ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF ALLIED HEALTH SCIENCE
DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Comparative study on the magnitude of intestinal parasites, Salmonella and Shigella species and Antimicrobial Susceptibility pattern among HIV infected and non-infected patients with diarrhea in selected health facilities, Dessie town, Northeast Amhara Region, Ethiopia

By: Assefa Belay

Advisors: Aster Tsegaye (MSc, PhD)
: Berhanu Seyoum (MSc, PhD)
Co-advisor Melaku Ashagrie (BSc, MSc)

A research thesis submitted to the Department of Medical Laboratory Sciences, School of Allied Health Science, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology)

June 2018
Addis Ababa Ethiopia

Addis Ababa University

School of Graduate Studies

This is to testify that the thesis is prepared by Assefa Belay, which is entitled "**Comparative study on the magnitude of intestinal parasites, Salmonella and Shigella species and Antimicrobial Susceptibility pattern among HIV infected and non-infected patients with diarrhea in selected health facilities, Dessie town, Northeast Amhara Region, Ethiopia"** and submitted in partial fulfillment of the requirements for the degree of Master of Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

External Examiner_____ Signature _____ Date_____

Internal Examiner _____ Signature _____ Date_____

Advisor Dr. Aster Tsegaye_ Signature _____ Date_____

Advisor Dr Berhanu Seyoum Signature _____ Date_____

_____ _____ _____

Chairman of the Department or Graduate program coordinator signature Date

Acknowledgement

My special gratitude goes to Addis Ababa University, College of Health Science, and Department of Medical Laboratory Sciences for giving me the opportunity to do my research on this valuable title.

I would also like to express my deepest gratitude to my advisors Aster Tsegaye (MSc, PhD), Birhanu Seyoum (MSc, PhD), and Co-adviser Melaku Ashagrie for their unreserved advice and fruitful comments starting from title selection.

My heartfelt gratitude also goes to Dessie Regional Health Research Laboratory (DRHRL) staffs especially microbiology Laboratory staffs, Dessie Dessie Health Science collage Laboratory staffs, Referral hospital laboratory staffs especial Yemata Endalew, Boru Hospital laboratory head, Dessie health center, Banbua wuha Health center nurses and laboratory staffs for their support to the study, data collection and for all their help during the study period. The study participants are gratefully acknowledged for their kind collaboration.

I would like to express my heartfelt thanks to unlimited help of God, and encouragement and support of my friends, relatives. My wife deserves special appreciation for her support, encouragement and endurance of the burden during the three years of my education.

Table of Contents

List of Abbreviations

AIDS:	Acquired immune deficiency syndrome
AML:	Amoxicillin
AMP:	Ampicillin
ART:	Antiretroviral therapy
AST:	Antimicrobial Susceptibility Testing
CAF:	Chloramphenicol
CDC:	Center for disease prevention and control
CIP:	Ciprofloxacin
CLSI:	Clinical Laboratory Standards Institute
CSAE:	Central Statistical Agency of Ethiopia
EU:	European Union
HIV:	Human immunodeficiency virus
KIA:	Kligler Iron Agar
MDR:	Multi- drug resistance
NTS:	Non typhoid salmonella
OPD:	Outpatients Department
SPI:	Salmonella Pathogenicity Island
SPSS:	Statistical package for social science
SXT:	Sulfamethoxazole Trimethoprim
T3SS:	type III secretion systems
UNICEF:	United Nations International Children Emergency Fund
USA:	United States of America
WHO:	World health organization
XLD:	Xylose Lysine Deoxycholate agar

List of figures

List of tables

Abstract

Diarrhea is a common complication and manifestation of HIV/AIDS, occurring in almost 90% of HIV/AIDS infections both in adults and children in developing countries like Ethiopia. Salmonella and Shigella infections are major global public health problems causing mild to severe forms of intestinal tract infection and diarrhea; Intestinal parasites are also additional burden in developing countries.

Objective: To determine the prevalence of intestinal parasites, *Salmonella* and *Shigella* Species and their antimicrobial susceptibility pattern among HIV infected and non-infected patients with diarrhea in selected health facilities, Dessie town, Northeast Amhara Regional State.

Methods: Health facility based cross sectional study was conducted at Dessie Referral Hospital, Dessie Health Center and Banbua wuha Health Center from January 2018 to April 2018. A total of 354 HIV infected and non-infected diarrheic outpatients were included using convenient sampling technique. Data on socio-demographic and clinical characteristics were collected using interview that employed structured questionnaire. Freshly voided stool specimen was used for investigate intestinal parasite, culturing and antimicrobial susceptibility testing. Data was entered and analyzed using SPSS software version 22 (IBM).

Results: Among 354 study participants, 112 were HIV infected and 242 were non HIV infected with diarrhea. The prevalence of Intestinal parasite and Bacterial infection among HIV infected was 26(23.2%) and 8(7.1%), respectively. Of the bacterial isolates, *Salmonella* accounts 6(5.4%) followed by *Shigella* 2(1.8%) among bacterial isolates. Moreover, the most prevalent parasite was *C. parvum* 9(8%), *E. histolytica/dispar* 8(7.1%), 4(3.6%) and 1(0.9%) co-infection of *C. parvum* and *C. catananyesis*. The bacterial isolates from diarrheal patients were 100% susceptible to Ceftriaxone 95.4% susceptible to Ciprofloxacin However, the isolates 100% resistant to Ampicillin and Amoxicillin.

Conclusion: High prevalence of enteric pathogens and high resistance of isolates to commonly prescribed antibiotics were observed from HIV infected and non-infected patients. Therefore, Prevention methods should be given attention to reduce the infection rate and AST should be practiced in the health facilities to select appropriate antimicrobial agent and prevent emergence of drug resistant bacteria.

Key words: Salmonella, Shigella, Antimicrobial susceptibility test, Multidrug resistance

1. Introduction

1.1. Background

Diarrhea is a passage of three or more loose or liquid stools per day, or more frequently than is normal for an individual according to the World Health Organization (WHO) definition (1). It is caused by bacterial, viral, and parasitic organisms and is usually a symptom of gastrointestinal infection (1,2, 3). Immunosuppression, a consequence of human immunodeficiency virus (HIV) infection favors the occurrence of multiple opportunistic infections responsible for a high mortality. Among these diseases, intestinal parasites are the main cause of severe chronic diarrhea such as Coccidia (*Cryptosporidium parvum*, *Isospora belli*, Cyclospora sp) and amoebae (*Entamoeba histolytica,* etc.) (4) and *Salmonella* and *shigella* are potential enteric bacterial causes of diarrhea in both immune competent and immune compromised patients (5). It is one of the leading causes of morbidity and mortality worldwide especially in areas with poor hygiene and sanitation practice and with limited access to safe water commonly in developing countries (6).

In developing countries, diarrheal disease accounts for an estimated 17.5–21% of all deaths in children under the age 5 years which is estimated as (1.5 million deaths per year). Of all child deaths from diarrhea, more than three fourths of death occur in the African region and South-East Asian regions, Those regions are also disproportionately burdened with infant and childhood HIV infections(7). Diarrhea is a common complication and manifestation of HIV/AIDS (acquired immune deficiency syndrome), occurring in almost 90% of HIV/AIDS infections both adults and children in developing countries that includes Ethiopia (2,8). Persistent diarrhea occurs with increased frequency in HIV-infected children and is associated with 11-fold increase in mortality compared to uninfected children (2).

Salmonella and *Shigella* infections are major global diarrhea causing public health problems that cause mild to severe forms of intestinal tract infection commonly associated with intake of a variety of food. More than a billion cases of diarrhea due to non typhoidal Salmonella (NTS),in which three million deaths occur annually (9). *Salmonella typhi* is another major cause of food and water borne gastroenteritis in human and remains an important health problem globally. The WHO estimates 16 million new cases and 600,000 deaths of typhoid fever occurred each year worldwide (10).

About 99% of the 200 million cases and more than 650,000 deaths per year due to infection with *Shigella* occur commonly in developing countries including Ethiopia, primarily among children and adults (9). *Shigella* is the predominant organism in bloody diarrhea in developing countries, and WHO has estimated that it causes 10% of acute diarrhea in children below five years. *Campylobacter* and non-typhoidal Salmonella are also commonly identified agents of dysentery in the developing world (7). Few studies have linked HIV infection to dysentery in children, and there is little evidence of a different spectrum of etiological agents in bloody diarrhea in HIV-infected individuals (11).

Children below the age of five years, elderly people and patients with immunosuppression are more susceptible to *Shigella* and *Salmonella* infection than healthy individuals (12).Rates of Gram-negative bacteria enteric infections are at least 10-fold higher among HIV-infected adults than in the general population, HIV infection increases the risk of Salmonella bacteremia 20- to 100-fold and mortality as much as seven-fold compared to counterparts (11).

Development of antimicrobial resistance by enteric pathogens like *Shigella* and *Salmonella* species against easily accessible and commonly prescribed antibiotic has become a major concern around the globe. This burden is widely spread in developing countries including Ethiopia (13). In the past two, three four decades *salmonella* and *Shigella* species have become progressively resistant to most of the first-line drugs used and the prevalence of multi drug resistant strains is an important concern of treatment (14).Changing antibiotic resistance patterns, rising antibiotic costs and the insignificant introduction of new antibiotics have made selecting optimal antibiotic regimens more difficult now than ever before (15).In most of the developing countries, laboratory investigations of *Shigella* and *Salmonella* are diagnostic challenges due to lack of adequate facilities that enable culture and antimicrobial susceptibility testing. Thus, there is a limited awareness of the prevalence of those infections and antimicrobial resistance. Thus calls for assessing the magnitude and antimicrobial susceptibility pattern in such settings. (16)

1.2. Statement of the problem

Diarrheal diseases caused by bacterial, viral, or parasitic pathogens are a major public health problem. Estimations by WHO indicate that the world population suffered from 4.5 billion incidences of diarrhea causing 1.8 million deaths in the year 2002. Approximately, 99% of the cases occurred in developing countries (17).An estimated 2.5 million deaths occur each year, in Asia, Africa and Latin America (18).

Intestinal parasitic organisms are common pathogens among HIV patients worldwide and have been known to cause severe and life-threatening diarrhea in such subjects (19).The infection rate is remarkably high in Sub-Saharan Africa where most cases are common among HIV positive patients. The incidence of parasitic infection was 50% in developed countries while it reached 95% in developing countries (4).

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 (17).A total of 69% of all episodes and 61% of all deaths attributable to shigellosis involved children less than 5 years of age (20).

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 (21), Enteric fever is an invasive, life-threatening, systemic disease with an estimated over 27 million cases, resulting in more than 200,000 deaths (22). 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 (23).

Studies conducted in different parts of Ethiopia showed different prevalence rates of *Salmonella* and *Shigella*. 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%),Butajira (10.5%, 4.5%) for *salmonella* and *Shigella*, respectively(9, 13, 16, 24,25). 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 (26,27).

With the increasing prevalence of HIV/AIDS and immunosuppressive chemotherapy, diarrhea in immunocompromised patients has become a growing challenge (28).Absence and delay in the

detection of diarrhea causing bacteria such as *Salmonella* and *Shigella* is associated with prolonged hospital stay, increased morbidity and mortality particularly in HIV infected patients and the rest of the community. Salmonellosis has been estimated to be nearly 20 times as common and 5 times more often bacteraemic in patients with HIV/AIDS than in those without the disease (28). As a result, frequent and consistent evaluation and study of the prevalence, etiologic agents, and predisposing factors of enteric fever is necessary in developing countries like Ethiopia in order to reduce its devastating effects.

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 (29)**.** Even though some studies were conducted from other parts of Ethiopia, published study was not found regarding the prevalence and antimicrobial susceptibility patterns of Salmonella and Shigella species from Dessie city (North east Amhara region). Therefore, this gap caught the attention of an investigator to understand the burden and antimicrobial susceptibility patterns of *Salmonella* and *Shigella* species and intestinal parasites in Dessie town and its vicinity.

Moreover, in health facilities in the study area, same as other similar settings in Ethiopia, routine culture and antibiotic susceptibility testing are not performed as an essential part of outpatient diagnosis and the treatment is on empirical basis.

1.3. Significance of the Study

- .This study provides recent information the magnitude of *Salmonella*, *Shigella* and Intestinal parasites for HIV infected and non-infected diarrheic patients
- It provided recent information antimicrobial resistance patterns *Salmonella* and *Shigella*.
- The study also used as a base line for further studies of other enteric diarrheagenic pathogens in the study area.
- It could also provide basic information for local health authorities to prevention and control of enteric pathogens.

2. Literature review

2.1 General characteristic and classification

Salmonella was first discovered and isolated from the intestines of pigs infected with classical swine fever, by Theobald Smith in 1855. There are over 2500 Salmonella serotypes that have been identified and more than half of them belong to Salmonella enteric a subspecies enterica, classified into two species, *Salmonella enteric*(type species) and *Salmonella bongori*, based on differences in their 16S rRNA sequence analysis (21).Based on the clinical patterns Salmonella strains can be grouped into typhoid Salmonella(*S. typhi* (most serious form) and (*S. paratyphi* A, B, C) and non-typhoid *Salmonella* (NTS) has different serotypes (*S.enteriditis* and *S.typhimurium* are the most common serotypes (21).

Genus *Salmonella* are a member of enterobactericeae, non-lactose fermenters, motile and gas producer gram negative rods (1). Salmonella are facultative anaerobic bacilli possessing three major antigens H or flagella antigen, O or somatic antigen and VI(capsular) antigen(possessing only few serovars). It is originally characterized by their ability to metabolize citrate as a sole carbon source and lysine as a nitrogen source, as well as their ability to produce hydrogen sulfide (30,31).

The genus *Shigella* has four species: *Shigella dysenteriae* (also Group A), *Shigella flexneri* (Group B), *Shigella boydii* (Group C), and *Shigella sonnei* (Group D).Each species may be further divided into serotypes on the basis of reactivity with hyper immune serum: *S. dysenteriae*(15 serotypes), *S. flexneri* (6 serotypes and 2 variants), and *S. boydii* (20serotypes) shigella is a non -motile and glucose fermenting bacteria, Gram-negative rod belonging to the family Enterobacteriaceae. (31).It is a bacterium found exclusively in intestinal tract of human. The incubation period ranges from one to seven days and a low infectious dose of 10 to 100 organisms (32).

2.2 Epidemiology

Salmonella and *Shigella* infections are major global public health problems that cause mild to severe forms of intestinal tract infection (16) and a common source of foodborne diseases that cause morbidity and mortality worldwide (33). Many Asian countries, including China, India, Vietnam, Pakistan and Indonesia, have high incidence rates of enteric fever, exceeding 100 cases

per 100,000 populations annually. Among these Pakistan and India have the highest incidence rates of 451.7 cases and 214.2 cases per 100,000 populations, respectively. In many developing countries, especially in sub-Saharan Africa, the limited diagnostic resources and proper surveillance tools result in poor characterization of the burden of enteric fever (21).

Salmonella species are found worldwide in humans, domestic and wild animals, including reptiles, birds and insects (34). Enteric fever is endemic in the developing world in regions that lack clean water and adequate sanitation and Non typhiod *Salmonella* occur worldwide (35). Treating *Salmonella* infection in humans is expensive; for example, in the USA, it causes illness in approximately 1.2 million patients annually, resulting in estimated medical costs of $365 million (36). In the European Union (EU), salmonellosis is the second most commonly reported gastrointestinal infection, with a confirmed case rate of 20.4 cases per 100,000 individuals in 2011 (37). In China, *Salmonella* causes an estimated 22.2% of foodborne diseases, and salmonellosis ranks fourth among the most prevalent foodborne diseases caused by microbial agents (33).

The global incidence of *Shigella* infections has been estimated at 80–165 million episodes annually. An estimated 99 % of episodes occur in the developing world and children aged less than 5 years and immune compromised individuals. (38). Shigellosis is a worldwide problem with *Shigella sonnei* predominating in Europe and US and *Shigella flexneri* more prevalent in Asian and African countries (24).

A research conducted in Rosario, Argentina (2016) studies the prevalence and virulence genes of *Shigella* species isolated from Patients with diarrhea. Findings revealed that prevalence of *Shigella* in 1022 diarrheic patients was 100(9.8%) and the isolation frequency was 74% for *S. flexneri*, the predominant species, and 26% for *S. sonnei* (39).

A case control study conducted in Lima, Peru, to identify etiologies and manifestations of persistent diarrhea in adults with HIV-1 Infection involved a total of 322 case and control subjects. The prevalence of *Cryptosporidium* species was 29 (20%) , 10 (7%) , *Giardia lamblia* 18 (12%), 4 (3%) , *Microsporidium* species 7 (5%),3 (2%) , Other parasites 14 (10%), 4 (4%) , *Shigella* species 12 (8%),7 (5%) , *Aeromonas* species 7 (5%) 0 , *Campylobacter* species 5 (4%),

0, ETEC 5 (4%), 3 (2%) , *Salmonella* species 3 (2%) 0, for cases and controls, respectively (40).

Another Case control study was conducted in pune India, among 45 HIV positive patients, 27(60%) who presented with diarrhea, and 18(40%) without any complaints of diarrhea. Overall, 36 diarrheal pathogens were detected in the 45 patients in the study. Of these, 24 pathogens were identified in the patients with diarrhea, while 12 were identified in patients without diarrhea. Parasites accounted 14(58.33%), bacteria 7 (29.17%), and fungi 3(12.50%).*Isospora* spp. was the most common parasite 7(25.9%) followed by *Cryptosporidium* spp. 4(14.8%). Other parasites included *Cyclospora* spp., *Strongyloides stercoralis*, and *Entamoeba histolytica* 1(3.7% each). *Escherichia coli* (18.5%).Other isolates were *Shigella flexneri* 1(3.7%) and *Mycobacterium tuberculosis* 1(3.7%) (8).

Study conducted in Kampala, Uganda showed that a total of 190 children, 47 were HIV positive. The prevalence rates of the pathogens in HIV-infected and -uninfected children were 19% (9/47) and 27 %(38/143), respectively; Bacterial pathogens were a significant cause of acute diarrhea in the HIV infected and the HIV-negative children. Among these 9 bacterial infection of HIV positive children 6 *E.coli*, 2 *Salmonella*, and 1 *Shigella* species and of the 38 bacterial pathogens of HIV negative children 24 were *E.coli*, 9 *Salmonella* and 5 *shigella* species (41).

Similar Study conducted in Nigeria in 2015 on prevalence and antimicrobial susceptibility patterns of *Salmonella* and *Shigella* in a total of 150 HIV seropositive patients having diarrhea. The prevalence of *Salmonella* and *Shigella* recovered from the stool samples examined were found to be 10(6.6%). *Salmonella* and *Shigella* accounted for 2(1.3%) and 8(5.3%), respectively. The species Salmonella enteric serovar Enteritidis for *Salmonella* and *S. flexneri* (n=7), *S. dysenteriae* (n=1) for *shigella* were the most common species type (42).

Another study conducted in Gaborone, Botswana, isolated 43(21%)Shigella from 221 and *Salmonella* 8/221 (3%) from rectal swabs of children under 5 years with diarrhoea.Whereas, *Shigella* is found in two of 100 specimens from children without diarrhoea. *S. Boydii* (13%) was the most prevalent *Shigella* species followed by *S. Fexneri* (6%) and *S. sonnei* (2%) (43).

A study conducted in Wester Cameroon, in a total of 396 people revealed an overall prevalence of intestinal parasites which was 14.64%. Out of 42 HIV/AIDS patients, 59.5% (25/42) were infected with intestinal parasites, while only 9.32% (33/354) of the HIV negative patients were infected with intestinal parasites. The parasites detected in study population included *Crystosporidium parvum* (2.53%), *Entamoeba histolytica* (7.52%), *Entamoeba coli* (4.04%), *Giardia lamblia* (0.25%), *Trichuris trichura* (0.25%), *Strongyloides stercoralis* (0.25%) and *Taenia* spp. (0.25%). In the HIV infected group, *Crystosporidium parvum* (19.04%), *Entamoeba histolytica* (19.04%), *Entamoeba coli (*21.42%), *Giardia lamblia* (2.38%), *Strongyloides stercoralis* (0.25%) and *Taenia* spp. (0.25%) were found (4).

In a study conducted at the Yaoundé Central Hospital, Cameroon, a total of 207 HIV positive people were recruited. Eighty (38.65%) were male and 127 (61.35%) were female. The overall prevalence of intestinal parasite infections was 57.48% (119/207). The parasites detected in the study population were, *Entamoeba coli* (22.68%), *Ascaris lumbricoïdes* (22.68%), *Entamoeba histolytica*(15.93%), Cryptosporidium spp (12.60%), *Isospora belli* (10.08%), *Trichuris trichiura*(7.60%), *Strongyloïdes stercoralis* (5.88%), *Ancylostoma duodenale* and *Necator americanus* (2.52%)(44)

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. Of 2517 children with NTS bacteremia in Malawi during 1998 to 2004, 85% were aged less than 36 months and an estimated 19% to 35% were HIV infected. During the same period, 2439 adults with NTS bacteremia were identified, and an estimated 95% to 98% were HIV infected (23).

Cross-sectional study was conducted in Jimma, Ethiopia, a total of 176 stool specimens from both adult and pediatric out-patients were collected and the findings of that study indicated that prevalence of *Salmonella* and *Shigella* were 19(10.8%) and 2(1.1%), respectively (9).

Another study conducted in Jimma health center, Jimma, Ethiopia (2014), indicated that from a total stool specimens of 260 diarrheal children collected and examined for the presence of *Salmonella* and *Shigella* species, a total of 22 (8.5%) samples were positive for *Shigella* species, 6 (2.3%) and Salmonella species, 16 (6.2%), respectively (45).

In a similar study conducted in Harar, Ethiopia, a total of 244 diarrheal stool samples were collected and the prevalence of *Salmonella* was 28 (11.5%) and 17 (6.7%) *Shigella* (16).

In another cross sectional study conducted in Hawassa, Ethiopia, facal samples collected from a total of 158 under-five children with diarrhea, of these 158 patients, 35(22.2%) bacterial pathogens were isolated. The isolated bacteria were *Campylobacte*r species, 20 (12.7%), *Shigella* species, 11 (7.0%), and *Salmonella* species, 4 (2.5%) (13).

Health institutional based cross sectional study was conducted in Gondar; samples were collected from 372 study subjects. Of the total of 372 stool cultures, 17(4.57%) *Shigella* species and 4(1.08%) *Salmonella* species were isolated. Most commonly isolated strains of *Shigella* were *S. flexneri* 11(64.7%) followed by *S. dysenteriae* 3(17.65%), *S. boydii* 2(11.77%) and *S. sonnei* 1(5.88%) (24).

A study was conducted in Butajira, central Ethiopia on a total of 382 stool samples from diarrheic patients were examined by culture and among them 40 (10.5%) were positive for *Salmonella* and 17 (4.5%) had *Shigella* species confirmed by biochemical and serotyping tests. Among 17 Shigella species *Shigella sonnei* founded as 6 (35.3%) followed by *Shigella flexneri* 5 (29.5%), *Shigella dysenteriae* 3(17.6%) and *Shigella boydii* 3 (17.6%) (25).

Study conducted in Gonder, Ethiopia, on HIV infected and non-infected individuals, Out of the 391 subjects included in the study, 199(63.8%) HIV seropositive and 113 sero negative patients had acute and chronic diarrhea respectively. While 79 was HIV seropositive without diarrhea. Of the 27 (8.7*%) Shigella* isolates taken from the diarrhea patients, 11 (3.5%) were from HIV positive subjects. while 16 (5.1%) were from the HIV negative ones (26).

Similar a study conducted in Jimma, Ethiopia, recruited a total of 372 HIV-infected and HIV-non-infected patients. Among the 99 HIV-infected patients with diarrhea, 25 (25.0%) of them had enteric bacteria among which 8(8.1%) were *Salmonella*, 4(4.0%) *Shigella* and 13(13.1%) *Campylobacter species*. Mycobacterium species were identified in 3(3.0%) of stool specimens obtained from HIV-infected patients with diarrhea and another 3 species were detected in HIV-infected patient without diarrhea. Salmonella species were isolated with higher prevalence in HIV-infected than in HIV non-infected patients (27).

Study conducted in Addis Ababa, Ethiopia, on sero negative diarrheal patients and HIV seropositive individuals without diarrhea. Of 147 AIDS patients with chronic diarrhea, 74 (50.3%) were infected with one kind or more of parasites. Out of 56 non-AIDS (seronegative) diarrheal patients, 41.1% (23/56) and out of the 43 non-diarrheal (seropositive) patients, 41.9% (18/43) were infected by a variety of intestinal protozoa and helminthes. Among these oocysts of *Cryptosporidium* species were isolated from 38/147 (25.9%) AIDS patients with chronic diarrhoea. In addition, among the opportunistic parasites frequently associated with AIDS, 2/147(1.4%) *Isospora belli* and 1/147 (0.7%) *Blastocystis* species were found in 74/147 (50.3%) of the AIDS patients with chronic diarrhea (46).

A study was conducted on 372 HIV infected and non-infected adult patients in southwestern Ethiopia to determine the prevalence of intestinal parasites. The prevalence of intestinal parasite in HIV infected and non-infected was 44.8% and 34.4% respectively. Among the opportunities protozoan parasites *C. parvum, I. beli, C. catyenesis* Oocyst were isolated from 6(11%), 4(7.4%), 2(3.7%) HIV infected patients with chronic diarrhea respectively (47).

Another study conducted at Addis Ababa, Ethiopia. A total of 253 diarrhea patients were participated, Of these 190 entropathogens were isolated. Sixty one (24.1%) were *E. coli,* (9.1%) were *Shigella* followed by (3.95%) *Salmonella* and *Citrobacter* species and 86 (34.0%) were parasites (48).

A study was conducted among HIV/ AIDS patients in Bahir dar northwest Ethiopia, out of 248 enrolled in the study, 171(69.0%) (90 males and 81 females) were infected with one or more intestinal parasites. The highest rate of intestinal parasites were observed among HIV/AIDS patients (80.3%, 151/188), and the infection rate of HIV negative individuals was 33.3% (20/60). *Cryptosporidum parvum* (43.6%), *Isospora belli* (15.5%) and *Blastocystis hominis* (10.5%) were opportunistic parasites that were found only in HIV/AIDS patients (49).

Study conducted based on medical records of selected ART centers at Adama, Afar and Dire-Dawa in Ethiopia, involves a total of 200 HIV positive patients on and without-ART. The prevalence was 16% for *Giardia lamblia,* 13% for *Entamoeba histolytica/ E. dispar,* 8% for *Cryptosporidium spp,* 5% for *Isospora belli,* 1.5% for *Blastocystis hominis*,2.5% for *Ascaris lumbricoidesand* 2% for *Hymnolepis nana* (50).

A study was conducted at Hawassa Teaching and Referral Hospital focusing on HIV positive individuals, Among HIV positive subjects, 59.8% (128/214) were infected with one or more intestinal parasites compared with 48.8% (80/164) of HIV negatives. The overall prevalence was 55.0% (208/378) of the study subjects were positive for at least one intestinal parasite. The most frequently detected parasites were *E. histolytica/dispar* (24.8%), *A. lamircoides* (12.2%) *cryptosporidium species* (20.1%) *I beli* (12.2%) *G.lambelia* (11.2%) in HIV positive patients (51).

A study conducted in Ethiopia Dessie referral hospital revealed overall prevalence of intestinal parasite in pre-ART and on-ART was 39% and 17.6%, respectively and from these the prevalence of protozoan, helminthic and both protozoan and helminthes were 31%, 7.4% and 0.7%; respectively. The prevalence of opportunistic intestinal parasites were 2.2% and from these 1.5% for *Cryptosporidium* species followed by *Isospora belli* 0.7% (52).

2.3. Pathogenicity

The severity of *Salmonella* and *Shigella* infections in humans varies depending on the serotype involved and the health status of the human host. Almost all strains of Salmonella are pathogenic and they have an ability to invade, replicate and survive in human host cells, their consequence in potentially fatal disease. When the bacteria enter the gastrointestinal tract via contaminated water or food, they penetrate the epithelial cells lining of the intestinal wall (17).*Salmonella* pathogenicity islands (SPIs) encodes for type III secretion systems (T3SS), multi-channel proteins that allow Salmonella to inject its effectors across the intestinal epithelial cell membrane into the cytoplasm (20).

Salmonella have five SPI (1-5) of them, SPI 1andSPI 2 are encoded by T3SS. The SPI-1 encoded T3SStranslocates effector proteins into cytosol of the host cells that is an ability invasion (gain access) of non-phagocytic human host cells and entropathogensis. Whereas the SPI2 encoded T3SS require for intercellular survival in murine macrophages. The remodeled vacuole blocks the fusion of the lysosomes and this permits the intracellular survival and replication of the bacteria within the host cells. The ability of Salmonella strains to persist in the host cell is crucial for pathogenesis, as strains lacking this ability are non-virulent (20).

Shigella is highly infectious, less than 100 microorganisms are sufficient to cause disease. This low infectious dose can at least partially be attributed to the presence of effective acid resistance systems, which enable *S. flexneri* to survive the acidic environment in the stomach (17,53,54). Furthermore, it was shown that *Shigella* species are able to down regulate the expression of antimicrobial peptides, which are important antibacterial effectors constantly released from the mucosal surfaces of the intestinal tract. After passage through the stomach and small intestine, the bacteria reach the large intestine, where they establish an infection (55).

The severe tissue destruction caused by *Shigella* spp. results in an impaired adsorption of water, nutrients, and solutes, which might cause the watery diarrhea as well as the blood and mucus in stools characteristic of shigellosis. Moreover, Shiga toxin, which is produced only by *Shigella dysenteriae* serotype 1, is cytotoxic for a variety of cell types and is responsible for the development of vascular lesions in the colon, the kidney, and the central nervous system. Due to the high toxicity of Shiga toxin, infections with *S. dysenteriae* serotype 1 are frequently associated with life-threatening complications (56,57).

2.4 Risk factors

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 (21, 31).

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 (9). In both S*almonella* and *Shigella* species immunocopromised individuals, children, aged people and HIV patients especially those with low CD4 count are highly at risk both *Salmonella* and *Shigella* species including adults. It has been shown that patients with CD4

counts <200 cells/mm3 or a history of AIDS-defining illness are at the greatest risk of enteric illnesses (11).

2.5 Clinical manifestation

The severity of Salmonella infections in humans varies depending on the serotype involved and the health status of the human host. The elderly, infants, and immune suppressed individuals are more likely to develop severe illness. *Salmonella* have four different clinical manifestations. These are enteric fever, gastroenteritis, bacteremia and other extra intestinal complications, as well as chronic carrier state (21). Enteric fever with bacteremia caused by *S. Typhi* (most serious form) and *S. Paratyphi* A, B, C. Diarrheal disease (enterocolitis): *S. Typhimurium* and *S. Enteritidis* are common causes in developing countries. The main sign and Symptoms in *Salmonella* infection include diarrhea (sometimes bloody), fever, abdominal cramps and vomiting. Mild infections are self-limiting. However, sometimes, the infection may lead to septicemia or more severe diarrhea with associated dehydration that can be life-threatening. NTS bacteremia is also the most common case and increase in the frequency and severity in those co-infected with HIV (21,30).

Shigella is a pathogen that primarily infects the lower intestinal tract. Patients with *Shigella* gastroenteritis typically present with high fever, abdominal cramps, and bloody, mucoid diarrhea. The approximate prevalence of these signs and symptoms is: Fever (30 to 40 %); abdominal pain (70 to 93%), mucoid diarrhea (70 to 85%), bloody diarrhea (35 to 55%), watery diarrhea (30 to 40%), vomiting (35%).The incubation period ranges from one to seven days, with an average of three days. Frequency of stools typically is 8 to 10 per day but may increase to up to 100 per day (58).

2.6 Laboratory Diagnosis

The laboratory investigation of microbial causes of diarrhea diseases like parasitic and bacteria used to detect, isolate, and identify pathogens or their products using microscopy, culture techniques, and biochemical methods (31).

2.6.1 Collection of fecal specimen for parasitic examination: a fresh fecal specimen is required. It should be uncontaminated with urine and collected into a suitable size, clean, dry, leak-proof

container. The container need not be sterile but must be free of all traces of antiseptics and disinfectants. A large teaspoon amount of feces is adequate or about 10 ml of a fluid specimen (59).

2.6.2 Microscopic Examination of stool: is used to identify motile parasites (trophozoit) eggs, cysts and oocysts of parasitic infection. Diarrheal disease caused by *I. belli, C. parvum and C. cayetanenisis* diagnosed by finding oocysts in fecal specimens. Specimens are usually watery and often have an offensive smell. Pus cells are not found. Oocysts of I. belli can usually be identified in direct wet preparations, prepared and examined as. If required the oocysts can be concentrated by the formol ether oocyst concentration technique. Oocysts of Cryptosporidium and *C. cayetanensis* can be detected in wet preparations but they are more easily identified in smears stained by the modified Ziehl-Neelsen (Zn) method following concentration by the formol ether oocyst concentration technique (59).

2.6.3 Concentration techniques, It may also be required: to detect *Strongyloides* larvae, the eggs of *Taenia*, cysts of *G. lamblia*, and to make it easier to detect small parasites, e.g. small fluke eggs, or the oocysts of intestinal coccidia prior to staining, to check whether treatment has been successful and to quantify intestinal parasites. The common concentration techniques are:

1. Sedimentation techniques in which parasites are sedimented by gravity or centrifugal force, e.g. formol ether concentration method which is the most frequently used technique because it concentrates a wide range of parasites with minimum damage to their morphology.

2. Floatation (also spelt flotation) techniques in which parasites are concentrated by being floated in solutions of high specific gravity, i.e. solutions that are denser than the parasites being concentrated. Examples include the zinc sulphate method and saturated sodium chloride method (59).

2.6.4 Culturing of Stool

Culture is the "conventional method" for the diagnosis of *Salmonella* and *Shigella* infection for the isolation and identification of the pathogen from stool specimen. MacConkey or Eosin-methylene blue and Hektoen, Salmonella-Shigella, or Xylose Lysine-Deosxycholate agar increase the likelihood of successful isolation of salmonella and shigella. Salmonella are non-

lactose fermenting characteristic on these agars and glucose positivity (usually with production of gas), H2S positive blackening the media and motility. *Shigella* non-lactose-fermenting colonies can be identified directly by standard commercial systems on the basis of four major characteristics: these are glucose positivity (usually without production of gas), lactose negativity, H_2S negativity, and lack of motility (29).

2.7 Treatment and susceptibility pattern

The antibiotics amoxicillin, Cotromoxazole, Chloramphenicol are commonly used drugs for the treatment of *Salmonella* and *Shigella* as tradition first line drugs. The first single antibiotic resistance incidence of Salmonella against chloramphenicol was reported early 1960s (60).Since then, 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 (21).

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 (Rplasmids). A commonly isolated plasmid carries resistance against ampicillin, chloramphenicol, tetracycline, sulfonamides, streptomycin and trimethoprim (61).

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 (14).

In a study conducted in Hawassa, Ethiopia, 11 *Shigella flaxneri* and 4 salmonella species were isolated. Among these all *Shigella flexneri* showed multiple drug resistance (MDR)

(resistance to two or more drugs), and of these, 3 (27.3%), 3(27.3%) and 1(9.1%) of *Shigella flexneri* showed high resistance against Amoxicillin (100%), Erythromycin (90.9%) and Ampicillin (63.6%). However, low resistance rate was observed against Gentamicin (27.3%) and Chloramphenicol (9.1%) and there was no resistance rate observed against Ciprofloxacin, Nalidixic acid, and Cotrimoxazole. The overall rate of resistance of *Salmonella* species was high for Erythromycin (100%) and Ceftriaxone (75%). But, lower resistance rate was observed against Nalidixic acid (25%). Among *Salmonella* serogroups, Serogroup B showed high resistance against Erythromycin (100%), and Ceftriaxone (100%). Similarly Serogroup A showed 100% resistance against both Erythromycin, and Nalidixic acid (13).

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 (16).

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 norflaxacin 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) (9).

The various studies reviewed above indicated the magnitude of the problem which this study tries to address in a less investigated area of the country in Northeastern Ethiopia.

2.8. Conceptual frame work

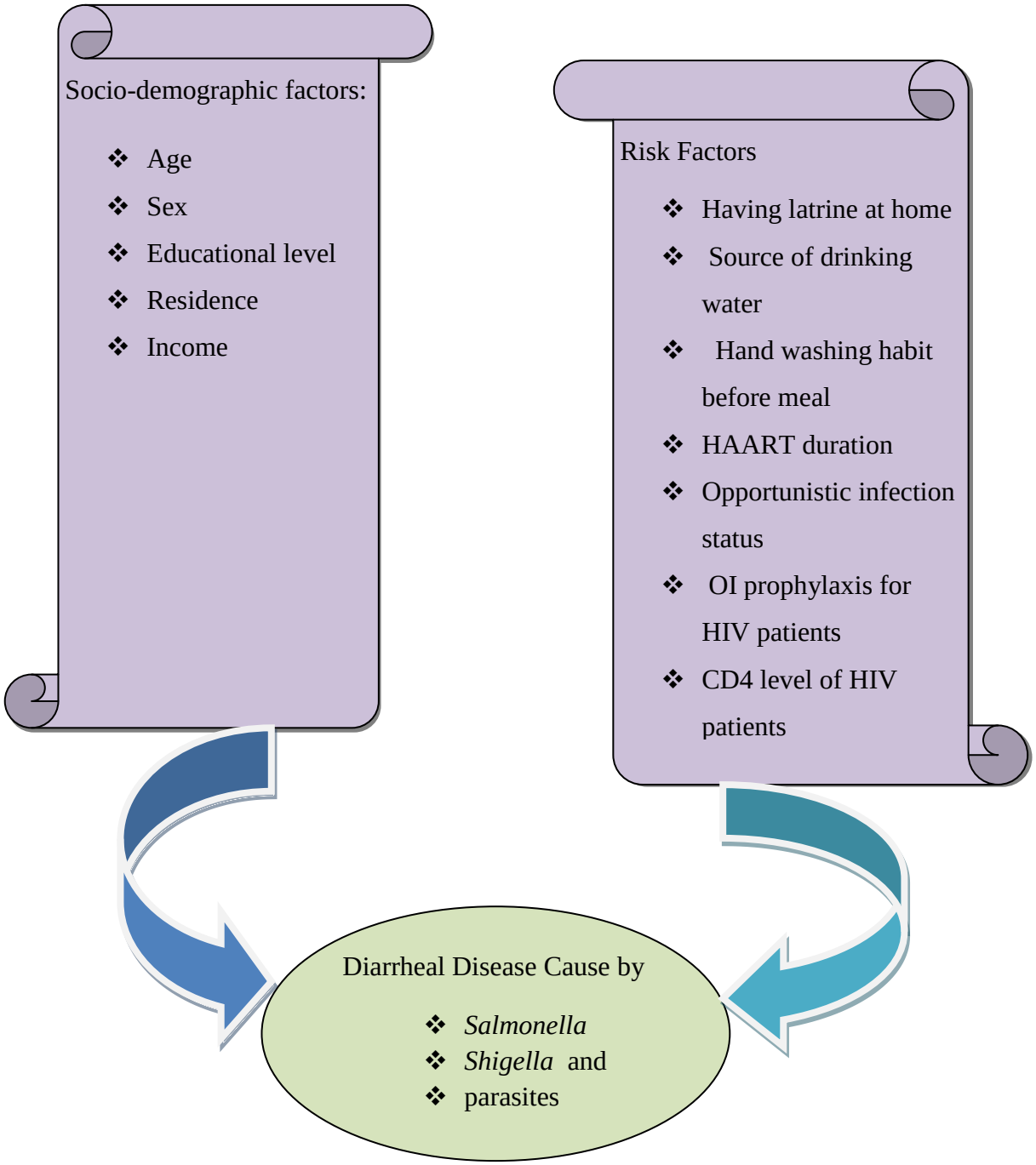

Figure 1: Conceptual framework of the study

3. Objectives

3.1. General Objective

To determine the prevalence of Intestinal parasite, *Salmonella* and *Shigella* Species and antimicrobial susceptibility patterns among HIV infected and non-infected patients with diarrhea in selected health facilities, Dessie town, Northeast Ethiopia.

3.2. Specific Objectives

- To assess the prevalence of *Salmonell*a and *Shigella* species among HIV infected and non-infected patients.
- To determine antimicrobial susceptibility pattern of *Salmonella* and *Shigella* species isolates
- To determine the prevalence of intestinal parasite among HIV infected and non-infected patients.
- To identify risk factors associated with bacterial infection and intestinal parasite among study participants.

4. Materials and methods

4.1. Study area

This study was conducted at Dessie Referral Hospital, Dessie Health center and Banbua wuha health center, which are located in Dessie town administration, Amhara Regional state, Ethiopia. Dessie town is found in the Northeastern part of Ethiopia, 401kms north of the capital city, Addis Ababa. The city is one of the fastest growing urban areas in the country and covers an area of 15.08 square kilometers. Based on the 2007 census conducted by the Central Statistical Agency of Ethiopia (CSA), Dessie has a total population of 151,174, of whom 72,932 are men and 78,242 women; 120,095 or 79.44% are urban inhabitants living in the town of Dessie, the rest of the population is living at rural kebeles around Dessie. The city has one referral hospital, one general hospital (Boru Meda), six health centers (Dessie, BanbuaWuha, Segno Gebeya, Tita, Kurkur, Meytero), four private hospitals and several private clinics. Dessie Referral Hospital is one of the largest hospitals in the region which is providing all patient diagnosis and treatment service for the community. Dessie Referral Hospital is governed by Regional Health Bureau. The hospital serves patients from all parts of the region (which comprises about 4 million people) and other neighborhood regions such as Afar and Oromia regional states. This research was conducted in Dessie referral hospital, and two randomly selected health centers namely, Dessie health center and BanbuaWuha health center (62).

4.2. Study design and period

Health facility based cross sectional study was conducted from January 2018 to April 2018 G.C in Dessie Ethiopia.

4.3. Study population

4.3.1 Source population:

All HIV infected and non-infected individuals who develop diarrhea and attended their care in public health facility of Dessie town.

4.3.2. Study population

All HIV infected and non-infected who have diarrhea and attending public health facilities of Dessie town during the study period and fulfilling the eligibility criteria.

4.4. Inclusion and exclusion criteria

4.4.1 Inclusion criteria

HIV infected and non-infected patients of both sexes and age greater than or equal to 15 year with diarrhea, volunteering to participate in the study were included.

4.4. 2 Exclusion criteria

- Critically ill or Patients on antibiotic treatments within the last 14 days

4.5 Study Variables

4.5.1 Dependent Variable

- Prevalence of *Salmonella* and *Shigella*
- Prevalence of intestinal parasite
- Antimicrobial susceptibility pattern

4.5.2. Independent Variable

Socio demographic variables include:

- Age
- Sex
- residence
- educational status
- income

Risk factor

- Having latrine at home and utilized
- Source of drinking water
- Hand washing habit before meal
- CD4 level of HIV patients

4.6. Measurement and Data collection

4.6.1. Sample size calculation

Sample size was determined using two population proportion formula as applied in estimated another studies (46,47).

$$\text{Study participants: } n = \frac{[p_1(1 - p_1) + p_2(1 - p_2)]}{(p_1 - p_2)^2} \times cp,power$$

The sample size for the study was calculated epi info by considering a 95% level of confidence, 80% power of studies done in Addis Ababa prevalence of 50% intestinal parasites of HIV positive (46) and Jimma 34% prevalence of HIV negative (47) respectively.

By this calculation, the sample size is 354, included 10% contingency in the study. The proportion of HIV infected and non HIV infected study participants was 1:2. As a result, 112 HIV infected and 242 none HIV infected diarrheic patients were included in the study.

4.6.2. Sampling technique

A convenient sampling technique was used to enroll consecutive outpatients with acute or chronic diarrhea attending the health facilities during the study period fulfilling the eligibility criteria until the required sample size was achieved.

4.6.3. Data collection procedure

Interview methods were used to obtain information. Data was collected from outpatients with acute or chronic diarrhea attending the medical and ART clinic of Dessie referral Hospital, Dessie health center and Banbua wuha health center. Demographic characteristic and risk factors such as educational status, having latrine at home, source of drinking water, hand washing habit before meal, history of HIV status, and CD4level, duration of diarrhea was collected by data collectors.

4.6.4. Sample Collection, Handling and Transport

Patients included in the study was given pre-labelled (with date, time, identification code, age), leak proof, wide mouth, sterile, screw-capped, sterile tube having or not Carry Blaire transport media to bring stool specimen after appropriate collection instruction. Fresh stool specimen was collected and then transported in ice cold box to Dessie Health Research Regional laboratory and processed within two hours if not used transport media.

4.6.5. Bacterial Culture and Identification

Freshly passed stool was collected; the sample was immediately transported to the laboratory after collection. If there is a delay in transporting fecal specimens, placed in Cary

Blair transport medium unless transported to the laboratory and processed within two hours of collection. For isolation of *Shigella* and *Salmonella* species, specimens were placed in Selenite F enrichment broth by using calibrated wire loop (0.001ml) and incubated at 37°C for 24 hours, then sub cultured onto xylose lysine deoxycholate (XLD) agar, *Salmonella* and *Shigella* (SS) agar and then incubated at 37°C for 18-24 hours. The growth of Salmonella and Shigella species was detected by their characteristic appearance on XLD agar (*Shigella*: red colonies, *Salmonella* red with a black center). On the other hand, on SS agar(colorless colony for shigella, colorless colony with black center) for salmonella. Control strains were *Salmonella typhimerium*(ATCC14028; *Shigella flexneri* ATCC12022, *Escherichia .coli* 25922 inhibited or pink colony in SS agar, Yellow colony on XLD agar, *Enterococcus facalise* 29213 inhibited were performed parallel to test to assure the isolation. The suspected colonies were further tested through a series of biochemical tests to identify *Shigella* and *Salmonella* species. The isolates were characterized based on the following standard biochemical tests. Indole Test, Urea test, (hydrogen sulphide production, gas production test, lactose fermenting (LF) and non-lactose fermenting (NLF) colonies) using triple sugar iron agar, Citrate Utilization test, Motility test, lysine decarboxylase test(LDC) (31).

4.6.6 Stool analyses of intestinal parasites

Fresh specimens were first examined as wet mounts using normal saline followed by Formal-ether concentration. After wet mounts examination, followed Formal-ether concentration. 1g of stool was placed in a clear conical centrifuge tube containing 7 ml formalin ether by using applicator stick. The resulting suspension was filtered through a sieve into another conical test tube. After adding 3 ml of diethyl ether to the formalin solution, the content was centrifuged at 3000 rpm for 5 minutes. The supernatant was poured away. Finally, smear was prepared from the sediment and observed under light microscope with a 40 objectives for *Isospora beli*. Air-dried smears were stained by modified Ziehl-Neelsen technique for *Cryptosporidium* and *cyclospora* oocysts. The smears were fixed in methanol for 2-3 minutes, stained with carbol fuschin for 15 minutes, decolorized in 1% acid alcohol for 15-30 seconds, washed in running tap water, counter-stained with 0.5% methylene blue for 30 seconds, washed in running tap water, and air

dried. Examine the smear microscopically for oocysts, using a low power magnification to detect the oocysts and the oil immersion objective to identify them (59).

4.6.7 Antimicrobial Susceptibility Testing

The antimicrobial susceptibilities of all identified bacterial isolates were performed according to the criteria of Clinical and Laboratory Standards Institute (CLSI) 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 after drying the plate for 3-5 minutes and incubated at 37^{o}C for 24 hours. Diameters of the zone of inhibition around the discs were measured using a digital meter caliper. The reference strains used as control were *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Shigella flexneri* ATCC 12021, *Salmonella typhimurium* ATCC 14028.

The following antimicrobials ware used with their respective concentration: Amoxicillin (AML, 25µg), Ampicillin (AMP, 10µg), Tetracycline (TTC 30-µg), Trimethoprim-sulfame thoxazole (SXT, 1.25/23.75µg), Chloramphenicol (CAF, 30-µg), Naldixic acid (NAL, 30µg) 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* in the study area. The results then interpreted according to CLSI guidelines antimicrobial susceptibility breaking points 2018 recorded as sensitive (s), intermediate (I) or resistance(R) according to CLSI 2018 (63). All antibiotics were obtained from Abtek Ltd.

4.7. Data quality assurance

To generate quality and reliable data, all quality control checks were done before, during and after data collect. All the questions in structured questionnaire were prepared in a clear and precise way and translated into local language (Amharic). Data collectors were informed

how to collect; the entire questionnaire were checked for completeness, during and after data collection by the principal investigator. Moreover, all laboratory assays were done by maintaining the quality control procedures. The raw data (the laboratory, clinical and demographic data) were checked for completeness and representativeness prior entry to the database.

Standard Operating Procedures (SOP) were strictly followed verifying that media meet expiration date and quality control parameters per CLSI guideline. Visual inspections of cracks in media or plates, contamination were performed. QC was performed to check the quality of medium. Cary-blair transport media was checked for its viability for one week by inoculating control strains and successful to store salmonella or shigella species up to one week 2-8^{o}C.Used control organisms for each isolated salmonella and shigella Control strains were Salmonella spp ATCC13076; Shigella spp ATCC12022. The isolated pathogens were identified using conventional biochemical tests. The microbiology media were purchased from OXOID, HIMEDIA laboratories pvt Ltd, Liofilchem Ltd and all antimicrobial disks were purchase Abtek biological Ltd with expiration date 2020.

4.8 Data analyses

The data generated was entered in to the Microsoft-Excel spreadsheet 2010 (Microsoft Cop., USA) every day. The data was imported and analyzed by Statistical Package for Social Sciences (SPSS) version 22.0. (IBM, USA). Descriptive statistics was computed and data was presented using figures and tables. Binary logistic regression was used to show the association of different variables with the dependent variable. Moreover, a multivariate analysis was computed to identify factors that are independently influence the occurrence of dependent variables. P-value less than 0.05 was considered statistically significant in all analysis.

4.9 Ethical considerations

This study obtained ethical approved from the Department Research Ethics Review Committee (DRERC) of Department of Medical Laboratory Sciences, school of College of Allied Health Sciences, collage of health, Addis Ababa University(AAU) and ethical clearance was obtained. Official cooperation letters was we obtained all levels such as AAU, Amhara Regional Health Bureau and zonal health offices. 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 were 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 were informed to physicians and the participants get their results and treatment duly as required.

4.10. Dissemination plan for results

The result of this study will be disseminated to concerned bodies including the hospital, health centers, zonal and regional health offices. The results will be submitted 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.

4.11 Operational Definitions

Diarrhea: - at least three loose stools in 24 hours, any number of watery, bloody and bloody mixed with watery stool accompanied by at least one of the following symptoms: nausea, vomiting, abdominal cramps or fever.

Acute diarrhea: -diarrhea that starts quickly and lasts a short time, usually 1 to 3 days. It can last up to 2 wks (14days). There may be cramp pain, or feel like patients are passing water. Patients may not be able to control their diarrhea. It diarrhea usually stops on its own.

Bloody diarrhea: - a type of diarrhea potentially critical in which there is blood mixed with loose, watery stools. The blood can arise from anywhere along your digestive tract, from the mouth to the anus. it is often a sign of gastrointestinal bleeding due to injury or disease.

Watery diarrhea: -often characterized as frequent and watery bowel movement and is an intestinal disorder. It is causing abnormal and excessive loosening of stools, infrequent and urgent bowel movements and continence.

Mucoid diarrhea: diarrhea with the presence of considerable mucus in the stools.

Mucus +blood: - the presence of mucus mixed with blood within the diarrheal stools.

Multidrug resistance: - a bacterium that is simultaneously resistant for two or more antimicrobials belonging to different chemical classes.

5. Results

5.1 Socio-demographic characteristics

A total of 354 study participants were included in this study. Out of these, 112(31.6%) were HIV infected and 242(68.4%) were non HIV infected diarrhea case patients enrolled from three health facilities in this study. Above half of the participants 185(52.2%) were Dessie Referral Hospital followed by 121(34.2%) from Dessie Health Center and 48(13.6%) from Banbau waha Health Center, with 100% response rate. As shown in the Socio-demographic characteristics table of HIV infected and non HIV infected patients (Table 1) the mean age was 35.33±13.11 (SD) years. Comparatively, in the HIV infected and non-infected groups the distribution by sex revealed a predominance of female cases in HIV infected 76(67%) and 127 (52.5%) male in non HIV infected cases. Majority of the HIV infected and non HIV infected (73%) and (65.7%) were urban residents; respectively.

The assessment of educational status of HIV infected and non-infected involved in the survey showed (47.3.9%), 25.2% not formally educated, 35(31.3%), 54(22.3%) primary, 17(15.2), 65(26.6%) secondary and 7(6.3%), 62(25.6%) higher education status respectively. Moreover, 37(33%) of HIV infected and 72(29.6%) of non HIV infected study participants had < 500 birr monthly income (table 1).

Table 1: Distribution of Socio-demographic characteristics of HIV infected and non HIV infected diarrheal patients from selected health facilities of Dessie town, Amhara Regional State, Northeastern Ethiopia, From January to April 2018

Variables		HIV Status		Total N/% N =354
		N=112 HIV + N/%	N=242 HIV – N/%	
Health facilities	Dessie R Hospital	67(59.8)	118(77.7)	185(52.2)
	Buanba wuha HC	10(8.9)	38(15.7)	48(13.6)
	Dessie HC	35(31.3)	86(35.5)	121(34.2)
Age Group years	15-24	19(16.9)	66(27.3)	85(24)
	25-34	33(29.5)	62(25.6)	95(26.8)
	35-44	38(33.9)	55(22.7)	93(26.2)
	>44	22(19.6)	59(24.4)	81(22.8)
Sex	Male	36(32.1)	127(52.5)	163(46)
	Female	76(67.8)	115(47.5)	191(54)
Residence	Urban	82(73.2)	159(65.7)	241(68)
	Rural	30(26.8)	83(34.3)	113(31.9)
Education statuse	Not formally learn	53(47.3)	61(25.2)	114(32.2)
	Primary	35(31.3)	54(22.3)	89(25.1)
	Secondary	17(15.2)	65(26.9)	82(23.2)
	Collage/ University	7(6.3)	62(25.6)	69(19.4)
Occupational statuse	Civil servant	20(17.9)	44(18.2)	64(18)
	Private	32(28.6)	64(25.4)	96(27)
	Unemployed	49(43.6)	84(34.7)	133(37.5)
	Farmer	11(9.8)	50(20.7)	61(17.2)
Monthly income(Eth birr	< 500	37(33)	72(29.6)	109(30.8)
	501-1000	28(25)	35(14.5)	63(17.8)
	1001-1500	9(8.0)	44(18.2)	53(15.0)
	1501-2000	17(15.2)	32(13.2)	49(13.8)
	>2000	21(18.6)	59(24.4)	80(22.6)

5.2 Etiologic agents of diarrhea

In this cross-sectional comparative study among diarrheal patients at selected health facilities in Dessie Ethiopia, the overall prevalence of enteric pathogens in stool samples was found to be 100(28.2%). Among these 76(21.5%) were intestinal parasite and 24(6.8%) were bacterial

infections. The prevalence of intestinal parasite and bacterial infection among HIV infected was 26(23.2%) and 8(7.1%) respectively. Of the bacterial isolates, the predominately isolated bacteria was *Salmonella* 6(5.4%) followed by *Shigella* 2(1.8%). Moreover, the most prevalent parasite was *C. parvum* 9(8%), *E. histoletica/dispar* 8(7.1%), followed by *G.lamblia* 4(3.6%) while 1(0.9%) patients had co-infection of *C. parvum* and *C. catananyesis*.

The prevalence of intestinal parasites and bacterial infection in non HIV infected patients was 50(20.7%) and 6.6% respectively. The most prevalent intestinal parasite in non HIV infected patients was *E. histoletica/despar* 39(16.1%) and *G. lambelia* accounted for 8(3.3%).

Table 2: Prevalence of bacterial and intestinal parasite in HIV infected and uninfected patients selected health facilities in Dessie Ethiopia, between January to April 2018.

Enteric Pathogen	HIV +(N = 112) No. (%)	HIV – (N =242) No. (%)	Total (N=354) No. (%)
Bacteria	**8(7.1)**	**16(6.6)**	**24(6.8)**
Salmonella sps	6(5.4)	11(4.5)	17(4.8)
Shigella sps	2(1.8)	5(2.1)	7(2.0)
Intestinal parasites	**26(23.2)**	**50(20.7)**	**76(21.5)**
E. histolyetica/despar	8(7.1)	39(16.1)	47(13.3)
G.lambelia	4(3.6)	8(3.3)	12(3.4)
Teania sps	2(1.8)	0(0)	2(0.56)
Cryptospordium parvum	9(8.0)	0	9(2.5)
C. parvum & C. catanyase	1(0.9)	0	1(0.3)
Ascaris. lumbricoides	0(0)	2(0.8)	2(0.56)
E. vermicularis	2(1.8)	1(0.4)	3(0.8)
Total	**34(30.4)**	**66(27.3)**	**100(28.2)**
Co-infection	**3(2.8)**	**1(0.4)**	**4(1.1)**
Shigella & C.parvum	2(0.6)	0	2(0.6)
Salmonella & A.labricoides	0	1(0.3)	1(0.3)
Salmonella & Teania	1(0.3)	0	1(0.3)

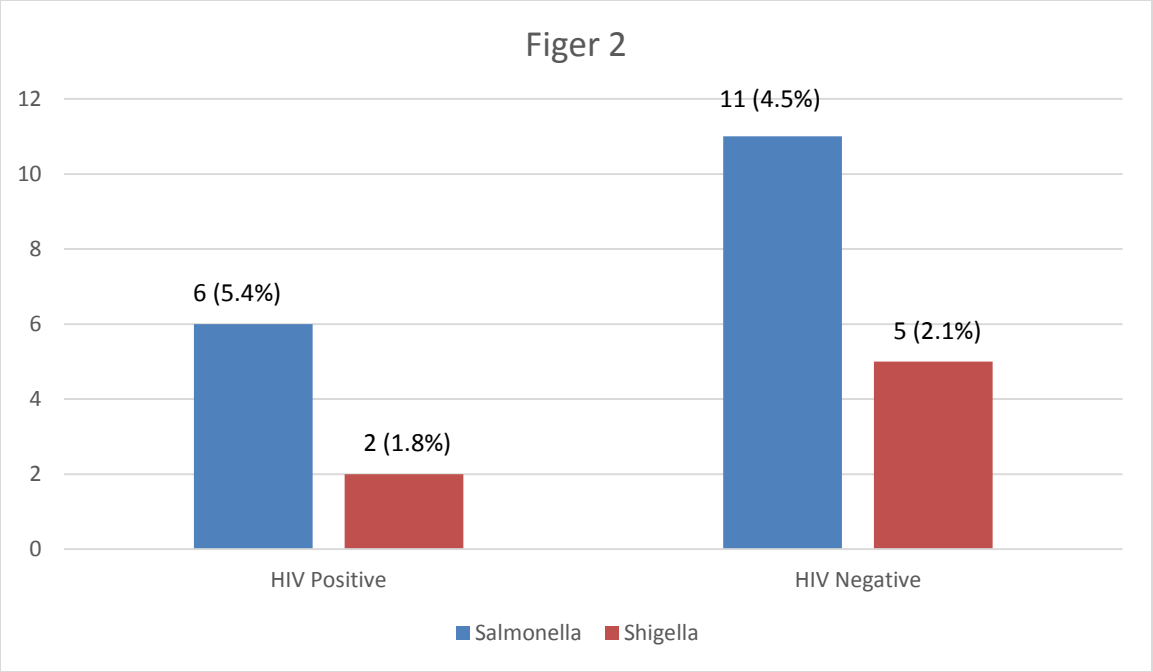

Figure 2: Prevalence of bacterial in HIV infected and uninfected patients selected health facilities in Dessie Ethiopia, between Januarys to April 2018.

5.3 Risk factors of bacterial infection

In this study, 13 independent variables were considered during the bivariate analysis of risk factors for bacterial diarrheal patients. Relatively higher prevalence of bacteria were found among the age group of 15-24 years, educational status of illiterates, farmers, not utilized latrine, who drink river or spring water and had mucoid plus bloody diarrhea. In multivariate analysis, patients had bloody + mucoid diarrhea (AOR= 5.982, 95%CI: (1.41-68.833), P<0.026), did not utilize latrine (AOR =6.407 95%CI: (1.139-36.024) P=0.035 and use river or spring water for drinking (AOR = 8.641, 95%CI: (1.983-37.656), P=0.004) were found to have statistically significant association with bacterial infection. Out of a total of 24 diarrheic patients who had statically significant bacteria, 4(16.7%) had a bloody plus Mucoid diarrhea (P<0.025), 16(66.7%) were not utilizing latrine (P=0.035) and 17(70.8%) river or spring water for drinking (P=0.004). There was no statically significant association between bacterial infection and adult's age, sex, residence, occupation, monthly family income, diarrhea duration, CD4 level and hand wash habit before meal (Table 3).

Table 3: Associations of risk factors for bacteria agents among study participant attending selected health facilities of Dessie, Ethiopia, between January to April 2018.

Variable	Bacterial infection		P.V	COR(95% C.I)	P.V	AOR(95%CI)
	Positive	Negative				
Residence						
Urban	11(4.6)	230(95.4)	1		1	
Ruler	13(11.5)	100(88.5)	0.019	2.718(1.178-6.275)	0.95	0.271(0.58-1.257)
Occupation						
C. servant	1(1.6)	63(98.4)	1		1	
Privet	4(4.2)	92(95.8)	0.37	2.74(0.29-25.85)	0.865	0.795(0.057-11.17)
Unemployed	12(9.0)	121(91.0)	0.08	6.25(0.79-49.15)	0.794	0.682(0.038-12.15)
Farmer	7(11.5)	54(88.5)	0.05	8.2(0.97-68.49)	0.156	0.095(0.004-2.446)
Education status						
Illiterate	13(11.4)	101(88.6)	0.04	8.8(1.12-68.47)	0.534	2.62(0.127-54.06)
Primary	5(5.6)	84(94.4)	0.20	4.05(0.46-35.48)	0.935	1.13(0.058-22.16)
Secondary	5(6.1)	77(93.9	0.18	4.42(0.50-38.74)	0.427	3.16(0.185-54.12)
Higher	1(1.4)	68(98.6)	1		1	
Monthly Income Ethio. Birr						
<500	14(12.8)	95(87.2)	0.04	3.78(1.05-13.6)	0.549	1.974(0.24-18.224)
501-1000	4(6.3)	59(93.7)	0.47	1.74(0.375-8.07)	0.814	0.765(0.082-7.152)
1001-1500	1(1.9)	52(98.1)	0.55	0.49(0.05-4.876)	0.597	0.480(0.032-7.261)
1501-2000	2(4.0)	47(96.0)	0.92	1.09(0.176-6.78)	0.686	0.611(0.056-6.644
>2000	3(3.8)	77(96.2)	1		1	
Diarrhea consistence						
Watery	11(4.4)	237(95.6)	1		1	
Mucoid	7(13.2)	46(86.8)	0.02	3.239(1.208-8.902)	0.238	1.983(0.636-6.182)
Bloody	2(6.3)	30(93.70	0.65	1.436(0.304-6.793)	0.476	0.532(0.094-3.017)
Blood + mucoid	4(19.0)	17(81.0)	**0.11**	**5.07(1.46-17.6)**	**0.026***	**5.982(1.41-68.833)**
CD4 Level						
<200cell/m^3	1(14.3)	6(85.7)	0.280	3.778(0.339-42.154)	0.749	1.974(0.24-12.254)
200-500cell/mm^3	4(11.8)	30(88.2)	0.164	3.022(0.637-14.344)	0.814	0.765(0.082-7.142)
>500cell/m^3	3(4.2)	68(95.8)	1			
Hand wash before meal						
Hand wash without soap	18(13.8)	112(86.2)	0.00	5.839(1.459-17.618)	0.539	1.624(0.345-7.638)
Hand wash with soap	6(2.7)	218(97.3)	1		1	
Latrine utilization						
Utilized	8(2.8)	280(97.2)	1		1	
Not utilized	16(24.2)	50(75.8)	**0.00**	**11.2(4.552-27.56)**	**0.035***	**6.407(1.139-36.024)**
Water source						
Pipe	7(2.4)	279(97.6)	1		1	
Not pipe	17(25)	51(75.0)	**0.00**	**13.286(5.245-33.654)**	**0.004***	**8.641(1.983-37.656)**

Note: *Statistically significant at P<0.05.AOR = adjusted odds ratio, COR = crude odds ratio, 1 = reference group, 95% CI = 95% confidence interval.

5.4 Associated risk factors of parasitic infection

In this study, 13 independent variables were considered during the bivariate analysis of risk factors for intestinal parasites in diarrheal patients. Relatively higher prevalence of intestinal parasites were found among the age group of 15-24 years, rural residence, educational status of illiterates, occupational status of unemployed, among study participants not utilized latrine, who drink river or spring water, monthly income below 500 birr and duration of diarrhea elapsed 6-10 days. In multivariate analysis, participants who did not learn formal education (illiterate) (AOR 5.8, 95%CI (1.62-21.04), (P=0.007), education primary level (AOR=3.7 95%CI(1.00-13.744), (P= 0.05), duration of diarrhea elapsed 6-10 days (AOR = 2.039, 95%CI(1.094-3.80), (p= 0.025), CD4 level between 200-500 (AOR=6.48(2.144-19.592), (P=0.001) and those who did not wash hand properly with soap (AOR=3.02 95%CI(1.5-6.23) P=0.003 were found to have statistically significant association with intestinal parasites. Out of a total of 76 diarrhea patient who had significant intestinal parasite, 37(48.7%) were not educate formal educated (illiterate) (P=0.007), 29(38.2%) had diarrhea elapsed 6-10 days, 13(38.2%) had CD4 level between 200-500 (AOR=6.48(2.144-19.592)(P=0.001 and 47(61.8%) did not wash hands properly with soap (P=0.003). There was no statically significant association between intestinal parasite and adult age, sex, residence, occupation, monthly family income, diarrhea consistence, HIV status, latrine utilization, water source (table 4).

Table 4 : Associations of risk factor for intestinal parasites among study participant attending selected health facilities of Dessie, Ethiopia, between Januarys to April 2018

Variable	Intestinal parasite		P.V	COR(95% C.I)	P.V	AOR(95% C.I)
	Positive	Negative				
Residence						
Urban	42(17.4)	199(82.6)	1		1	
Ruler	34(30.0)	79(70.0)	0.007	2.04(1.21-3.44)	0.874	0.947(0.48-1.866)
Occupation						
C. servant	5(7.8)	59(92.2)	1		1	
Privet	15(15.6)	81(84.4)	0.151	2.2(0.75-6.35)	0.901	1.078(0.332-3.495)
Unemployed	32(24.0)	101(76.0)	0.009	3.7(1.4-10.12)	0.882	1.093(0.335-3.563)
Farmer	24(24.0)	76(76.0)	0.000	7.6(2.68-21.8)	0.240	2.421(0.553-10.59)
Educational status						
Higher	3(4.3)	66(95.7)	1		1	
Illiterate	**37(32.5)**	**77(67.5)**	**0.00**	**10.6(3.2-35.9)**	**0.007***	**5.8(1.62-21.04)**
Primary	**21(23.6)**	**68(76.4)**	**0.003**	**6.79(1.9-23.86)**	**0.05***	**3.7(1.00-13.744)**
Secondary	15(18.3)	67(81.7)	0.015	4.93(1.36-17.8)	0.060	3.5(0.95-13-744
Monthly Income(Ethi birr)						
<500	36(36.3)	73(73.7)	0.000	5.14(2.15-12.3)	0.308	1.738(0.60-5.031)
501-1000	14(22.2)	49(77.8)	0.028	2.98(1.12-7.91)	0.669	1.276(0.418-3.897)
1001-1500	9(17.0)	44(81.0	0.160	2.13(.74-6.13)	0.907	1.072(0.337-3.411)
1501-2000	10(20.4)	39(79.6)	0.064	2.67(.94-.57)	0.372	1.667(0.543-5.118)
>2000	7(8.8)	73(91.2)	1		1	
Diarrhea duration						
1-5	45(17.3)	215(82.7)	1		1	
6-10	**29(34.9)**	**54(65.1)**	**0.001**	**2.56(1.48-4.47)**	**0.025***	**2.039(1.094-3.80)**
>10	2(18.2)	9(81.8)	0.940	1.06(.222-5.08)	0.563	1.629(0.312-8.50)
CD4 Level						
<200cell/m^3	3(42.9)	4(57.1)	0.690	4.575(0.88-23.57)	0.118	4.058(0.701-23.499)
200-500c/mm^3	13(38.2)	21(61.8)	**0.007**	**3.776(1.44-9.883)**	**0.001***	**6.48(2.144-19.592)**
>500cell/m^3	10(14.1)	61(85.9)	1		1	
Hand wash before meal						
Hand wash with soap	29(12.9)	195(87.1)	1		1	
Hand wash without soap	47(36.2)	83(63.8)	**0.001**	**3.8(2.24-6.47)**	**0.003**	**3.02(1.5-6.23)**
Latrine utilization						
Utilize	53(18.4)	235(81.6)	1		1	
Not utilize	23(34.8)	43(65.2)	0.004	2.37(1.32-4.27)	0.529	1.86(0.501-3.836)

Note: *Statistically significant at P<0.05.AOR = adjusted odds ratio, COR = crude odds ratio, 1 = reference group,95% CI = 95% confidence interval,

5.5 Antimicrobial susceptibility pattern

The present study obtained susceptibility status of both *Salmonella* and *Shigella* isolates to Ceftriaxone 24(100%), Ciprofloxacin 24(100%) and Nalidixic acid 17(70.8%) however, all 24(100%) showed antimicrobial resistance to ampicillin and Amoxicillin. All 17(100%) *Salmonella* spp isolates were resistant to Ampicillin and amoxicillin, while all 17(100%) sensitive to ciprofloxacin and ceftriaxone. All *shigella* spp isolates 7(100%) were resistant to Ampicillin and amoxicillin while 6(85.7%) were susceptible to ciprofloxacin and ceftriaxone 7(100%). Moreover, tetracycline, cotrimoxazole, chloramphenicol and nalidixic acids showed variable antimicrobial resistance as shown in table 5.

Table 5: Antibiotic susceptibility of Shigella and Salmonella isolates among diarrheic patients in Dessie, Ethiopia, between January to April 2018.

		Antimicrobial Agents							
Isolates (n)	pattern	AMP	AMX	TTC	CAF	COT	NAL	CRX	CIP
Salmonella(17)	S	0(0.0)	0(0.0)	9(52.9)	11(64.7)	10(58.8)	14(82.4)	17(100)	1(100)
	I	0(0.0)	0(0.0)	6(35.4)	3(17.6)	3(17.6)	2(11.8)	0(0.0)	0(0.0)
	R	17 (100)	17 (100)	2(11.8)	3(17.6)	4(23.5)	1(5.9)	0(0.0)	0(0.0)
Shigella(7)	S	0(0.0)	0(0.00)	4(57.1	5(71.4)	4(53.1)	4(53.4)	7(100)	6(85.7)
	I	0(0.0)	0(0.0)	1(14.3)	1(14.3)	(0.0)	1(14.3)	0(0.0)	0(0.0)
	R	7(100)	7(100)	2(28.6)	1(14.3)	3(46.9)	2(28.6)	0(0.0)	1(14.3)
Total(24)	S	0(0.0)	0(0.0)	13(54.2)	16(66.7)	14(58.3)	17(70.1)	24(100)	23(95.8)
	I	0(0.0)	0(0.0)	7(29.1)	4(16.7)	3(12.5)	4(16.7)	0(0.0)	0(0.0)
	R	24(100)	24(100)	4(16.7)	4(16.7)	7(29.2)	3(12.5)	0(0.0)	1(4.2)

Key:

S-Susceptibility I- Intermediate, R-Resistance, Ampicillin-AMP, Amoxicilline-AMX, Tetracycline-TTC, chloramphenicol- CAF, Nalidixic acid, Ceftriaxone-CRX, Ciprofloxacin-CIP

5.6 Multi-drug resistant isolates

Among *Salmonella* isolates, 13(76.5%) of 17 were multidrug resistant, four spp (23.5%) of which showed resistance to two to three antimicrobials, seven (41.2%) of them showed resistance to four antimicrobials and two spps (11.8%) were resistant to six antimicrobials. Regarding, *Shigella* isolates, 6(85.7%) of 7 showed multidrug resistance, one (14.3 %) of the isolates showed resistance of two antimicrobial, two (28.6%) of the isolates showed resistance to three to four antimicrobials and one (14.3%) was resistant to six antimicrobials. Multidrug resistance (MDR) was seen in 19(79.2%) of 24 isolated bacterial infections as shown in table 6.

Table 6: Multi-drug resistance of Salmonella and shigella isolates from diarrheal patients in selected health facilities of Dessie, Ethiopia, from January- April, 2018.

Bacteria		**Antimicrobial resistance pattern**							
isolated	**Total**	R0	R1	R2	R3	R4	R5	R6	MDR
Salmonella	17(70.8)	0(0.0)	0(0.0)	4(23.5)	4(23.5)	7(41.1)	2(11.8)	0(0.0)	13(76.5)[a]
Shigella	7(29.2)	0(0.0)	0(0.0)	1(14.3)	2(28.6)	3(37.5)	0(0.0)	1(14.3)	6(85.7)[b]
Total	24(100)	0(0.0)	0(0.0)	5(20.8)	6(25)	10(41.7)	2(8.3)	1(4.2)	19(79.2)[c]

R0 = No antibiotic resistance, R1 = Resistance to one, R2 = Resistance to two, R3 = Resistance to three, R4 = Resistance to four, R5 = Resistance to five, R6=Resistance to six and more drugs, MDR = Multi-drug resistant, a= Percent is computed from total number of salmonella, b= Percent is computed from total number of shigella isolates, c= Percent is computed from total number of isolates based on which MDR definition is applied.

6. Discussion

In this study among diarrheal patients at selected health facilities in Dessie Ethiopia, the prevalence in terms of HIV infected and non HIV infected patients were (23.2%), (21.7%) for intestinal parasites and (7.1%), (6.6%) for bacterial infections respectively.

The prevalence of *Shigella* in HIV infected patients was found to be 1.8%. This result was relatively consistent with studies done in Gondar northwest part of Ethiopia (26), Pune India (8), Kampala Uganda (41) and where (3.5%), (3.7%), (2.1%) Shigella isolates have been reported, respectively. However, our finding was lower compared to a 4%,5.3%, 8.1%, prevalence of *Shigella* species isolated from a study done in Jimma southwest part of Ethiopia, Lima Peru and Nigeria respectively (26,40,42). This variation might be due to age group, socioeconomic factor, the nature of the public water supply and seasonal variations.

Of the study participants who were HIV infected 6 (5.4%) were found to be infected with *Salmonella* spp. This finding was in line with a study from Kampala Uganda (4.3%) (41). and higher than study done in Nigeria (1.3%) and Lima Peru (2%) (42, 40). However; our finding was lower than studies from southwest part of Ethiopia Jimma (8.1%) (26).

The prevalence of bacterial infection among non HIV infected patients was 16(6.6%). Among these *Salmonella* accounts 11(4.5%), this study was in line with studies done in Hawassa, southern parts of Ethiopia, Gaborone Botswana and Kampala Uganda where 2.5%, 3% and 6.3% respectively (13, 43, 41). Our finding was higher than studies done in Gondar 1.08% (24), 1.04% (14). However, our finding was lower than studies done in Harar and Butajira (11.5%), (10.5%) respectively (16,25). This might be due to those studies done more than four years ago and the awareness of population now a day better than before four year because health extension works and health professionals increased in number and health facilities are available.

The isolation rate of *Shigella* species among HIV negative 5(2.1%) in our study was relatively comparable to the study done in Jimma (2.3%) and Kampala Uganda (3.5%) (45,41). Our result is lower when compared to the previouse studies conducted in North west Ethiopia (24), in Harar (16), in Gondar (14) in Ethiopia and Gaborone Botswana(43) in Africa country, in which the prevalence rates 8.7%, 6.7%, 16.9%, 21% *Shigella* isolate have been reported respectively. This might be due to differences in awareness of the people about personal and environmental hygiene from the continuous health education made by the different health

educators in the different health institutions against of shigellosis.

Intestinal parasites were detected in 23.2% of the HIV/AIDS patients and 20.6% of the HIV negative patients. The prevalence of intestinal parasites detected in the HIV positives was slightly higher than patients in HIV negative. Our finding was low compared to studies conducted in Addis Ababa (50.3%, 41.1%) (46), southwest Ethiopia (44.8%, 37.8%)(47), Bahir Dar (80.3%, 33.3%)(49), Hawassa (59.8%, 48.8%)(51), Cameroon (59.5%. 9.32%)(4) in Africa continent and Lima Peru (55%, 21%)(40) in Latin America, for HIV positive and HIV negative reports respectively. The reports of Cameroon were low on HIV negative and reports of Lima Peru were in line with compare with our finding. The low rate of isolation as observed in the present study might be due to the increasing awareness of the people about personal and environmental hygiene made by the health institutions and other partners.

In this study, the prevalence of *cryptosporidium* spps was 9(8.0%). This result was nearly similar with findings from selected ART centers of Adama, Afar and Dire-Dawa (8%), southwestern Ethiopia (11%), Cameroon in Africa (12.6%) and Pune India(14.8%) in Asia continents (50, 47,44,8). This result was higher compared to reported from Dessie Ethiopia (1.5%) (52). However, our finding was lower compared to Addis Ababa (25%), Bahir Dar (43.6%), Hawassa (20%), in Ethiopia, Cameroon (19%) in African continent and Lima Peru (20%) in Latin America (46, 49, 51, 4, 40). This low prevalence in this study might be due to early starting ART, time gap where those studies were done but nowadays there is a better awareness of the patients about ART treatment, intestinal parasite infection and their cause.

This study showed that bacterial agents of *Salmonella* and *Shigella* infections have been statistically significant associated with bloody plus mucoid diarrhea in which Salmonella and *Shigella* infected individuals had 5.98 time higher association with bloody plus mucoid diarrhea than with watery diarrhea. This is might be due to the ability of the those bacteria to destruct tissue, invade and replicate in cells lining the colon and rectum. The bacteria serotype is also another cause of bloody plus mucoid diarrhea in the stool samples.

Absence of latrine at home or not utilizing latrine and source of drinking water was the predisposing factors of bacterial infection as evidenced by multivariate analysis of this study.

Patient who did not utilize latrine had been 6.407 more likely to have bacterial infections of *Salmonella* and *Shigella* than patients who were utilizing latrine. This was due to the individuals defecated everywhere outside their environments (ineffective feces disposal) and then flies and other insects carry those wastes and transmit those diseases. This finding was supported by study done in Mekelle which showed 7.5 time risk in those who did not have toilet in their home than those who had toilet in their home (29).

Drinking water source was another factor that exposed patients in *Salmonella* and *Shigella* bacterial infections. Patients who drink river or spring water (untreated water) 8.641 were more likely to be associated with *Salmonella* and *Shigella* bacterial infections than who drink treated pipe water. This was due to those bacteria mainly transmitted person-to-person by the fecal-oral route, primarily by people with contaminated hands that was not washed with soap after toilet and drinking contaminated water or non-available of safe water.

In this study, participants who did not learn formal education (illiterate) was showed were 5.8 times more likely infected with intestinal parasite than patients who were learn in higher education and patient who were primary level more than 3 times likely to be exposed than who attained higher education This might be due to people who not learn formal education and primary level had low awareness about the transmission route of intestinal parasite.

Patients who had diarrhea elapsed for 6-10 days had more than two fold significant association with intestinal parasite positivity. As can be expected, patients who did not wash hands properly with soap before meal had 3 times higher risk of having intestinal parasitic infection than patients who wash hands properly with soap. This might due to most intestinal parasites had not sever and urgent case and so they persist long time with their.

In this study, the susceptibility pattern for all bacterial strains showed resistance to at least two drugs. The majority of the bacterial pathogens were resistant to two or more drugs tested, with ampicillin, amoxicillin, cotrimoxazole and tetracycline were not effective drugs. The main reason could be the frequent use of these antibiotics. Among the seventeen *Salmonella* spp isolates, the overall rate of resistance was high for Amoxicillin and Ampicillin (100%),

Tetracycline (47%), Cotrimoxazol (41%), Chloramphenicol (35.2%) and Nalidixic acid(17.6%) whereas 17 *Salmonella* spp were 100% sensitive to Ceftriaxone and Ciprofloxacin. This finding was comparable with the results of Gondar (26) Ampicillin (75%) Amoxicillin (100%), Cotrimoxazole (50%), Nalidixic acid (25.0%) resistance, Jimma (9) (100%) Ampicillin, (47%) Tetracycline, (26%) Nalidixic acid, Harar (16) (100%) resistance to Ampicillin and Amoxicillin, This study was high in contrast to the findings of Sudan that were 100% sensitive to chloramphenicol and 100% sensitive to tetracycline, 64% sensitive to ampicillin (6), and all isolates of *Salmonella* susceptible to all antibiotics like Ampicillin, Tetracycline, Cotriamoxole and Chloramphenicol reported in Gondar (14).

In this study, highest resistance was showed to Ampicillin 7(100%), Ampicillin 7(100%), 3(46%) Cotriamoxazol and 0.0% ceftriaxone and 14.3% Ciprofloxacin for *shigella* species. Similar findings have been reported by studies done at Harar (16), 100% 100%, Gondar (26), 94.1%, 88.2%, Mekelle (29) 100% Ampicillin and Amoxicillin resistance was reported, respectively. This could be due to the over use of these drug for many years. According to this study, therefore: Ampicillin, Tetracycline, Amoxicillin and SXT are no longer effective for the treatment of Shigellosis and Salmonellosis in the study area On the other hand, lower resistance (higher rate of sensitivity) were observed against ceftriaxone and ciprofloxacin which is an indicative of possible use of these drugs as an empiric therapy particularly in the study area. The possible justification for such low level resistance might be attributable to infrequent prescription of these drugs. Hence, they could be considered as alternative options in the treatment of *Salmonella* and *Shigella*.

5.1 Limitation

Due to lack of availability of antisera, serotyping was not performed to differentiate the isolates *Shigella* species (*S. dysentery, S. flexneri, S. boydii* and *S. sonnei*) and salmonella serogroups.

6. Conclusion and Recommendations

6.1 Conclusion

The prevalence of intestinal parasite and bacterial infection was high in HIV infected and non-infected diarrheal complaint patient especially *E.histoletca/dispar,* Cryptosporidium spps and salmonella were predominantly isolated in HIV infected diarrheic patients and E. *histolytic/despar* following *Giardia lamella* and Salmonella was high prevalent in HIV negative patients in this study. Drinking water source, absence of latrine, hand wash habit was statistically significantly associated with enteric pathogens. So treated water source supply, availability of latrine in the community to utilize properly and increasing awareness to improve hand wash habit could minimize prevalence of these enteric pathogens. On the other hand CD4 was statically significant association with intestinal parasite. So that frequent health education is important to raise patients CD4 level in the health facility

The most frequently prescribing drugs Ampicillin, Amoxicillin Tetracycline and Cotriamoxazole showed high resistance for *Salmonella* and *Shigella* isolates in the study. On the other hand Ceftriaxone and Ciprofloxacin was the best drug of choice for the treatment of diarrhea caused by *Salmonella* and *Shigella* in the study area.

6.2 Recommendation

Based on the findings, the following recommendations were forwarded:

- Patients who were not utilizing latrine, no habits of hand wash with soap before meal, and drank untreated water statistically significant association to enteric pathogen. We recommend attention to be improvement latrine utilization and availability, availability of safe water sources, hand wash habit improve and health education.
- We recommend to emerging routine modified techniques in ART clinic to screening opportunistic infection.
- Expanding culture facilities in district hospitals and general hospitals important to monitor antibiotic resistance.
- Prescribing Ciprofloxacin and Ceftriaxone are effective in areas where there is no culturing and antimicrobial susceptibility testing service availability.
- Further studies are important confirm serotyping characteristics of *Salmonella* and *Shigella.*

7. References

1.World Health Organization, Diarrhoeal disease fact sheet. Geneva, Updated 2017. Available from http://www.who.int/mediacentre/factsheets.

2. UNICEF/WHO, Diarrhoea: Why children are still dying and what can be done, 2009, ISBN 978-92-806-4462-3

3. Kibet SC, Matiru VN, Kibaba PW, Mucheke A and Louis H. Prevalence and Antibiotic Susceptibility Patterns of Shigella and Salmonella Causing Diarrhoea in Children Below 5 Years at Thika Level Five District Hospital. *wnofns.* 2017; 11: 28-36

4 . Nkenfou CN, Nana CT, Payne VK. Intestinal Parasitic Infections in HIV Infected and Non-Infected Patients in a Low HIV Prevalence Region, West Cameroon. *PLoS ONE* 2013; 8(2): e57914.

5. Dikman AE, Schonfeld E, Srisarajivakul NC, Poles MA. Human Immunodeficiency Virus-Associated Diarrhea: Still an Issue in the Era of Antiretroviral Therapy. *Dig Dis Sci*. 2015; 60:2236–2245.

6. Saeed A, Abd H and Sandstrom G. Microbial etiology of acute diarrhoea in children under five years of age in Khartoum, Sudan. *Journal of Medical Microbiology*. 2015; 64, 432–437.

7 . World Health Organization, Recommendations on the management of diarrhoea and pneumonia in HIV-infected infants and children: integrated management of childhood illness (IMCI), 2010, ISBN 978 92 4 154808 3.

8. Shah S, Kongre V, Kumar V. A Study of Parasitic and Bacterial Pathogens Associated with Diarrhea in HIV-Positive Patients. *Cureus* .2016; 8(9): e807.

9.Lamboro T, Ketema T, and Bacha K. Prevalence and Antimicrobial Resistance in Salmonella and Shigella Species Isolated from Outpatients, Jimma University Specialized Hospital, Southwest Ethiopia. *African J. Basic & Appl. Sci.* 2017; 9 (3): 118-125.

10 .Dagnew M, Tiruneh M, Moges F, Gizachew M. Bacterial Profile and Antimicrobial Susceptibility Pattern among Food Handlers at Gondar University Cafeteria, Northwest Ethiopia. *J Infect Dis Ther. J Infect Dis Ther.* 2013;1:2.

11.AIDSinfo, Guidelines for Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents. 2017; 1-12. https://aidsinfo.nih.gov/guidelines

12.Girma G. Prevalence, Antibiogram and Growth Potential of Salmonella and Shigella in Ethiopia: Implications for Public Health: A Review, *Res. J. Microbiol.* 2015; 10 (7): 288-307.

13.Getamesay, Getenet G, Ahmed Z, prevalence of shigella, salmonella and compylobacter species and their susceptibility patters among under five children with diarrhea in Hawassa town, south Ethiopia. *Ethiop J Health Sci*.2014; 24(2): 101-108

14.Huruy K, Kassu A, Mulu A, Gebretsadik S, Andargie G,Tadesse T, et al. High Level of Antimicrobial Resistance in Shigella Species Isolated From Diarrhoeal Patients in University of Gondar Teaching Hospital, Gondar, Ethiopia. *Pharmacology online.*2008; 2: 328-340

15. John Hopkins hospital Antibiotic Guidelines, Treatment Recommendations For Adult Inpatients. 2015-2016;1-5.

16. Reda AA, Seyoum B, Yimam J, Andualem G, Fiseha S. and Vandeweerd J, Antibiotic susceptibility patterns of Salmonella and Shigella isolates in Harar, Eastern Ethiopia. *J. Infect. Dis. Immunol*.2011; 3 (8):134-139.

17. Schroeder GN, Hilbi H, Molecular Pathogenesis of Shigella species, Controlling Host Cell Signaling, Invasion, and Death by Type III Secretion, *Clin.Microbiol.Rev.* 2008; 21(1) 134–156.

18.Tambe AB, Nzefa LD and Nicoline NA. Childhood Diarrhea Determinants in Sub-Saharan Africa: A Cross Sectional Study of Tiko Cameroon, *Challenges.* 2015; 6:229-243.

19.Samie A, Makuwa S, Mtshali S, Potgieter N, Thekisoe O, Mbati P et al, parasitic infection among HIV/AIDS patients at bela-bela clinic, limpopo province, south africa with special reference to cryptosporidium. *Southeast Asian J trop Med public health.* 2014; 45(4):783-795.

20. Kotloff KL, Winickoff JP, Ivanoff B, Clemens JD. Swerdlow DL, Sansonetti PJ, et al, Global burden of Shigella infections: implications for vaccine development and implementation of control strategies. *Bulletin of the World Health Organization.* 1999; 77(8): 651-666.

21. Eng SK, Pusparajah P, Mutalib NS, Ser HL, Chan KG & Lee LH. Salmonella: A review on pathogenesis, epidemiology and antibiotic resistance, *Frontiers in Life Science.* 2005; 8:3, 284-293.

22. Crump JA, Luby SP and Mintz ED. The global burden of typhoid fever. *Bull. World Health Organ.* 2004; 82: 346–353

23. Morpeth SC, Ramadhani HO, and Crump JA. Invasive Non-Typhi Salmonella Disease in Africa. *Food Safety CID.* 2009; 49: 606–11.

24 .Andualem TD,Tiruneh MW, Moges FY, Muluye DF, Ayalew GG. Prevalence and Antimicrobial Susceptibility Patterns of Shigella and Salmonella Species among Patients with Diarrhea Attending Gondar Town Health Institutions, Northwest Ethiopia. *Science Journal of Public Health.* 2014; 2(5): 469-475.

25.Mengistu G, Mulugeta G, Lema T, Aseffa A. Prevalence and Antimicrobial Susceptibility Patterns of Salmonella serovars and Shigella species. *J Microb BiochemTechnol.* 2014; 2:1-7.

26.Andualem B, Kassu A, Diro E, Moges F, Gedefaw M. The prevalence and antimicrobial responses of Shigella Isolates in HIV-1 infected and uninfected adult diarrhoea patients in north west Ethiopia. *Ethiop.J.Health Dev.* 2006; 20(2):100-105.

27.Awole M, Gebre-Selassie S, Kassa T, Kibru G, Isolation of potential bacterial pathogens from the stool of HIV-infected and HIV-non-infected patients and their antimicrobial susceptibility patterns in Jimma Hospital, south west Ethiopia. *Ethiop Med J.* 2002; 40(4):353-64.

28. World Health Organization, Basic Laboratory procedures in clinical bacteriology, Second edition, Geneva 2003.

29.Gebrekida A, Asmelash TD, Kahsay G and Gebreysus AW.Prevalence and antimicrobial susceptibility patterns of Shigella among acute diarrheal outpatients in Mekelle hospital, Northern Ethiopia. *BMC Res Notes.* 2015; 8: 611.

30.European Centre for Disease Prevention and Control, Surveillance of seven priority food and waterborne diseases in the EU/EEA. Stockholm: ECDC; 2015; 53-113.

31 Cheesbrough M, Medical Laboratory Manual for Tropical Countries Part 2. 2nd Ed., Cambridge, UK: Cambridge University Press; 2006;179-186

32 Dekker J, Frank K. Salmonella, Shigella, and Yersinia. *Clin Lab Med.* 2015; 35(2): 225–246.

33.Kuang X, Hao H, Dai M, Wang Y, Ahmad I, Liu Z, et al. Serotypes and antimicrobial susceptibility of Salmonella spp. isolated from farm animals in China. *Front. Microbiol.* 2015;6: 602.

34.Majowicz SE., Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ et al.The global burden of non typhoidal Salmonella gastroenteritis. *Clin. Infect. Dis. Off. Publ. Infect.* 2010; 50: 882–889.

35. Pasquali P. HIV infections and zoonosis, animal production and health Isitituto Superiore di Sanità Rome, Italy FAO 2004.

36.U.S. department of health and human service Centers for Disease Control and Prevention (CDC),Antibiotic Resistance Threats in the United States, 2014; 65-73.

37.European Centre for Disease Prevention and Control (ECDC), Annual epidemiological report, reporting on 2011 surveillance data and 2012. Stockholm. 2013;103–108.

38. Ram PK, Crump JA, Gupta SK, Miller MM and Mintz ED. Analysis of data gaps pertaining to Shigella infections in low and medium human development index countries 1984–2005 part II, *Epidemiol. Infect.* 2008;136: 577–603.

39. Casabonne C, Gonzáalez A, Aquili V, and Balaguáe C. Prevalence and Virulence Genes of Shigella spp. Isolated from Patients with Diarrhea in Rosario, Argentina, *Jpn. J. Infect. Dis.* 2016; 69, 477–481.

40. Ca´rcamo C, Hooton T, Mark HW, Noel SW, Gilman R, Arevalo J. Etiologies and Manifestations of Persistent Diarrhea in Adults with HIV-1 Infection: A Case-Control Study in Lima, Peru. *JID.* 2005; 191:11–19

41. Musiime V, Kalyesubula I, Kaddu-Mulindwa D, Byarugaba J. Enteric Bacterial Pathogens in HIV-Infected Children With Acute Diarrhea in Mulago Referral and Teaching Hospital, Kampala, Uganda. *jiapac.* 2009; 8(3):185-190

42.Temitope OA, Opeyemi BO, Olaniran O, Edoghogbo RH, Olawole DA, Bolatito O et al. Prevalence and antimicrobial susceptibility patterns of Salmonella and Shigella in HIV patients in Ile-Ife, Osun State. *IJBAR*. 2015; 6(05): 413-417.

43. Urio EM, Collison EK, Gashe BA, Sebunya TK and Mpuchane S. Shigella and Salmonella strains isolated from children under 5 years in Gaborone, Botswana, and their antibiotic susceptibility patterns. *Tropical Medicine and International Health.* 2001; 6(1): 55-59.

44. Vouking MZ, Enoka P, Tamo CT, Tadenfok CN. Prevalence of intestinal parasites among HIV patients at the Yaoundé Central Hospital, Cameroon.Pan African Medical Journal. 2014;18:136.

45. Beyene G. and Tasew H., Prevalence of intestinal parasite, Shigella and Salmonella species among diarrheal children in Jimma health center, Jimma southwest Ethiopia. *Ann. Clin. Microbiol. Antimicrob*., 2014;13(1):10.

46 Fisseha B, Petros B,Woldemichael T and Mohammed H. Diarrhoea-associated parasitic infectious agents in AIDS patients within Addis Ababa Hospitals. *Ethiop J Health Dev.*1999; 13:163-173.

47. Awole M, Gegre-Selassie S, Kassa T and Kibtu G.Prevalence of intestinal parasites in HIV-infected adult parasites in southwestern Ethiopia. *Ethiop J Health Dev.*2003; 17(1):71-78.

48. Mamuye Y, Metaferia G, Birhanu A, Desta K, Fantaw S. Isolation and Antibiotic Susceptibility Patterns of Shigella and Salmonella among Under 5 Children with Acute Diarrhoea: A Cross-Sectional Study at Selected Public Health Facilities in Addis Ababa, *Ethiopia. Clin Microbiol*. 2015;4(1):186.

49. Alemu A, Shiferaw Y, Getnet, Yalew A, Addis Z. Opportunistic and other intestinal parasites among HIV/AIDS patients attending Gambi higher clinic in Bahir Dar city, North West Ethiopia. *Asian Pacific Journal of Tropical Medicine.*2011:661-665.

50. Adamu H, Petros B. Intestinal protozoan infections among HIV positive persons with and without antiretroviral treatment (ART) in selected ART centers in Adama, Afar and Dire-Dawa, *Ethiopia.Ethiop J Health Dev.* 2009:23(2):133–140.

51. Assefa S, Erko B, Medhin G, Assefa Z, Shimelis T. Intestinal parasitic infections in relation to HIV/AIDS status, diarrhea and CD4 T-cell count. *BMC Infectious Diseases*.2009; 9:155

52. Missaye A, Dagnew M, Alemu A and Alemu A. Prevalence of intestinal parasites and associated risk factors among HIV/AIDS patients with pre-ART and on-ART attending dessie hospital ART clinic, Northeast Ethiopia. *AIDS Research and Therapy* 2013; 10:7.

53. Santos RL, Tsolis RM, Bäumler AJ, Adams LG. Pathogenesis of Salmonella-induced enteritis. *Braz J Med Biol Res.* 2003; 36(1):3-12.

54. Dupont HL, Levine MM, Hornick RB, Formal SB. Inoculum size in shigellosis and implications for expected mode of transmission. *J. Infect. Dis.*1989; 159:1126–1128.

55. Gouin E, Welch MD, and Cossart P. Actin-based motility of intracellular pathogens. *Curr. Opin.Microbiol.* 2005; 8: 35–45.

56. Islam D, Bandholtz L, Nilsson J, Wigzell H, Christensson B, Agerberth,et al. Down regulation of bactericidal peptides in enteric infections: a novel immune escape mechanism with bacterial DNA as a potential regulator. *Nat. Med.* 2001; 7:180–185

57.Cherla RP, Lee SY,Tesh VL, Shiga toxins and apoptosis. *FEMS Microbiol Lett.* 2003; 228:159–166.

58.Rabia A, Marcia B G, Clinical manifestations and diagnosis of Shigella infection in children. *Fevereiro*. 2006.

59 Cheesbrough M, Medical Laboratory Manual for Tropical Countries Part 1. 2nd Ed., Cambridge, UK: Cambridge University Press; 2009; 192-199.

60. Montville T J and Matthews K R, Food Microbiology: An Introduction. 2nd Ed., ASM Press, Washington USA, 2008; 507.

61 Bhattacharya S, Khanal B, Bhattarai NR and Das MR. Prevalence of Shigella species and their antimicrobial resistance patterns in Eastern Nepal. *J. Health, Popul. Nutr.* 2005; 23:339-342.

62.Central Statistical Agency, Population and Housing Census Report-Amhara region. 2007; CSA- Ethiopia.

63. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility testing. USA: Wayne, PA. 2018; M02-S25

9. Annexes

Annex I: Participant Information Sheet

I. Participant Information Sheet (English version)

Title of the project: "Comparative study on the magnitude of Salmonella and Shigella Species and Antimicrobial Resistance pattern among HIV infected and non-infected patients with diarrhea in selected health facilities, Dessie town, Northeast Amhara Region, Ethiopia"

Principal Investigator: Assefa Belay

Advisor: Dr. Aster tsegaye (PhD, Associate Professor) and Dr. Berhanu Seyoum (Msc,PhD)

Department: Clinical Laboratory science, College of Health Sciences, Addis Ababa University.

Introduction: My name is Assefa Belay and I am Msc student in Diagnosis and public Health microbiology at Addis Ababa University College of Health science, department of clinical laboratory science . I am doing a research on Comparative study on the magnitude of Salmonella and Shigella Species and Antimicrobial Resistance pattern among HIV infected and non-infected patients with diarrhea in selected health facilities, Dessie town, Northeast Amhara Region, Ethiopia.

Purpose of the study:

The purpose of this study is to determine prevalence of salmonella and shigella infection, determining antimicrobial susceptibility and associated risk factors among HIV infected and HIV non-infected patients in Dessie selected health facilities. 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 will assess

the prevalence of salmonella and shigella infection, antimicrobial resistance pattern and associated risk factors among HIV infected and HIV non infected patients.

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 demographic and clinical information related to diarrheal Infection will be filled on the questionnaire. stool sample will be collected for laboratory analysis. You will 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 will be kept confidential.

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

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

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

Results Dissemination:

There will be a report which is written about the finding of the study, either through publication or any other means. The result will 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: Mr. Assefa Belay (Candidate of MSc, Diagnosis and Public Health Microbiology), department of Medical Laboratory Science, school of Allied Health Science, College of Health Sciences, Addis Ababa University

Addis Ababa, Ethiopia

Cell phone: +251911095032

E-mail: assefab12@gmail.com

Or

If you want more information and check about this project you can contact the following people

Addis Ababa University, Medical faculty institutional review board

Tell: +251-11-5-53-87-34

Fax: +251 -11-5-51-15-1-30-99

Email: aaumfirb@yahoo.com

II. Participant information Sheet (Amharic version)

የጥናቱ ተሳታፊዎች የመረጃ ቅጽ፡- የጥናቱ ርዕስ፡ በደሴ ከተማ፣ በተመረጡ የጤና ተቃማት ዉስጥ የተቅማጥ በሽታ አምጭ ባክቴሪያዎች ኤች.አይ.ቪ ቫይረስ በደማቸው ውስጥ ባለባቸውና ቫይረሱ ደማቸው በሌለባቸው በሽተኞች ስርጭቱን ማወቅ እና ለመድሃኒት ያለው ቁርኝት በሚል የሚደረግ ጥናት ነው፡፡

አጠቃላይ መረጃ፡ በጥናቱ በመሳተፍዎ ከልብ እያመሰገን ከመወሰንዎ በፊት፡- ይህንን ቅጽ በትክክል ያንብቡ ወይም ሲነበብልዎ በትክክል ያድምጡ፤ እንዲሁም ግልፅ ያልሆነለዎትን ነገር በሙሉ በነፃነት ይጠይቁ።

መግቢያ፡- አሠፋ በላይ እባላለሁ፡፡ የአዲስ አበባ ዩንቨርሲቲ የህክምና ማይክሮ ባዮሎጅ የ2ኛ ድግሪ ተማሪ ነኝ፡፡ በደሴ ከተማ በተመረጡ ጤና ተቃማት ቫይረሱ በደማቸው ውሰጥ ባለባቸውና በሌለባቸው የተቅማጥ በሽታ አምጭ ባክቴሪያዎች ስርጭት ለማወቅ የሚካሄድ ጥናት ነው፡፡

ፈቃደኝነት፡- እርስዎንና ሌሎችንም በጥናቱ በሙሉ ፍቃደኝነት እንዲሳተፉ እየጠየቅን በጥናቱ በመሳተፍ ፍቃደኛ ከሆኑ ለሚቀርብለዎትን መጠይቅ ምላሽ ከሰጡ በኋላ የሰገራ ናሙና እንዲሰጡ ይጠየቃሉ::

ሚስጥራዊነት፡- የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኃላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡ በጥናቱ ላይ እያሉ በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አለዎት፡፡

የሚያገኙት ጥቅም፡- በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም:: ነገር ግን ዉጤታቸዉ ለሚከታተላቸዉ ሀኪም እንዲደርሰዉ ይደረጋል፡፡ የእርሰዎ በዚህ ጥናት ተሳታፊ መሆን ለክልሉ እንዲሁም ለሃገር ጠቀሜታ አለው፡፡

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት እና ተዛማጅ ችግር፡- በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም፡፡ የሚወሰደው ናሙና ሰገራ ብቻና እራሰዎ ያለ ምንም ተጨማሪ መሳርያ የሚሰጥ ስለሆነ የሚያመጣዉ ችግር የለም፡፡

ውጤቱን ስለመጠቀም፡- ከዚህ ጥናት በኋላ የበሽታውን ስርጭት በተመለከተ ሪፖርት ይፃፋል፡፡ ሆኖም የእርስዎን ማንነት የሚገልፅ መረጃ የማይካተት ሲሆን ችግሩን ለማሳወቅ ብቻ የሚውል ነው፡፡

አድራሻ፡

ማንኛውም ጥያቄ ወይም ጥርጣሬ ካለዎት ይህንን አድራሻ ይጠቀሙ፡

የዋናው ተመራማሪ አድራሻ

አሰፋ በላይ ስልክ፡- 09-11-09-50-32

ኢ-ሜይል: assefab12@gmail.com

(ዲያግኖሲስና ፐብሊክሄልዝ ማይክሮ ባይሎጅ ክፍል፣ ክሊኒካል ላቦራቶሪ ሳይንስ፣ የጤና ሳይንስ ኮላጅ፣ አዲስ አበባ ዩንቨርሲቲ፣ አዲስ አበባ፣ ኢትዮጵያ)

ስልክ፡- +251-11-5-53-87-34

Fax: +251 -11-5-51-15-1-30-99

ኢ-ሜይል: **aaumfirb@yahoo.com**

Annex II: Informed consent form

I: Informed consent English version

Serial no……..

Subject Code…..…….....

This study is prepared by Mr. Assefa Belay in Addis Ababa University, College of health sciences for the purpose of MSc thesis work. The objective of this study is to determine the prevalence and antimicrobial susceptibility pattern of salmonella and Shigella among HIV infected and non-infected outpatients of all age groups with acute diarrhea at Dessie referral hospital, Dessie health center and Banbua wuha health center . I am requesting you to participate in the study, which would require your response to an interview, and to provide samples (stool) for laboratory examination. Your role in participation is important to know the current status of salmonella and shigella infection and drug resistance patterns in health facilities in Dessie and helps in the selection of appropriate treatment for patients. Its procedure is not invasive and I don't think that any problems will happen to you as part of this study and does not have direct benefit to you. You have full right to accept or reject participation, your confidentiality is kept and you will not have payment for participation. The result will be communicated with the physician. You have to participate after you have understood what you will be doing for this study, have had all your questions answered; agree to takepart in this research

Contact address:

Addis Ababa University, College of Health Science, Office

Principal Investigator's Name: Assefa Belay

Tel: +251911095032 Email: assefab12@gmail.com

Advisor's Name and Address: Dr. Berhanu seyoum (Msc, PHD)

Tel:+251911677774 Email: seyoumbe07@gmail.com

and Dr. Aster Tsegaye (PhD, Associate Professor))

Tel: +251 911 69 60 85 Email: tsegayeaster@yahoo.com

Assent form Adult

I understand clearly about the study and I have agreed to participate in the study.

Participant Name _____ Signature_____, Date_____

II. Informed Consent (Amharic version)

¾ðnÅ˜ƒ SÖ¾mÁ pϑ ፡- ÃI Ø“ƒ ¾T›Å[Ñ¨< ¾›Ç=e ›vv ¿”y`c=+ ¾Ö?“ dÃ”e ¦T] uJ’<ƒ u›„ ›có uLÃ ¾G<K¦— Ç=Ó] KSS[mÁ êG<õ c=J” ›LT¨< uÅc? ]ð^M Jeø‚ϮM‡ uÅc? Ö?“ ×u=Á“ uՂ‚ղՂ ¨<H Ö?“ ×u=Á ¦pTØ ›Uß uJ’<ƒ cMV’@L“ g=Ñ@L ν¡ϯ]Á-‹ zÃ[c< uÅT†¨< νKν†¨<“ uK?Kν†¨< ui¦™‹ e`ß~” KT¨p“ KSÉH’›ƒ ÁK¨<” Óƒ`’ƒ Å[Í Td¾ƒ uT›M `°e LÃ KTØ“ƒ u¦SKŸ¦ uT›Å[Ó Ø“ƒ LÃ KSd¦õ SJ’< ¾Ø“~ ›LT“ ØpU ¦ÑMëM—M፡ u¦ÚT] Ø“~ ¨<eØ ›KSd¦õ Swϯ °ÅJ’“ uT”—¨<U Ñ›²? ŸØ“~ u^c? ¨<d’@ S¨<×ƒ °ÅT›‰M“ u²=lU U¡”Áƒ U”U ›Ã’ƒ SÑ<LLƒ °”ÅTÃÅ`ew˜ uT›Ñν ¦[É‰KG<፡፡cKJ’U G<’@¦¨<” uT›Ñν uTÖ?” uðnÅ˜ƒ uU`U\ LÃ KSd¦õ K¦S^T]¨< ðnÅ˜Ϯ” cØ‰KG<፡፡ u¦ÚT] T”—¨<”U ÁMÑν˜ ’Ñ` ¾SÖ¾p °ÉM ¦cØ„˜ uT›Ñν˜ Ϯ”Ϯ SMe ›ÓˆKG<፡፡ ÃI” G<K< uTÑ“²w U`U\ LÃ eK’@ S[Í“ ¾cÑ^ “S<“ KSeÖƒ ¦cTT‰KG< ÃI””U uò`T¾ ›[ÒÓ×KG<፡፡

¾¦d‚ϮÒ¨< eU፡ _____ ò`T _____ k” _____

S[Í¨<” Áe[Ç¨< ›"M _____ ò`T _____ k” _____

Annex III: Questionnaire

I English Version Questionnaire

This questionnaire is prepared by Mr. Assefa Belay in Addis Ababa University, College of Health Sciences that aimed to determine the prevalence of salmonella and Shigella and their antimicrobial susceptibility patterns among HIV infected and non-infected of all ages with acute diarrhea at Dessie selected health facilities, Northeast Ethiopia.

Date_____

Subject ID_____

English Version Questionnaire

Part I: Socio-demographic characteristics				
Identification code _____				
No.	Questionnaire on identification of the respondents	Alternative choice for Responses	Skip	Code
101	Age	_____ year		

102	Sex	A. Male B. Female		
103	Residence(From where did you come)	A. Urban B. Rural		
104	What is your occupation?	A. None Employee B. Seville servant C. Merchant D. Farmar		
105	What is your educational status?	A.Illiterate B. Only read & write C. Primary school completed D. Secondary school completed E. College or university		
106	Monthly family income in ETB?	A. ≤500 B. 501-1000 C. 1001-1500 D. 1501-2000 E. >2000		
Part II: Clinical data				
201	Duration of diarrhea	_____Days		
202	2. Consistency of diarrhea	A. Watery B. Mucoid C. Bloody D. Mixed (Mucoid + blood)		
203	History of HIV/AIDS	A. Yes B. No		
204	If yes, how much CD4 number	_____Cells/uL		
III. Risk factors				

301	Presence of latrine at home: Utilized	A. Yes B. No		
302	If "No" number 301, where do you defecate?	A. Public latrine B. Open Field C. River		
303	Source of drinking water	A. Private Pipe water B. Public pipe water C. River water(spring) D. Well water		
304	Hand washing habit before meal:	A. Yes B. No		
305	If "Yes", for number 304, do you use soap	A. With soap B. Without soap		

II መጠይቅ (Amharic Version)

ÃI SÖÃp ¾¦²ÒË¨< ¾›Ç=e ›vv ¿"y`c=+Ã ¾Ö?" dÃ"e ¦T] uJ'<ƒ u›„ ›có uLÃ ¾G<K¦— Ç=Ó] KSS[mÁ êG<õ c=J" ›LT¨< ucT@" Ue^p uÅc? ›Ÿ"vu= ¦pTØ ›Uß ¾J'<ƒ" cMV'@L" g=Ñ@L ¾T>vK< v¡ቱ]Á-‹" e`ß~" KT¨p" KSÉH'>ƒ ÁK¨<" Óƒ`'ƒ Å[Í Td¾ƒ uT>M zÃ[c< uÅT†¨< vKv†¨<" uK?Kv†¨< ui¦™‹ ¾T>Å[Ó SÖÃp ¨<::

ክፍል 1: የግለሰቡ ማህበራዊ እና ኢኮኖሚያዊ መረጃ				
የተሳታፊው መለያ ቁጥር _____				
ተ.ቁ	የግለሰቡ አካላዊ ማህበራዊ እና ኢኮኖሚያዊ መጠይቅ ዝርዝር	የመልስ አማራጮች	ይለፍ	መለያ ቁጥር
101	እድሜ	_____ አመት		
102	ë¦	ı. ¨"É 2. c?ƒ		
103	**የመኖሪያአድራሻ**	ı. ከተማ 2. ገጠር		
104	**ስራ?**	ı. የመንግስትሰራተኛ 3. የቤትእመቤት **2.** የግልስራ 4. ተማሪ		

105	**የትምህርት ሁኔታ?**	**1**. ያልተማረ 2. መጻፍና ማንበብ ብቻ 3. የመጀመሪያ ደረጃ ት/ትያጠናቀቀ 4.ሁለተኛደረጃ ት/ት ያጠናቀቀ 5. የኮሌጅ/ ዩኒቨርሲቲ		
106	**በአማካይ የቤተሰቦ ወርሃዊ ገቢ ስንት ነው?**	**1. ≤500 2. 501-1000** **3. 1001-1500 4. 1501-2000** **5. >2000**		
ክፍል II: ከጤና ጋር የተያያዙ መረጃዎች				
201	}pTÖ< KU" Á¡M Ñ>²? q¾wl/i	_____ቀን		
202	}pTÖ< U" ›Ã'ƒ ¨"<;	1 ¨<HT ðdi 2. SÓM ¾}kLkKuƒ 3 ÅU ¾}kLkK 4. ÅU" SÓM		
203	**አች.አይ. ቪ ቫይረስ በደምዎ ይገኛል;**	1. **አዎ 2. የለም**		
204	}.l 205 ›**ዎ** ŸJ' ¾CD4 SÖ"l/i e"ƒ ¨"< ?	_____ሴሎች በማይ ክሮሊትር		
ክፍል: III ›ÒLß G<'@ታ©‹				
301	KSÖØ ¾UƒÖkS<ƒ ¨<H Ÿ¾ƒ ¨"< ¾U}Ñ–<ƒ;	1 ¾ÓM Ù"Ù 2. ¾l'w Ù"Ù 3 ¾¨"'' ¨<H 4. ¾Ñ<ÉÔÉ ¨<H		
302	¾ÓL‹G< i"ƒ u?ƒ ›L‹G<;	1 ›**ዎ** 2. ¾K'U		
303	}.l 302 ¾K'U ŸJ' i"ƒu?ƒ ¾ƒ ¨" ¾UƒçÇÆƒ;	1 ¾l'w i"ƒ u?ƒ 2. T@Ç LÃ 3. ¨"'		
304	UÓw ŸSSÑv‹G< uòƒ °Í‹G<" ƒታÖvL‹G<;	1. ›**ዎ** 2. ›"}ÖwU		
305	}.l 302 ›¨ ŸJ' uUÉ" ¨"< ¾Uƒታ Öu<ƒ;	1. udS<" 2. ÁKdS<"		

ስለትብብርዎ እናመሰግናለን !

II. Informed Consent (Amharic version)

¾ðnÅ˜ƒ SÖ¾mÁ pϑ :- ÃI Ø"ƒ ¾T›Å[Ñ¨< ¾›Ç=e ›vv ¿"y`c=+ ¾Ö?" dÃ"e }T] uJ'<ƒ u›„ ›có uLÃ ¾G<K}—Ç=Ó] KSS[mÁ êG<õ c=J" ›LT¨< uÅc?]ð^M Jeøታ M‡ uÅc? Ö?" ×u=Á" uÙ.ንÙ. ¨<H Ö?" ×u=Á }pTØ ›Uß uJ'<ƒ

cMV'@L“ g=Ñ@L vį'ƀ]Á-‹ zÃ[c< uÅT†¨< vKv†¨<“ uK?Kv†¨< uì¦™‹ e`ß~” KT¨p“ KSÉH'>ƒ ÁK¨<” Ófˇ'ƒ Å[Í Td¾ƒ uT>M `°e LÃ KTØ“ƒ u¦SKŸ¦ uT>Å[Ó Ø“ƒ LÃ KSd¦õ SJ'< ¾Ø“~ ›LT“ ØpU ¦ÑMëM—M:: u¦ÚT] Ø“~ ¨<eØ ›KSd¦õ Sw'ƀ °ÅJ'“ uT”—¨<U Ñ>²? ŸØ“~ u^c? ¨<d'@ S¨<×ƒ °ÅT>‰M“ u²=lU U¡”Áƒ U”U ›Ã'ƒ SÑ<LLƒ °”ÅTÃÅ`ew˜ uT>Ñv ¦[É‰KG<::cKJ'U G<'@¦¨<” uT>Ñv uTÖ?” uðnÅ˜ƒ uU`U\ LÃ KSd¦õ K¦S^T]¨< ðnÅ˜'ƀ” cØ‰KG<:: u¦ÚT] T”—¨<”U ÁMÑv˜ 'Ñ` ¾SÖ¾p °ÉM ¦cØ„˜ uT>Ñv˜ Ψ”Ψ SMe ›Ó˜‰KG<:: ÃI” G<K< uTÑ“²w U`U\ LÃ eK'@ S[Í“ ¾cÑ^ “S<“ KSeÖƒ ¦cTU‰KG<:: ÃI”"U uò`T¾ ›[ÒÓ×KG<::

¾¦d;'ò¨< eU:_____ ò`T _____ k” _____

S[Í¨<” Áe[Ç¨< ›"M _____ ò`T _____ k” _____

Annex IV Laboratory procedures

1. COLLECTION AND TRANSPORT OF FAECES

Faeces for microbiological examination should be collected during the acute stage of diarrhoea.

1. Give the patient a clean, dry, disinfectant-free bedpan or suitable wide-necked container in which to pass a specimen. The container need not be sterile. Ask the patient to avoid contaminating the faeces with urine.
2. Transfer a portion (about a spoonful) of the specimen, especially that which contains mucus, pus, or blood, into a clean, dry, leakproof container. Worms and tapeworm segments: When the specimen contains worms or tapeworm segments, transfer these to a separate container and send them to the laboratory for identification.

3. Write on the request form the colour of the specimen and whether it is formed, semiformed, unformed, or fluid. Report also if blood, mucus, worms, or tapeworm segments are present.
4. Label the specimen and send it with a request form to reach the laboratory within 1 hour (if a delay longer than 1 hour is anticipated, collect the specimen in Cary-Blair medium,

Samples in transport media should be kept cold and arrive at the laboratory within 48 hours. If appropriate transport media is not available, unpreserved feces may be submitted. The unpreserved fecal sample must be submitted in a clean, container with no soap or disinfectant residue. The sample must be kept cold and transported to the lab within 8 hours of collection.

2. Describe the appearance of the specimen

- Colour of the specimen.
- Whether it is formed, semiformed, unformed or fluid.
- Presence of blood, mucus or pus
- Presence of worms, e.g. Enterobius vermicularis, Ascaris lumbricoides, or tapeworm segments e.g. Taenia species

3. Examine the specimen microscopically Saline and eosin preparations to detect parasites

1. Place a drop of fresh physiological saline. Using a piece of stick or wire loop, mix a small amount of fresh specimen (especially mucus and blood) with each drop. Cover each preparation with a cover glass.
2. Examine the preparations using the 10x and 40x objectives with the condenser iris closed sufficiently to give good contrast.
3. Look especially for motile E. histolytica trophozoites containing red cells, motile G. lamblia trophozoites, motile Strongyloides larvae, and the eggs and cysts of parasitic pathogens.

Formol ether concentration technique

Principle

In the Ridley modified method, feces are emulsified in formol water, the suspension is strained to remove large feces particles, ether or ethyl acetate is added, and the mixed suspension is centrifuged. Cysts, oocysts, eggs, and larvae are fixed and sedimented and the faecal debris is separated in a layer between the ether and the formol water. Feces fat is dissolved in the ether.

Required

- Formol water, 10% v/v.
- Diethyl ether or ethyl acetate.
- Sieve(strainer) with small holes, preferably 400–450m in size.*

Method

1. Using a rod or stick, emulsify an estimated 1 g (pea-size) of faeces in about 4 ml of 10% formol water contained in a screw-cap bottle or tube.
2. Add a further 3–4 ml of 10% v/v formol water, cap the bottle, and mix well by shaking.
3. Sieve the emulsified faeces, collecting the sieved suspension in a beaker.
4. Transfer the suspension to a conical (centrifuge) tube made of strong glass, copolymer, or polypropylene. Add 3–4 ml of diethyl ether or ethyl acetate.
5. Stopper the tube and mix for 1 minute. If using a Vortex mixer, leave the tube unstoppered and mix for about 15 seconds (it is best to use a boiling tube).
6. With a tissue or piece of cloth wrapped around the top of the tube, loosen the stopper (considerable pressure will have built up inside the tube).
7. Centrifuge immediately at 750–1000 g (approx. 3000 rpm) for 1 minute. After centrifuging, the parasites will have sedimented to the bottom of the tube and the faecal debris will have collected in a layer between the ether and formol water
8. Using a stick or the stem of a plastic bulb pipette, loosen the layer of faecal debris from the side of the tube and invert the tube to discard the ether, faecal debris, and formol water. The sediment will remain.

9. Return the tube to its upright position and allow the fluid from the side of the tube to drain to the bottom. Tap the bottom of the tube to resuspend and mix the sediment. Transfer the sediment to a slide, and cover with a cover glass.
10. Examine the preparation microscopically using the 10 objective with the condenser iris closed sufficientlyto give good contrast. Use the 40 objective to examine small cysts and eggs. To assist in the identification of cysts, run a small drop of iodine under the cover glass.
11. If required, count the number of each species of egg in the entire preparation. This will give the approximate number per gram of faeces.

Formol ether oocyst concentration technique

Follow steps 1 to 6 of the above method. Continue as follows:

7. Centrifuge immediately at low speed, i.e. RCF 300–400 g (about 1000 rpm) for 1 minute.

8. Using a plastic bulb pipette or Pasteur pipette, carefully remove the entire column of fluid below the faecal debris and ether and transfer this to another centrifuge tube.

9. Add formol water to make the volume up to10–15 ml. Centrifuge at RCF 750–1000g (about 3000 rpm) for 5–10 minutes.

10. Remove the supernatant. Tap the bottom of the tube to resuspend and mix the sediment. Transfer the sediment to a slide and examine for oocysts using the 40x objective.

4. Culture and bacterial identification

Xylose Lysine Desoxycholate Agar (XLD Agar)

PRINCIPLES: XLD Agar is both a selective and differential medium. It contains yeast extract as a source of nutrients and vitamins. It utilizes sodium desoxycholate as the selective agent and, therefore, is inhibitory to gram-positive micro-organisms. Xylose is incorporated into the medium since it is fermented by practically all enterics except for the

shigellae and this property enables the differentiation of Shigella species. Lysine is included to enable the Salmonella group to be differentiated from the non-pathogens since without lysine, salmonellae rapidly would ferment the xylose and be indistinguishable from nonpathogenic species. After the salmonellae exhaust the supply of xylose, the lysine is attacked via the enzyme lysine decarboxylase, with reversion to an alkaline pH which mimics the Shigella reaction. To prevent similar reversion by lysine positive coliforms, lactose and sucrose are added to produce acid in excess.

To add to the differentiating ability of the formulation, an H2S indicator system, consisting of sodium thiosulfate and ferric ammonium citrate is included for the visualization of the hydrogen sulfide produced, resulting in the formation of colonies with black centers. The nonpathogenic H2S- producers do not decarboxylate lysine; therefore, the acid reaction produced by them prevents the blackening of the colonies which takes place only at neutral or alkaline pH.

PROCEDURE

Specimen Types

This is a selective differential medium for the isolation of Salmonella and Shigella from stool specimens or rectal swabs of patients suspected to have a bacterial enteric infection or from non-clinical materials

Test Procedure

1. Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora.
2. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
3. A less selective medium such as MacConkey Agar should also be inoculated to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.

4. Incubate plates, protected from light, at 35 ± 2°C for a minimum of 18 to 24 h. Colonies on XLD Agar may require 48 h incubation for full color development.
5. XLD Agar may be used as a medium for subculturing from Selenite F Broth.

Look for colonies that could be Shigella or Salmonella. Shigella and H2S negative strains of Salmonella produce 1–2 mm diameter red colonies on XLD agar. Red colonies with black centres are produced by H2S positive salmonellae, e.g. strains of S. Typhimurium

Tests used to identify presumptively shigellae and salmonellae

					KIA Medium Reactions			
	Motility	Urea	Indole	LDC	Slope	Butt	Black(H2S)	Cracks(Gas)
SHIGELLAE		-						
Shigella dysenteriae	-	-	D	-	R	Y	-	-
Shigella flexneri	-	-	D	-	R	Y	-	-1
Shigella boydii	-	-	D	-	R	Y	-	-2
Shigella sonnei	-	-	-	-	R	Y	-	-
Salmonella		-						
S. Paratyphi A	+	-	+	-	R	Y	-3	+
S. Paratyphi B	+	-	-	+	R	Y	+	+
S. Paratyphi C	+	-	-	+	R	Y	+ weak	+
S. Typhi	+	-	-	+	R	Y	+	+

Key: KIA Kligler iron agar, LDC Lysine decarboxylase, d different strains give different results, R Red-pink (alkaline reaction), Y Yellow (acid reaction).

Notes:1 Some strains of serotype 6 produce gas. 2 Serotypes 13 and 14 produce gas. 3 About 12% of strains produce H2S weakly. 4 A minority of strains do not produce H2S. 5 Salmonella Pullorum and Salmonella Gallinarum are non-motile. 6 A minority of strains do not produce H2S. (31)

Indole test

Testing for indole production is important in the identification of enterobacteria. Most strain of E. coli, P. vulgaris, P. rettgeri, M. morganii, and Providencia species break down the amino acid tryptophan with the release of indole.

Principle

The test organism is cultured in a medium which contains tryptophan. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4 (p) –dimethylamino benzaldehyde. This reacts with the indole to produce a red coloured compound. The indole test also can be performed by culturing the organism in tryptone water or peptone water containing tryptophane, and detecting indole production by adding Kovac's or Ehrlich's reagent to an 18-24h culture.

Method

1. Transfer about 1ml of the test organism (tryptone water) into test tube.
2. Add 3-5 drops of Indole reagent (modified kovac's reagent).

Results

- Positive test: Red colour
- Negative test : No red colour

Kligler's Iron Agar (KIA)

- KIA reaction is based on the fermentation of lactose and glucose, and the production of hydrogen sulphide.
- Glucose is present at low concentration in the medium (0.1%) as compared to lactose (1%).
- Red-pink slope and Yellow butt Fermentation of glucose only.

Example; Salmonella and shigella

- Slope pink due to a reversion of the acid reaction under aerobic conditions.
- Yellow slope & Yellow butt: Fermentation of lactose and possibly glucose.
- Red-Pink slope and butt: No fermentation of glucose and lactose

- ❖ Blackening along the stab line or throughout the medium hydrogen sulphide (H2S) production

Example; Salmonella typhi produces small amount whereas Salmonella typhimurium causes extensive blackening

5 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

Antimicrobial Agent		Disk content	Zone diameter nearest whole mm			
Test			R	I	S	Comment
A	Ampicillin	10ug	≤ 13	14-16	≥ 17	Results of ampicillin testing cab used to predict results of Amoxacillin
C	Tetracycline	30ug	≤ 11	12-14	≥ 15	
B	Trimethoprime-selfamethazole	1.25ug/23.75ug	≤ 10	11-15	≥ 16	
C	chloramphinicol	30ug	≤ 8	13-17	≥ 18	
O	Nalidixic acid	30ug	≤ 19	14-18	≥ 13	

B	Ceftriaxone	30ug	≤ 19	20-22	≥ 23	
B	Ciprofloxacin	5ug	≤ 20	21-30	≥ 31	A Ciprofloxacin (breakpoint for salmonella only
B	Ciprofloxacin	5ug	≤ 15	16-20	≥ 20	A Ciprofloxacin (breakpoint for non salmonella

Laboratory report format of AMR :

Code: _____ 1. stool Culture and identification:

XLD(SSA): Positive :_____ Negative _____

Isolated Bacteria _____

2. Antimicrobial susceptibility testing	S (mm)	I (mm)	R(mm)
Amoxicillin	------	------	------
Ampicillin	------	------	-------
TMP-SMX	-------	-------	-------
Ceftriaxone	-------	-------	-------
Tetracycline	-------	-------	-------
Chloramphenicol	-------	-------	-------
Ciprofloxacin	-------	-------	-------

Comments: _____

Addis Ababa University

School of Graduate Studies

Declaration

I the under signed agree to accept all responsibilities for the scientific and ethical conduct of the research project. I will provide timely progress report to my advisor and seek the necessary advice and approval from my advisors in the course of the research. I will communicate timely to my advisors all stakeholders involved in the study including any source of funding for this research.

Name of the student:

Name Assefa Belay (Bsc) Signature_____ Date _____

This proposal has been submitted for ethical review with my approval as university advisor. Name of the advisors:

Aster Tsegaye (BSc, Msc, PhD) Signature_____ Date_____

Berhanu Seyoum (BSc, Msc, PhD) Signature _____Date_____