5 times more likely to be positive for intestinal parasites compared to those who washed their hands always [AOR = 15.313, 95% CI = 15.523-66.566]. Those food handlers who didn't take ant-helminthic/protozoa drugs within the past six months were 12.9 times more likely to be positive for

intestinal parasites compared to those who take ant-helminthic/protozoa within the past six month [AOR = 12.956, 95% CI =3.398,19.405]

Table 3: The association between intestinal parasite infection and risk factors on food handlers at selected governmental hospitals of Jimma zone, southwest, Ethiopia 2022

Predictor Variables	Intestinal Parasites		COR [95% CI]	AOR [95% CI]	p-value
	Positive	Negative			
Education					
Unable to read and write	6(66.7%)	3(33.3)	5(1.163,21.500)	1	
Primary	4(22.2%)	14(77.8%)	0.71(0.215,2.373)	3.047(0.495,18.755)	0.230
Secondary and above	36(30.5%)	82(69.5%)	1	0.571(0.512,2.138)	0.405
Medical Checkup in the last 6 months					
Yes	7(16.3%)	36(83.7%)	1	1	
No	29(38.7%)	46(61.3%)	3.242(1.275,8.246)	**3.711(1.149,11.989)**	**0.028**
Hand washing after toilet by soap and water					
Always	23(23.5%)	75(76.5%)		1	
Sometimes	13(65.0%)	7(35.0%)	6.056(2.160,16.976)	**15.313(3.523,66.566)**	**<0.001**
Taken ant-helminthic/protozoa drugs within the past six months					
Yes	8(14.5%)	47(85.5%)	1	1	
No	28(44.4%)	35(55.6%)	4.700(1.912,11.553)	**12.956(3.398,19.405)**	**<0.001**
Prepare food when suffering from disease like diarrhea					
Yes	24(27.3%)	64(72.7%)	0.563(0.236,1.340)	0.827(0.273,2.504)	0.736
No	12(40%)	18(60%)	1	**1**	

5.4. Prevalence of *Shigella* and *Salmonella* species

Out of 118 food-handlers screened, stool cultures revealed 13 (11%) *Salmonella* isolates. These bacterial isolates were identified from 7(53.8%) food handlers who age below 26 years and remaining in above 26 years of age. No Shigella species was isolated from any of the stool samples obtained from Food handlers.

Table 4: Frequency of *Salmonella* species and demographic factors among food handlers in Selected Governmental Hospitals of Jimma Zone, Southwest, Ethiopia 2022

Characteristics	**Characteristics**	Salmonella	
		Positive	Negative
Sex	Male	2(13.3)	13(86.7)
	Female	11(10.7)	92(89.3)
Age	Below 26 years	7(13.7%)	44(86.3%)
	Above 26 years	6(9%)	61(91%)
Educational Status	Unable to read and write	3(33.3%)	6(66.7)
	1-8 grade	2(11.1%)	16(88.9%)
	Grade 9 and above	8(8.8%)	83(91.2%)
Service year	Below 1 year	2(16.7%)	10(83.3%)
	1-2 years	7(11.9%)	52(88.1%)
	Above 2 years	4(8.5%)	43(91.5%)
Area of work	JUMC	8(8.4%)	87(91.6%)
	SGGH	2(25%)	6(75%)
	SCPH	1(20%)	4(80%)
	AGH	2(20%)	8(80%)

5.5. Factors Associated with *Salmonella* Isolates

The findings from the multivariate logistic regression analysis showed that two variables were significantly and independently associated with the occurrence of salmonella at a $p < 0.05$ and the 95% confidence interval. The analysis showed that Food handlers with untrimmed fingernail status were 8.8 times more likely to be positive for Salmonella compared with those who trimmed their finger nails [AOR = 8.810, 95% CI =1.262,41.508]. Similarly, the odds of being positive for Salmonella were 4.03 times higher among food handlers who sometimes wash their hand with soap and water after toilet, compared with those who always wash their hand with soap and water after toilet [AOR = 4.03, 95% CI =1.046,15.558]. But there was no association among other characteristics like educational status and certificate in food preparation (p value > 0.05)

Table 5: Factors associated with *Salmonella* infections in food handlers from selected Governmental hospitals of Jimma zone, southwest, Ethiopia 2022

Predictor Variables	Salmonella		COR [95% CI]	AOR [95% CI]	p-value
	Carrier	Non-carrier			
Education					
Unable to read and write	3(33.3%)	6(66.7)	0.93(0.040,0.921)	2.862(0.445,18.423)	0.268
Primary	2(11.1%)	16(88.9%)	0.771(0.150,3.927)	1.585(0.244,10.313)	0.630
secondary and above	8(8.8%)	83(91.2%)	1	1	1
Certificate in safe food preparation					
Yes	2(28.6%)	5(71.4%)	1	1	
No	11(9.9%)	100(90.1%)	3.636(0.629,21.011)	0.22.(0.030,1.608)	0.136
Hand washing after toilet with soap and water					
Always	6(5.1%)	92(94.9%)	1	1	
Sometimes	7(28%)	18(72.0%)	5.639(1.694,18.773)	**4.035(1.046,15.558)**	**0.043**
Trimmed finger nail					
Trimmed	10(8.9%)	102(91.1%)	1	1	
untrimmed	3(50%)	3(50%)	10.200(1.814,57.366)	**8.810(1.262,41.508)**	**0.028**

5.6. Antimicrobial susceptibility pattern of *Salmonella* isolated

From the identified 13 Salmonella species tested against 6 selected antimicrobial agents as followed in Table 8. Antimicrobial susceptibility test pattern of Salmonella isolates identified from 13 food hadlers showed highest resistance to Ampicillin 13(100%). However, among all Chloramphenicol 2(15%) shows the highest intermediate. Whereas ceftriaxone 13(100%), Amoxicillin/clavulanic acid ciprofloxacin 11(85%), and chloramphenicol 10(77%), were detected susceptible respectively (Table 6).

Table 6: Antimicrobial susceptibility testing of Salmonella isolates among the food handlers in Governmental Hospitals of Jimma Zone, Southwest, Ethiopia, 2022(n = 13).

	Salmonella isolates (n= 13)		
	Sensitive n (%)	**Intermediate n (%)**	**Resistant n (%)**
Ciprofloxacin	11(85%)	0%	2(15%)
Ceftriaxone	13(100%)	0%	0%
Tetracycline	6(46%)	0%	7(54%)
Ampicillin	0%	0%	13(100%)
Chloramphenicol	10(77%)	2(15%)	1(8) %
Amoxicillin/Clavulanic acid	11(85%)	1(8%)	1(8%)

5.7. Multiple drug resistance patterns of the isolates

Overall, 13 (100%) Salmonella isolates were resistant to at least one antimicrobial agent (Table 7). Multidrug resistance (MDR = non-susceptible to ≥1 agent in ≥3 antimicrobial drugs) (83). The results of multiple drug resistant (MDR) patterns of *Salmonella* isolate 53.84% were found to be multiple drug resistant (resistant to three and above antimicrobial drugs). (Table 7).

Table 7: MDR pattern of Salmonella isolates from stool specimen of food handlers in Selected Governmental Hospitals of Jimma Zone, Southwest, Ethiopia, 2022(n = 13).

Number of antibiotics resisted	**MDR Pattern**	**Salmonella isolates N (%)**
R2	AMP, TET	6(46.15%)
R3	AMP, CIP, TET	2(15.38%)
	AMP, CIP, C	2(15.38%)
R4	AMP, C, TET, CIP	2(15.38%)
	AMP, C, TET, AUG	1(7.69%)
R5	AMP, C, TET, CIP, AUG	0(0%)
R6	AMP, C, TET, CIP, AUG, CRO	0(0%)

MDR multidrug resistance, C Chloramphenicol, TET Tetracycline, CIP Ciprofloxacycline, AMP Ampicillin, CRO Ceftriaxone, AUG Amoxcillin/Clavulic acid

5.8. Knowledge Practice and Attitude of Food Handlers on Food Hygiene and Safety

5.8.1. Knowledge of food handlers on Food hygiene and safety

Almost the majority of Food handlers' knowledge correctly answered in the categories of self-hygiene, washing and possible safety and quality. This shows that their level of knowledge is in excellent conditions since they answer well for all questions with a score nearer to 91.7%%. Food handlers were good knowledge in the health status of workers should be evaluated before employment (100%), Washing hands before food contact reduce food contamination (87.3%), and Dysentery be spread by contaminated food (100%). Majority of participants answered it correctly the results indicate personal awareness is good in handling of food. (Table 8)

Table 8:: Knowledge assessment of food handlers in Governmental Hospitals of Jimma Zone, Southwest, Ethiopia, 2022

Questions on the knowledge of food handlers on food safety	Yes	No/Don't know
Ever heard of foodborne diseases?	116 (98.3)	2 (1.7)
Can an infected food handler transmit pathogens to the consumers?	84 (71.2)	34 (28.8)
Washing hands before food contact reduce food contamination?	103 (87.3)	15 (12.7)
Using glove to handle food reduces the risk of food contamination?	85 (72.0)	33 (28)
Properly washing of utensils reduce the risk of food contamination?	118 (100)	0 (0)
Dysentery be spread by contaminated food?	118 (100)	0 (0)
Typhoid fever can be transmitted by food	112 (94.9)	6(5.1)
Is a microbe found on the skin of asymptomatic food handlers?	118 (100)	0 (0)
Contaminated food show change in color, smell, or taste?	115 (97.5)	3 (2.5)
The health status of workers should be evaluated before employment	118 (100)	0 (0)
Rodents / vectors can spread food borne diseases	103 (87.3)	15 (12.7)

5.8.2. Attitude of food Handlers on Food hygiene and safety

About 74.6% of the participants agreed with Always personal cleanness is highly important in preparation of food. Most respondents (71.2%) agreed that Food handlers suffering from food borne diseases should

not be allowed to go to work and 0.8% were disagree with Food handlers suffering from food borne diseases should not be allowed to go to work. Nearly 49.2% of respondents strongly agree with Food makers who have wounded fingers and hands can handle food only, if they correctly cover their cuts. About 60.2% of the participants agreed with Food handlers should have proper short nails and clean hands are important during food preparation and 1.7% were disagree with proper short nails and clean hands are important to food preparation with the statement. (Table 9)

Table 9: Attitude assessment of food handlers in Governmental Hospitals of Jimma Zone, Southwest, Ethiopia, 2022

Food safety statement	**Strongly agree**	**Agree**	**Uncertain**	**Dis-Agree**	**Strongly disagree**
Always personal cleanness is highly important in preparation of food	25(21.2)	88(74.6)	5(4.2)	0(0)	0(0)
Food handlers suffering from food borne diseases should not be allowed to go to work	23(19.5)	84(71.2)	10(8.5)	1(0.8)	0(0)
Food makers if wounded fingers and hands can handle food only. if they correctly cover their cuts	58(49.2)	48(40.7)	12(10.2)	0(0)	0(0)
Food makers must wear gloves, clothes, cap before start preparation	56(47.5)	26(22)	35(29.7)	1(0.8)	0(0)
Food handlers should have proper short nails and clean hands are important to food preparation	32(27.1)	71(60.2)	13(11)	2(1.7)	0(0)
It is important to wash hands right after unhygienic Practice	39(33.1)	65(55.1)	13(11)	1(0.8)	0(0)
Food handlers should use a clean hand towel to wipe their hands after washing them	61(51.7)	35(29.7)	18(15.3)	3(2.5)	1(0.8)

5.8.3. Practice of food Handlers on Food hygiene and safety

Table 10 illustrates the food safety practices of the food handlers. Among the interviewed participants, Majority (83.1%) of the food handlers wash hands reguraly with soap and water after returning from toilet. The result of this study revealed that more than half 63.6% never had been take regular medical checkup and 36.3% of them had regular medical checkups. In addition, from the study participants, 74.6% of them had prepare food when suffering from disease like diarrhea, but 25.4% of them didn't prepare food when suffering from disease like diarrhea. All 100% wash hands before preparing and serving any food. The overall food safety level of knowledge, Practice, and Attitude of food hadlers in this study is shown 91.7%, 86.7% and 57.9% respectively with respect to food safety among the food handlers among study participants.

Table 10: Practice assessment of food handlers in Governmental Hospitals of Jimma Zone, Southwest, Ethiopia, 2022

Questions on the practice of food handlers on food safety	Yes	No/Don't know
Do you wash your hands with soap and water after returning from toilet?	98 (83.1)	20 (16.9)
Habit of eating uncooked raw meat?	22(18.6)	96(81.4)
Have you taken ant-helminthic/protozoa within the past six month?	55 (46.6)	63 (53.4)
Do you take regular medical checkup?	43 (36.4)	75 (63.6)
Certified in food preparation and handling?	7(5.9)	111(94.1)
Do you wear proper clean suitable uniform before working?	118 (100)	0 (0)
Do you wash your hands before preparing and serving any food?	118 (100)	0 (0)
Do you prepare food when suffering from disease like diarrhea?	88 (74.6)	30 (25.4)
The practice of using common knife for cutting raw flesh food and other food.	112 (94.9)	6 (5.2)
Trimmed finger nails	112 (94.9)	6 (5.1)

6. Discussion

Food Handlers may be a potential carrier to a wide range of enteric pathogens and they have been responsible in the transmission of many microorganisms. The spread of those Intestinal Parasites, Salmonella, Shigella species are come from various risk factors. Therefore, this study was undertaken to identify Intestinal Parasites, *Salmonella*, *Shigella* species from food handlers in selected governmental hospitals of Jimma Zone, southwest, Ethiopia. Food handlers in catering establishments may be infected with a variety of enteropathogens, which has been linked to the spread of various GIT illnesses among university students and the general public. Infectious illness transmission by food handlers is a widespread and ongoing issue everywhere.(2)

This study has attempted to identify the prevalence of intestinal parasites and enteric bacteria mainly Salmonella and Shigella from food handlers in selected governmental hospitals of Jimma Zone, southwest, Ethiopia

In this study the prevalence of intestinal parasitic infection prevalence was 30.5% CI: (22.4–39.6) This prevalence of parasitic infection among food handlers was in agreement with the findings of other studies conducted in Ethiopia like Gondar town 29.1% (67), Haramaya 25.2% (84), in Khartoum, Sudan 30.5% (85), and 23.7% [41] in Kenya (53). The higher prevalence rate of parasites was reported in Ethiopia from 41.1% from Bahir Dar (33), 45.3% from Addis Ababa (2), Yebu town 44.1% (56), Gambia 46.3% (86), and Swat Pakistan 83.3% (86) when compared with this study. However, lower prevalence was reported in Axum town 14.5% (9), Bahir Dar university 12.9% (87), Nairobi 15.7% (53), and Northern Iran 3.7% (88). This discrepancy may be caused by differences in epidemiologic distribution, personal hygiene habits and environmental cleanliness, sampling, appropriate utensil cleaning, and regular medical check-ups, and different settings of socio demographic characteristics of the target population (2).

In this study, from 36 total positive samples, the predominant parasite from food handlers was *Ascaris lumbricoides* (14.4%) was the predominant parasite identified followed by *Giardia lamblia* (7.6%) and *Entamoeba histolytica/dispar* (3.4%). Similar findings have been reported in previous studies done in Ethiopia 17.8%(89) in Yebu(56) 18.1% in Gondar but the study conducted in Addis Ababa (70.8%)(2) reported *Entamoeba histolytica* as the main parasite. The protozoa account for about 36.1% of IPIs in this study. Thus, food handlers harboring these protozoan parasites that do not need environmental maturation might contaminate food and water and spread the parasites directly to customers in food establishments. A high frequency of amoebiasis and giardiasis indicates poor food and water handling practices and a lack of personal cleanliness among research participants (90).

Food handlers who have Medical Checkup in the last 6 months were identified as significantly associated risk factors of food handlers being infected by IPs. The odds food handlers who have intestinal parasites were high among food handlers who did not undergo a medical check-up in the 6 months prior to the survey. This study is in line with that medical check-ups of food handlers are associated with the prevalence of intestinal parasites Mekelle

(54), and western Iran (47). This is because food handlers who did not know about their health conditions before employment and while working have lower likelihood of taking treatment and mass drugs, and as a result they may have new or existing infections or reinfections by the parasites.

Regular hand washing after visiting toilets had a statistically significant association with intestinal parasitic infection. Food handlers who didn't practice regular hand washing after visiting toilets were about 15 times more likely to be infected by intestinal parasites than their counterparts. This finding appeared to be consistent with those of other studies that noted statistically significant results for the same predictor variables. These studies include East and West Gojjam prison (91), Chagni town (92), Harmaya university (84), and Arbaminch (93). This proved that those who handle food improperly have no idea how to avoid contaminating it. Additionally, the transmission chain of intestinal parasites is broken by appropriate hand cleanliness practices. The findings of this study did not agree with those of a study conducted at Wollo University, which failed to provide statistically significant results for the same predictor variables (94). This discrepancy could be research methodology difference, research time, sample size difference, epidemiological and environmental distribution difference, improved personal hygiene practices, environmental sanitation, and ignorance of health promotion practices

Food handlers who had never been dewormed showed a statistically significant higher prevalence of infection by intestinal parasite. This was in agreement with the previous study in Bogota Colombia(95). Our results may indicate the efficacy of deworming programs in food handlers as we could show a declining prevalence of intestinal parasites. the recommendation of preventative deworming programs in food handlers is very important. This was because deworming might treat affected people, reduce community burden, and stop the spread of new diseases.

In this study, the prevalence of Salmonella species was 11% which was in agreement with the finding reported in Sodo town (8.8%) (96) but higher than the study conducted in Addis Ababa 3,5% (2) and Haramaya (65). On the contrary to our study, higher findings were reported in Nigeria 31.5% (97). However, no Salmonella isolate was reported in Gondar (67), Hawassa (98), and Jordan (99). These bacterial isolates may differ in different places due to a variety of factors, including personal cleanliness, environmental sanitation, epidemiologic pathogen dispersion, and sample size. For example, in Jordan, they used only XLD culture media and did not use any enrichment media The differences may be due to the environmental condition, study area, and the laboratory method used for the bacterial identification.

Untrimmed fingernail status and hand washing practice after toilet were the associations with the occurrence of salmonella. Finger nail status had significant association with the isolation rate of pathogens in this study. The associations were in line with studies conducted at Arba Minch (64) and Yebu (56). Due to the fact that the area beneath a fingernail retains the majority of the organisms and is challenging to clean, untrimmed fingernails may

contain these bacteria isolates and may operate as a conduit for the transportation of organisms from the source to the meal.

In this study, the odds of *Salmonella* were higher among food handlers who did not wash hands after the toilet visits with soap than those who did. The finding was in agreement with other similar studies Jimma (100) and Lagos, Nigeria (101). This is due to the reason that proper hand washing practices break the chain of transmission. Also, hand washing before a meal reduces intestinal parasites and pathogens by preventing ingesting of the infective stage by 68%(72).

In this study no Shigella species were isolated from the stool culture which is similar with the results from Hawassa (102), Addis Ababa (2), and Jordan (99). However, the prevalence of Shigella species were reported from Gondar 3.1% (89), and Haramaya 1.4%, Eastern Ethiopia 0.9% (65). Regional disparities in the prevalence of Salmonella among food handlers may be attributable to the socio demographic features of the studied population, better educational standards, and variances in regional variation.

Antimicrobial resistance has been recognized as an rising around the world issue both in created and creating nations (103). In this study, all (100%) of the Salmonella isolates tested were resistant to ampicillin, this finding was in line with the studies conducted in Bahir Dar(12) ,Addis Ababa(2) and Nigeria(97). The high proportion of resistance found in ampicillin may be due to the truth that it has wide antimicrobial scope, the less costly orally managed anti-microbial and is promptly accessible over the counter in numerous settings. About 8% of Salmonella isolates were resistant to amoxicillin-clavulanate. this was in line with the study conducted in Nigeria (101). Salmonella was (100%) susceptible to ceftriaxone is in line with studies conducted in Addis Ababa 100% (2) which might be due to the recently available drugs in the country

The low resistant rate of Salmonella isolates to chloramphenicol, 8%, could be the reason why physicians stopped to prescribe the drug long time ago and once again the strains started to become sensitive (26). According to this study, ampicillin and tetracycline are no longer effective for the treatment of salmonellosis in the study area. MDR isolates observed in this study might be due to organization of numerous antimicrobials for contaminations and indiscriminate utilize of anti-microbials. A study conducted in Addis Ababa appeared that all Salmonella isolates were multidrug resistant. In this manner, doctors and clinicians ought to utilize the individual drugs as the first-line anti-microbials for the treatment of salmonellosis within the study area. The magnitude of MDR Salmonella species in this study was 53.84%, which is comparable with the previous findings in South Ethiopia (96) but lower than in study conducted in Addis Ababa University which is 100% resistance (2). The high MDR rate of Salmonella isolates for most of the antibiotics currently used could limit our antibiotic option for empirical therapy.

This study also suggests that 91.7%, 86.7% and 57.9% of the participants (food handlers) had good level of knowledge, practice and Attitude towards food safety and hygiene respectively. The study is in keeping with a study conducted in Dangila town (104) and India (105).

7. Strength and Limitation of the study

7.1. Strength

- Study participants that have positive findings for enteric pathogens was instructed and referred to their respective medical center for appropriate treatments and check up

7.2. Limitation

- Species identification for Salmonella isolates was not done.
- *Entamoeba histolytica* and *E. dispar* were not differentiated

8. Conclusion

In this study, 30.5% of stool specimens were positive for different intestinal parasites. Salmonella isolation rate was 11%, of which 100% were resistant to ampicillin, and >85% sensitive to Amoxicillin/clavulanic acid, Ciprofloxacin and ceftriaxone. *A. lumbricoides* among intestinal parasites were the predominant isolates. Among the total isolates, multidrug resistance was recorded in salmonella isolates. Of the checked risk factors, irregular medical checkups, Poor Hand washing practice and deworming status were significantly associated with Intestinal parasites and also Irregular Hand washing after visiting toilet and untrimmed nail were associated with Salmonella Species. Therefore, constant surveillance, improvement of personal hygiene, and taking anti helminthic/protozoa drug and periodic medical check-up are recommended to control pathogens infection in food handlers.

9. Recommendation

Based on the results of the study the following are recommended:

I. For food handlers

- Food handlers should practice hand washing at critical times especially hand washing after visiting toilets and before eating a meal.
- Food handlers should be trimming their fingernails regularly and improve their hygiene practice.

II. For Jimma University Medical center, SGGH, SCPH and AGH

- Hospital administration should provide food preparation and handling training, 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
- Continuous Medical checkup of food handlers should be mandatory to alleviate the problem by the concerned body
- The deworming programs should be better to include food handlers.

III. **For researchers**

- Further research is recommended to validate the source and point of enteropathogenic infection as well as molecular characterization, and serotyping of Salmonella and Shigella is important

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APPENDEX

Annex-I: English version Information Sheet and Consent Form

Title of the Research: - A Prevalence of Salmonella, Shigella, and Intestinal Parasites infection among Food Handlers working in selected **governmental** hospitals of Jimma zone, southwest Ethiopia.

Name of Investigator: - Tsion Melaku

Name of University: -School of Medical Laboratory Sciences, Institute of Health, Jimma University.

Introduction: - The above-named researcher is Master of Science Degree in Medical Parasitology student at School of Medical Laboratory Sciences, Institute of Health, Jimma University. They was be carrying out a research project as part of their graduation requirements (Master Science), you are invited to participate in the study which ties to assess your knowledge, attitude, practice of your jobs and taking 5g of stool sample.

Your Participation: -it is on voluntary basis. If you refuse to participate it was not involve any penalty and punishment related to your work. Thus, your rights was be respected of whether you choose to participate or not. Even if you have decided to participate, you was be free to change your mind and you may with draw your consent and discontinue participation in the study at any time.

In order for you to decide if you want to participate or not, this brief information sheet has been prepared to help you understand the purpose of the research. Please read it and if you have questions, you may contact any of the investigators whose names are provided above. Their contact information has also been included in the information sheet.

Purpose of the study: The purpose of this study is to determine the prevalence of Salmonella, Shigella, And Intestinal Parasites Among Food Handlers of Governmental hospitals of Jimma zone. In order to design treatment and preventive strategies, the explanation of the prevalence, antimicrobial resistance and associated risk factors of these common infection is crucial; therefore this study was assess the prevalence of salmonella, shigella and intestinal parasitic infection, antimicrobial resistance pattern and associated risk factors food handlers.

Procedure and Participation: For this study to be successful we need your participation. And I am asking you to participate voluntarily in this study. If you are voluntary to participate in this study, you are

expected to understand and sign the informed consent. Then Socio a demographic and other related question was be filled on the questionnaire. stool sample was be collected for laboratory analysis. You was be given instruction how to collect the stool samples in clean/sterile container by data collectors.

Confidentiality: All personal information you give and data obtained from laboratory analysis was be kept confidential.

Expected benefits: your participation in this study was benefit for the Hospital and the nation as a whole. If there is any positive finding in laboratory examination the result was be reported to your physician (concerned body) for appropriate treatment and management

Risks: there is no any risk for participating in this study except that you was spend a maximum of 20 minutes for interview and you was give a small amount of stool sample for laboratory analysis.

Incentives: there are no special incentives that you was be given for participating in this research.

Results Dissemination: There was be a report which is written about the finding of the study, either through publication or any other means. The result was not bear any information relevant to your personality in anyway.

Freedom to withdraw: You have the right to withdraw or leave the study.

Person to Contact: If you have question or problem related with the present study, you can contact the principal investigator at any time using the following address:

Address of principal Investigator: Mss. Tsion Melaku (Candidate of MSc, Medical Parasitology), department of Medical Laboratory Science, Institute of Health, Jimma University, Ethiopia

Cell phone: +251924124542

E-mail: tsionmelaku02@gmail.com

Annex II : English version of consent form

I_____ I have been requested to participate in this study which involves collection of stool samples from me and in which I was answer few questions. The purpose of this study and sample collection procedure has been explained for me. I have also read the information sheet (or it has been read to me); I have asked some questions and clarification has been given to me. I have given my consent on behalf of myself to participate in study and I hereby confirm my agreement with my signature.

Signature _____ Date_____

Thank you for your participation in this important study.

N.B: If you want to request additional information about the study, you can contact me by

+251924124542/tsionmelaku02@gmail.com

Annex III: Questionnaire I

English Version Questionnaire

Questionnaire on the assessment of knowledge, attitude and practice of food handlers on food borne pathogens

Date_____

Subject ID_____

	PART-I:- Socio Demographic Characteristics of the Worker	
1.	Sex	-Male □ -Female □
2.	Age	_____
3.	Educational status	- Illiterate □ -Elementary □ -High school and above □
4.	For how many years you have worked as food handler in this hospital?	- ≤1 □ - 1–2 □ - > 2 □
5.	Institution	1. JMC 2. SGGH 3. SCPH 4. AGH
6	Job division	-cleaning utensils □ -food handling □ cooking □
PART-II:-Knowledge, Attitude and practice based assessment towards their work situation		
Knowledge related Question of Food handlers		
1.	Ever heard of food borne diseases?	-Yes □ -No □

2.	Have you ever heard of any of the following as a problem in food	• Salmonella □ Yes □ No • Shigella □ Yes □ No • Giardia □ Yes □ No • Amoeba □ Yes □ No • Other _____
3.	Infected food handler transmit food borne diseases to the customers	□ Yes □ No
4.	Washing hands before work reduces the risk of food contamination	-Yes □ -No □
5.	Using gloves while handling food reduces the risk of food contamination	-Yes □ -No □
6.	Proper cleaning and sanitization of utensils reduces the risk of food contamination	-Yes □ -No □
7.	Typhoid fever can be transmitted by food	-Yes □ No □
8.	Bloody diarrhea can be transmitted by food	-Yes □ -No □
9.	Microbes are on the skin, in the nose and mouth of healthy food handlers	-Yes □ -No □
10.	Contaminated foods always have some change in color, odor or taste	-Yes □ -No □
11	The health status of workers should be evaluated before employment	-Yes □ -No □
12	Rodents / vectors can spread food borne diseases	-Yes □ -No □
	1-10Attitude related Question in Food hygiene and Safety issues	
1.	Always personal cleanness is highly important in preparation of food and One main responsibility of my job is to handle food safety	-Strongly Agree □ -Agree □ -Uncertain □ -Disagree □

		-Strongly disagree □
2.	Food handlers suffering from food borne diseases should not be allowed to go to work	-Strongly Agree □ -Agree □ -Uncertain □ -Disagree □ -Strongly disagree □
3.	Food makers if wounded fingers and hands can handle food only. if they correctly cover their cuts.	-Strongly Agree □ -Agree □ -Uncertain □ -Dis Agree □ -Strongly dis agree □
4.	Food makers must wear gloves, clothes, cap before start preparation	-Strongly Agree □ -Agree □ -Uncertain □ -Dis Agree □ -Strongly dis agree □
5.	Food handlers should have proper short nails and clean hands are important to food preparation	-Strongly Agree □ -Agree □ -Uncertain □ -Dis Agree □ -Strongly dis agree □
9.	It is important to wash hands right after unhygienic Practice	-Strongly Agree □ -Agree □ -Uncertain □ -Dis Agree □ -Strongly dis agree □

10.	Food handlers should use a clean hand towel to wipe their hands after washing them	-Strongly Agree □ -Agree □ -Uncertain □ -Dis Agree □ -Strongly dis agree □
	1-10 Practice related question in Food hygiene and Safety issues	
1.	Do you regularly wash your hands after returning from toilet?	-Yes/always □ -No □
2.	. If yes?	-with water only □ -with water and detergent □
3.	Habit of eating uncooked raw meat?	-Yes □ -No□
4.	Have you taken ant-helminthic/protozoa within the past six month?	-Yes □ -No □
5.	Source of water	-pipe □ other source □
6.	Certified in food preparation and handling?	-Yes □ -No □
7.	Do you take regular medical checkup last 6 month?	-Yes □ -No □
8.	Do you wear proper clean suitable uniform before working?	-Yes □ -No □
9.	Do you wash your hands before preparing and serving any food?	-Yes □ -No □
10.	Do you prepare food when suffering from disease like diarrhea?	-Yes □ -No □
11	The practice of using common knife for cutting raw flesh food and other food.	-Yes □ -No □
12	Trimmed finger nails	Yes □ -No □

Annex II : Amharic version of information and consent form

አባሪ-I፡ የመረጃ ሉህ እና የስምምነት ቅጽ

የጥናቱ ርዕስ ፡ - በደቡብ ምዕራብ ኢትዮጵያ በጅማ ዞን በሚገኙ የመንግስት ሆስፒታሎች የምግብ ተቆጣጣሪዎች መካከል የሳልሞኔላ፣ የሺጌላ እና የአንጀት ጥገኛ ተውሳኮች ስርጭት ።

የተመራማሪው ስም ፡- ጽዮን መላኩ

የዩኒቨርስቲ ስም ፡ -የህክምና ላብራቶሪ ሳይንስ ትምህርት ቤት፣የጤና ተቋም፣ጅማ ዩኒቨርሲቲ።

መግቢያ ፡ - ከላይ የተጠቀሰው ተመራማሪ በጅማ ዩኒቨርሲቲ የጤና ተቋም የህክምና ላቦራቶሪ ሳይንስ ትምህርት ቤት በሜዲካል ፓራሲቶሎጂ ማስተር አፍ ሳይንስ ተማሪ ነው። የመመረቂያ መስፈርታቸው አካል የሆነ የምርምር ፕሮጀክት ያካሂዳሉ (ማስተር ሳይንስ) እርስዎ ዕውቀትዎን ፣ አመለካከትዎን ፣ የስራ ልምምድዎን ለመገምገም እና 5g የሰገራ ናሙና በመውሰድ በየትኛው ትስስር ላይ እንዲሳተፉ ተጋብዘዋል።

የእርስዎ ተሳትፎ : - በፈቃደኝነት ላይ የተመሰረተ ነው. ለመሳተፍ ፈቃደኛ ካልሆኑ ከስራዎ ጋር የተያያዘ ማንኛውንም ቅጣት እና ቅጣት አያካትትም። ስለዚህ፣ ለመሳተፍ ወይም ላለመሳተፍ መብትዎ ይከበራል። ለመሳተፍ ወስነህም ቢሆን፣ ሃሳብህን ለመለወጥ ነፃ ትሆናለህ እናም ፈቃድህን ወስደህ በማንኛውም ጊዜ በጥናቱ መሳተፍ ማቋረጥ ትችላለህ።

ለመሳተፍ ወይም ላለመሳተፍ ለመወሰን፡ ይህ አጭር የመረጃ ሉህ ተዘጋጅቶ የጥናቱን ዓላማ ለመረዳት ይረዳዎታል። እባኮትን ያንብቡ እና ጥያቄዎች ካሉዎት፣ ስማቸው ከላይ ከተጠቀሱት መርማሪዎች ማናቸውንም ማነጋገር ይችላሉ። የእነርሱ አድራሻ መረጃ በመረጃ ወረቀቱ ውስጥም ተካትቷል።

የጥናቱ ዓላማ፡- የዚህ ጥናት ዓላማ በጅማ ዞን በሚገኙ የመንግስት ሆስፒታሎች የምግብ አያያዝ ባለሙያዎች መካከል የሳልሞኔላ፣ የሺጌላ እና የአንጀት ጥገኛ ተውሳኮችን ስርጭት ለማወቅ ነው። ሕክምናን እና የመከላከያ ስልቶችን ለመንደፍ የእነዚህን የተለመዱ ኢንፌክሽኖች ስርጭት ፣ ፀረ-ተሕዋስያን የመቋቋም እና ተያያዥ የአደጋ መንስኤዎች ማብራሪያ አስፈላጊ ነው ። ስለዚህ ይህ ጥናት የሳልሞኔላ፣ የሺግላ እና የአንጀት ጥገኛ ተውሳክ ኢንፌክሽን፣ ፀረ-ተህዋስያን የመቋቋም ዘይቤ እና ተያያዥ የአደጋ መንስኤዎች የምግብ ተቆጣጣሪዎች ስርጭትን ይገመግማል።

ሂደት እና ተሳትፎ ፡- ይህ ጥናት ስኬታማ እንዲሆን የእርስዎን ተሳትፎ እንፈልጋለን። እናም በዚህ ጥናት በፈቃደኝነት እንድትሳተፉ እጠይቃችኋለሁ። በዚህ ጥናት ላይ ለመሳተፍ ፈቃደኛ ከሆኑ ፣ በመረጃ ላይ የተመሰረተ ስምምነትን ተረድተው መፈረም ይጠበቅብዎታል። ከዚያ የሶሺዮ ዲሞግራፊ እና ሌሎች ተዛማጅ ጥያቄዎች በመጠይቁ ላይ ይሞላሉ። የሰገራ ናሙና ለላቦራቶሪ ምርመራ ይሰበሰባል. የሰገራ ናሙናዎችን በንፁህ/ንፁህ እቃ ውስጥ እንዴት እንደሚሰበስቡ በመረጃ ሰብሳቢዎች መመሪያ ይሰጥዎታል።

ምስጢራዊነት ፡ ሁሉም የሚሰጡት የግል መረጃ እና ከላቦራቶሪ ትንታኔ የተገኘው መረጃ በሚስጥር ይጠበቃል።

የሚጠበቁ ጥቅሞች ፡ በዚህ ጥናት ላይ መሳተፍዎ ለሆስፒታሉ እና ለሀገር በአጠቃላይ ይጠቅማል። የላብራቶሪ ምርመራ ላይ ምንም አይነት አወንታዊ ውጤት ካለ ውጤቱ ለህክምና እና ለህክምናው ለሐኪምዎ (ለሚመለከተው አካል) ሪፖርት ይደረጋል።

አደጋ የለም ለቃለ መጠይቅ ቢበዛ 20 ደቂቃዎችን ከማጥፋት በስተቀር እና ለላቦራቶሪ ትንታኔ ትንሽ መጠን ያለው የሰገራ ናሙና ከመስጠት በስተቀር.

ማበረታቻዎች ፡ በዚህ ጥናት ላይ ለመሳተፍ የሚሰጣችሁ ልዩ ማበረታቻ የለም።

በህትመትም ሆነ በሌላ መንገድ ስለ ጥናቱ ግኝት የተጻፈ ሪፖርት ይኖራል። ውጤቱ በማንኛውም ሁኔታ ከእርስዎ ስብዕና ጋር ተዛማጅነት ያለው ማንኛውንም መረጃ አይሸከምም።

የመውጣት ነፃነት ፡ ጥናቱን የመተው መብት አልዎት።

የሚገናኘው ሰው ፡ ከአሁኑ ጥናት ጋር የተያያዘ ጥያቄ ወይም ችግር ካጋጠመዎት በሚከተለው አድራሻ በማንኛውም ጊዜ ዋናውን መርማሪ ማነጋገር ይችላሉ።

የዋናው ተመራማሪ አድራሻ ፡ ወይዘሮ ጽዮን መላኩ (የኤምኤስሲ፣ ሜዲካል ፓራሲቶሎጂ እጩ)፣ የጅማ ዩኒቨርሲቲ ጤና ኢንስቲትዩት የሕክምና ክፍል ትምህርት ክፍል

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አባሪ II ፡ የአማሪኛ ቅጂ የስምምነት ቅጽ

ከእኔ የሰገራ ናሙናዎችን መሰብሰብን በሚያካትት እና ጥቂት ጥያቄዎችን በምመልስበት በዚህ ጥናት እንድሳተፍ ተጠየቅኩ ። የዚህ ጥናት አላማ እና የናሙና አሰባሰብ ሂደት ተብራርቶልኛል። እኔም የመረጃ ወረቀቱን አንብቤአለሁ (ወይ ተነበበኝ)፤ አንዳንድ ጥያቄዎችን ጠይቄ ማብራሪያ ተሰጥቶኛል። በራሴ ስም በጥናት ለመሳተፍ ፈቃዴን ሰጥቻለሁ እናም በዚህ ፊርማ ላይ መስማማቴን አረጋግጣለሁ።

ፊርማ _____ ቀን_____

በዚህ ጠቃሚ ጥናት ላይ ስለተሳተፉ እናመሰግናለን።

ማሳሰቢያ ፡ ስለ ጥናቱ ተጨማሪ መረጃ ለመጠየቅ ከፈለጉ በ እኔን ማግኘት ይችላሉ።

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አባሪ III፡ መጠይቅ I

በምግብ ወለድ በሽታ አምጪ ተህዋሲያን ላይ የምግብ ተቆጣጣሪዎች የእውቀት፣ የአመለካከት እና የተግባር ግምገማ ላይ መጠይቅ

ቀን_____

የርዕሰ ጉዳይ መታወቂያ_____

	ክፍል- I: - የሰራተኛው ማህበራዊ ስነ-ሕዝብ ባህሪያት	
1.	ፆታ	- ወንድ □ - ሴት □
2.	ዕድሜ	_____
3.	የትምህርት ደረጃ	- መሃይም □ - አንደኛ ደረጃ □ - ሁለተኛ ደረጃ ትምህርት ቤት እና ከዚያ በላይ □
4.	በዚህ ሆስፒታል ውስጥ ለምን ያህል አመታት በምግብ ተቆጣጣሪነት ሰርተዋል?	- ≤1 □ - 1–2 □ - > 2 □
5.	የስራ ቦታ	1. ጅማ ሆስፒታል 2. ሸነንጊበ ሆስፒታል 3. ሰቃ ጨቆርሳ ሆስፒታል 4. አጋሮ ሆስፒታል
.6	የሥራ ክፍፍል	- የጽዳት ዕቃዎች □ - የምግብ አያያዝ □ ምግብ ማብሰል □
ክፍል- II፡- በእውቀት፣ በአመለካከት እና በተግባር ላይ የተመሰረተ የስራ ሁኔታ ግምገማ		
ከእውቀት ጋር የተያያዘ የምግብ ተቆጣጣሪዎች ጥያቄ		
1.	ስለ ምግብ ወለድ በሽታዎች ሰምተው ያውቃሉ ?	- አዎ □ - አይ □

2.	በምግብ ውስጥ እንደ ችግር ከሚከተሉት ውስጥ አንዱን ሰምተው ያውቃሉ	• ሳልሞኔላ □አዎ □አይደለም • Shigella □አዎ □አይደለም • ጃርዲያ □አዎ □አይደለም • አሜባ □አዎ □አይደለም • ሌላ _____
3.	የተበከለው ምግብ ተቆጣጣሪ የምግብ ወለድ በሽታዎችን ለደንበኞች ያስተላልፋል	□አዎ □አይደለም
4.	ከስራ በፊት እጅን መታጠብ የምግብ መበከል አደጋን ይቀንሳል	- አዎ □ - አይ □
5.	ምግብ በሚይዙበት ጊዜ ጓንቶችን መጠቀም የምግብ ብክለትን አደጋ ይቀንሳል	- አዎ □ - አይ □
6.	ዕቃዎችን በትክክል ማጽዳት እና ማጽዳት የምግብ ብክለትን አደጋ ይቀንሳል	- አዎ □ - አይ □
7.	ታይፎይድ ትኩሳት በምግብ ሊተላለፍ ይችላል	- አዎ □ - አይ □
8.	በደም ውስጥ ያለው ተቅማጥ በምግብ ሊተላለፍ ይችላል	- አዎ □ - አይ □
9.	ማይክሮቦች በቆዳ ላይ, በአፍንጫ እና በአፍ ውስጥ ጤናማ ምግብ ተቆጣጣሪዎች ናቸው	- አዎ □ - አይ □
10.	የተበከሉ ምግቦች ሁልጊዜም በቀለም፣በመአዛ ወይም በጣዕም ላይ የተወሰነ ለውጥ አላቸው።	- አዎ □ - አይ □
11	የሰራተኞች የጤና ሁኔታ ከቅጥር በፊት መገምገም አለበት	- አዎ □ - አይ □

12	አይጦች / ቬክተሮች በምግብ ወለድ በሽታዎችን ሊያስተላልፉ ይችላሉ	- አዎ □ - አይ □
	1-10 ከአመለካከት ጋር የተያያዘ ጥያቄ በምግብ ንፅህና እና ደህንነት ጉዳዮች ላይ	
1.	በምግብ ዝግጅት ውስጥ ሁል ጊዜ የግል ንፅህና በጣም አስፈላጊ ነው እና አንዱ ዋና ሀላፊነቴ የምግብ ደህንነትን መቆጣጠር ነው።	- በጠንካራ ሁኔታ እስማማለሁ □ - እስማማለሁ □ - እርግጠኛ ያልሆነ □ - አልስማማም □ - በጠንካራ ሁኔታ አልስማማም □
2.	በምግብ ወለድ በሽታዎች የሚሰቃዩ ምግብ ተቆጣጣሪዎች ወደ ሥራ እንዲሄዱ መፍቀድ የለባቸውም	- በጠንካራ ሁኔታ እስማማለሁ □ - እስማማለሁ □ - እርግጠኛ ያልሆነ □ - አልስማማም □ - በጠንካራ ሁኔታ አልስማማም □
3.	ጣቶች እና እጆች ከቆሰሉ ምግብ ሰሪዎች ምግብን ብቻ መቆጣጠር ይችላሉ። ቁርጥራጮቻቸውን በትክክል ከሸፈኑ.	- በጠንካራ ሁኔታ እስማማለሁ □ - እስማማለሁ □ - እርግጠኛ ያልሆነ □ -ዲስ እስማማለሁ □ - በጠንካራ ሁኔታ አልስማማም □
4.	ምግብ ሰሪዎች ዝግጅት ከመጀመራቸው በፊት ጓንት፣ ልብስ፣ ኮፍያ ማድረግ አለባቸው	- በጠንካራ ሁኔታ እስማማለሁ □ - እስማማለሁ □ - እርግጠኛ ያልሆነ □ -ዲስ እስማማለሁ □ - በጠንካራ ሁኔታ አልስማማም □

5.	አጭር ጥፍር ሊኖራቸው ይገባል እና ንጹህ እጆች ለምግብ ዝግጅት አስፈላጊ ናቸው	- በጠንካራ ሁኔታ እስማማለሁ □ - እስማማለሁ □ - እርግጠኛ ያልሆነ □ -ዲስ እስማማለሁ □ - በጠንካራ ሁኔታ አልስማማም □
9.	ከንጽህና ጉድለት በኋላ ወዲያውኑ እጅን መታጠብ አስፈላጊ ነው	- በጠንካራ ሁኔታ እስማማለሁ □ - እስማማለሁ □ - እርግጠኛ ያልሆነ □ -ዲስ እስማማለሁ □ - በጠንካራ ሁኔታ አልስማማም □
10.	ምግብ ተቆጣጣሪዎች እጃቸውን ከታጠቡ በኋላ ለማጽዳት ንጹህ የእጅ ፎጣ መጠቀም አለባቸው	- በጠንካራ ሁኔታ እስማማለሁ □ - እስማማለሁ □ - እርግጠኛ ያልሆነ □ -ዲስ እስማማለሁ □ - በጠንካራ ሁኔታ አልስማማም □
	1-10 በምግብ ንፅህና እና ደህንነት ጉዳዮች ላይ ተዛማጅ ጥያቄን ተለማመዱ	
1.	ከመጸዳጃ ቤት ከተመለሱ በኋላ እጅዎን ይታጠቡ?	- አዎ □ - አይ □
2.	. እሺ ከሆነ?	- በውሃ ብቻ □ - በውሃ እና ሳሙና □
3.	ያልበሰለ ጥሬ ሥጋ የመብላት ልማድ?	- አዎ □ - አይ □

4.	ባለፈው ስድስት ወር ውስጥ አንንት-ሄልሚንቲክ/ፕሮቶዞኣን ወስደዋል?	- አዎ □ - አይ □
5.	የውሃ ምንጭ	- ቧንቧ □ - ሌላ ምንጭ □
6.	በምግብ ዝግጅት እና አያያዝ የተረጋገጠ?	- አዎ □ - አይ □
7.	መደበኛ የሕክምና ምርመራ ያደርጋሉ?	- አዎ □ - አይ □
8.	ከመሥራትዎ በፊት ትክክለኛ ንፁህ ዩኒፎርም ይለብሳሉ?	- አዎ □ - አይ □
9.	ማንኛውንም ምግብ ከማዘጋጀት እና ከማቅረብዎ በፊት እጅዎን ይታጠቡ?	- አዎ □ - አይ □
10.	እንደ ተቅማጥ ባሉ በሽታዎች ሲሰቃዩ ምግብ ያዘጋጃሉ?	- አዎ □ - አይ □
11	ጥሬ ሥጋን እና ሌሎች ምግቦችን ለመቁረጥ የተለመደ ቢላዋ የመጠቀም ልማድ.	- አዎ □ - አይ □

Annex-III Afaan oromoo version of the questionnaire

Gaafannoo Afaan Oromoo

Dabalata-I: Waraqaa Odeeffannoo fi Unka Hayyamaa

Mata duree Qorannichaa:- Tatamsa'ina dhukkuba Saalmooneelaa, Shigeelaa, fi Paaraasaayitii Garaachaa Namoota Nyaata Qaban Giddugala

Maqaa Qorataa: - Tsion Melaku

Maqaa Yuunivarsitii: - Mana Barumsaa Saayinsii Laaboraatoorii Meedikaalaa, Inistiitiyuutii Fayyaa, Yuunivarsiitii Jimmaa.

Seensa: - Qorataan maqaan isaa armaan olitti ibsame kun, Yuunivarsiitii Jimmaa, Inistiitiyuutii Fayyaa, Mana Barumsaa Saayinsii Laaboraatoorii Meedikaalaatti barataa Digirii Mastersii Saayinsii Paaraasitolojii Meedikaalaati. Isaan akka qaama ulaagaa eebba isaanii (Master Science)tti pirojektii qorannoo ni raawwatu, qorannoo irratti akka hirmaattan affeeramtaniittu kunis beekumsa, ilaalcha, shaakala hojii keessanii madaaluu fi saamuda sagaraa 5g fudhachuuf hidhata.

Hirmaannaa Keessan: -tola ooltummaadha. Yoo hirmaachuu didde adabbii fi adabbii hojii kee waliin walqabatu tokkollee hin dabalatu. Haala kanaan hirmaachuu filachuu fi dhiisuu kee mirgi kee ni kabajama. Yoo hirmaachuuf murteessitellee yaada kee jijjiiruuf bilisa ta'a akkasumas hayyama kee fudhachuun yeroo barbaaddetti qo'annicha irratti hirmaachuu addaan kutuu dandeessa.

Hirmaachuu barbaadduu fi dhiisuu kee murteessuuf, waraqaa odeeffannoo gabaabaa kun kaayyoo qorannichaa hubachuuf si gargaaruuf qophaa'eera. Mee dubbisaa gaaffii yoo qabaattan qorattoota maqaan isaanii armaan olitti ibsame kamiyyuu qunnamuu dandeessu. Odeeffannoon quunnamtii isaaniis waraqaa odeeffannoo irratti hammatameera.

Kaayyoo qorannichaa: Kaayyoon qorannoo kanaa baay'ina dhukkuba Salmonella, Shigella, Fi Parasites Garaachaa Namoota Nyaata Qaban Giddugala. Tooftaalee wal'aansaa fi ittisaa dizaayinii gochuuf ibsi tamsa'ina, dandeettii farra maaykiroobiyaanii fi sababoota balaa infekshinii waliigalaa kanaa waliin walqabatan murteessaadha; kanaaf qorannoon kun baay'ina saalmooneelaa, shigeelaa fi infekshinii paraasitii garaachaa, akkaataa ittisa farra maaykiroobiyaanii fi sababoota balaa kanaan walqabatan namoota nyaata qaban ni madaala.

Adeemsaafi Hirmaannaa: Qorannoon kun akka milkaa'uuf hirmaannaa keessan nu barbaachisa. Akkasumas qorannoo kana irratti tola ooltummaadhaan akka hirmaattan isin gaafachaa jira. Yoo fedhiidhaan qorannoo kana irratti hirmaatte, hayyama beekumsa qabu hubachuu fi mallatteessuun si irraa eegama. Sana booda gaaffilee Hawaasummaa dimogiraafii fi kanneen biroo kanaan walqabatan gaaffilee irratti ni guutamu. saamuda sagaraa xiinxala laabraatooriidhaaf ni fudhatama. Akkaataa saamuda sagaraa meeshaa qulqulluu/sterile keessatti walitti qabuu dandeessan qajeelfamni siif kennama.

Iccitii: Odeeffannoon dhuunfaa ati kennitu hundi fi daataa xiinxala laabraatoorii irraa argatte iccitii ta'ee ni eegama.

Faayidaa eegamu: qorannoo kana irratti hirmaachuun keessan Hospitaalichaa fi akka waliigalaatti sabaaf bu'aa qabaata. Qorannoon laabraatoorii keessatti argannoon gaariin yoo jiraate bu'aan isaa wal'aansaa fi bulchiinsa sirrii ta'eef hakiima keessaniif (qaama dhimmi ilaallatu) ni gabaafama

Balaa: qorannoo kana irratti hirmaachuuf balaan tokkollee hin jiru yoo baay'ate daqiiqaa 20 af-gaaffiidhaaf dabarsuu fi saamuda sagaraa xiqqoo xiinxala laabraatooriidhaaf kennuu malee.

Onnachiiftuu: qorannoo kana irratti hirmaachuuf onnachiiftuu addaa siif kennamu hin jiru.

Bu'aa Babal'isuu: Gabaasni waa'ee argannoo qorannichaa barreeffame ni jiraata, karaa maxxansaa yookiin karaa biraa kamiinuu. Bu'aan isaa odeeffannoo dhuunfaa kee wajjin walqabatu kamiyyuu hin qabatu.

Bilisummaa ofirraa baasuu: Qo'annoo irraa of baasuu ykn dhiisuuf mirga qabda.

Nama Qunnamtu: Qorannoon ammaa kanaan walqabatee gaaffii ykn rakkoo yoo qabaattan, teessoo armaan gadii fayyadamuun yeroo barbaaddetti qorataa muummee qunnamuu dandeessu:

Teessoo Qorataa muummee: Aadde Tsion Melaku (Kaadhimamaa MSc, Medical Parasitology), kutaa Saayinsii Laaboraatoorii Meedikaalaa, Inistiitiyuutii Fayyaa, Yuunivarsiitii Jimmaa, Itoophiyaa

Dabalata II ፡ Unka hayyamaa Afaan Oromootiin qophaa'e

Ani_____ Qorannoon saamuda sagaraa narraa walitti qabuu of keessaa qabuu fi gaaffii muraasaaf deebii itti kennu kana irratti akkan hirmaadhu na gaafatameera. Kaayyoon qorannoo kanaa fi adeemsa saamuda fudhachuu naaf ibsamee jira. Waraqaa odeeffannoos dubbiseera (ykn naaf dubbifamee jira); Gaaffii tokko tokko gaafadhee ibsi naaf kennameera. Qo'annoo irratti akka hirmaadhuuf bakka bu'ee hayyama koo kanan kenne yoo ta'u, mallattoo kootiin waliigaltee koo kanaan mirkaneessa.

Mallattoo _____ Guyyaa_____ .

Qo'annoo barbaachisaa kana irratti hirmaachuu keessaniif galatoomaa.

N.B: Waa'ee qorannichaa odeeffannoo dabalataa gaafachuu yoo barbaaddan karaa

+251924124542/tsionmelaku02@gmail.com irratti bilbilaa

Dabalata III: Gaaffii I

Gaaffii Gosa Afaan Oromoo

Gaaffii madaallii beekumsaa, ilaalchaa fi shaakala namoota nyaata qabatanii dhukkuboota nyaataan daddarban irratti giddugala yaalaa

Guyyaa_____

ID mata duree_____ .

	KUTAA-I:- Amaloota Hawaasummaa Dimoogiraafii Hojjetaa	
1.	Walqunnamtii saalaa	-Dhiira □ -Dubartii □
2.	Umurii	_____
3.	Haala barnootaa	- Dubbisuu fi barreessuu hin dandeenye □ -Mana sadarkaa tokkoffaa □ -Mana barumsaa sadarkaa lammaffaa fi isaa ol □
4.	Hospitaala kana keessatti waggaa meeqaaf nyaata qabattee hojjetteetta?	- ≤1 □ - 1–2 □ - > 2 □
5.	Ragaa Fayyaa qabduu	- Eeyyee □ Lakki □
.6	Qoodinsa hojii	- meeshaalee qulqulleessuu □ - nyaata qabachuu □ bilcheessuu □

KUTAA-II:-Madaallii beekumsaa, Ilaalchaa fi shaakala irratti hundaa'e haala hojii isaanii irratti

Beekumsa waliin walqabatu Gaaffii warra Nyaata qabatanii

1.	Dhukkuboota nyaataan daddarban dhageessanii beektuu?	-Eeyyee□ -Lakki □
2.	Kanneen armaan gadii keessaa tokko akka rakkoo nyaataatti dhageessanii beektuu	• Salmonella □ Eeyyee □ Lakki • Shigella □ Eeyyee □ Lakki • Giardia □ Eeyyee □ Lakki Amoeba □ Eeyyee □ Lakki Kan biraa

3.	Namni nyaata qabate dhukkuba nyaataan daddarbu maamiltootaaf dabarsu	-Eeyyee □ -Lakkii □
4.	Hojii dura harka dhiqachuun carraa faalama nyaataa hir'isa	-Eeyyee □ -Lakkii □
5.	Nyaata yeroo qabattan guwaantii fayyadamuun carraa faalama nyaataa hir'isa	-Eeyyee □ -Lakkii □
6.	Meeshaalee sirnaan qulqulleessuu fi qulqulleessuun balaa faalama nyaataa hir'isa	-Eeyyee □ -Lakkii □
7.	Dhukkubni taayifooyidii nyaatadhaan daddarbuu danda'a	-Eeyyee □ -Lakkii □
8.	Garaa kaasaa dhiigaa nyaatadhaan daddarbuu danda'a	-Eeyyee □ -Lakkii □
9.	Maaykiroobii gogaa irratti, hidhii fi afaan namoota nyaata fayya qabeessa qaban keessa jiru	-Eeyyee □ -Lakkii □
10.	Nyaatni faalame yeroo hunda halluu, urgooftuu ykn dhandhamaa irratti jijjiirama tokko tokko qaba	-Eeyyee □ -Lakkii □
11	Haalli fayyaa hojjettootaa qaxarrii dura madaalamuu qaba	-Eeyyee □ -Lakkii □
12	Hantuutni / veektaroota dhukkuboota nyaataan daddarban babal'isuu danda'u	-Eeyyee □ -Lakkii □
	1-10Gaaffii ilaalchaan walqabatu dhimmoota qulqullina nyaataa fi Nageenyaa keessatti	
1.	Yeroo hundumaa qulqullinni dhuunfaa nyaata qopheessuu keessatti barbaachisaa ta'uu isaa fi Itti gaafatamummaa hojii koo inni guddaan tokko nageenya nyaataa to'achuudha	-Cimsee walii gala □ -Walii galuu □ -Mirkanaa'aa hin taane □ -Walii hin galu □ -Cimsee walii hin galu □

2.	Namoonni nyaata qaban dhukkuba nyaataan daddarban qabatanii gara hojiitti akka deeman hayyamamuu hin qabu	-Cimsee walii gala □ -Walii galuu □ -Mirkanaa'aa hin taane □ -Walii hin galu □ -Cimsee walii hin galu □
3.	Warri nyaata hojjetan yoo qubaafi harki madaa'an nyaata qofa qabachuu danda'u. yoo ciccitaa isaanii sirritti haguugan.	-Cimsee walii gala □ -Walii galuu □ -Mirkanaa'aa hin taane □ -Walii hin galu □ -Cimsee walii hin galu □
4.	Warri nyaata hojjetan qophii jalqabuu dura guwaantii harkaa, uffata, kophee uffachuu qabu	-Cimsee walii gala □ -Walii galuu □ -Mirkanaa'aa hin taane □ -Walii hin galu □ -Cimsee walii hin galu □
5.	Namoonni nyaata qaban cimdii gabaabaa sirrii ta'e qabaachuu kan qaban yoo ta'u, harki qulqulluun qophii nyaataaf barbaachisaa dha	-Cimsee walii gala □ -Walii galuu □ -Mirkanaa'aa hin taane □ -Walii hin galu □ -Cimsee walii hin galu □
9.	Shaakala qulqullina hin qabne booda battalumatti harka dhiqachuun barbaachisaa dha	-Cimsee walii gala □ -Walii galuu □ -Mirkanaa'aa hin taane □ -Walii hin galu □ -Cimsee walii hin galu □
10.	Namoonni nyaata qaban erga dhiqatanii booda harka isaanii haxaa'uuf haalluu harkaa qulqulluu fayyadamuu qabu	-Cimsee walii gala □ -Walii galuu □ -Mirkanaa'aa hin taane □ -Walii hin galu □ -Cimsee walii hin galu □

	1-11 Gaaffii shaakala waliin walqabatu dhimmoota qulqullina nyaataa fi Nageenyaa keessatti	
1.	Erga mana fincaanii irraa deebitee booda harka dhiqattuu?	-Eeyyee □ -Lakkii □
2.	. Yoo eeyyee ta'e?	-bishaan qofaan □ -bishaanii fi saamunaan qulqulleessituun □
3.	Amala foon qalamaa hin bilchaanne nyaachuu?	-Eeyyee □ -Lakkii □
4.	Ji'a jaha darban keessatti qoricha ant-helminthic/protozoa fudhattaniittuu?	-Eeyyee □ -Lakkii □
5.	Madda bishaanii	-tuuboo □ -madda biraa □
6.	Qophii nyaataa fi qabachuu irratti mirkanaa'aa?	-Eeyyee □ -Lakkii □
7.	Yeroo hunda qorannoo fayyaa ni gootu?	-Eeyyee □ -Lakkii □
8.	Hojii hojjechuu dura uffata sirrii qulqulluu mijaawaa sirrii ta'e uffattuu?	-Eeyyee □ -Lakkii □
9.	Nyaata kamiyyuu qopheessuu fi dhiheessuun dura harka dhiqattuu?	-Eeyyee □ -Lakkii □
10.	Yeroo dhukkuba akka garaachaatiin rakkattu nyaata qopheessituu?	-Eeyyee □ -Lakkii □
11	Nyaata foon qalamaa fi nyaata biroo muruuf cirracha waliigalaa fayyadamuu.	-Eeyyee □ -Lakkii □

Annex IV-Laboratory Protocol

Protocol to the manuscript entitled prevalence of Salmonella, Shigella, and intestinal parasites among food handlers: A cross-sectional study in Jimma zone governmental hospital, south west Ethiopia, 2022

This protocol has been provided by the authors to give readers additional information about the laboratory examination of intestinal parasites and bacterial isolates from stool specimens

1. Laboratory Diagnosis of Intestinal Parasitic Infections

Laboratory diagnosis of intestinal parasitic infections can be carried out by detection and identification of the parasites or their particular stages (ova/egg, cyst, larva or trophozoite) in the stool specimen. For this study we used 10% (v/v) formalin solution to maintain the morphological characteristics of parasites.

1.1.Direct wet mount examination

Reagents and equipment to be used

- Normal Saline (0.85% NaCl)
- Lugol's Iodine
- Glass microscope slides
- Cover slips (22 mm by 22 mm size)
- Pipettes
- Examination Gloves
- Microscope
- Applicator wooden stick

Procedure

1. About 2 mg (the size of match stick head) of stool sample mixed with a drop of saline and a drop of iodine placed on a slide
2. then carefully covered the stool with a cover slip to avoid bubble formation

3. Finally, the slide was examined for the presence of motile larva, trophozoites and ova of intestinal parasites under light microscope at 10× and 40× magnifications.

1.2.Formalin-Ethyl Acetate Sedimentation Concentration

Reagents and equipment

- Formol-water, 10% v/v
- Diethyl ether
- Tea strainer
- Centrifuge

Procedure

1. About 1 g (pea-size) specimen has been mixed with 4 ml of 10% formol-water well.
2. Then, further of 3 ml 10% formalin solution was added and strained the fecal suspension through the tea strainer into a 15 ml conical centrifuge tube.
3. Add 4 ml of ethyl acetate, stopper the tube, and shake vigorously in an inverted position for 1 minute and immediately centrifuged at 3000 revolutions per minute (rpm) for 1 min.
4. Carefully remove the stopper free the plug of debris from the top of the tube by ringing the sides with an applicator stick. Decant the top layers of supernatant.
5. Use a cotton-tipped applicator to remove debris from sides of the centrifuge tube and smear was prepared using a slide from the sediment
6. Finally, the slide was examined under a microscope with magnification power of 10x objective first and then 40x objective.

1.3.Auramine-O staining Method

Auramine is the fluorochrome dye that forms a complex with mycolic acids found in the acid-fast cell wall of organisms that resist decolorization by acid-alcohol. Potassium permanganate, counterstain renders tissue and its debris nonfluorescent, therefore reducing the possibility of artifacts. The cellular structures visualized under U-V appear bright yellow green.

1. Prepare a smear and air dry
2. Fix in absolute methanol for 1 min, then air dry before proceeding with staining
3. Flood the slide with Auramine-phenol solution leave the solution on the slide for 20 minutes
4. Rinse with tap water and drain excess water from the slide.
5. Flood the slide with 0.5% acid ethanol and leave the destaining solution on the slide for 2 minutes.
6. Rinse with tap water and drain excess water from the slide.
7. Flood the slide with 0.1 % Potassium permanganate and leave the counterstain fluid on the slide for 2 minutes.
8. Rinse with tap water and air dry.
9. Examine with 20x and 40x objectieves.

1.4. **Biochemical Identification Of Shigella And Salmonella**

Test principles

Isolation and identification remain the gold-standard for the diagnosis of infections due to *Salmonella* and *Shigella.* Theoretically, culture is 100% specific and unlike rapid tests, yields an isolate which may be subjected to further characterization (e.g. antimicrobial susceptibility testing).

Virtually all isolation protocols for *Salmonella spp.* and *Shigella spp*. include the use of selective and differential media to enhance recovery of the targeted organisms. Selective media are formulated to suppress background flora. These media also provide preliminary, macroscopic, differentiation of enteric organisms on the basis of colony color and morphology.

When interpreting *Salmonella* and *Shigella* cultures, it is important to remember that colony morphology on selective agar is not diagnostic. Colony morphology is used simply as a means to identify colonies for additional testing. Colonies that produce *Salmonella*-like or *Shigella*-like morphology ("suspect colonies") on selective agar must be subjected to additional biochemical (and serological) testing to confirm the identification.

As other *Enterobacteriaceae* may look similar to *Salmonella* or *Shigella* on selective media, the presence of suspect colonies alone cannot be considered diagnostic. A final genus / species level identification requires additional testing for confirmation. Similarly, isolates presumptively identified as suspect-*Salmonella spp*. or suspect-*Shigella spp*. on the basis of agglutination with polyvalent antisera must be subjected to biochemical confirmation. Similarly, polyvalent antisera can be a useful screening

tool; however, some O and H antigenic types are found in multiple genera among the *Enterobacteriaceae*, so reaction with any given antiserum without adequate biochemical testing is not diagnostic.

1.5.Salmonella Shigella (SS) Agar

Principle

The presence of bile salts mixture and dyes (brilliant green) inhibits the growth of gram-positive species to a varying degree. Differentiation of enteric organisms is achieved by the incorporation of lactose in the medium. Organisms which ferment lactose produce acid which, in the presence of the neutral red indicator, results in the formation of red/pink colonies. Lactose non-fermenters form colorless colonies. The latter group contains the majority of the intestinal pathogens, including Salmonella and Shigella. The sodium thiosulfate and ferric citrate enable the detection of hydrogen sulfide production as evidenced by colonies with black centers.

Preparation

- Suspend 60.0 grams of *Salmonella Shigella* Agar in 1000 ml distilled water.
- Mix well
- Heat to boiling with frequent agitation to dissolve the medium completely.
- Mix well and pour into sterile Petri plates.
- Let the agar solidify and store in the refrigerator (avoid freezing). Prepared culture media can be kept for at least a week in refrigeration.

Procedure

1. Allow the plates to warm to room temperature and the agar surface to dry before inoculating.
2. Heavily inoculate and streak the specimen as soon as possible after collection.
3. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface.
4. Streak for isolation with a sterile loop.

5. Incubate plates aerobically at 36 °C 24 hours.
6. Examine colonial morphology.

Interpretation of results

Lactose fermenter: If lactose fermentation occurs, the medium was turn red due to the acidic pH

Non-Lactose fermenter: Salmonella and Shigella appear as transparent or translucent colorless colonies. Colonies of *Salmonella spp.* may appear with or without black centers (depending on the species isolated).

Control species

Salmonella typhimurium ATCC 14028 = Colorless colonies with black center

Shigella flexneri ATCC 23354 = Colorless colonies

2. Biochemical tests

2.1.Triple Sugar Iron Agar (TSI)

Preparation of TSI agar medium

1. Combine the ingredients, and adjust the pH to 7.3
2. Boil to dissolve the agar and dispense into tubes.
3. Sterilize by **autoclaving at 121 °C for 15 minutes**
4. Cool in a slanted position to give a 2.5 cm butt and a 3.8 cm slant.

Interpretation of Triple Sugar Iron Agar Test

1. If lactose (or sucrose) is fermented, a large amount of acid is produced, which turns the phenol red indicator yellow both in the butt and in the slant. Some organisms generate gases, which produces bubbles/cracks on the medium.
2. If lactose is not fermented but the small amount of glucose is, the oxygen-deficient butt was be yellow (remember that butt has comparatively more glucose than slant i.e. more media more glucose), but on the slant the acid produced (less acid produces in slant as media in slant is less)

was be oxidized to carbon dioxide and water by the organism and the slant was be red (alkaline or neutral pH).

3. If neither lactose/sucrose nor glucose is fermented, both the butt and the slant was be red. The slant can become a deeper red-purple (more alkaline) as a result of the production of ammonia from the oxidative deamination of amino acids (remember peptone is a major constituent of TSI agar).

4. if H_2S is produced, the black color of ferrous sulfide is seen.

Organism	**Slant**	**Butt**	**Gas**	**H_2S**
Shigella	Alkaline (K)	Acid (A)	Neg (-)	Neg (-)
Salmonella	Alkaline (K)	Acid (A)	Pos (+)	Pos (+)

2.2.Motility-Indol-Ornithine Agar (MIO)

Procedure

1. Allow medium to warm to room temperature prior to inoculation.
2. Using a straight needle, select isolated colonies from a pure 18-24 hours culture and stab the center of the medium to about one-half its length.
3. Incubate the tubes aerobically at 35 degrees C. for 18-24 hours.
4. Caps should be loose during incubation.
5. Examine for motility and ornithine production

Interpretation of results

Motility: Positive motility is denoted when turbidity or cloudy growth extends from the line of inoculation. Growth only along the stab line is indicative of a negative motility test.

Indole test: A positive test for indole is denoted when a pink to red color band is formed at the top of the medium after addition of Kovacs Reagent. A yellow color denotes a negative indole test after addition of Kovacs Reagent.

Ornithine test: A positive test for ornithine is denoted by a dark, turbid purple color in the medium. A yellow color throughout the medium denotes a negative ornithine result.

2.3.Urea test

Procedure

1. A small amount of growth is harvested with a sterile (1 µL) loop or needle.
2. Lightly inoculated the surface of the agar slant.
3. Tubes are incubated under aerobic conditions at 36°C with caps loosened.
4. Tubes should be examined and results recorded at 24 hours, 48 hours, and 5-7 days.

Interpretation of results

Positive- intense pink color on the slant

Negative- no color change

2.4.Simmons citrate agar

Procedure

1. A small amount of growth was harvested with a sterile (1 µL) loop.
2. Lightly inoculated the surface of the agar slant.
3. Tubes were incubated under aerobic conditions at 36°C with caps loosened.
4. Tubes were examined and results recorded at 24 hours, 48 hours, and 3-5 days.

Interpretation of results

Positive - intense blue color (initially the color change may only occur on the agar slant)

Negative - agar remains green

2.5.Lysine Iron Agar (LIA)

Principle of Lysine Iron Agar (LIA)

- Lysine iron agar contains lysine, peptones, a small amount of glucose, ferric ammonium citrate, and sodium thiosulfate.

- The medium has an aerobic slant and an anaerobic butt. The medium is stabbed to the base of the butt and streaked on slant.
- When glucose is fermented, the butt of the medium becomes acidic (yellow).
- If the organism produces lysine decarboxylase, cadaverine is formed. Cadaverine neutralizes the organic acids formed by glucose fermentation, and the butt of the medium reverts to the alkaline state (purple).
- If the decarboxylase is not produced, the butt remains acidic (yellow).

Interpretation of results

Lysine Decarboxylation (detected in butt):

- Positive Test: Purple slant/purple butt (alkaline)
- Negative Test:Purple slant/yellow butt (acid), fermentation of glucose only

Lysine Deamination (detected on slant):

- Positive Test:Red slant
- Negative Test:Slant remains purple

Antimicrobial susceptibility testing

Modified Kirby-Bauer Antimicrobial susceptibility testing technique: Method:

1. Using a sterile wire loop, touch 3–5 well-isolated colonies of similar appearance to the test organism and emulsify in 3–4 ml of sterile physiological saline or nutrient broth.
2. In a good light match the turbidity of the suspension to the turbidity standard (0.5 McFarland standard). Mix the standard immediately before use). When comparing turbidities, it is easier to view against a printed card or sheet of paper.
3. Using a sterile swab, inoculate a plate of Mueller Hinton agar. Remove excess fluid by pressing and rotating the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions, rotating the plate approximately 60^{o} to ensure even distribution.

4. With the Petri dish lid in place, allow 3–5 minutes (no longer than 15 minutes) for the surface of the agar to dry.

5. Using sterile forceps, needle mounted in a holder, or a multidisc dispenser, place the appropriate antimicrobial discs, evenly distributed on the inoculated.

Note: The discs should be about 15 mm from the edge of the plate and no closer than about 25 mm from disc to disc. No more than 6 discs should be applied (90 mm dish). Each disc should be lightly pressed down to ensure its contact with the agar. It should not be moved once in place.

6. Within 30 minutes of applying the discs, invert the plate and incubate it aerobically at 37^{o}C for 16–18 hour

7. After overnight incubation, examine the control and test plates to ensure the growth is confluent or near confluent. Using a ruler on the underside of the plate, measure the diameter of each zone of inhibition in mm. The endpoint of inhibition is where growth starts.

Zone Diameter (mm) interpreted as: S (Susceptible), I (Intermediate) or R (Resistant)

Zone Diameter Interpretive Standards for Enterobacteriaceae, in mm Testing conditions Media: Mueller-Hinton agar.

Use maximum 12 disks on a 150 mm plate;

Use maximum 6 disks on a 100-mm plate. Disks should be placed no less than 24 mm apart, center to center. Number of disks to test = 12 Inoculum: direct colony suspension equivalent to

0.5 McFarland standards

Incubation: 35 +/- 2oc, ambient air 16-18 hours

Laboratory report format of AMR :

Antimicrobial Agent		Disk content	Zone diameter nearest whole mm			
			R	I	S	Comment
	Ampicillin	10ug	≤ 13	14-16	≥ 17	
	Tetracycline	30ug	≤ 11	12-14	≥ 15	
	chloramphenicol	30ug	≤ 8	13-17	≥ 18	
	Ceftriaxone	30ug	≤ 19	20-22	≥ 23	
	Ciprofloxacin	5ug	≤ 20	21-30	≥ 31	A Ciprofloxacin (breakpoint for salmonella only

Code:_1. stool Culture and identification:

XLD(SSA): Positive :_____ Negative ____

Isolated Bacteria ____

2. Antimicrobial susceptibility testing	S (mm)	I (mm)	R(mm)
Amoxicillin/Clavulanic acid	------	------	------
Ampicillin	------	------	-------
Ceftriaxone	-------	-------	-------
Tetracycline	-------	-------	-------
Chloramphenicol	-------	-------	-------
Ciprofloxacin	-------	-------	-------

JIMMA UNIVERSITY

INSTITUTE OF HEALTH

FACULTY OF HEALTH SCIENCES

SCHOOL OF MEDICAL LABORATORY SCIENCES

APPROVAL SHEET

This is to certify that the proposal entitled "**Prevalence of *Salmonella, Shigella* and Intestinal Parasites Infection Among Food Handlers Working in Selected Governmental Hospitals of Jimma Zone, Southwest Ethiopia 2022**" submitted to faculty of health science school of medical laboratory science in partial fulfillment of the requirement for masters of science degree in medical parasitology, is a record of original research proposal prepared by Tsion Melaku Tefera(BSc), under my supervision and no part of the proposal work has been submitted for any other degree. The assistance and help received during the course of proposal development have been duly acknowledge. Therefore, I recommend that it be accepted as fulfilling the proposal requirements.

_____	_____	_____
Name of first Advisor	**signature**	**date**
_____	_____	_____
Name of second Advisor	**signature**	**date**
_____	_____	_____
Name of Examiner	**signature**	**date**