ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

BACTERIAL ETIOLOGIES AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS AMONG PRISONER WITH ACUTE GASTROENTERITIS AT KALITY PRISON ADDIS ABABA, ETHIOPIA

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A THESIS SUBMITTED TO DEPARTMENT OF MEDICAL LABORATORY SCIENCES, SCHOOL OF ALLIED HEALTH SCIENCES, COLLEGE OF HEALTH SCIENCES, ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS IN CLINICAL LABORATORY SCIENCES (DIAGNOSTIC AND PUBLIC HEALTH MICROBIOLOGY SPECIALTY)

November, 2017

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"Bacterial etiologies and antimicrobial susceptibility patterns among prisoner with acute gastroenteritis at Kality prison Addis Ababa, Ethiopia"

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List of Abbreviations

CLSI----------Clinical and Laboratory Standards Institute

DRERC ----------Department Research and Ethical Review Committee

EPHI ----------Ethiopia public health institute

LF----------Lactose fermenting

LIA ----------Lysine Iron Agar

MAC ----------MacConkey Agar

NLF ----------Non lactose fermenter

OPD ----------Outpatient Department

SMAC----------Sorbitol MacConkey agar

Spp----------Species

SPSS ----------Statistical Package for Social Sciences

SIM ----------Sulfide Indole Motility medium

SFB----------Selenit F broth

TSIA----------Triple Sugar Iron agar

WHO ----------World Health Organization

XLD----------Xylose-Lysine Desoxycholate

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Abstract

Background: Diarrhea among prisoners caused by enteric bacteria is a public health problems worldwide, especially in tropical and developing countries; Antimicrobial resistance of enteropathogenic bacteria has profound clinical implications. Poor sanitation and restriction to water access may favor the spread of enteropathogenic bacteria, which is one of the leading causes of morbidity in the world.

Objectives: To determine the prevalence, antimicrobial susceptibility patterns and associated risk factors of acute gastroenteritis among prisoners at Kality prison, Addis Ababa, Ethiopia.

Methods: Cross-sectional study was conducted from January, 2017 to September, 2017 on a total of 238 prison inmates having acute gastroenteritis. All study participants; fresh stool specimen was collected by using Caryp-Blair transport media. Samples were transported to EPHI bacteriology laboratory at 2-8^{0}c for bacteriological analysis and each specimen were inoculated onto XLD agar, MAC agar, SMAC agar and SFB. Pure isolates were characterized based on bacterial colony morphology and standard biochemical procedures. Antimicrobial susceptibility testing was done on Muller-Hinton agar using disk diffusion. Socio-demographic and associated risk factors data were gathered using a predesigned structured questionnaire. Socio-demographic, clinical and laboratory data was entered and analyzed using SPSS version 23.

Results: The overall prevalence of entropathogenic bacteria in this study was 20.6% (n=49/238). Out of this 55%, 28.6% and 16.3% of the isolates were positive for *E.coli O157H7*, *Shigella spp*. and *Salmonella spp* respectively. *E.coli 0157; H7* was highly resistance to ampicillin (96.29%), while they showed lower level of resistance to cotrimoxazole and Ceftriaxone. *Shigella* and *Salmonella* isolates showed100% resistance to ampicillin. In the other hand, all isolates of *E. coli 0157*; H7 were 100% susceptible to Nalidixic Acid and Ciprofloxacin. Isolates of *Salmonella* spp. were 100% susceptible to Ceftriaxone, Nalidixic Acid, cotrimoxazole, and Ciprofloxacin.

Conclusions: Enteropathogenic bacteria from acute gastroenteritis were high among prisoner inmates. Multidrug resistance was common among *shigella spp* and *E.coli O157H7*. Ampicillin and cotrimoxazole showed high resistance to *E.coli O157H7* and *Shigella* isolates in this study. Ciprofloxacin was susceptible for both *Salmonella* and *Shigella* isolates.

Key terms: prisoner, gastroenteritis, Bacterial isolates, antimicrobial resistance pattern, Multidrug resistance, Kality prison, Addis Ababa, Ethiopia

Introduction

1.1. Background

Acute gastroenteritis is a severe infection of the gastrointestinal tract (GI) which is characterized by diarrhea, stomach pain, nausea, vomiting, fever or feeling unwell (1). Gastroenteritis usually passes in less than 24 hours but can continue for several days (1). Acute gastroenteritis can be caused by a wide range of bacteria, virus and parasites. The commonest bacterial pathogens that cause acute gastroenteritis are: *Campylobacter* species, *Salmonella* species, *Shigella* species, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Vibrio cholera* and *Yesinia enterocolitica*. The common route of infection by these pathogens is the ingestion of contaminated foods and drinks (2).

Shigellosis and salmonellosis are still global health problems, especially, in developing countries where poor sanitation, lack of clean water supply and proper sewage disposal system exist. The emergence of increased antimicrobial resistance of *Shigella* and *Salmonella* species are global challenges, particularly in developing countries like Ethiopia where increased misuse of antimicrobial agents by human beings occur (3).

A high proportion of the prisoner population comes from disadvantaged groups in the community in which the prevalence of both communicable and non-communicable diseases is higher than in the general population. The environment of the prison itself carries inherent risks for communicable disease transmission. In many countries, poor water and sanitation, overcrowding and poor food safety standards are among the risk factors that led to outbreaks of infectious diseases in prison (4).

Factual information regarding antimicrobial resistance shows that the factors leading to misuse of antibiotics and its contribution to bacterial resistance are knowledge, attitude and behavior toward the use of antibiotics, role of prescriber and amateur health practitioners in prescribing antibiotics, patient expectations and patients past experience, economic enticements, diagnostic uncertainty, poor drug quality, unsanitary conditions accounting for spread of resistant bacteria and inadequate surveillance(5). There are numerous unnecessary prescriptions of antibiotics seen in many developing countries for cases of acute diarrhea (5). There are many reasons for prescribing unnecessary antibiotics among which there is no standard laboratory test before

prescribing drugs, instead the physician assumes it is a bacterial infection, second patients demand antibiotics causing pressure on physicians to prescribe antimicrobial medications(5).

Many bacterial diseases could, until recently, be treated with inexpensive antimicrobial agents, but treatment has recently been made more expensive and less successful by the emergence and spread of resistant organisms (6). Antimicrobial drug resistance is a large and growing problem among organisms that cause diarrheal disease. Although most diarrheal diseases are self-resolving and should not be treated with antimicrobial agents; recent reviewed data from Gabon, Nigeria, and Tanzania suggest that resistance among causative organisms of these infections, such as enterotoxigenic, enteropathogenic, and enteroaggregative *Escherichia coli*, is high and appears to be rising (6). Bacteria become drug resistant in several ways. A particular type of mechanism is not confined to a single class of drugs. Two bacteria may use different resistant mechanism to withstand the same chemotherapeutic agent. Furthermore, resistant mutants arise spontaneously and are then selected. Mutants are not created directly by exposure to a drug (7).

Diarrheagenic bacteria in the prison inmates associated with lack of clean water, poor sanitation, inadequate food storage and handling, and deficient cleaning of materials and installations used for food preparation, hygiene of inmates (hand washing, treating water) (8). The disease is transmitted faeco-orally, the commonest modes being person-to-person contact and contaminated food and water (8). Infected food handlers can spread the disease; flies can breed in infected faeces and contaminate food. It is a disease of overcrowding, insanitary conditions and poor personal hygiene, and affects mostly prison inmates of developing countries (8). However, there are no available data in Ethiopia on the prevalence of enteropathogenic bacteria, drug resistance pattern and associated factors among prisoner. Therefore, this study was aimed to determine the prevalence, antimicrobial susceptibility patterns and associated risk factors among acute gastroenteritis at Kality prison, Addis Ababa, Ethiopia.

1.2. **Statement of the Problem**

Diarrheal diseases have long been established as a leading cause of morbidity and mortality throughout the world. Globally, diarrhea is the third largest cause of morbidity and the sixth largest cause of mortality among population of all ages. Where an estimated 3-5 billion diarrheal illness and 5-10 million diarrhea-related deaths occur annually among those living in Africa, Asia, and Latin America (9). According to WHO, approximately one billion cases of diarrhea occur each year worldwide casing a burden that was about 99.2 million lost (9).

Diarrheal diseases caused by bacterial pathogens are a major problem worldwide especially in developing countries in conditions of poor environmental sanitation, inadequate water supplies, poverty and limited education (10). Acute gastrointestinal illness rank second only to acute respiratory illness as the most common disease worldwide (10). From studies of stool cultures performed in U.S Hospitals the most commonly isolated bacterial pathogens are *campylobacter* (42%) of isolates, *Salmonella* (32%), *Shigella* (19%), and *Escherichia coli* (7%).

Shigellosis has a worldwide distribution with an estimated 600,000 deaths occurring annually throughout the world. Secondary attack rates can be as high as 40% in households and among close contacts (11). Outbreaks can result from person-to-person transmission and/or contaminated food and water. There is an increased risk in certain populations, in conditions of crowding where personal hygiene may be poor; such as prisons (11). Most prisons were constructed to maximize public safety, not to minimize the transmission of disease or to efficiently deliver health care. The probability of transmission diarrheal disease is increased by crowding; delays in medical evaluation and treatment, rationed access to soap, water, and clean laundry, insufficient infection control expertise. The immediate transfer of inmates from one location to another further complicates the diagnosis of infection, interruption of transmission, recognition of an outbreak, performance of a contact investigation, and eradication of disease. Many prisons lack adequate information technology, and clinical information sharing between facilities and the different jurisdiction responsible for the care of inmates is often poor (12).

Antimicrobial resistance is one of the world's most serious public health problems, many of the microbes that cause infectious disease no longer respond to common antimicrobial drugs. The prevalence rate of antimicrobial resistance all overall the world of diarrheal shigellosis is 10-90% for ampicillin and 5-95% for trimethoprim/sulfamethoxazole (13). Acquired bacterial resistance

is common in isolates from healthy persons and from patients with community acquired infections in developing countries where the need for antibiotics is driven by the high incidence of infectious disease (14). Among isolates of enteric pathogens resistance is increasing particularly to first line, inexpensive, broad spectrum antibiotics. Furthermore introductions of newer drugs have been followed relatively quickly by the emergence and dissemination of resistant strains (15).

Poor sanitation and restriction to water access may favor the spread of communicable diseases, especially infectious diarrhea, which is one of the leading causes of morbidity in Ethiopia. Investigation of bacterial etiologic agents causing diarrhea is important for treatment and prevention of diarrheal disease. Increasing resistance in bacterial diarrheaogenic pathogens is an important and emerging public health problem. Among prisoners, the most frequent reasons for visiting health facilities are diarrhea. However, there are no available data in Ethiopia on the prevalence of enteropathogenic bacteria, drug resistance pattern and associated factors among acute gastroenteritis prisoners. Hence, the aim of this study was to determine the prevalence, antimicrobial susceptibility patterns and associated risk factors among acute gastroenteritis at Kality prison, Addis Ababa, Ethiopia.

1.3. **Significance of the study**

Investigation of bacterial etiologic agents causing diarrhea is important for treatment and prevention of diarrheal disease. Increasing resistance in bacterial diarrheaogenic pathogens is an important and emerging public health problem. This needs regular monitoring of the antibiotic susceptibility of diarrheaogenic pathogens in a particular area. In brief the study will

- Increases the level of understanding on the prevalence of common enteric pathogens and expand them in routine microbiology laboratory activity.
- Provides updated information on susceptibility pattern of the isolates to avoid extensive use and misuse of antimicrobial drugs which have favored the emergence and survival of resistant strains of micro-organisms.
- Increases awareness towards enteric pathogens and antibiogram for empiric treatment.
- Be used as a baseline for next studies in this line.
- Be a source of information for policy makers or decision makers in this area.

2. Literature review

Diarrheal diseases caused by bacterial pathogens are a major problem worldwide especially in developing countries in conditions of poor environmental sanitation, inadequate water supplies, poverty and limited education (16). According to WHO report, diarrheal disease was the 3rd cause of death in Ethiopia, approximately killing (6%) out of 413,000 people in 2012 (17). There is limited studies conducted on patterns of enteric pathogen in prison inmates but various studies done worldwide have shown changing patterns in the etiology of diarrhaeogenic bacterial pathogen in general community (hospital setting, town, specifically in children<5) some of them reviewed below.

A study conducted in Palestine showed that the prevalence of enteric pathogens from acute gastroenteritis Patients was 9.1%. Of these enteropathogenic bacteria *Salmonella, Campylobacter coli/jejuni,* and *Aeromonas hydrophilia* were constituted 25% each. *Shigella,* 16.7% and *Yersinia enterocolytica* constituted 16.7% and 8.3% respectively (18)**.** Higher prevalence (50.3%) of enteropathogenic agents were isolated from 564 specimen., out of these isolates *E coli* and *Shigella* species constituted 54% and 27.8% respectively. The predominant *E. coli* was Shiga toxin-producing *E. coli* (105 isolates [34.5%]) and the predominant *Shigella* serotype was *Shigella sonnei* (88 isolates [56.1%]) (19).

Study conducted in Gonder, Ethiopia on 372 diarrheic patients reported that 4.57% and 1.08% of *Shigella* and *Salmonella* spps respectively. The most commonly isolated strains of *Shigella* were *S. flexneri* which constituted 64.7%, *S. dysenteriae* 17.65%, *S. boydii* 11.77% and *S. sonnei* 5.88% (3). Another cross sectional study conducted on 384 diarrheic patients in Hiwot Fana Hospital 14.58% of enteropathogenic bacteria. The isolates were proved to be positive for *Shigella* species (20). High prevalence of enteropathogenic bacterial isolates was reported by Asrat *et al* (2008). Among the 76 isolates of *Shigella* species, serogroup B (*Sh. flexeneri*) was the most prevalent (54.0%). Furthermore, 37 *Salmonella* species were isolated. Serogroup B was the most prevalent (81.1%) (21).

Study conducted in Karubanda prison, southern province of Ruwanda showed that among 23 samples collected for culture30.4% were positive for *Shigella* species out of thes isolates85.7% of the isolates were typed as *Shigella flexneri* and 14.3% for *Shigella dysenteriae* type-2 (22)**.** Another study conducted in prison inmates reported multiple sero-types of Salmonella outbreaks

in two state prisons, Arkansas. The isolated *Salmonella* species showed that from stool specimens of 7 inmates experiencing diarrhea identified 3 serotypes: Anatum, Cerro, and Heidelberg. The *Salmonella* species reported from prison B was Salmonella sero-type Anatum (23).

The enteric bacteria that cause acute gastroenteritis are very different from one another. They cause quite different clinical syndromes; their ecology, epidemiology and modes of transmission are distinct; and they are widely separated genetically. The fact that those different organisms are becoming increasingly antibiotic resistant underlines the occurrence of the pressures that lead to the emergence and spread of resistance (24). The prevalence rate of antimicrobial resistance all overall the world of diarrheal shigellosis is 10-90% for ampicillin and 5-95% for trimethoprim/sulfamethoxazole (25). For this reason the antibiotics resistance is receiving increasing attention in light of the increasing incidence of human bacterial infections resistant to antibiotic treatment. The resistance of enteropathogenic bacteria to commonly prescribed antibiotics is increasing both in developing as well as in developed countries; resistance has emerged even to newer, more potent antimicrobial agents (26).

A study conducted in Palestine on antimicrobial resistance for enteric pathogens isolated from acute gastroenteritis patients in Gaza strip showed that, among 132 diarrheal patients, 9.1% of enteropathogenic bacteria were isolated. High antimicrobial resistance were reported among these isolates; Campylobacter *coli/jejuni* (52.4%), *Aeromonas hydrophilia* (49.2%), *Yersinia enterocolytica* (42.9%), *Shigella* (26.2%) and *Salmonella* spp. (22.2%) (18). Similarly, high antibiotic resistance was reported from study conducted in Tehran. Among the isolates 75.5% of *E. coli* was resistance to amoxicillin and tetracycline and 5.2% of E. coli isolates were resistance to more than six antibiotics. Most *Shigella* isolates (95%) were resistant to tetracycline and trimethoprim-sulfamethoxazole (91.7%), with greatest antibiotic resistance observed among *S. sonnei* (60.2%) isolates (19).

Study conducted in Iran showed that, antibiotic resistance rates are increasing among *S. sonnei* strains in pediatric patients. From eighty nine *Shigella* isolates, *S. sonnei* was the most prevalent species (60.7%) followed by, *S. flexneri* (31.5%). Eleven antimicrobial resistance patterns (R1-R11) were identified among *S. sonnei* isolates. The majority of the strains were resistant to trimethoprim-sulfamethoxazole, tetracycline and streptomycin. All isolates were susceptible to

ciprofloxacin, ceftizoxime and chloramphenicol (27). Study conducted in the republic of Rwanda in Karubanda prison, *Shigella* species were sensitive to ciprofloxacin, cefotaxime, nalidixic acid, cefotazidine and gentamycin. However, the species were resistant to the commonly prescribed antibiotics chloramphenicol, ampicillin and cotrimoxazole (28).

Study conducted on diarrheic patients attending Gondar town health institutions showed that, *Shigella* isolates were presented high resistance rate to ampicilin (94.1%), amoxicillin (88.2) and tetracycline (88.2%). *Salmonella* species were 100% resistance to tetracycline and amoxicillin and 75% for ampicillin. However, all isolates of both *Shigella* and *Salmonella* were 100% susceptible to ciprofloxacin and norfloxacin (3). Other study conducted in Harar on antibiotic susceptibility patterns of *Salmonella* and *Shigella* isolates showed that; Salmonella isolates were 100% resistance to ampicillin and amoxacillin 14.2% to tetracycline, 28.6% to chloramphenicol, 89.3% to norfloxacin, and 92.8% to gentamicin. *Shigella* species showed 100% resistant to ampicillin andamoxicillin, 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. The organisms developed complete resistance to ampicillin and amoxicillin (30).

Diarrhea acquired via contaminated water and foods are important determinants for the occurrence of acute gasteroenteritis. Poor storage of drinking water (e.g. obtaining water from storage containers by dipping, no drinking water storage facility), use of unsafe water sources (such as rivers, pools, dams, lakes, streams, wells and other surface water sources) were the known determinant factors (31). Some epidemiological studies have revealed that not washing hand before meals or after defecation (32), unhygienic (kitchen, living room, yard), unsafe food storage, presence of flies inside the house, were associated with risk of diarrhea morbidity (33).

In Ethiopia there is no study conducted to show the risk factors associated with the occurrence of enteric pathogen in prison inmates. However, study conducted in republic of Rwanda in Karubanda Prison showed there was an irregular distribution of soap in the prison with an attack rate of 0.2%, whereas in other part of the prison there were shortage of water with the attack rate was 0.6 % (31). This indicated that the availability of adequate water and soap associated with enteropathogenic bacterial pathogens.

3. Objectives

3.1. General objective

To determine the prevalence of bacterial etiologies with their antimicrobial susceptibility pattern and associated risk factors among prisoner with acute gastroenteritis at Kality prison, Addis Ababa, Ethiopia.

3.2. Specific objectives

- To determine the prevalence of bacterial causing acute gastroenteritis in prison inmates
- To determine antimicrobial susceptibility patterns of bacteria isolates from acute gastroenteritis.
- To identify associated risk factors for contracting diarrhea.

4. **Research question**

What are the most common pathogenic bacteria isolates, their antibiotic resistance pattern and associated risk factors for acute diarrheal disease among patients experienced gastroenteritis in prison inmate at Kality prison?

5. Methods and Materials

5.1. Study area

The study was conducted at Kality Prison which is located in Akaki Kality subcity woreda 7 around 11 km from central Addis Ababa, Ethiopia. It is the Southernmost sub-city of the nation's capital. The prison serves as the main prison of Ethiopia. Which is divided into male and female zones, comprising around 4,000 sentenced prisoners currently, of which 3,400 were males and 600 were females The prison has a clinic and a general hospital, The clinic gives service only for prisoners whereas the hospital serves both staffs and high risky prisoners (34).

5.2. Study design and period

An institutional based cross-sectional study was conducted from January 2017 to Sept /2017.

5.3. Source population

All prisoners' at Kality prison who sought medical services during the study period.

5.4. Study Population

All prisoners who had acute diarrhea and sought medical services during the study

5.5. Inclusions and Exclusions criteria

5.5.1. Inclusions Criteria

- ✓ All prisoners who had acute diarrhea during the study period.
- ✓ All prisoners volunteer to give informed consent to participate on the study.

5.5.2. Exclusion Criteria

- ✓ Patients who took antibiotics currently within the last10 days.
- ✓ Patients who had chronic diarrhea (greater than 14 days).

5.6. Sample size and sampling procedure

5.6.1. Sample size

The sample size of this study was calculated based on previous study conducted in Gondar town, Ethiopia. The prevalence of bacterial isolates among diarrheal patients from this study was 16.9% (29).Using 95 % of confidence interval with 5% of margin of error the sample size

was calculated as follows.

$$n = \frac{(Z\alpha/2)^2 P (1-P)}{d^2}$$

Where:

n = No of sample that will be included

α= confidence level

P= prevalence from the previous study.

d= acceptable difference

$$n=\frac{1.96^2(0.169)(1-0.169)}{0.05^2}$$ n= 216

Adding the 10 % contingency 238

So, the sample size was **238**

5.6.2. Sampling procedure

A total of 238 study participants were recruited using convenient sampling technique.

5.7. Study Variables

Dependent Variables-

- Antimicrobial susceptibility patterns
- Prevalence of bacteria isolates

Independent variables-

- Age
- Sex
- Illiteracy
- Clinical sign and symptom
- Sharing of drinking utensils
- Kinds of water storage utensils
- Absence of regular hand washing habit,
- Hand washing without soap
- Use of water from unprotected source
- Absence of latrine
- Latrine cleaning frequency

5.8. Data Collection

5.8.1. Socio-demographic and clinical data

Socio-demographic and clinical data were collected using structured questionnaire. The structured questionnaire contains information such as age, sex, educational status, source of drinking water, latrine usage, hand washing and clinical sign and symptoms.

5.8.2. Stool Sample Collection and transportation

Fresh stool specimens were collected from each study participants for bacteriological analysis. The specimens were coded with patients ID and information and transported using Cary-Blair transport media to Ethiopian Public Health Institute clinical bacteriology laboratory at 2-8^{0}c.

5.9. Identification of bacterial isolates

Each stool sample directly cultured onto MacConkey agar (MAC), Selenit F broth (SFB), and Sorbitol MacConkey agar (SMAC) Medias. Approximately 1 g of each sample inoculated into 10 ml of Selenite F broth. The tubes and plates were incubated for 24 hours at 37^{0}c. From enrichment Selenite F broth subcultured onto Xylose-Lysine Desoxycholate (XLD) agar then the plates incubated at 37°C for 18 to 24 hrs. The suspected bacteria were identified by colony morphology and biochemical characteristics. On MAC agar suspected enteric bacteria appeared as transparent or colorless colonies. *Salmonella spp.* appeared on XLD agar as red colonies with black centre owing to H_2S production. *Shigella spp*. colonies identified on XLD agar the colonies appeared as transparent red and also *E.coli O157H7* on SMAC sorbitol negative showed transparent colorless colonies. Standard biochemical tests like Triple Sugar Iron agar (TSI), Sulfide Indole Motility medium (SIM), Lysine Iron Agar (LIA), Urea agar, Citrate, Indole test and specific antisera were used for the confirmation of *Salmonella, Shigella* and *E.coli O157H7* (35, 36).

Specific antisera

Confirmation of the results was performed for *Salmonella, Shigella and E.coli O157H7* isolates by specific antisera.

Serotyping of Shigella spp. And E.coli O157:H7

Serological identification of *Shigella* begins with the use of polyvalent antisera, which is used to identify the species (i.e. S. dysenteriae, S. boydii, S. flexneri, or S. sonnei). If agglutination is observed with polyvalent antisera, the isolates are then tested with the individual monospecific serum found in the polyvalent antisera and *E.coli O157:H7* confirmed by slide agglutination test using commercially available antisera. (37).

Serotyping of Salmonella *Spp.*

Serotyping of *Salmonella* strains is carried out by identification of surface antigens (lps, O-antigens) and flagella antigens (proteins, H-antigens). Most commonly, strains of *Salmonella* express two phases of h- antigens but aphasic, monophasic and triphasic variants are known (38).

5.10. **Antimicrobial susceptibility test for bacterial isolates**

The disk diffusion method was performed and after 16-18hours of incubation at 37^{o}C zone of inhibition was measured and interpreted as recommended by the Clinical and Laboratory Standards Institute (CLSI). Using a sterile wire loop, 3-5 pure colonies were picked and emulsified in nutrient broth. Standard inoculums adjusted to 0.5 McFarland was swabbed onto Muller-Hinton agar. Drug susceptibility testing of all bacteria isolates was performed using disk diffusion method incubating at 37^{o}C for 18 hours against Ampicillin(10 µg), Ceftriaxone(10 µg), Nalidixic Acid(10µg), Trimethoprim/sulfamethoxazole(25µg), and Ciprofloxacin(5µg). The zone of inhibition was measured to the nearest millimeter and isolates were classified as sensitive and resistant according to the standardized table supplied by CLSI (39).

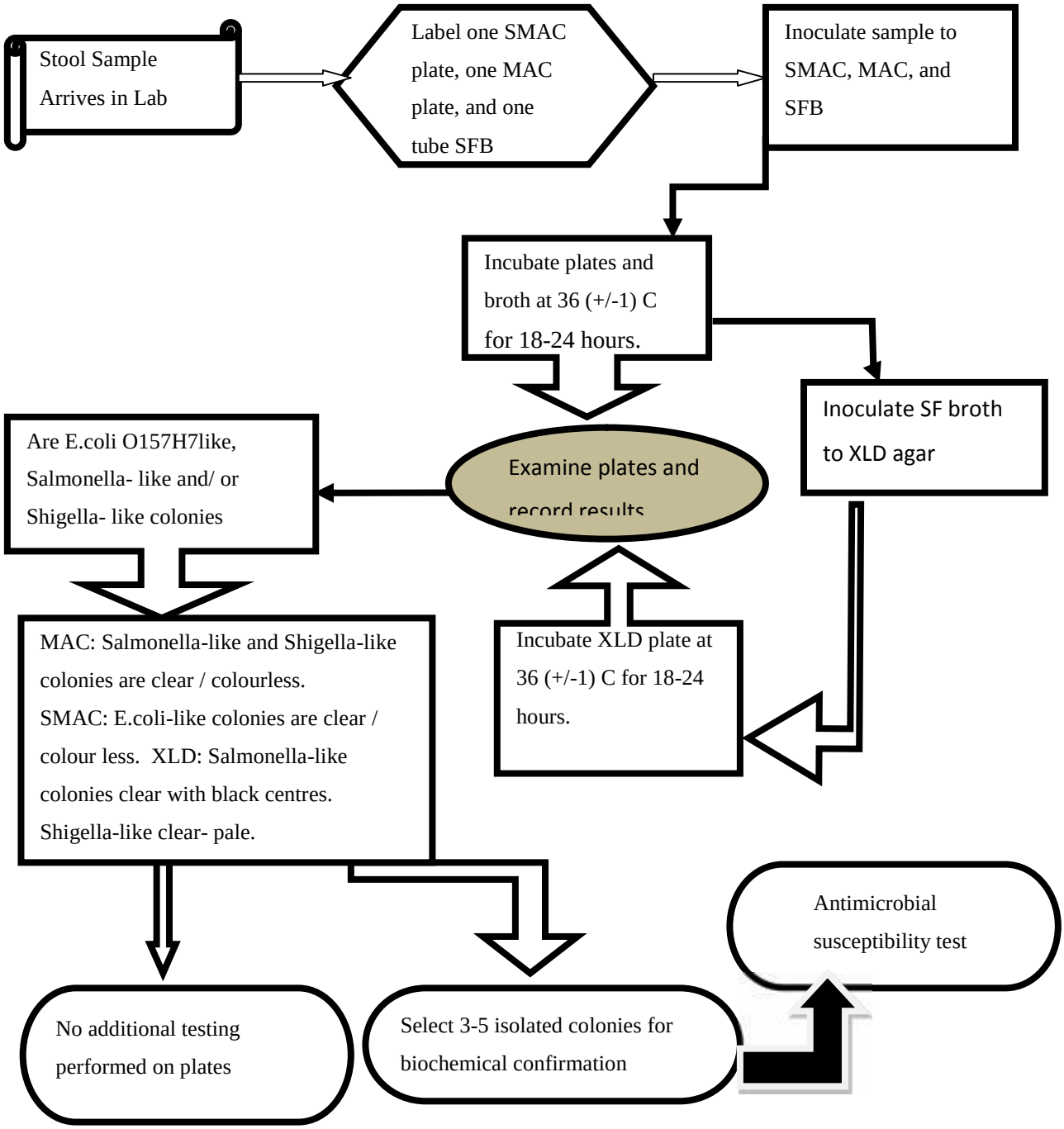

Figure 5.10: Flow chart for identification of enteric pathogens and antimicrobial susceptibility among prisoner with acute diarrhea in Kality prison clinic, Addis Ababa, 2017

5.11. **Quality control**

A standard control strains *E. coli* ATCC25922, *Salmonella spp* ATCC13076; *Shigella spp* ATCC12022 were used and verify that the patient identifiers on the specimen match those on the accompanying requisition. Ensure that all media and supplies were used passed the required QC and were used before their expiration date.

5.12. **Statistical analysis and interpretation**

Socio-demographic, clinical and laboratory data were entered and analyzed using SPSS version 20. Descriptive data was explained by tables and figures. Univariate and multivariate analysis was used to assess the associated risk factors. P-value < 0.05 was considered statistically significant.

5.13. **Data quality Assurance**

All stool samples for culture and antimicrobial susceptibility patterns was performed in accordance with EPHI bacteriology laboratory SOPs. Completed questionnaires, culture and antimicrobial susceptibility test results was coded by numbers, recorded carefully and entered in a computer software SPSS version 20.The data was also stored in a CD as a backup.

5.14. Operational definition

Prison: - is a building in which people are legally held as a punishment for a crime they have committed or while awaiting trial.

Prisoners:-Prisoners are inmates confined in long-term facilities run by the state or federal government. They are typically criminals who have received a sentence of incarceration. Sentence length may vary by state because a few states have one integrated prison system in which both prison and jail inmates are confined in the same types of facilities.

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

5.15. **Ethical clearance**

The study was approved by Department Research and Ethical Review Committee (DRERC) of the Department of Medical Laboratory Science, School of Allied Health Sciences, College of

Health Sciences, Addis Ababa University. Permission letter was also obtained from the study site. The purpose and procedures of the study was explained to the study participants within the study period. Those patients give informed consent were selected and enrolled as the participants of the study. A patient result was communicated only to the attending physicians.

6. **Results**

6.1. Socio-demographic characteristics of diarrheic prisoner

A total of 238 study participants were included in this study, of which 76.1% (n=181/238) were males. The ages of the study participants ranged from 19 to 81 years with a mean age of 33.61 with +/-SD 9.85. Among the study participants154 (64.7%) lived in urban areas and 34.5% (n=82/238) of prisoners were attended primary school.

Table 6.1:- Distribution of socio-demographic characteristics of diarrheic prisoner attending Kality prison clinic, Addis Ababa, Ethiopia, 2017.

Characteristics	**Number**	**Percent**
Gender		
Male	181	76.1
Female	57	23.9
Age in year		
18-24year	19	8.0
25-45year	188	79.0
Above 45	31	13.0
Educational status		
Illiterate	9	3.8
1-8grade	82	34.5
9-12	99	41.6
Higher education	48	20.2
Residence before impersonation		
Urban	154	64.7
Rural	84	35.3
Length of imprisonment		
Months	53	22.3
Years	185	77.7
No of prisoner /cell, room		
<100	21	8.8
101-200	127	53.4
>200	90	37.8

6.2. Clinical features of acute gastroenteritis

The clinical features of the diarrheal patients were indicated as in Table 6.2. In this study, out of the total of 238 diarrheal patients, 229(96.2%), 95(39.9%) and 74(31.1%) had abdominal pain, fever, and vomiting, respectively. With regard to consistency of stools, 75(31.5%), 33(13.9%), 78(32.8%) and 52(21.8%) were watery, mucoid, bloody and dysentery stool samples, respectively. The study also revealed that duration of diarrhea ranged from 1-5 days, 6-10 days, and 11-14 days in 209(87.8%), 26(10.9%) and 3(1.3%) of the selected diarrheal patients, respectively (Table 6.2).

Table6.2:- Clinical features of the study subjects who visited kality prison clinic Addis Ababa, Ethiopia, 2017

Symptoms	Number	Percent
Fever		
Yes	95	39.9
No	143	60.1
Vomiting		
Yes	73	30.7
No	165	69.3
Abdominal pain		
Yes	109	45.8
No	129	54.2
Tenasemess		
Yes	104	43.7
No	134	56.3
Consistency of stool		
Watery	61	25.6
Bloody	31	13.0
Mucoid	92	38.7
Dysentery	54	22.7
Duration of diarrhea		
1-5 day	133	55.9
6-10 day	86	36.1
11-14 day	19	8.0
Frequency of diarrhea per day		
1-3	34	14.3
3-5	155	65.1
>5	49	20.6

6.3. Food and hygiene related variables

Of the total 238 study participants, 100% of the patients responded that they were using modern types of latrine with dissimilar cleaning practice such as:- every time of use 21(8.8%), every day 156(65.5%) and1-2 time a week 61(25.6%).with regard to source of drinking water, 208(87.4%) of acute diarrhea patients that they were using pipe water for drinking purpose. Data revealed that 93 (39.1%) of the patients responded that they were sharing of water drinking utensils in group. Furthermore, the data revealed that162 (68.1%), 62(26.1%) and 14(5.9%) of the study subjects were obtaining their foods from prison only, prison and family and family only respectively.

Table 6.3:- Environmental and behavioral variables of the study subjects who visited Kality prison clinic Addis Ababa, Ethiopia, 2017

Environmental and behavioral variables	Number	Percent
How often cleaned		
Every time	21	8.8
Every day	156	65.5
1-2 time a week	61	25.6
Hand washing habit		
Yes	234	98.3
No	4	1.7
Hand washing by		
Water only	97	40.8
Water and soap	140	58.8
Source of meal		
Family only	14	5.9
prison only	162	68.1
prison and family	62	26.1
Store food		
Yes	92	38.7
No	146	61.3
Cooked food stored by		
Refrigerator	2	.8
In ladder	14	5.9
Disk-cover	57	23.9
plastic/pestal	20	8.4
For how long food stored		
1day	83	34.9
2day	9	3.8
>2 day	1	.4
Source of drinking water		
Pipe water	208	87.4
Packed Water	30	12.6
Kinds of water storage utensils		
Bucket	16	6.7
Jerrycan	57	23.9
Highland	165	69.3
Share water drinking utensils		
Yes	93	39.1
No	145	60.9

6.4. Bacterial Etiologies among prisoner

The overall prevalence of bacterial isolates was 20.6% (49/238). The commonest isolates among this enteropathogenic bacteria was enterohemorrgic *E. coli O157H7* 55% (n=27/49) followed by *Shigella species* 28.6% (n=14/49), and *Salmonella spp* 16.3% (n=8/49).

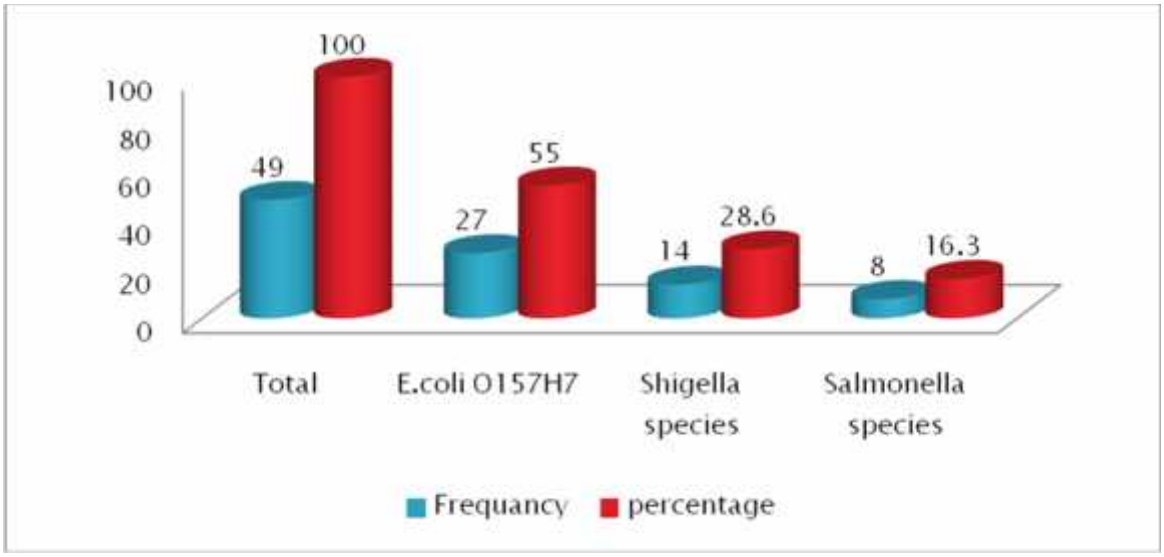

Figure 6.4:-Frequency of bacterial pathogens isolated from fecal sample of prisoner with acute diarrhea at Kality prison clinic from Jan-Sept 2017, Addis Ababa, Ethiopia

6.5. Antimicrobial susceptibility pattern

The overall antimicrobial resistance profiles for all isolates were 27.8%. The antimicrobial resistance profiles of the 49 isolates were shown in table 6.5. *Shigella species* showed high resistance rates for all antibiotics (37.1%), followed by *E.coli O157H7* (24.4%) and *Salmonella spp*. (20%). Resistance rates of ampicillin, trimethoprim/sulfamethoxazole, nalidixic acid, ceftriaxone and ciprofloxacin were (97.9%), (14.6 %), (6.3%), (16.7%) and (4.2%) respectively.

Table 6.5: Antimicrobial susceptibility pattern of enteric bacterial pathogens identified from Kality prison clinic, Addis Ababa, Ethiopia, 2017.

Enteropathogenic bacteria (n=49)		**E.coli "O157H7" (n =27)**	**Shigella species (n=14)**	**Salmonella species (n=8)**	**percent**
AM	R	96.3%(26)	100%(14)	100%(8)	97.9
	S	3.7%(1)	0	0	2.1
NA	R	0	21.4%(3)	0	6.1
	S	100%(27)	78.6%(11)	100%(8)	93.9
SXT	R	14.8%(4)	21.4%(3)	0	14.3
	S	85.2%(23)	78.6%(11)	100%(8)	85.7
CI	R	14.8%(4)	28.6%(4)	0	16.3
	S	85.2%(23)	71.4%(10)	100%(8)	83.7
CIP	R	0	14.3%(2)	0	4.1
	S	100%(27)	75.7%(12)	100%(8)	95.9
Percent		24.4	37.1	20	

Key S-Susceptibility,R-Resistance, Ampicillin (AM), Ceftriaxone (CI), Nalidixic Acid (NA), Trimethoprim/sulfamethoxazole (SXT), Ciprofloxacin (CIP).

Enterohemorrhagic *E.coli 0157; H7* isolates were 100% susceptible to Nalidixic Acid and Ciprofloxacin. Moreover, 85.2% of the isolates were susceptible to Trimethoprim/ sulfamethoxazole and Ceftriaxone. However, 96.3% of the isolates were resistant to ampicillin.

Shigella spp isolates were susceptible to 85.7% to ciprofloxacin, 78.6% to trimethoprim-sulphamethoxazole, and nalidixic acid,71.4% to ceftriaxone and -75.7% to Ciprofloxacillin. In the contrary, the isolates were 100% resistant to ampicillin.

Salmonella spp isolates were 100% susceptible to Ceftriaxone (CI), Nalidixic Acid (NA), Trimethoprim/sulfamethoxazole (SXT) and Ciprofloxacin (CIP). However, the isolates showed 100% resistant to ampicillin.

6.6. Multi-drug resistant isolates

Among the total isolates (n=49) multidrug resistance were recorded in 8(16.3) of all the three bacterial isolates. *Shigella spp* and *E.coli O157:H7* isolates showed 28.6% (n=4/14) and 14.8% (n=4/27) multiple drug resistance respectively. Out of isolated MDR *Shigella*, 7.1% were resistance to three antimicrobials, 14.3% were resistance to four antimicrobials and 7.1% were resistance to five antimicrobials. Regarding, *E coli O157:H7* isolates, 14.8% of the isolates showed multidrug resistance to three antimicrobials. The other enteric bacteria, eight (100%) of Salmonella were not showed multidrug resistance it is summarized on table 6.6.

Table 6.6:- Resistance antibiogram of isolates from stool specimen in acute diarrheagenic prisoner, Kality, Addis Ababa, Ethiopia, 2017.

Number of antimicrobial resistance	**Resistance antibiogram**	**Resistance isolates n (%)**		
		Salmonella spp(n=8)	Shigella spp (n=14)	E coli O157:H7 (n=27)
RO	None	0	0	1(3.7)
R1	Amp	8(100)	10(71.4)	21 (77.8)
R3	Amp ,CRO,SXT AMP,CRO,CIP	0	1(7.1)	4 (14.8)
R4	Amp ,SXT,CRO,NA	0	2(14.3)	0
R5	Amp, CIP, SXT,CRO,NA	0	1(7.1)	0

Key: Amp-Ampicillin, SXT-Cotrimethoxazole. CRO-ceftriaxone, CIP-ciprfloxacillin, RO-none resistance, R1-resistance for one antimicrobial, R2-resistance for two antimicrobial, R3-resistance for three antimicrobial, R4-resistance for four antimicrobial, R5-resistance for five antimicrobial.

6.7. Bivariate analysis of potential risk factors associated with acute diarrhea

6.7.1. Socio-demographic characteristics

Among the major socio-demographic factors that were included in this study, age, sex, educational status, length of imprisonment, number of prisoner per cell/ room, and place of residence before impersonation. The association of these variables with detection of bacterial enteropathogens was assessed and large number of prisoner per cell (>200) was significantly associated with acute diarrhea (p=0.038, AOR=6.74, 95%CI [1.76, 25.9]).

6.7.2. Clinical variables

Patients having bloody and/or watery diarrhea were statistically associated with identification of enteropathogens and acute diarrheal disease with p value (p=0.024) and AOR=5.33, 95%CI [1.63, 44.9] and (p=0.030) AOR=1.18, 95%CI [1.02, 2.22] respectively. Further the identification of enteropathogens was found to be 5.3 and 1.18 times more likely to be associated with bloody and watery diarrhea than dysentery. The frequency of diarrhea >5 per days has significantly associated with identification and isolation of enteric pathogens (p=0.02 AOR=2.4, 95CI [1.61, 5.12]) and was found to be 2.4 times more likely associated with occurrence of diarrhea compared to the frequency of diarrhea 1-3 per days. Among the clinical manifestations of the study prisoner with acute diarrhea, abdominal pain was statistically significant association for the detection of enteropathogens from acute diarrhea stool (p=0.041, AOR=2.34,95%CI[1.88, 33.3]), and found to be 2.3 times more likely to associated with acute diarrhea than compare to those who have fever and vomiting as shown in Table 6.7.

6.7.3. Food and hygiene related variables

Detection of enteric pathogens was significantly associated with patients who share water drinking utensil (p=0.029) and hand washing habit only with water (p=0.010). Sharing water drinking utensil was about 3.5 times more likely to associate with enteric pathogens detection as compared with those who did not share water drinking utensil AOR=3.509, CI [3.49, 9.17] and hand washing with water only was about 3 times more likely to associate with enteric pathogens detection as compared to those who wash their hand with water and soap (AOR=3., CI [2.68, 9.425]). However, there was no statistical significant association between sources of drinking water, kinds of water storage utensils with the detection of enteric pathogens.

Table 6.7: Bivariate analysis of potential risk factors associated with enteropathogenic bacteria among study participants attending Kality prison clinic, Addis Ababa, Ethiopia, 2017.

Variables	Enteric pathogen	No Enteric pathogen	p-value	COR(95 %CI)	p-value	AOR(95 %CI)
Gender						
Male	34	147	.35	1.41(.693,2.861)	.45	.51(.087, 2.935)
Female	14	43		1		1
Age in year						
18-24year	2	17		1		1
25-45year	40	148	.28	.44(.097, 1.963)	.23	.232(.207, 65.29)
Above 45	6	25	.42	.49(.088, 2.723)	.99	.99(
Educational status						
Illiterate	4	5	.11	.288(.064, 1.29)	.29	5.35(.230, 124.4)
1-8grade	14	68	.81	1.121(.44,2.828)	.24	.332(.053, 2.081)
9-12	21	78	.73	.857(.359, 2.05)	.71	.732(.146, 3.67)
Higher education	9	39		1		1
Residence Before Impersonation						
Urban	30	124	.72	.887(.460, 1.71)	.39	1.76(.479, 6.522)
Rural	18	66		1		1
Length Of Imprisonment						
Months	10	43		1		1
Years	38	147	.789	1.112(.512,2.41)	.61	.681(.158, 2.941)
No Of Prisoner /Cell, Room						
<100	3	19		1		1
101-200	36	128	.59	1.566(.309,7.94)	.99	.746(.017, 33.43)
>200	9	43	.01	1.44(1.032,1.66)	.04	6.74(1.76, 25.92)
Fever						
Yes	18	77	.70	1.136(.592, 2.2)	.02	.118(.020, .691)
No	30	113		1		1
Vomiting						
Yes	15	59	.14	.606(.314, 1.17)	.37	.543(.141, 2.087)
No	33	131		1		1
Tenasmess						

Yes	48	181	.00	.010(.001, .076)	.00	392.1(19, 7869)
No	0	9		1		1
Abdominal pain						
Yes	15	89	.05	1.939(1.98, 3.8)	.04	2.34(1.05, 3.94)
No	33	101		1		1
Consistency of stool						
Watery	10	65	.025	2.38(1.113,5.09)	.03	1.18(1.02, 1.22)
Bloody	8	25	.001	32.3(4.11,254.2)	.02	5.33(1.63, 44.9)
Mucoid	21	57	.025	4.20(.196, .899)	.09	1.00(.194, 5.19)
Dysentery	9	43		1		1
Duration of diarrhea						
1-3 day	43	166		1		1
4-5 day	5	21	.25	.469(.129,1.702)	.75	3.141(.13,72.46)
>5 day	0	3	.31	2.12(.494, 9.07)	.78	.636(.025, 16.25)
Frequency of diarrhea / day						
1-3	12	36		1		1
4-5	36	152	.38	.397(.049, 3.18)	.35	.180(.005, 6.67)
>5	0	2	.03	2.01(1.01,3 .08)	.02	2.41(1.61, 5.12)
How often cleaned						
Every time	7	14		1		1
Every day	26	130	.07	2.50(.919, 6.79)	.09	8.49(.734, 98.38)
1-2 time a week	15	46	.44	1.53(.522, 4.51)	.33	2.23(.442, 11.18)
Hand washing habit						
Yes	46	188	.17	4.08(.56, 29.78)	.92	1.17(.048, 28.89)
No	2	2		1		1
Hand washing by						
Water only	21	59	.01	2.1(1.048, 2.35)	.01	3.02(2.68, 9.415)
Water &soap	27	131		1		1
Source of meal						
Family only	5	9		1		1
prison only	35	127	.05	.267(.071, 1.00)	.51	.390(.024, 6.352)
prison & family	8	54	.14	.538(.234, 1.24)	.83	1.187(.253, 5.57)
Store food						
Yes	15	77	.24	1.49(.763, 2.95)	.98	1.07(.008, 139.8)
No	33	113		1		
Cooked food stored by						
Refrigerator	1	1		1		1
In ladder	2	12	.26	.176(.009, 3.65)	.99	.000
Disk-cover	9	48	.95	1.059(.153, 7.3)	.94	1.128(.05, 25.35)

plastic/pestal	3	17	.93	.941(.228, 3.89)	.95	.935(.122, 7.177)
For how long food stored						
1day	15	71		1		1
2day	0	6	1.0	.000	1.0	.000
>2 day	0	1	1.0	1.000	1.0	.000
Water storage utensils						
Bucket	1	15		1		1
Jerrycan	6	41	.61	.567(.063, 5.08)	.97	.935(.033, 26.51)
Highland	41	134	.13	.202(.026, 1.57)	.68	1.443(.247, 8.42)
Share drinking utensils						
Yes	18	68	.003	3.71(1.94, 7.1)	.03	3.509(3.49, 9.17)
No	30	122		1		1
Source of drinking water						
Pipe water	43	174	.04	.12(.016, .89)	.96	1.08(.041, 28.75)
Packed Water	5	16		1		1

**Significance <0.05,COR-crude odd ratio in bivariate logistic regression ,AOR-adjusted odd ratio in multivariate logistic regression and 95% confidence interval for association of risk factors to dependent variable in the study. Reference category is denoted by number 1 selected due to less association factor to dependent variable.*

7. Discussion

In this study the overall prevalence of entheropathogenic bacteria among prison inmates was (20.6%). The isolation rate of *Shigella spp*. 28.6%(n=14/49) was almost in line with studies conducted in Iran (27.8%) (19). Higher prevalence rate than studies conducted in Iran (5.1%) (22), Republic of Rwanda (0.6 %) (22) and Palestine (1.5%) (18). Rate of *Shigella* was also higher than previous studies conducted on other community setup (other than prisoner) in Ethiopia: Harar (14.58%) (20), Harar (6.7%) (40), Gondar 16.9% (29) and 4.57 %(3). 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. This finding was lower than studies done in Thailand (40.4%) (15). A low rate of isolation in the present study may be due to differences in the method of sample collection, isolation and identification.

This study showed that *S. dysentriae* (57.14%) was the predominant sero-group, which was found to be higher than the finding reported in Abadan, Iran (5.5%) (19). Study conducted in Gondar shows (17.65%) (3) and Addis Ababa (17.65%) (41), whereas prevalence of *S .flexneri* current finding indicated that rate (24.57%) was lower than the findings reported in Iran (52.7%) (31), Gondar (64.7%) (3), overall Ethiopia 54% (21) and in line with a report in Tehran, Iran (30.57%) (19) The current finding indicated that *S. boody* (14.28%) were the least frequently isolated spp. which was in line with the study conducted in the Gondar town 11.7% (3) Majority of *Shigella* isolates were found in dysentery 8(57.2%), within Share drinking utensils 9 (64.3%), using pipe water as source of drinking 14 (100%) and using Highland as drinking utensil 14(100%). However, it was not statistically significant associated with an increasing of infection with *Shigella*.

In the current finding the isolation rate 16.3 %(n=8/49) of Salmonella spp. was found to higher than studies done in Palestine Refugee (0.76%)and Tehran,Iran in children (7.4%) . However, it was lower than the findings reported in Mexico (27%) among prisoner. The rate of *Salmonella spp* was also consistent with previous studies conducted on other community setup (other than prisoner) in Ethiopia: Harar (11.5%) (40), Gondar (19.5%) (3). However, it was higher than the findings reported in Hawassa (2.5%) (26) and Dilla(0.93%) (42). This might be due to the increasing awareness of the people about personal and environmental hygiene made by the

health institutions and other partners or might be sample size, geographical variation and epidemicity of the disease.

Numerous types of *E. coli* that causes diarrheal disease have been described, including enterotoxigenic strains, Enteropathogenic strains, enteroinvasive strains, enterohemorrhagic strains, and enteroaggregative strains. Of these different types, only enterohemorrhagic strains of serotype O157:H7 can be routinely detected in most clinical microbiology laboratories because a specific selective medium, Sorbitol-MacConkey agar is widely available. The geographic distribution of these strains varies, and media for detection may be available in some laboratories and not in others. Sorbitol-negative strains can be further identified with specific serotyping reagents.

In this study 55% (n=27/49) *E. coli O157:H7* were isolated which is higher than study conducted in Iran rate of 34.5% in children (19), the study conducted in Bahir Dar town showed an overall isolation rate of 48.3% *E. coli* in children aged under five with acute diarrhea of which 28.9% E. coli O157:H7 was isolated (43), a study conducted in Jimma town isolated 1.8% *E. coli O157:H7* food handlers (44) and the study conducted in Nigeria, Lagos with the prevalence of 5.1% of EHEC associated with watery diarrhea (45).

The emergences of increased antimicrobial resistances are global challenges, particularly in developing countries. In this study, the susceptibility pattern for all bacterial strains showed resistance to at least one drugs as shown in (table 6.6). The majority of the bacterial pathogens were resistant to three or more drugs tested, with ampicillin, cotrimoxazole and Ceftriaxone being the most ineffective drugs similar to the study conducted in Zambia (46). This finding shows antimicrobial resistance pattern of *Shigella spp.* against Ampicillin, trimethoprim-sulphamethoxazole, Nalidixic Acid, Ciprofloxacin and Ceftriaxone were 100%,21%,21%,14.3% and 28.6% resistance respectively. The highest resistance of Ampicillin (100%) was comparable to the report from Harar 96.4% (21) and Meklele 100% (47). Study in Hawassa, Southern Ethiopia also showed 63.6% resistance to Ampicillin but there was no resistance rate observed against Ciprofloxacin, Nalidixic acid, and Cotrimoxazole (25), in the same study area 56% to trimethoprim-sulphamethoxazole resistance reported (43). This indicates that the resistance of ampicillin increasing through time. While a study done in Addis Ababa showed resistance level

of 78.7% to ampicillin and 45.3% to trimethoprim-sulphamethoxazole of *Shigella spp*. (41) which indicated that ampicillin resistance was increased by 21.3% within the last Twelve years.

Another enteric bacterial pathogen *Salmonella spp*. also showed 100% antimicrobial resistance to ampicillin this finding was higher compared to other studies from Addis Ababa (21) showing ampicillin resistance of (81.2%), trimethoprim- sulphamethoxazole (75.7%) and very low resistance reported to ampicillin in Gaza strip, Palestine (22.0%)(18) . this result shows 100% susceptible to Ceftriaxone, Nalidixic Acid , Trimethoprim/sulfamethoxazole, Ciprofloxacin. This might be due to variation in number of strains and different batch of antimicrobial disk used.

The antibiogram of *E. coli O157:H7* showed resistance of 96.29% to ampicillin, 14.8% trimethoprim-sulphamethoxazole, and ceftriaxone this finding shows 100% susceptible to Nalidixic Acid and Ciprofloxacin. The isolates had similar resistance pattern report in keney show ampicillin (83.9%) but higher reissuance to trimethoprim-sulphamethoxazole (95.7%) (48). In the same way with the study done in Bahirdar in which high levels of antimicrobial resistance to ampicillin (86.8%) and on the contrary this study higher rate of cotrimoxazole resistance (76%) was documented and low levels of resistance to ciprofloxacin (6.9%) was included (43). On the other hand, the study conducted in Addis Ababa the resistance showed the following level of resistances to the antibiotics ampicillin (77.3%), trimethoprim-sulphamethoxazole(68.2%), ciprfloxacillin and ceftriaxone(23.1%) unlikely to the present study indicated no resistance was documented for ciprofloxacin and Nalidixic Acid (41).

Risk Factors Associated with enteropathogenic bacteria

As shown in Table 6.7, AOR was used to assess the association between selected risk factors and enteropathogenic bacteria. P-values less than 0.05 were considered as statistically significant. Among the major socio-demographic factors that were included in this study, age, sex, educational status, length of imprisonment, and place of residence before impersonation did not show significant association with the prevalence of enteropathogenic bacteria at 95% confidence interval. A study reported that the role of various socio-demographic characteristics has practical implications for behavior, public health, and disease control that need to be considered as risk factors for infection with enteropathogenic bacteria (50).

High morbidity and mortality from infectious diarrhea in Gaza strip, and outbreaks of bloody diarrhea are closely related to inadequate sanitation and hygiene, crowding with lack of household ventilation and personal indoor air pollution are all-important living conditions that promote diarrheal diseases in Gaza strip. In addition, refugees and internally displaced persons are at especially high risk (48). The risk of transmission of endemic communicable diseases, such as acute respiratory infections and diarrheal diseases is increased in displaced populations due to associated crowding, inadequate, unsafe drinking water, and sanitation and poor access to health care (49).

Access to drinking water means that the source is less than 1 kilometer away from its place of use and that it is possible to reliably obtain at least 20 liters per member of a household per day. Safe drinking water is water without microbial, chemical and physical characteristics that meet WHO guidelines or national standards on drinking water quality. Access to safe drinking water is the proportion of people using improved drinking water sources. About 1.1 billion people in world have no access to any type of improved drinking source of water. As a direct consequence, 1.6 million people die every year from diarrheal diseases attributable to lack of access to safe drinking water and basic sanitation, mostly in developing countries (51).

This study was not showed statistically significant correlation between the current water access in the prison and diarrhea. As showed in able 6.7, 91.2% responds the current drinking water access in the prison was tap water and 8.8% use packed water. This means not to say, there is no possibility of contamination of tap water in kality prison. Even though there was no significant association between drinking water access and enteropathogenic bacteria, there is habit of sharing water drinking utensils, which was significantly associated enteric bacterial pathogen in the prison which was 3.5 times more associated for detection compared to not share water drinking utensils.

A study conducted in Nigeria showed that there is association between domestic water sourcing practice and the risk of developing diarrhea. It is therefore recommended that high premium be placed on improving access to water and improved household hygiene as a way of helping to curb diarrhea (52). Endemic dysentery is associated with fecal contamination of water sources (53). Reviewed study conducted 2009 showed: Parbatia, Orissa, Eastern India, an outbreak of cholera associated with an unprotected contaminated well (54). Researchers in Kenyan showed

that diarrhea risk was higher among shallow well users. Chlorinating stored water, latrines, and rainwater use all decreased diarrhea risk; combined interventions may have increased health impact (55).

This study showed, diarrhea is more frequent in crowded houses, 53.4% from cases living in houses with 101-200 prisoner per cell/room, and most of cases 37.8% live in houses with 101-200 prisoner per cell/room. Moreover more enteropathogenic bacteria were isolated from crowded houses, 73.4% of enteropathogenic bacteria isolated from cases live in house with >200 prisoner per cell/room. These results suggest that there were significant difference between diarrhea and houses with low crowded houses. The risk of diarrhea was associated with having large number of prisoner per cell **(**>200) and was found to be 6.74 times more likely to be infected with enteropahogens compared to those having number of prisoner per cell (<100).

Severity of sign and symptoms of diarrheal patients varies from patient to patient depending on the cause and other factors (56). Diarrhea may be accompanied by mucous, chills, vomiting, and fever and lost of weight or an urgent need to use the bathroom. Most studies revealed that high number of patients had these sign and symptoms, one of them showed that 76.1% presented with acute watery diarrhea, (20%) with loose stool, (3.3%) with bloody diarrhea and 0.6% cases with mucoid diarrhea, vomiting was a predominant clinical feature in 77.7% cases and 35% suffered from abdominal pain (57). In another study, Cleary showed that bacterial gastroenteritis usually characterized by the presence of bloody diarrhea, mucous in the stools and a high fever (58). Most studies revealed that high number of patients with gastroenteritis had high fever during diarrhea. The second most frequent symptom was vomiting, reported in 78.6%. Of whom, 28.6% had three or more episodes in the previous 24 hours, fever was measured or presumed by guardians in (59%) (59).

In the present study mucous diarrhea were predominant (65.2%), followed by vomiting and loss of weight (50%), fever (40.9%), chills (31.1%), bloody diarrhea (14.4%). This study supports the conclusions from other studies that bacterial enteropathogens induce a clinical illness characterized by fever, mucous diarrhea, chills, vomiting, bloody diarrhea, loss of weight, or various combinations of these symptoms (57).

In this study when comparing sign and symptoms with isolated enteropathogenic bacteria, abdominal pain were predominant(45.7%), tenasmess (43.7%) fever (39.9%), mucoid diarrhea

(38.7%), vomiting (30.7%),watery diarrhea(25.6%), bloody diarrhea (13%), blood mixed with mucus (22.7%). factors assessed on the type of diarrhea in which bloody and watery were 5.33and 1.18 times more likely associated with detection of enteropahogens compared to mucoid diarrhea respectively. With regard to clinical manifestation Abdominal pain and fever was statistically significant associated with identification of enteric pathogens in acute diarrheic stool. Moreover, enteric pathogens causing diarrhea were also associated with the Frequency of diarrhea per day in which relatively occurrence of diarrhea >5/ days were 2.41 times more associated for detection compared to lower occurrence of diarrhea(1-3) per days.

8. Limitations of the study

Due to scarcity of reference material regarding on bacterial etiologies and antimicrobial susceptibility patterns among prisoner with acute gastroenteritis most reviewed data conducted on other group of community (other than prison).

Yersinia entricolitica, *microaerophilic Campylobacter* spp and anaerobic *Clostridium difficle* were not isolated. In addition, viruses that causes acute gastroenteritis did not included in this study.

9. Conclusion and Recommendation

9.1. **Conclusions**

Bacterial infections are common public health problems among prisoner inmates. The overall prevalence of bacterial isolates in this study was 20.6%. *E. coli O157H7* was the commonest isolates among these enteropathogenic bacteria.

Multidrug resistance was common among *shigella spp*. The most frequently prescribing drugs ampicillin, and trimethoprim- sulphamethoxazole showed high resistance for *E.coli O157H7* and *Shigella* isolates in this study. But it was found that *Salmonella* and *Shigella* species were susceptible for ciprofloxacin.

Prisoner with abdominal pain, fever, bloody and watery diarrhea had the highest incidence of enteric pathogens. Episode of diarrhea >5 per day and hand washing without soap were associated with the detection of enteropathogenic bacteria. In addition, high percentages of enteropathogenic bacteria (79.2%) were isolated from crowded houses with >200 prisoner per cell.

9.2. **Recommendations**

- Further multi-site studies in prison inmates are required for isolation and burden of potential enteropathogenic bacteria from acute gastroenteritis patients.
- The alarming of antimicrobial resistance in enteric bacterial pathogens was a threat for prisoner, so it is important to continue periodic surveillance on these organisms in terms of antimicrobial susceptibility patterns.
- Sensitization of prisoners and prison authorities explaining the importance of improve hygiene inmates (hand washing and treating water), increase the number of hand washing points, and educate inmates on the importance of hand washing with soap before and after eating and using latrines.
- Distribute soap to inmates at regular basis.
- Decrease the number of prisoners per cell based on the standards.
- Improvement of laboratories to increase their ability to isolate all types of enteropathogen.

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Annexes

Annex I: Information and Consent Sheet

1. Study Information Sheet

Hello, my name is ----------and I am working with Abraham fujaga on this study. The study aims in identifying the bacterial pathogens and determining their antimicrobial susceptibility causing diarrhea in person inmate. Hereby, provides useful information for the management of diarrhea in these patients. Currently; I am here & would like to ask you some questions and request you to bring a small amount of stool specimen (3-5mg). I will tell you how you can collect the stool and bring safely. Though it seems something procedural the study will help improve health of person inmates. Your name will not be asked and unique identification is not required. If you want to withdraw from the study anytime along the process, you will not be obliged to continue or give reasons for doing so.

Refusing to participate or withdrawing from the study along the process will not have any consequences on you and the services provided to you. I would like to appreciate your help. If you have any question or anything that is not clear, please direct to Abraham Fujaga, School of Medical Laboratory Sciences, AAU.

Cell phone 0923-12-41-26;/E-mail: abfujaga@gmail.com

Do you want to participate in the study? Yes, I want to participate ☐

No, I do not want to participate ☐

If you are clear with the information provided and agree to participate please sign on the consent form.

2. Consent Form

I, the undersigned individual, am oriented about the objective of the study. I have informed that all of my information will be kept confidential and used solely for this study. In addition, I have been well informed that my name will not be asked and unique identification is not required. If I want to withdraw from the study anytime along the process, I will not be obliged to continue or give reasons for doing so. However, my agreement to participate in this study is with the assumption that, the information and the specimen that I provide will help greatly to the management of acute diarrhea prisoner patients.

Signature:-_____ Date:-_____

Annex II: Amharic Version of Study Information and Consent Form

1. መረጃ ለጥናቱ ተሳታፊዎች

ጤና ይስጥልኝ! _____ እባላለሁ፡፡ በአዲስ አበባ ዩኒቨርሲቲ አብርሀም ፉጃጋ በኩል በሚደረገው ጥናት አብሬ እሰራለሁ፡፡ የዚህ ጥናት ዋና አላማ ብዙውን ጊዜ በማረሚያ ቤት ተቅማጥ አምጪ ረቂቅ ተህዋሳትን በመለየት በሽታ አምጪ ተህዋሲያኑ ሊያክም የሚችል መድሃኒት መምረጥ በዚህም በማረሚያ ቤት ተቅማጥ አንዱ የጤና እክል የሆነውን የዚህን ኢንፌክሽን በአግባቡ መቆጣጠር እንዲቻል ማድረግ ነው፡፡ እዚህ አጠገባችሁ የሆንኩት የተወሰኑ ጥያቄዎች ለመጠየቅና መጠነኛ የሆነ የሰገራ ናሙና (3-5mg) እንድትሰጡኝ ነው፡፡ የሰገራ ናሙና ለዚሁ ጥናት በተዘጋጀ እቃ ውስጥ አድርጋችሁ በጥንቃቄ የምታመጡበትን መንገድ እነግራችኋለሁ፡፡ ይህን ስናደረግ የርስዎ ስምም ሆነ የተለየ እርስዎን የሚለይ ሚስጥራዊ ቁጥር አንጠቀምም፡፡ በዚህ ጥናት እየተሳተፉ ባሉበት ድንገት ማቋረጥ ቢፈልጉ የማቋረጥ መብትዎ የተጠበቀ ነው፡ ፡ ለምን ማቋረጥ እንደፈለጉ ምክንያት እንዲያቀርቡም ሆነ ጥናቱ እንዲቀጥሉ አይገደዱም፡፡

በጥናቱ መሳተፍ ባለመፈለግዎ በእርስዎ ላይም ሆነ በሚያገኙት አገልግሎት ላይ የሚያመጣው ምንም አይነት ችግር አይኖርም፡፡ የእርስዎ በጥናቱ መሳተፍ ግን ለሚደረገው ጥናት ትልቅ እገዛ እንደሚሆን ሳልጠቁምዎት አላልፍም፡፡ ስለትብብርዎ ከልብ አመሰግናለሁ፡፡ ስለጥናቱ ለሚኖሮት ማንኛውም አይነት ጥያቄ አቶ **አብርሀም ፉጃጋ** በስልክ ቁጥር **0923-12-41-26** ደውለው መጠየቅ ይችላሉ፡፡

ስለጥናቱ የተሰጠው መረጃ ግልፅ ከሆነልዎ እና በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ እባክዎን የስምምነት መግለጫ ፎርም ላይ ይፈርሙ፡፡

2. የስምምነት ማረጋገጫ ፎርም

እኔ ፊርማዬ በስተመጨረሻው ላይ የሚገኘው ግለሰብ የዚህ ጥናት አላማ ተገልፆልኛል፡፡ በተጨማሪም እኔ የምሰጠው መረጃም ሆነ ናሙና ለዚህ ጥናት ብቻ እንደሚዉልና በሚስጥር እንደሚያዝ ተገልፆልኛል፡፡

በዚህ ጥናት ለመሳተፍ ስምና ሌላ አድራሻ መግለፅ እንደማያስፈልገኝ ተረድቻለሁ፡፡ ከዚህ በተጨማሪም በጥናቱ ላለመሳተፍ መወሰን ወይንም በፈለግኩት ጊዜ ማቋረጥ እንደምችልና ሳቋርጥም ለማቋረጥ የፈለግኩበትን ምክንያት ለማስረዳት እንደማልገደድ እንዲሁም በጥናቱ ለመሳተፍ ፈቃደኛ አለመሆኔ ወይም በጥናቱ ሂደት ላይ ተሳታፊ ከሆንኩ በኋላ አቋርጬ መውጣቴ በእኔ ላይ የሚደርሰው አንዳችም ተፅእኖ እንደሌለ ተረድቻለሁ፡፡

ሆኖም እኔ በዚህ ጥናት ላይ ተሳታፊ ለመሆን ስስማማ በሚገኘው ጠቃሚ መረጃ በማረሚያ ቤት ተቅማጥ ህመም ታራሚዎች ላይ እያመጣ ያለውን ጫና ለመቀነስ የሚረዳ መሆኑን ተስፋ ለማድረግ ነው፡፡

ፊርማ፡_____ ቀን፡_____

Annex III Questionnaire

I. Demographic and Socio-Economic Information

1. code number: _____
2. Age_____
3. sex (Put √ in the applicable box) Male ☐ Female ☐
4. Education: 1. Illiteracy 2. Primary (1-4) 3. Junior secondary (5-8) 4. Senior secondary (9-12) 5. Diploma & above
5. How many people are living in this house? _____
6. For how long did you live in this prison? _____

II. Clinical Data

1. Number of days with diarrhea: _____ days.
2. Stool frequency per day_____
3. Consistency of stool:- A) Watery B) Bloody C) Mucoid D) Mixed (blood and mucus)

   E) Others (if any)
4. Has vomit? Yes ☐ N☐ If yes, state vomiting frequency per day: _____
5. Has fever Yes ☐ No ☐
6. Has abdominal pain Yes☐ No ☐

III. Hygiene Practices

1. Is there a toilet (latrine) in functioning condition for prisoner? Yes ☐ No ☐

   If no, how do you defecate yourself?

   A. Directly excrete into fishpond
   B. Directly excrete on the ground
   C. Other: ……

   If yes, is it in use? Yes in use☐ Not in use ☐
2. What type of latrine or toilet is it?

   A. Flush or pour flush toilet
   B. Ventilated improved pit latrine

C. Pit latrine with slab

D. Pit latrine without slab/open pit.

E. Composting toilet

F. Other type of toilet

3. How often is the latrine cleaned?

A. Every time it is spoiled

B. Every day

C. 1-2 times a week

D. Not cleaned

4. .Where do you dispose of household garbage?

A. Refuse pit

B. Open surrounding

C. Other: ……..

5. Where do you dispose of waste water?

A. Sewage system

B. Pond

C. Garden

D. Other: …..…

6. Do you often wash your hands?

A. After going to toilet

B. Before eating

C. After eating

D. Never

E. Sometimes

F. Usually

7. How do you wash your hands?

A. Water only

B. Water and soap

C. Other:……

8. From where do you get your food?

A. From family

B. Prison

C. Prison and family

9. Do you store cooked foods for later used? Yes ☐ No ☐

10. If yes, how do you store the cooked foods?

A. In refrigerator

B. In food cupboard

C. In disk-cover

D. Other_____

11. Does this prison have water for basic functions of inmate? Yes ☐ No ☐

12. What is the most commonly used source of water?

A. Piped water

B. Hand pump

C. Well

D. River

E. Other(specify)

13. In the past one month, has this prison ever been without water for a full 24hours ☐es ☐ No

14. How long do you often keep the cooked food before reuse? …….

15. Do you often heat the cooked foods before reuse? Yes ☐No ☐

16. What do you use to clean utensils/containers used for feeding?

A. Water only

B. Hot water only

C. Water with soap

D. Hot water with soap

17. What kind of utensils do you use for storing water?

A. Storage containers without lid

B. Storage containers with lid

18. Type of collection container used?

A. Pot

B. Plastic bucket

C. Iron bucket

D. Jerry can

E. Other

Annex IV: Amharic Version Questionnaire

<u>የህሙማን ማህበራዊ ሁኔታ</u>

1/ መለያ ቁጥር _____

2/ እድሜ _____

3/ ፆታ ወንድ_____ ሴት_____

4 / የትምህርት ደረጃ ሀ/ 1-4 ለ/ 5-8 ሐ/ 9-12 መ/ ዲፕሎማ/ ድግሪ ሠ/ ከዛ በላይ

5/ ምን ያህል ታራሚ ይኖራል እርሶ ባሉበት ምድብ ውስጥ _____?

6/ ማረሚያ ቤት ውስጥ ምን ያህል ጊዜ ቆይተዋ._____?

7/ ቀደም ሲል የመኖሪያ አድራሻዎ የት ነው ሀ/ በገጠር ለ/ በከተማ

<u>የህመም ምልክት</u>

1/ ተቅማጥ ከጀመሮት ምን ያህል ቀን ሆነ _____ ?

2/ በቀን ምን ያህል ጊዜ አስቀመጦት _____ ?

3/ የሚያስቀምጦት ተቅማት

ሀ. ደም የተቀላቀለበት ነው

ለ. እንደ ውሀ የቀጠነ ነው

ሐ. ዝልግልግ ያለ ንፍጥ የሚመስል

መ. የተቀላቀለ

4/ ትውከት ነበረቦት/ አለዎት አዎ ☐ የለም ☐

5/ ትኩሳት አለዎት /ነበረቦት አዎ ☐ የለም ☐

6/ ቁርጠት (የሆድ ህመም አለዎት /ነበረቦት አዎ ☐ የለም ☐

7/ ሰገራ ሲቀመጡ ማሰማጥ አለዎት /ነበረቦት አዎ ☐ የለም ☐

<u>የግልና የአካባቢ ንፅህና</u>

1/ በመኖሪያ አካባቢ ሽንት ቤት አለ / አዎ ☐ የለም ☐

የለም ከሆነ የት ትፀዳዳላቹ/_____

አዎ ከሆነ አገልገሎት ይሰጣል አዎ ☐ የለም ☐

2/ የምትጠቀሙበት ሽንት ቤት አይነት /

ሀ/ ዘመናዊ

ለ/ አካባቢ ቁሳቁስ የተሰራ

ሐ/ ሌላ _____

3/ በምን ያህል ጊዜ ይፀዳል

ሀ/ በየግዜዉ ከተፀዳዳን በኃላ

ለ) በየቀኑ

ሐ) ከ 1 – 2 ጊዜ በሳምንት

መ) አይፀዳም

4) ከቤት ዉስጥ የሚወጣዉ ደረቅ ቆሻሻ የት ነዉ የሚጣለዉ?

ሀ) ቆሻሻ መጣያ ገንዳ

ለ) በተገኘዉ ክፍት ቦታ

ሐ) ሌላ _____

5) ፍሳሽ ቆሻሻ እንዴት ነዉ የሚያስወግዱት?

ሀ) የቆሻሻ ፍሳሽ መስመር

ለ) ወደ ኩሬ

ሐ) አትክልት ቦታ

መ) ሌላ_____

6)አብዛኛዉ ጊዜ እጆን የሚታጠቡት ምን ምን ካደረጉ በኃላ /በፊት ነዉ ?

ሀ) ከሽንት ቤት መልስ

ለ) ከምግብ በፊት

ሐ) ከምግብ በኃላ

መ) አልታጠብም

ሠ) አንዳንዴ

ረ) አልፎ አልፎ

7) እጆን በምን/ እንዴት ነዉ የሚታጠቡት?

ሀ) በዉሃ ብቻ

ለ) በዉሃ እና በሳሙና

ሐ)ሌላ_____

8) በአብዛኛው ጊዜ ምግብ ከየት ነዉ የሚያገኙት ?

ሀ) ከቤተሰብ

ለ) ከማረሚያ ቤቱ

ሐ) ከማረሚያቤቱ እና ከቤተሰብ

9) ሲመገቡ የተረፈ ምግብ ለቀጣይ ያስቀምጣሉ?

አዎ ☐ የለም ☐

10) መልሶ አዎ ከሆነ እንዴት ነዉ የሚያስቀምጡት ?

ሀ) ፍሪጅ ዉስጥ

ለ) ሎካር ዉስጥ

ሐ) ክዳን ሳህን

መ) ሌላ_____

11) ለመጠጥ አገልግሎት የሚዉል ዉሃ ከየት ነዉ የሚያገኙት?

ሀ) ቧንቧ ዉሀ

ለ) ወንዝ

ሐ) ኩሬ

መ) ጉድጓድ ዉሃ

ሠ) ሌላ_____

12) አብዛኛዉን ጊዜ የበሰለ ምግብ ለምን ያህል ጊዜ አቆይተዉ ይጠቀማሉ?_____

13) አብዛኛዉን ጊዜ በስሎ የቆየ ምግብ እያሞቁ ይጠቀማሉ? አ☐ የለም ☐

14)የተመገቡበት እቃ ለማጽዳት ምን ይጠቀማሉ?

ሀ) ዉሀ ብቻ

ለ)የሞቀ ውሀ ብቻ

ሐ)ውሀ እና ሳሙና

መ)የሞቀ ውሀ እና ሳሙና

15) ውሀ ለማጠራቀም ምን አይነት እቃ ይጠቀማሉ?

ሀ)ክዳን ያለው የውሀ ማጠራቀሚያ

ለ)ክዳን የሌለው የውሀ ማጠራቀሚያ

16) የትኛዉ የውሀ ማጠራቀሚ እቃ ይጠቀማሉ?

ሀ) እንስራ

ለ) ፕላስቲክ ባልዲ

ሐ) የብረት ባልዲ

መ) ጀረኪና

ሠ) ሌላ————————

Annex V Laboratory forms

For Laboratory Use Only

Code no _____

Identification steps for suspected colonies

1. Glucose and lactose fermentation _____
2. H 2S production_____
3. Lysine decarboxylase: _____
4. Motility test: _____
5. Indole test:_____
6. Citrate utilization test: _____

Antimicrobial susceptibility results (Resistant [R] or Sensitive [S])

1. Ampicillin (10 µg) _____
2. Naldic Acid (10µg) _____
3. Ciprofloxacin (5µg) _____
4. Cotrimoxazole (25µg) _____
5. Ceftriaxone (10 µg) _____

Sig._____ and Date_____

Annex VI:-Stool cultures SOP

1. Principle

Acute infectious diarrhea can be caused by a variety of different etiological agents: bacteria, viruses and protozoa. The common bacterial agents include *Salmonella* spp., *Shigella* spp., *Vibrio cholerae, Campylobacter* spp., enterotoxogenic and enterohemorrhagic *Escherichia coli, Aeromonas* spp., and *Plesiomonas shigelloides*. This SOP describes the procedure for the isolation of *Salmonella, Shigella,and E.coli O157H7*.

Salmonella and *Shigella* are screened by using differential and selective direct plating media, which are based on carbohydrate fermentation (eg. lactose and xylose) and H_2S production. Since some non-pathogenic, Gram-negative bacilli found in normal feces may produce the same reactions as enteric pathogens, biochemical screening and agglutination tests are necessary for identification. Culture for *E.coli* O157H7 also performed, using specialized media Sorbitol macConky agar. *E.coli* O157H7 is sorbitol negative.

2. Materials

   a. Culture media: MacConkey agar (MAC), Sorbitol MacConkey agar (SMAC), Xylose lysine deoxycholate agar (XLD), and Selenite F broth (SEL F).
   b. Biochemical Media: Triple Sugar Iron agar (TSI), Sulfide Indole Motility medium (SIM), Lysine Iron Agar (LIA), Urea agar , Citrate and Indole test
   c. Antisera for serotyping of *Salmonella, Shigella*

3. Specimen

Stool sample transported within 2 hours after collection. If there is a delay in transport, refrigerate the specimen or place in appropriate transport medium.

4. Quality Control (QC)

Process the specimen as soon after receipt as possible. If there is a delay in processing place the specimen in the refrigerator.

Verify that the patient identifiers on the specimen match those on the accompanying requisition.

Ensure that all media and supplies used have passed the required QC and are used before their expiration date.

5. Safety Precautions

Standard safety precautions for handling of specimens must be used when processing these specimens:

6. Procedure

Primary Inoculation:

Inoculate the following primary plating media: MAC, XLD, SMAC and SEL F

- Incubate MAC, XLD, and SMAC overnight at 35 ± 2°C.
- Incubate SEL F overnight at 35 ± 2°C with cap loosened.

Subculture SEL: Subculture one to two loopfuls of SEL F broth to an XLD plate after overnight incubation. Incubate inoculated plate overnight at 35 ± 2°C.

Culture Examination: Examine primary plates (MAC, XLD, and SMAC) after overnight incubation.

- MAC: Look for transparent or colorless colonies (NLF); select one of each type of NLF and inoculate biochemical screening media (TSI, SIM, LIA, Urea slant and Indol). Incubate inoculated tubes overnight at 35 ± 2ºC with caps loosened.
- XLD: Look for red colonies (NLF - with or without black centers); select one of each type of NLF and inoculate biochemical screening media (TSI, SIM, LIA, Urea slant and Indol). Incubate inoculated tubes overnight at 35 ± 2ºC with caps loosened
- SMAC: Look for transparent or colorless colonies (sorbitol negative/sorbitol Non fermenter)
- Confirm identification of *Salmonella, Shigella,* and *E.coli O157H7* with typing sera.

7. Interpretation

Identification of *Salmonella, and Shigella*: Refer to table of Most Frequently Encountered Reactions in Screening Biochemicals. Confirm identification with serotyping.

8. Reporting

For negative cultures, report as:

"No *Salmonella, Shigella,* or *E.coli O157H7* isolated."

For positive cultures, report as:

"*Salmonella* (indicate *serotype*) isolated."

"*Shigella* (*indicate species*) isolated."

"*E.coli O157H7* isolated." (40, 41, 42)

Annex VII: - Biochemical's test

Tabel-8 Most Frequently Encountered Reactions in Screening Biochemical's

Test	Organisms[a]		
	Shigella	*Escherichia*	***Salmonella***
Citrate utilization			
Triple sugar iron agar[b]	K/A-	A/AG-	**K/AG+**
Lysine iron agar[b]	K/A-	K/K-	**K/K+**
Lysine decarboxylation[c]	_	+	+
Motility[c]	—	+	+
Urea hydrolysis[c]	—	—	—
Indole production[c]	+or-	+	—
H_2S production			
Gas production			

Source: Laboratory methods for the diagnosis of Epidemic Dysentery and Cholera. Centers for Disease Control and Prevention (CDC),Atlanta, GA USA. 1999.

[a] *For each of these organisms, variable reactions may occur.*

[b] *Reactions expressed as "slant/butt"; K = alkaline; A = Acid; G = gas produced; + = hydrogen sulfide (H_2S) produced; (+) = weakly positive for H_2S production; - = no H_2S produced.*

[c] *+ = positive reaction; - = negative reaction.*

Annex VIII: Declaration

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

Name: Abraham Fujaga:-

Signature _____

Place_____

Date of submission _____

This proposal has been submitted with my approval as University advisor.

1. Name: Mr.Meles Hailu (BSC, MSC)
   Signature _____
   Place _____
   Date of submission _____
2. Name: Mr. Adugna Abera (BSc, MSc)
   Signature_____
   Place_____
   Date of Submission_____
3. Amete Miheret (BSc, MSc)
   Signature_____
   Place_____
   Date of Submission_____