

ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES, SCHOOL OF MEDICINE
DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY, AND PARASITOLOGY



**Extended-Spectrum β -Lactamase and Carbapenemase producing *Escherichia coli* O157:H7
among diarrheic patients in Shashemene, west Arsi, Ethiopia**

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**A RESEARCH THESIS SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY,
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DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY, AND PARASITOLOGY

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By

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ABBREVIATION AND ACRONYMS

| | |
|-------|--|
| AAU | Addis Ababa University |
| AIEC | Adherent Invasive <i>Escherichia coli</i> |
| AST | Antimicrobial Susceptibility Test |
| ATCC | American Type Culture Collection |
| CLSI | Clinical Laboratory Standard Institute |
| DAEC | Diffusely Adherent <i>Escherichia coli</i> |
| DDST | Double Disc Synergy Test |
| DNA | Deoxyribo Nucliec Acid |
| EAEC | Enteragravetive <i>Escherichia coli</i> |
| EHEC | Enterohemorrhagic <i>Escherichia coli</i> |
| EIEC | Enteroinvasisve <i>Escherichia coli</i> |
| ELISA | Enzyme-Linked Immunosorbent Assay |
| EPEC | Enteropathogenic <i>Escherichia coli</i> |
| ESBL | Extended Spectrum β -Lactamase |
| ETEC | Enterotoxigenic <i>Escherichia coli</i> |
| HACCP | Hazard Analysis Critical Control Points |
| HCF | Health Care Facility |
| HUS | Hemolytic Uremia Syndrome |
| IRB | Institutional Review Board |
| MDR | Multi-Drug Resistant |
| MHA | Muller Hinton Agar |
| MHT | Modified Hog Test |
| PCR | Polymerase chain Reaction |
| SPSS | Statistical Package for the Social Sciences |
| STEC | Shiga Toxin-Producing <i>Escherichia coli</i> |
| VTEC | Vero Toxin producing <i>Escherichia coli</i> . |
| WHO | World Health Organization |

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ABSTRACT

Background: *Escherichia coli* (*E. coli*) O157:H7 has been found in various sources across the globe, and until recently, it was uncommon for this pathogen to produce Extended Spectrum β -Lactamase (ESBL) and Carbapenemase. However, recent reports from different regions have shown that ESBL-producing *E. coli* O157:H7 is becoming more prevalent. In Ethiopia, while there is sufficient knowledge about the epidemiology of *E. coli* O157:H7 in the country's different food supply chains, there is a lack of information regarding the extent of disease caused by this pathogen and its production of ESBL and Carbapenemase.

Objectives: To isolate *E. coli* O157:H7, determine its antimicrobial susceptibility profiles, characterize its ESBL and Carbapenemase production from stool specimens collected among diarrheic patients and explore the association of *E. coli* O157:H7 infection with demographic and clinical features of diarrheal patients in Shashemene, west Arsi, Ethiopia.

Methods: A total of 423 study participants were included from July 1, 2022, to November 25, 2022, in this prospective healthcare facility-based cross-sectional study among all patients with diarrhea. The bacterial pathogen was isolated and identified by colony characteristics, Gram stain, and standard biochemical tests using API 20E as well as utilization of sorbitol and serotyping by antisera for O157 antigen. Each identified isolate was screened and tested for ESBL and Carbapenemase production phenotypically and further characterized at the molecular level for 2 Carbapenemase (*bla*NDM, *bla*KPC) and 3 ESBL coding genes (*bla*CTX-M, *bla*TEM, and *bla*SHV). The data were entered into SPSS version 26.0 software for analysis. Bi-variant and multi-variant analyses were employed using a logistic regression model for further analysis and were interpreted based on the odds ratio and level of statistical significance at a p-value <0.05.

Result: Upon laboratory investigation, *E. coli* O157:H7 strain was found in 38/423 (9%) study participants from this majority of the participants [262 (61.9%)] were males with a 1.6:1 male: female ratio and 81 (19.1%) of the participants were less than five years old and 14 (3.3%) of patients were elders aged above 55 years. Living in urban area, having domestic animal and having ≥ 5 family size were significantly associated with *E. coli* O157:H7 cases. High antimicrobial resistance was observed on Ampicillin [38, (100%)] followed by Amoxicillin with clavulanic acid [34, (89.5%)]. However, all isolates were sensitive to ciprofloxacin. Twenty-seven (71.1%) and 12 (31.6%) isolates were phenotypically confirmed to be ESBL and carbapenemase producers, respectively. The genotypic testing revealed that the most abundantly found ESBL genes were *bla*TEM group 15 (79%) followed by *bla*CTX-M group 12 (63%) and *bla*SHV group 2 (10%). Additionally, from the 12 carbapenemase-positive isolates, 8 (66.6%) were confirmed to have the *bla*KPC group gene and none of the isolates were positive for *bla*NDM group.

Conclusion: The *E. coli* O157:H7 isolates from this study exhibited a high level of resistance to some of the antimicrobials tested. The magnitude of ESBL and Carbapenemase production among these isolates was found to be high. High resistance of Ampicillin and Amoxicillin/Clavulanic acid was observed among ESBL, and carbapenemase-producing isolates and Ciprofloxacin was found to be the most effective drug against both ESBL producers and non-producers. *bla*TEM group gene was the most abundant ESBL coding gene found and *bla*KPC group gene was the only gene found in our isolates that code for Carbapenemase.

Keywords: Antimicrobial susceptibility, ESBL, Carbapenemase, Diarrhea, *E. coli* O157:H7, Shashemene

1. INTRODUCTION

1.1. Background

Foodborne illnesses are a major problem in developing countries, causing significant medical and social costs [1]. These illnesses can be caused by various infectious microorganisms, including bacteria, viruses, and parasites [2]. There are three main types of foodborne illness: infections, intoxications, and Toxin-mediated infections. Food infection is caused by ingesting food containing active pathogens, whereas food intoxication is caused by ingesting toxic substances generated by microorganisms [3]. Toxin-mediated infection, on the other hand, occurs as a result of pathogenic microorganisms producing enterotoxins through colonization and proliferation of cells in the gastrointestinal tract [4].

Shiga toxin-producing *E. coli* (serogroup O157:H7) is a particularly concerning pathogen that have gained increased attention in recent years [5]. This bacterium is more dangerous than other well-known foodborne pathogens because it affects all age groups, it needs a low infectious dose, and it is unusually acid-tolerant. Foods, like raw meat, raw milk, and undercooked ground beef are important vehicles for the transmission of this bacteria to people [6]. Cattle are considered a reservoir for this highly virulent pathogen [7].

In Ethiopia, foodborne illness is a common health issue, and it is among the leading cause of death among people of all ages in the country [8] with raw meat consumption and poor food handling practices contributing to the problem [9]. Low food handling and safety regulations, a lack of personal sanitation among meat supply chain staff, and a lack of awareness about *E. coli* O157:H7-caused infection are all recognized as major challenges in Ethiopia [10].

There are different studies conducted on *E. coli* O157:H7 from different food chain values but little is known regarding its significance in diarrheal disease. Furthermore, information is scarce regarding the epidemiology of the pathogen among diarrheagenic patients. Therefore, the aim of this study was to isolate *E. coli* O157: H7 and characterize their ESBL and Carbapenemase production.

1.2.Statement of the problem

Diarrheal disease is a significant global health issue, particularly in developing countries with poor sanitation and limited resources. According to the WHO, It is the third leading cause of morbidity and the sixth leading cause of death worldwide, with approximately one billion cases and 5-10 million deaths annually in Africa, Asia, and Latin America [11].

Bacteria, viruses, or parasites all can be the cause of diarrhea. Bacterial pathogens are a major cause of diarrheal disease, with *E. coli* being the most common agent of moderate-to-severe diarrhea in low-income countries [11, 12]. *E. coli*-focused research is critical because this organism lives in a wide variety of hosts, including humans and animals. The most common pathogens responsible for diarrheal disease are pathogenic *E. coli* specifically serogroup O157:H7 [2].

Antibiotic resistance is a major issue that is increasing every year. High antimicrobial resistance lowers therapeutic alternatives while increasing treatment costs and fatality rates [13-15]. ESBL and Carbapenemase production by Shiga toxin-producing *E. coli* was formerly uncommon; however, there are some reported cases since the first report of ESBL producing *E. coli* O157:H7 in 2013 Denmark [16]. Infections caused by ESBL-producing *E. coli* have a higher morbidity and fatality rate. Most ESBL-associated illnesses are resistant to multiple antibiotics, leaving only a few compounds as a treatment option [17].

Testing diarrheal sample for *E. coli* O157:H7- is not common in Ethiopia. Even though pathogenic *E. coli* is considered the most common cause of diarrhea, still the diagnostic tests focus only on the detection of *Salmonella*, *Shigella* and others. Any bacterial growth on diarrheal stools other than *Salmonella* or *Shigella* is commonly recorded as 'no *Salmonella* or *Shigella*' [18]. Several investigations on the prevalence and antimicrobial resistance patterns of Shiga toxin-producing *E. coli* from various food sources, including beef, [6, 19, 20], food handlers [21] meat [9], and street foods [10] have been undertaken in Ethiopia. However, there is limited study on clinical samples, as well as limitations in detecting their ESBL and Carbapenemase production.

Despite their extensive distribution, the prevalence of ESBL and Carbapenemase-producing organisms is understated since a vast majority of laboratories do not perform standard tests to detect ESBL. Infections caused by ESBL and Carbapenemase generating gram-negative bacteria are becoming more common. As a result, detecting ESBL and Carbapenemase generating bacteria

in feces is critical in understanding the colonization rate with those bacteria, which is useful in predicting the risk of infection. In Ethiopia, data on the prevalence of ESBL and Carbapenemase carriage are rare, and to the best of our knowledge, no investigation has focused specifically on ESBL and Carbapenemase-producing *E. coli* O157:H7. As a result, the goal of this study is to isolate *E. coli* O157:H7 and evaluate its production of ESBL and carbapenemase, as well as its connection with different socio-demographic statuses of diarrheic patients in Shashemene Ethiopia.

1.3. Significance of the study

Screening for ESBL and Carbapenemase-producing bacteria in the colon is crucial for predicting the risk of bacterial infection. The colon is a reservoir for many pathogenic infectious agents, making it important to understand the prevalence of these bacteria in diarrheal patients. Currently, there is no data on the prevalence of ESBL and Carbapenemase-producing *E. coli* O157:H7 carriage among diarrheal patients in Ethiopia. This study aims to provide baseline data on the magnitude of ESBL and Carbapenemase-producing *E. coli* O157:H7 carriage and associated risk factors among diarrheal patients in Ethiopia. This study will also increase health professionals' understanding of ESBL and Carbapenemase-producing *E. coli* O157:H7 and aid in the selection of treatment for infections caused by these bacteria. Additionally, this study will be helpful for epidemiological purposes and offer evidence-based data needed for the implementation of infection prevention and control methods. Moreover, this study will serve as a springboard for further large-scale evidence-based studies in Ethiopia.

2. LITERATURE REVIEW

2.1. General characteristics of *Escherichia coli*

Escherichia coli (*E. coli*) is a Gram-negative bacterium commonly found in the intestines of humans and animals. It is a facultative anaerobe, capable of growing both with and without oxygen. *E. coli* is a rod-shaped bacterium and exhibits motility through the presence of flagella. It is known for its ability to ferment glucose and other sugars. *E. coli* is a type of bacteria that can exist in various forms and can survive in different environments, with or without oxygen. Although many strains of *E. coli* are naturally present in the gastrointestinal tract of humans and animals, not all strains are harmless. Some strains can cause severe and even fatal infections in humans [22]. Commensal *E. coli*, which are non-pathogenic strains, are commonly found in the microflora of the intestine. However, there are several pathogenic strains of *E. coli* that can cause illnesses such as enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli*, enteroinvasive *E. coli*, enteroaggregative *E. coli*, diffusely adherent *E. coli*, and adherent invasive *E. coli*. The most significant pathotype of diarrheic *E. coli* is Shiga-toxin or Verotoxin producing EHEC, which can cause life-threatening diarrhea [23].

EHEC has become a major public health concern due to numerous food-borne outbreaks, including the first reported outbreak in 1982 in the United States that was linked to undercooked hamburgers [24-26]. Since then, *E. coli* O157:H7 outbreaks have been associated with various foods like spinach, lettuce, sprouts, unpasteurized milk, apple juice, apple cider, salami, and water sources frequently visited by animals. *Escherichia coli* O157:H7 is the main Shiga toxin-producing *E. coli* serotype that has been connected to several human disorders such as hemorrhagic colitis, thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome [27, 28].

2.2. Growth characteristics *E. coli* O157:H7

The inability to create -glucuronidase, which can hydrolyze a synthetic molecule, and the delayed D-sorbitol fermentation (>24 h). MUG (4-methylumbelliferyl-D-glucuronide) distinguishes *E. coli* O157:H7 from other *E. coli* strains [29].

Temperatures between 7 to 45°C are considered to be optimal for *E. coli* O157:H7 growth, with 37°C being ideal. Normal pasteurization techniques (> 60 °C) successfully eliminate *E. coli* O157:H7 because it is not very heat resistant. Growth prefers a pH near neutral but can tolerate

extremely acid pH and can thrive on diets with low pH values (3.6 - 4.0), especially at low temperatures [30, 31].

2.3.Epidemiology of *E. coli* O157:H7

2.3.1. Geographical distribution

Infections with *E. coli* O157:H7 have been reported on every continent except Antarctica [32, 33]. Even though the magnitude differs from area to area this pathogen is found in every part of the world including developed nations like USA [34]. Cases of *E. coli* O157:H7-related diarrhea have been documented in several African nations as well, including Cameroon [35], Egypt [36], Ghana [37], South Africa [38], Kenya, Nigeria [39], as well as Ethiopia [40]. Mostly the distribution of this pathogen is associated with areas that have communities with direct contact with soil and domestic animals [41]. *E. coli* O157:H7 has been reported in all parts of Ethiopia with varying magnitude[6, 19-21, 40, 42, 43] from clinical and non-clinical samples.

*2.3.2. Occurrence of *E. coli* O157:H7 in Ethiopia*

E. coli O157:H7 has been found in both human and animal populations in Ethiopia. *E. coli* O157:H7 research has mostly concentrated on milk products, meat products, slaughterhouses, butcher shops, and animal abattoirs [6, 19, 20, 43]. However, only a few investigations have been undertaken to estimate the incidence of STEC in clinical specimens, specifically serogroup O157:H7 [42]. Although the majority of these research are from central Ethiopia, there are reports from other regions of the country as well. Abattoirs, butcher shops, retail shops, restaurants, farms, milk dealers, and children under the age of five are all part of the research site. The table below summarizes the study population, study area, and prevalence reports.

*2.3.3. Reservoir of *E. coli* O157:H7 and mode of transmission*

E. coli O157:H7 is a bacteria present in the digestive systems of warm-blooded mammals, with cattle serving as the principal reservoir. However, it also has been discovered in other animals, birds, and fishes. This strain is especially harmful because it can be shed in infected animals' excrement, [7] contaminating any place that comes into contact with feces of those animals. Humans can get sick by drinking raw milk, or polluted water and eating raw meat contaminated with *E. coli* O157:H7 [44, 45].

The transmission of *E. coli* O157:H7 occurs through the fecal-oral route, primarily through contaminated food such as raw or undercooked ground beef, raw milk, and contaminated

vegetables like lettuce and spinach [4, 19, 46]. It can also spread through contaminated water sources [47], direct contact with infected animals at farms or petting zoos [48], poor sanitary conditions during food preparation in restaurants, and occasionally through occupational exposure for those working in slaughterhouses or in healthcare settings diagnosing and treating *E. coli* O157:H7 infections [49, 50].

2.4.Pathogenesis E. coli O157:H7

Even though *E. coli* O157:H7 can be found in different animals, it primarily causes illness in humans. Although it induces diarrhea in cattle and other animals, they still serve as a reservoir for the bacteria [51].

E. coli O157:H7 pathogenicity is dependent on virulence factors. The Locus of Enterocyte Effacement (LEE) encoded components (responsible for the generation of translocate intimin receptor and intimin), toxins (Shiga toxin), and the beginning of inflammatory cascades are the key virulence factors associated with its pathogenicity [52]. Adhesion is an important step for bacterial survival and colonization. Bacterial colonization, growth, and pathogenicity may be facilitated by adhesive factors. *E. coli* O157:H7 has the ability to cling to intestinal mucosal tissues and release a variety of proteins, enzymes, and toxins [2]. After the bacteria adheres to enterocytes (using translocated intimin receptors), it penetrates into the cell and releases its Shiga toxins (Stx1 and Stx2). Shiga toxins are the outstanding virulence factors for developing bloody diarrhea and HUS by halting the protein synthesis of the cell which leads to cell death of the enterocytes and peripheral organs cells like kidney as well as by activating inflammatory cascades, which further leads to distraction of intestinal and blood vessel walls. It also leads to deep venous thrombosis by activating clotting factors [53, 54]. (Figure. 1)

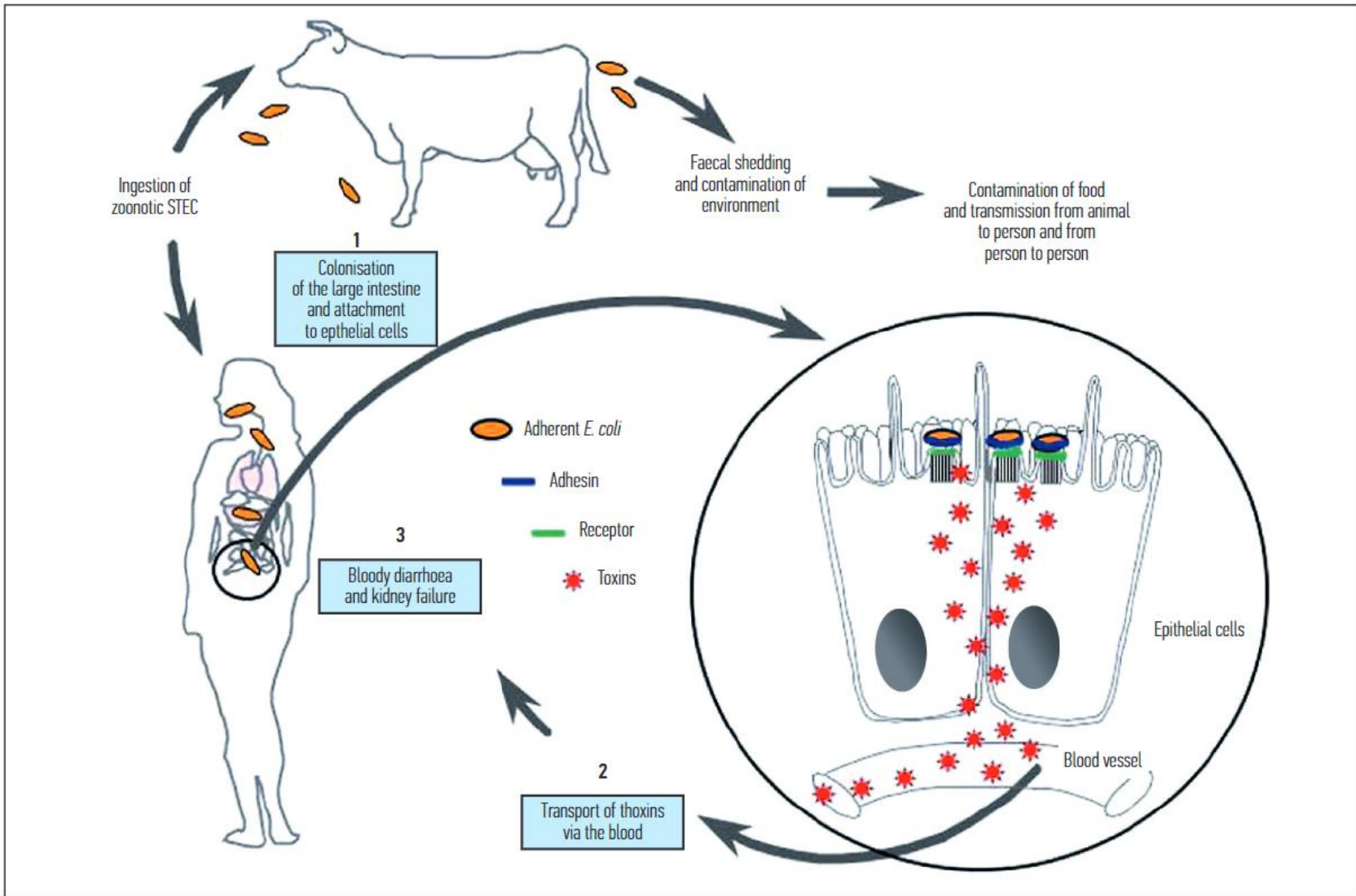


Figure 1: “The mechanism by which *E. coli* O157:H7 causes diarrhea and hemolytic uremic syndrome in humans.” [55]

2.5. Clinical signs and symptoms from *E. coli* O157:H7 infections

E. coli O157:H7 infection can cause severe illness in individuals. The onset of symptoms typically occurs within 2-5 days after consuming contaminated food or beverages and can persist for up to 8 days. Although the following symptoms are commonly associated with the infection, they may vary from person to person [56].

- ✓ Abdominal cramps,
- ✓ Severe bloody diarrhea,
- ✓ Non-bloody diarrhea,
- ✓ Little to no fever,
- ✓ Fatigue, Nausea,
- ✓ Hemolytic uremic syndrome (HUS)[53],
which is the most serious complication of
this bacteria that can lead to kidney failure
and death

2.6. Clinical and Laboratory Diagnosis of *E. coli* O157:H7 Infections

In clinical instances, *E. coli* O157:H7 infection is diagnosed by examining faecal samples, as well as food and environmental samples, to determine the source of infection. There is, however, no one method for isolating all *E. coli* serotypes. Infection symptoms range from mild diarrhoea to potentially catastrophic hemolytic uremic syndrome [53]. Diarrheic faeces and common dietary items are common diagnostic samples. Advanced techniques, such as immunoassays and polymerase chain reaction technologies, have made it possible to identify *E. coli* in faeces, food, and water more quickly. These molecular approaches provide great sensitivity, selectivity, and quick results [33, 57, 58]. The latex agglutination test is widely used together with Sorbitol MacConkey Agar for rapid detection of *E. coli* O157:H7, with a positive result showing agglutination or becoming milky and smooth [59].

2.7. Prevention and control of *E. coli* O157:H7 infections

To avoid the transmission of *E. coli* O157:H7 from animals to people, it is recommended that you wash your hands thoroughly with soap and running water for at least 20 seconds before eating food or drink, especially if you have handled contaminated goods or worked on a farm. Furthermore, it is critical to properly cook things like ground beef, pork, sheep meat, or sausage until the flesh is grey or brown throughout and any fluids run clear [60]. Hazard analysis critical control points (HACCP) implementation is also an effective food safety management system that can address various chemical, biological, and physical threats from raw material production to end product consumption [61]. A vaccination for cattle has been created that immunizes them against the *E. coli* O157:H7, that limits shedding and minimize bacteria transfer from animals to human [62, 63].

2.8. Treatment

Administering antibiotics to individuals with *E. coli* O157:H7 infections could potentially exacerbate the illness by causing the bacteria to lyse, leading to an increase in the production and secretion of Shiga toxins. This, in turn, may elevate the risk of hemolytic uremic syndrome (HUS) [64, 65]. In vitro studies have shown that antibiotics can induce the production of Shiga toxin by *E. coli* O157:H7. As a result, treatment is primarily supportive in nature, aimed at limiting symptom duration and preventing systemic complications. To prevent dehydration and electrolyte loss, individuals with diarrhea are advised to consume clear liquids [22].

2.9. Antimicrobial resistance

Although it is not recommended to use antibiotics to treat *E. coli* O157:H7 infections, there are situations where therapeutic or prophylactic administration of antibiotics may be necessary. There is also potential spread of antibiotic-resistant genes in the environment from widespread use in humans and farm animals. Therefore, it is important to determine the antimicrobial susceptibility status of *E. coli* O157:H7 [66, 67]. While antimicrobial resistance is increasing among food-borne bacteria globally, *E. coli* O157:H7 has shown a slightly lower prevalence. Antimicrobials are commonly used for disease prevention and growth promotion in food animals already known to carry *E. coli* O157:H7 [68], which can promote resistance in *E. coli* O157:H7 in animal intestinal tracts. Studies have found antimicrobial-resistant *E. coli* O157:H7 isolates in diarrheic patients, animal excreta, and animal-sourced foods.

2.10. ESBL and Carbapenemase production of *E. coli* O157:H7

ESBL and Carbapenemase production by Shiga toxin-producing *E. coli* was formerly uncommon. However, recently, there are some reports of ESBL and carbapenems-producing *E. coli* O157:H7 from different parts of the world. The first report of ESBL-producing *E. coli* O157:H7 was reported in Netherland from chicken isolates in 2003 [69] and the first report from a human clinical sample isolate was reported in Denmark, which originated from a fecal sample of a 2-year-old girl with bloody diarrhea who was part of a small outbreak in 2004. Until this year, there was no report of such scenario from human clinical isolates [16]. There is also one study conducted in Japan that shows ESBL production among isolates found in beef cattle [67]. Another study from Egypt found that 63.0% of *E. coli* O157:H7 isolated from meat and dairy products carry β -Lactamase-encoding genes

[70]. One study from Tanzania shows that 10% of isolate from cattle and 9.3% of isolate from human specimens have ESBL production capability [71].

Even though there is little information regarding ESBL production in other parts of the world, there is no available published data regarding carbapenemase production of this bacteria in any part of the world. Regarding our country, it is similar to the world where no published data on ESBL and Carbapenemase production of this bacteria is available in Ethiopia.

Table 1: Summary of the prevalence, study participant, and sample type of *E. coli O157:H7* in different parts of Ethiopia

| Authors | Study area | Study participant | Sample type | Prevalence % |
|----------------------|-------------------|---|-------------------------------------|---------------------|
| Beyi et al., 2017 | Central Ethiopia | Butcher shops and restaurant | Meat | 4.5% [19] |
| Gutema et al., 2021 | Bishoftu Town | Cattle, Beef, and Humans | Meat and stool | 7.1% [20] |
| Nahom Misikir, 2021 | Addis Ababa | Abattoirs and Butcher Shops | Meat | 0.95% [40] |
| Tadese et al., 2021 | Ambo Town | Abattoir and Retailer Shops | Meat | 9.1% [6] |
| Hile et al., 2017 | Jimma town | Abattoir | Bovine carcasses and cecal contents | 9.3% [43] |
| Hile et al., 2021 | Addis Ababa | Market | Retail Lettuce | 0.51% [72] |
| Minda et al., 2021 | Arsi | Catering Establishments | Food of Animal Origin | 2.1% [73] |
| Wada et al., 2017 | Wolaita Sodo | Food Handlers in Food-Handling Establishments | Stool | 6.03% [21] |
| Getaneh et al., 2021 | Eastern Ethiopia | Under-five children | Stool | 15.3% [42] |
| Ayenew et al., 2019 | Addis Ababa | Pediatric patients with diarrhea | Stool | 4.5% [74] |
| Adugna et al., 2015 | Bahir Dar | Children aged under five with acute diarrhea | Stool | 28.9% [75] |

3. OBJECTIVES

3.1. General objective

To investigate *Escherichia coli* O157:H7, its antibiogram profile, and ESBL and Carbapenemase production from stool specimens in diarrheic patients.

3.2. Specific objectives

- To isolate *Escherichia coli* O157:H7 from diarrheic samples.
- To determine the phenotypic antimicrobial susceptibility profiles of isolated *Escherichia coli* O157:H7 from diarrheic samples.
- To characterize ESBL production among *Escherichia coli* O157:H7 from diarrheic samples.
- To characterize Carbapenemase production among *Escherichia coli* O157:H7 from diarrheic samples.
- To explore the association of *Escherichia coli* O157:H7 infection with demographic and clinical features of diarrheal patients.

4. METHOD AND MATERIALS

4.1. Study area

This study was conducted in Shahemene Zuria Wereda and Shashemene Town. The study site was selected because there was a high report of diarrheal diseases previously from this area (unpublished report from AHRI). Shahemene is located 240 km from the capital Addis Ababa in the West Arsi zone, Oromia region of Ethiopia. It has a weather condition mostly long dry and hot season from November to March and a long rainy season from July to October. The area has a total population of 543, 597 (270,455 male and 273,142 female), according to the 2021 estimate of the Central Statistical Agency [76].

There are 15 public health care facilities; from these, 8 public health care facilities (Abosto, Awasho, Chebi, Toga, Harbate, Fajogole health centers and Melka oda hospital and Shashemene specialized hospital) were randomly selected as specific study area.

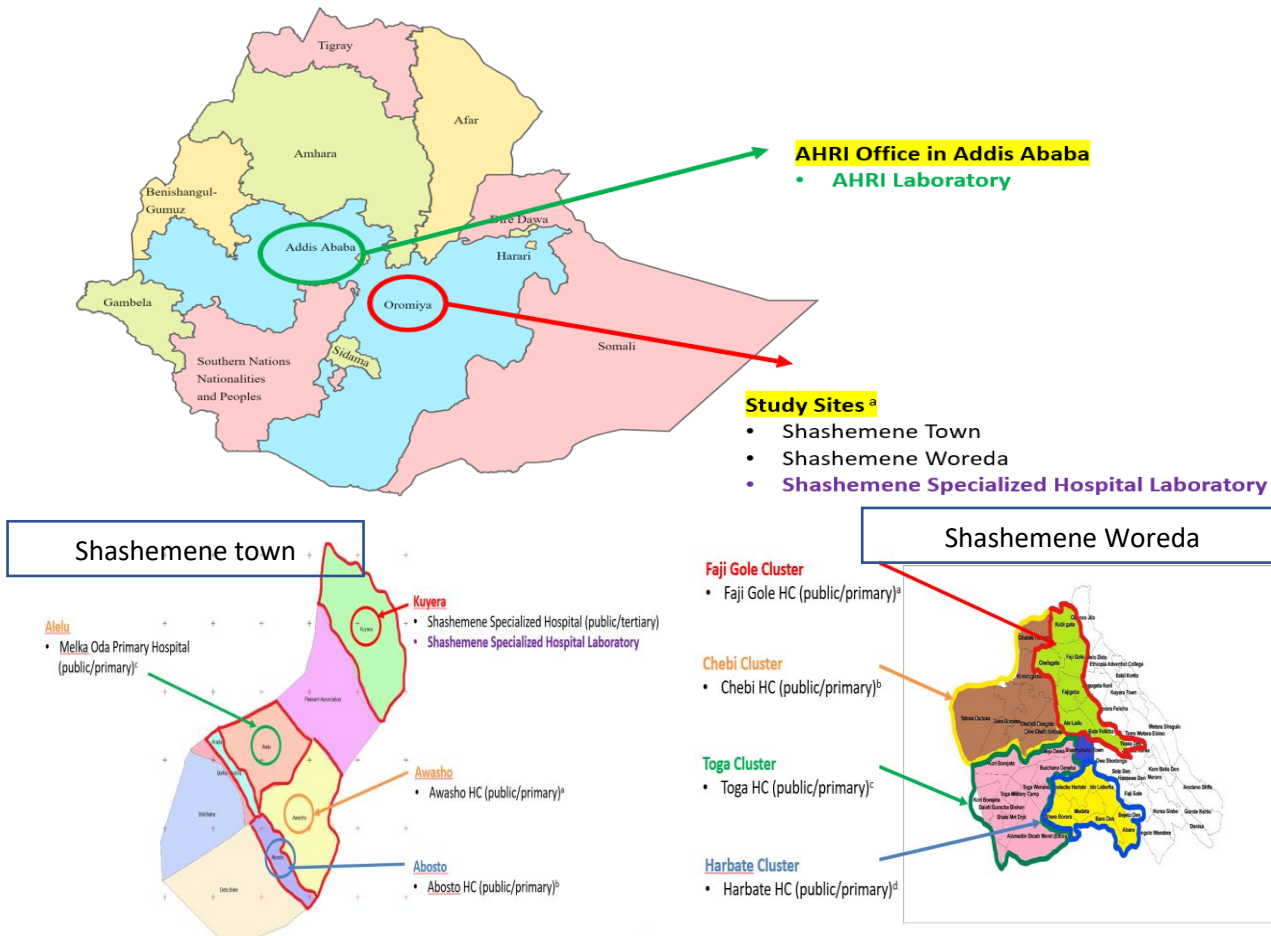


Figure 2: Study sites in Shashemene town and the surrounding Shashemene woreda (source: Google map)

4.2. Study population

Patients who visited selected health care facilities with acute diarrhea (that experience passage of three or more loose stools within the last 24 hours) or those with persistent diarrhea were considered in this study.

4.3. Sample size

The required sample size is calculated based on a single sample size estimation using the following formula as shown below.

$$n = \frac{Z^2 p(1 - p)}{d^2}$$

Where 'n' is the sample size, 'Z' is the level of confidence (with 95% confidence, the 'Z' value is 1.96), 'P' is the projected prevalence or proportion (P = 50%), and 'd' is the degree of freedom (d = 4.5%). Because there hasn't been a previous large-scale investigation of *E. coli* O157:H7 among diarrheic patients from the general population, the value of p is set at 50%.

$$n = \frac{(1.96)^2 \times 0.5(1 - 0.5)}{(0.05)^2} = \mathbf{384}$$

But there might be a 10% non-response rate.

$$N = \frac{1}{1 - \text{non response reate}} \times n$$

$$N = \frac{1}{1 - 0.1} \times 384 = \mathbf{427}$$

The total sample size determined is **427** study participants.

4.4. Study design and period

This study was a prospective cross-sectional healthcare facility-based (HCF-based) study under Ethiopian Cholera Control and Prevention (ECCP) project. This study was conducted from July 1st, 2022, to March 2, 2023.

4.5. Sampling techniques

From the total public health care facilities (HCFs) found in Shashemene, 8 public HCFs were selected randomly, and the sample size (427) was proportionally allocated to each public HCF based on the

previous year diarrheal case report; then samples were taken systematically to select individual study participants.

All patients visiting those selected public HCFs from their catchment areas were screened at each entry point of respective HCFs for symptoms of diarrheal diseases based on the inclusion criteria of the study. Patients with suspected diarrheal illness or other diarrhoea symptoms (acute watery diarrhoea, acute bloody diarrhoea, chronic diarrhoea) were eligible to participate in the study.

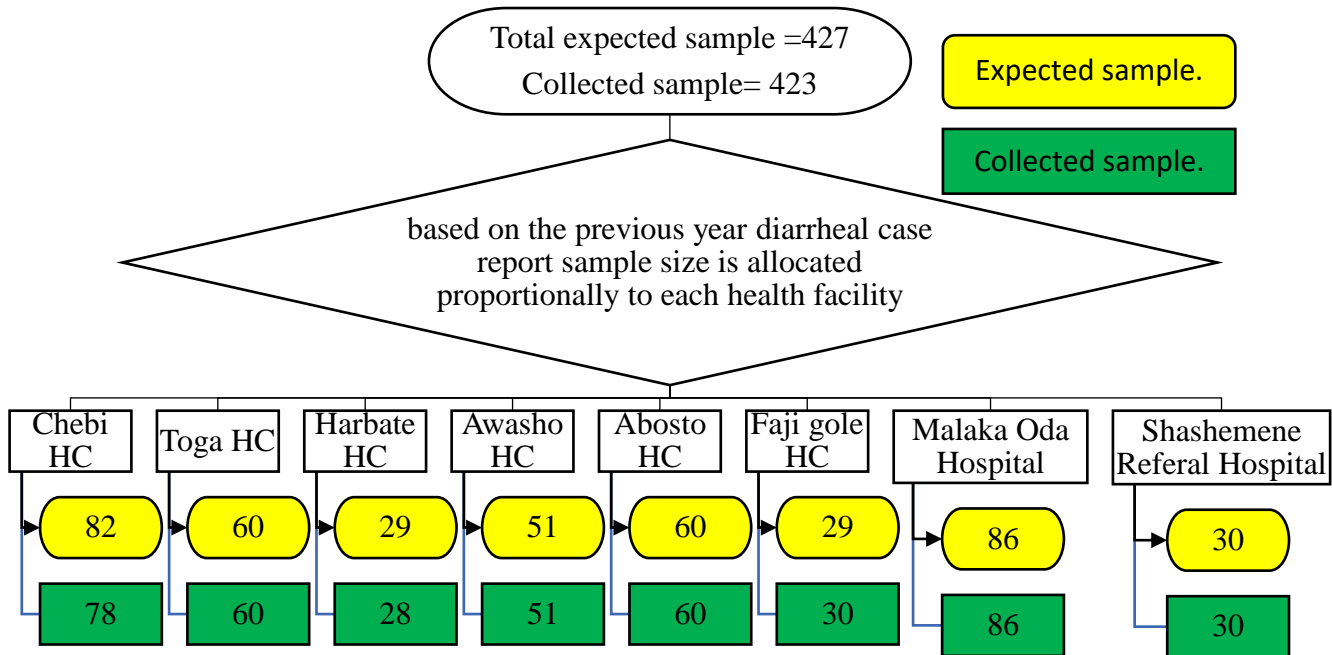


Figure 3: Sample proportional allocation to randomly selected healthcare facilities.

4.6. Inclusion and exclusion criteria

4.6.1. Inclusion criteria

Patients that have any type of diarrheas include:

- Acute watery diarrhea lasts several hours or days.
- Acute bloody diarrhea: also called dysentery.
- Persistent diarrhea: lasts 14 days or longer.

4.6.2. Exclusion criteria

- Patients who have been taking antibiotic treatment for the last two weeks

4.7. Study variables

4.7.1. Dependent variables

- Detection of *E. coli* O157:H7 from a stool sample.
- Phenotypic antibiotic resistance
- ESBL production
- Carbapenemase production

4.7.2. Independent variables

- Age
- Sex
- Educational status
- Residence area
- Occupation
- Source of drinking water
- Family size
- Eating habits of undercooked or uncooked food.
- Ownership of domestic animal
- Drinking raw milk

4.8. Data collection tool and procedures

Data were collected using a semi-structured questionnaire. Data collectors were trained and monitored before data collection and during their work. After collection and entry into data collection software, the data was rechecked for completeness, and any incomplete or miss-filled information was filled again if possible or excluded.

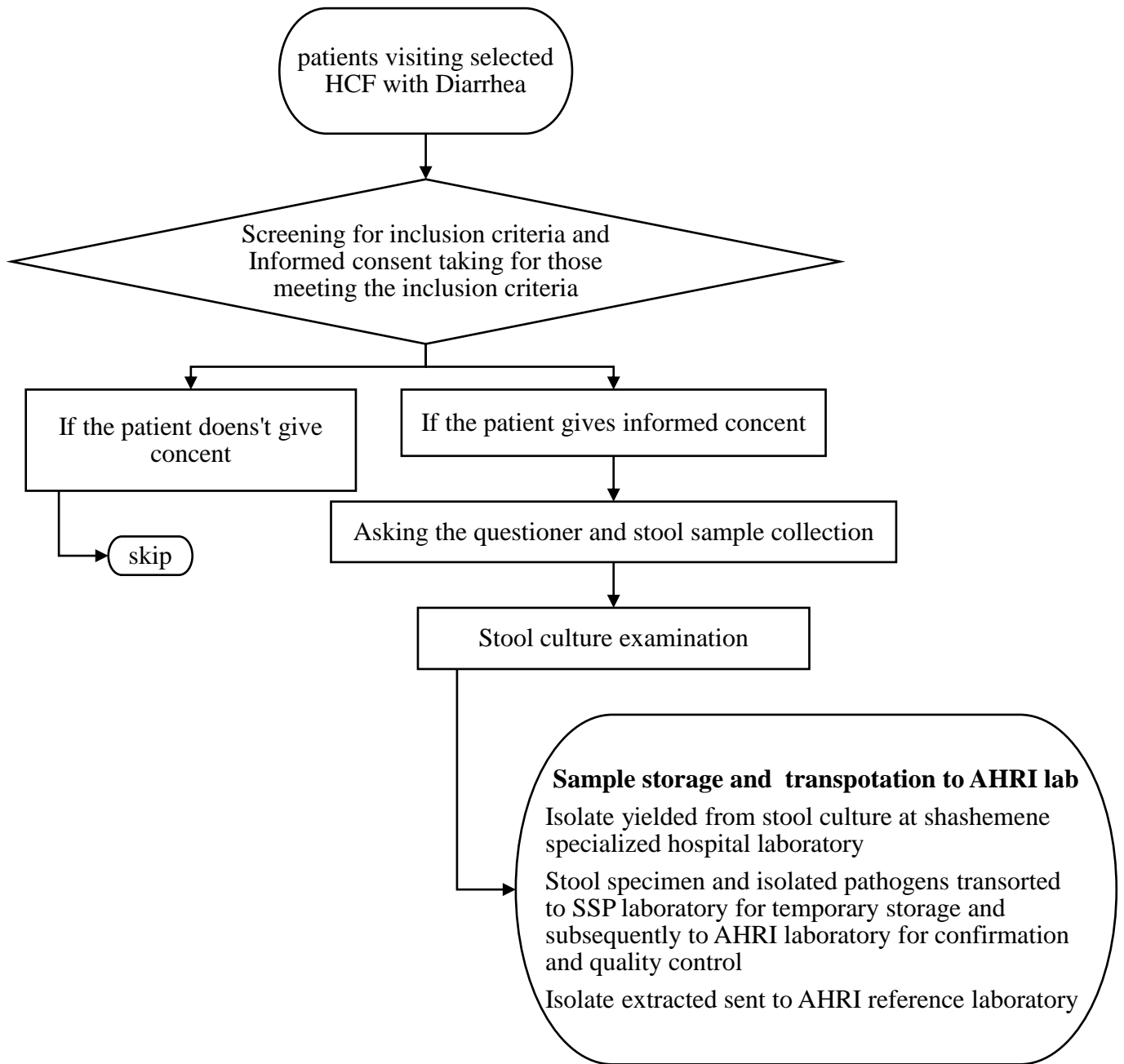


Figure 4: Data and sample collection procedures

4.9. Sample Collection

Faeces samples were collected from diarrheal patients using standard laboratory specimen collecting protocols (direct stool sample was collected from adult patients and rectal swab were collected from infants and children that were unable to give direct stool sample). All obtained samples were labelled with the sample ID, date of collection, and sample type. Using a Carry-Blair transport medium, the samples were carried to the Shashemane Specialised Hospital Microbiology laboratory for microbiological investigation utilising a cold chain. When the samples arrived, they were promptly inoculated onto the proper culture media for isolation, as illustrated in the flowchart below.

4.10. Laboratory diagnosis

4.10.1. Growth and Identification

Stool samples were collected and transported to the microbiology laboratory of Shashemane Specialized Hospital by using Cary Blair transporting medium. As soon as the samples arrived in the laboratory, they were inoculated onto MacConkey agar (Mac) plates and incubated at 37°C overnight aerobically. All positive cultures and Lactose fermenting bacteria were characterized by colony characteristics, Gram stain, and standard biochemical tests using API 20E to identify *E. coli*. Further identification for *E. coli* O157:H7 was done by sorbitol utilization test on Sorbitol MacConkey agar, and serogrouping was done using antisera for O157. A pure colony of *E. coli* O157:H7 was collected for Antibiotic Susceptibility Testing (AST) and further characterization of ESBL and Carbapenemase production.

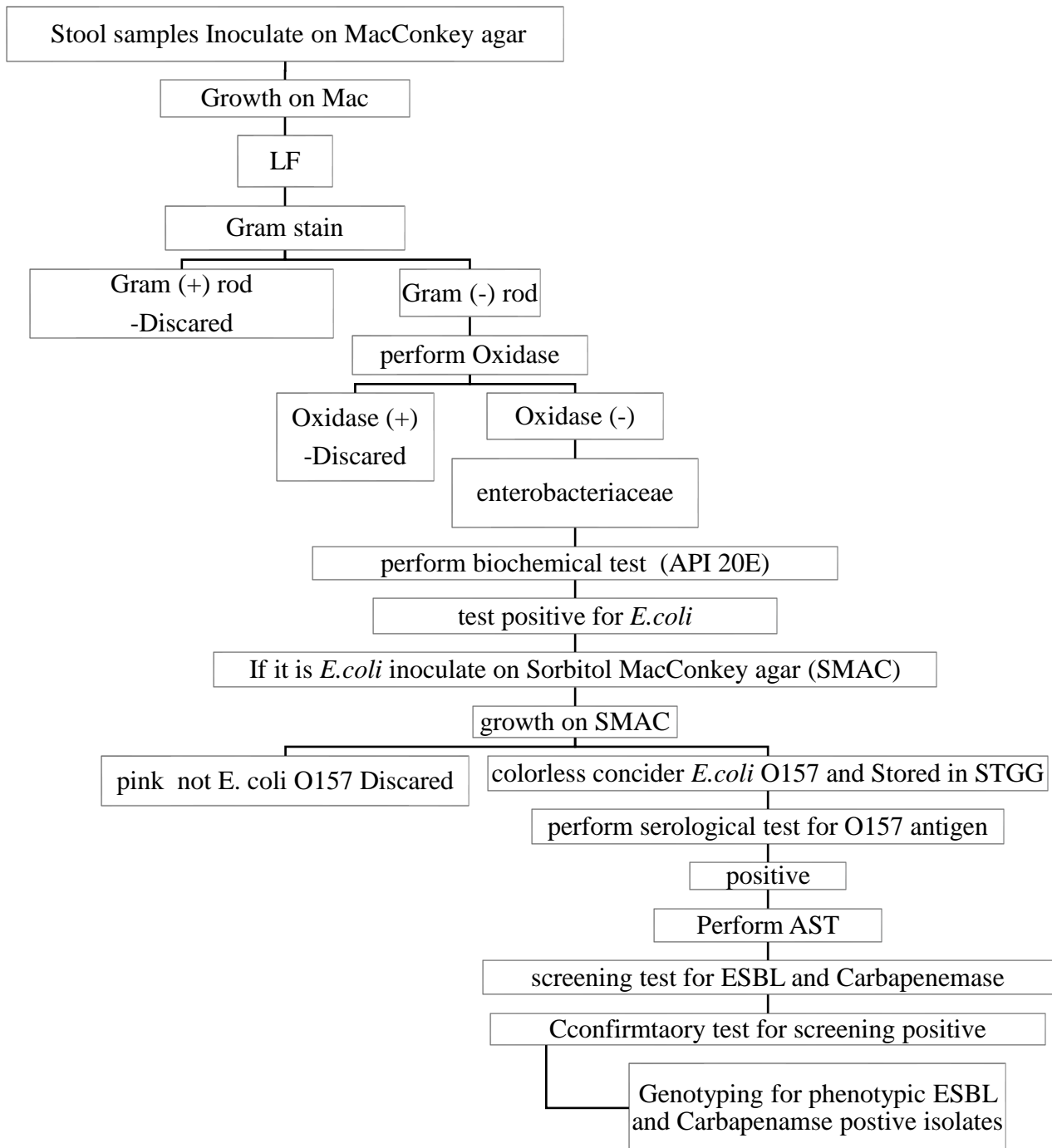


Figure 5. Sample processing flow-chart

4.10.2. Antimicrobial Susceptibility Testing

Using a sterile wire loop, pure colonies of *E. coli* O157:H7 were picked from MacConkey agar and emulsified in nutrient broth to prepare a 0.5 McFarland standard using McFarland Densitometer. From the standard, the test bacteria were inoculated onto Muller-Hinton agar for the AST tests by soaking inoculating swab one time to 0.5 McFarland suspension after swiping the overflowed sample on the mouth of the tube using Lawn or carpet culture. The AST was performed using the Kirby–Bauer disc diffusion method using different antibiotics listed below, and the results were analyzed and interpreted according to CLSI guidelines [77]. Antibiotic discs to be used for antimicrobial susceptibility tests are listed below.

- Ampicillin (10 µg),
- Amoxicillin-clavulanate (20/10 µg)
- Gentamicin (10 µg),
- Ceftriaxone (30 µg),
- Cefotaxime (30 µg),
- Ceftazidime (30 µg),
- Ciprofloxacin (5 µg),
- Trimethoprim-Sulfamethoxazole (1.25/23.75 µg),
- Chloramphenicol (30µg),
- Azithromycine (15 µg),
- Cefuroxime (30 µg),
- Tetracycline (30 µg)
- Imipenem(10µg),
- Ertapenem (10 µg), and
- Meropenem(10µg)

4.10.3. Extended Spectrum β -Lactamase production test

4.10.3.1. Screening for ESBL

Screening for ESBL was performed using ceftazidime (30 µg), ceftriaxone (30 µg), and cefotaxime (30 µg) and looking for a zone of inhibition. Zone of inhibition for ceftazidime < 22 mm, for ceftriaxone < 25 mm, and for cefotaxime < 27 mm, was considered as ESBL producer and were further confirmed by using the combined disk method [77].

4.10.3.2. Phenotypic confirmatory test for ESBL

A combined disc approach was used to conduct phenotypic confirmatory tests for ESBL formation using third generation cephalosporins alone (cefotaxime, ceftriaxone, and ceftazidime) and in combination with clavulanate. A zone diameter of 5 mm for any antimicrobial agent tested in conjunction with clavulanate compared to the agent's zone diameter when tested alone was judged positive for ESBL formation [77, 78].

4.10.4. Carbapenemase production test

4.10.4.1. Screening for Carbapenemase

Screening for Carbapenemase was performed by using meropenem (10 µg), ertapenem (10 µg), or imipenem (10 µg) and looking for a zone of inhibition. If the zone of inhibition for ertapenem is <19 mm and that for meropenem is <16 mm, it is indicative of Carbapenemase production. Further confirmation was done by using a modified carbapenem inactivation method (MCIM) technique [77].

4.10.4.2. Phenotypic confirmatory test for Carbapenemase

The modified carbapenem inactivation method (MCIM) was used to conduct phenotypic confirmatory testing for the capacity of carbapenem-resistant isolates to generate Carbapenemase. A 1-L loop of the tested isolate was emulsified in 2ml trypticase soya broth (TSB), and a 10-g meropenem disc was placed in the tube. After incubating for 4 hours 15 minutes, the meropenem disc in the suspension was removed from each TSB-meropenem disc suspension and transferred to a Mueller Hinton agar (MHA) plate that had been inoculated with *E. coli* ATCC 25922; the plate was then incubated at 35°C for 18-24 hours, where zone of inhibition measuring 6-15 mm and the presence of pinpoint colonies within 16-18 mm zone were considered positive for carbapenemase production [77, 78].

4.10.5. Molecular Characterization of ESBLs and Carbapenemase Genes

DNA Extraction

The boiling process, as described earlier, [79] was employed to extract DNA from new colonies of *E. coli* O157:H7 isolates. A suspension of 3 to 5 colonies from an overnight nutrient agar growth was made in 500 µL of 1x Tris-acetate-EDTA buffer and boiled for 15 minutes at 94 °C. The suspension was then frozen at 20°C for 10 minutes, followed by a one-minute incubation at ambient temperature and a 5-minute centrifugation at 13,000 g. The resultant supernatant (150 µL) was transferred to a nuclease-free Eppendorf tube and gel electrophoresis was used to assess DNA quality and amount before being stored at 20°C until analysis.

PCR Protocol

The conventional PCR technique was utilized to identify ESBL genes (*bla*CTX-M group, *bla*TEM group, and *bla*SHV group) and carbapenem resistance-determining genes (*bla*KPC group and *bla*NDM group) in the samples [80, 81]. The ESBL genes were detected in a single PCR reaction by combining the three ESBL genes in one tube, while the carbapenemase genes were detected in two separate uniplex PCR reactions using two separate tubes. The PCR was conducted in a Bio-Rad MJ

Mini PTC-1148 thermocycler with a final volume of 25 μ L containing 12.5 μ L 2 x HotStarTaq multiplex PCR Master Mix (QIAGEN), 1 μ L of each primer (2 μ L), 2 μ L of template DNA, and 8.5 μ L of nuclease-free water for both ESBL and carbapenemase genes. The PCR cycling parameters for both reactions were the same, with initial denaturation at 95°C for 15 minutes, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 58 °C for 90s, extension at 72 °C for 90s, and final extension at 72 °C for 10 minutes. Positive and negative controls were included in each step. The PCR products were analyzed using gel electrophoresis in a 1.5% agarose gel stained with ethidium bromide and visualized using a gel imaging system, GelDoc (Cleaver GelPro). A molecular weight marker of 100bp ladder (Promega) was used to determine the molecular weight of the amplified products.

4.11. Quality Assurance and Quality control

Prior to the data gathering procedure, stringent safeguards were put in place to assure data quality. Standardized data collection materials were used, which aided in the study's consistency and homogeneity. These materials were meticulously created and tested ahead of time to assure their dependability and efficacy.

Furthermore, after each phase of preparation, the sterility of the media used for sample inoculation was extensively verified. This procedure is critical to avoiding contamination that could taint the results. The integrity of the samples and subsequent analyses were maintained by guaranteeing the sterility of the medium, reducing the likelihood of false-positive outcomes. A positive American Type Culture Control (ATCC) was used to validate the functionality of each culture medium used. In addition, at each phase of the polymerase chain reaction (PCR) test for Extended-spectrum beta-lactamases (ESBL) and carbapenemases, both positive and negative controls were included.

4.12. Data management and statistical analysis

The collected data were coded and entered into SPSS version 27.0 software for analysis. Categorical covariates were summarized using frequencies and percentages and numerical variables were summarized with a median value based on the data distribution symmetry. Bi-variant analyses were employed using a logistic regression model and multi-variant analyses were employed using a logistic regression model for variables that have P-value ≤ 0.25 and further analyses were interpreted based on the odds ratio and level of statistical significance at a p-value < 0.05 .

4.13. Ethical consideration

The Departmental Research Ethics Review Committee (DRERC) of the Department of Microbiology, Immunology, and Parasitology (DMIP), College of Health Science, Addis Ababa University (EC approval document number DRERC/002/2022), the AHRI/ALERT Ethics Review Committee of Armauer Hansen Research (EC approval document number PO/11/21), and the National Research Ethics Review Committee (EC approval document number 7/2-512/00259/35) Institute under Ethiopian Cholera Control and Prevention (ECCP) project provided ethical approval. The data was solely used for this study. Before enrolling in the study, each study participant provided informed written consent in accordance with established ethical procedures. During data collection and analysis, anonymity was maintained.

4.14. Dissemination of results

The finding of this study will be presented to Addis Ababa University College of Health Sciences, School of Medicine, Department of Microbiology, Immunology, and Parasitology and will be submitted to the School of Medicine as partial fulfillment of a master's degree in medical microbiology. It will also be presented in seminars and conferences. Furthermore, a copy of the thesis will be submitted to the Shashemene town and Zuria Wereda health office. Dissemination of the finding to the scientific community will be done through publications in national/international peer-reviewed journals.

5. RESULT

5.1. *Socio-Demographic characteristics of the study participants*

During data collection from July to November 2022, a total of 41,563 patients visited all 8 healthcare facilities, of whom 1399 patients were screened for eligibility requirements and 1,388 fulfilled the inclusion criteria. Among these patients, 423 were selected systematically and enrolled in the study. The median age of the study participants was 18 years with an age range of 1 to 75 years. Eighty-one (19.1%) of the participants were less than five years old and 14 (3.3%) of patients were elders aged above 55 years. The majority of the participants [262 (61.9%)] were males with a 1.6:1 male: female ratio.

Out of 423 study participants, 258 (51.5%) of them live in an urban area and more than half of the participants [233 (55.1%)] attended only a primary school; and 126 (29.8%) study participants are farmers. In addition, the overwhelming majority of the participants [345 (81.6%)] had domestic animals. Tap water is the major source of drinking water for more than half of the participants [287 (67.8%)]. The majority (291 (68.7%)) of the participants have a habit of eating uncooked food (Table 2)

5.2. *Magnitude and associated factors of E. coli O157:H7-related diarrhea*

All the 423 participants selected for the study were presented with watery diarrhea, of whom 13.2% had mucus and 3.5 % had dysentery. Upon laboratory investigation, we have found [93.6% (396/423)] of isolates from diarrheal sample to be *E. coli* and *E. coli* O157:H7 strain was found in 38 (9%) of the study participants. Living in an urban area (AOR:0.135; 95%CI:0.050, 0.361), having a domestic animal (AOR:7.154; 95%CI:1.510, 33.890), and having ≥ 5 family size (AOR:0.275; 95%CI:0.120, 0.631) were significantly associated with *E. coli* O157:H7 cases (Table 2- only those variables with P-value of ≤ 0.25 on their Bivariant analysis are incorporated in the table).

Table 2. Socio-Demographic characteristics, magnitude, and associated factors of *E. coli* O157:H7 related diarrhea

| Variable | Category | Freq. N (%) | <i>E. coli</i> O157:H7 Positive. N (%) | Bivariant | | Multivariant | |
|----------------------------------|------------|-------------|--|-----------|-----------------------|---------------------|------------------------|
| | | | | P-value | COR (95%CI) | P-value | AOR (95%CI) |
| Age group | <=5 | 81 (19.1%) | 13 (34.2%) | 1 | | | |
| | 6-15 | 102 (24.1%) | 9 (23.7%) | 0.0141 | 0.506 (0.205, 1.021) | 0.313 | 0.497 (0.128, 1.930) |
| | 16-25 | 133 (31.4%) | 10 (26.3%) | 0.056 | 0.425 (0.177, 1.021) | 0.111 | 0.387 (0.121, 1.243) |
| | 26-35 | 60 (14.2%) | 4 (10.5%) | 0.101 | 0.374 (0.115, 1.210) | 0.156 | 0.339 (0.076, 1.510) |
| | 36-45 | 25 (5.9%) | 1 (2.6%) | 0.152 | 0.218 (0.027, 1.756) | 0.372 | 0.353 (0.036, 3.463) |
| | 46-55 | 8 (1.9%) | 1 (2.6%) | 0.793 | 0.747 (0.085, 6.595) | 0.858 | 0.808 (0.079, 8.267) |
| | >=56 | 14 (3.3%) | 0 (0%) | 0.999 | 0 | | |
| Residence | Urban | 218 (51.5%) | 28 (73.7%) | 1 | | | |
| | Rural | 205 (48.5%) | 10 (26.3%) | 0.006 | 0.348 (0.165, 0.736) | <0.001 ^a | 0.135 (0.050, 0.361) |
| Educational status | None | 122 (28.8%) | 14 (36.8%) | 1 | | | |
| | Primary | 233 (55.1%) | 20 (52.6%) | 0.381 | 0.724 (0.352, 1.490) | 0.305 | 1.780 (0.592, 5.353) |
| | Secondary | 47 (11.1) | 1 (2.6%) | 0.089 | 0.168 (0.021, 1.313) | 0.433 | 0.400 (0.040, 3.950) |
| | Collage | 15 (3.5%) | 2 (5.3%) | 0.833 | 1.187 (0.242, 5.816) | 0.252 | 3.184 (0.439, 23.105) |
| | University | 6 (1.4%) | 1 (2.6%) | 0.702 | 1.543 (0.168, 14.178) | 0.318 | 3.96 (0.267, 58.271) |
| Family Size | ≤ 4 | 185 (43.7%) | 24 (63.2%) | 1 | | | |
| | ≥ 5 | 238 (56.3%) | 14 (36.8%) | 0.013 | 0.419 (0.210, 0.836) | 0.002 ^a | 0.275 (0.120, 0.631) |
| Domestic animal ownership | No | 78 (18.4%) | 2 (5.3%) | 1 | | | |
| | Yes | 345 (81.6%) | 36 (94.7%) | 0.044 | 4.427 (1.043, 18.795) | 0.013 ^a | 7.154 (1.510, 33.890) |
| Source of drinking water | Tap | 297 (70%) | 19 (50%) | 1 | | | |
| | Spring | 31 (7.3%) | 1 (2.6%) | 0.492 | 0.488 (0.063, 3.773) | 0.828 | 1.283 (0.136, 12.122) |
| | Well | 72 (17%) | 16 (42.1%) | <0.001 | 4.180 (2.026, 8.626) | 0.004 ^a | 3.639 (1.506, 8.792) |
| | River | 23 (5.4%) | 2 (5.3%) | 0.669 | 1.393 (0.304, 6.391) | 0.167 | 3.508 (0.592, 20.771) |
| Drinking raw milk | No | 214 (50.6%) | 24 (63.2%) | 1 | | | |
| | Yes | 209 (49.4%) | 14 (36.8%) | 0.108 | 0.568 (0.285, 1.132) | 0.604 | 0.814 (0.374, 1.772) |
| Blood in diarrhea* | No | 408 (96.5%) | 32 (84.2%) | 1 | | | |
| | Yes | 15 (3.5%) | 6 (15.8%) | <0.001 | 7.833 (2.623, 23.397) | <0.001 ^a | 12.465 (2.824, 55.018) |
| Abdominal Cramp* | No | 323 (76.4%) | 24 (63.2%) | 1 | | | |
| | Yes | 100 (23.6%) | 14 (36.8%) | 0.048 | 2.028 (1.006, 4.090) | 0.337 | 1.626 (0.603, 4.385) |

AOR, adjusted odds ratio; COR, crude odds ratio; CI, confidence interval; 1, Reference; *, clinical condition; (Others: -Prisoners, commercial sex workers, laborers, housewives, and no job, ^a statistically significant)

5.3. Magnitude of ESBL and Carbapenamase producing *E. coli* O157:H7 and its distribution among different socio-demographic status of our study participants

From the total of 38 *E. coli* o157:H7 isolates, 27 (71.1%) were confirmed ESBL, and 12 (31.6%) were confirmed as carbapenamase producers phenotypically. All the 27 study participants with ESBL-producing isolates owned domestic animals and the source of drinking water for the majority of study

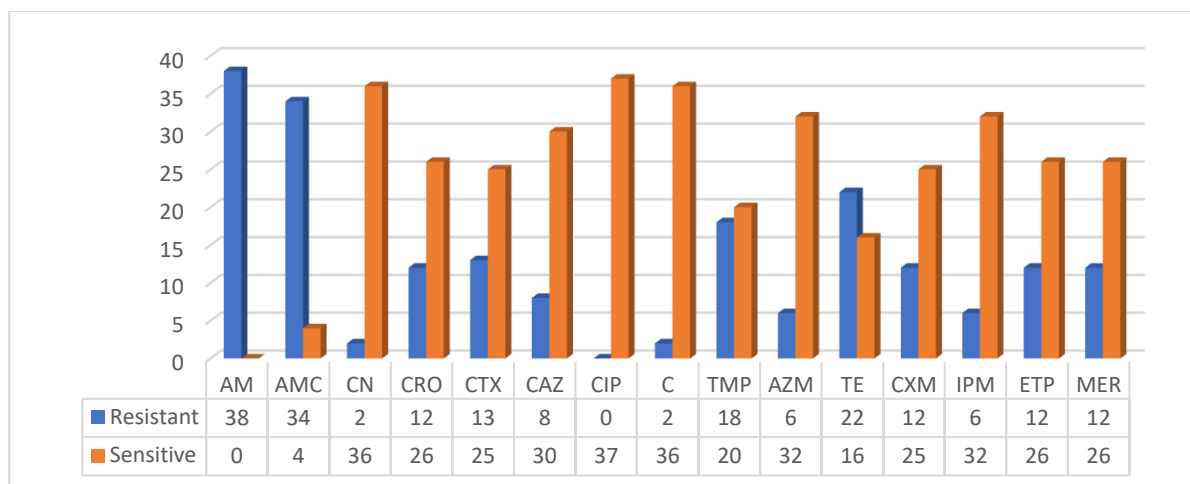
participants with ESBL-producing isolates [14/27 (52%)] was well water. The overall distribution of ESBL and carbapenemase-producing isolates among different socio-demographic status of our study participants are shown in the table below (Table 3). Even though there is a percentage difference all socio-demographic status of the participant is not significantly associated (P-value > 0.05) with ESBL and carbapenemase production.

Table 3. Magnitude of ESBL and Carbapenemase producing *E. coli* O157:H7 and its distribution among different socio-demographic status of our study participants

| Variables | Category | ESBL Positive (N=27) | Carbapenemase Positive (N=12) |
|---------------------------------|----------------------------------|----------------------|-------------------------------|
| Residence | Urban | 19 (70%) | 10 (83%) |
| | Rural | 8 (30%) | 2 (17%) |
| Gender | Male | 17 (63%) | 8 (67%) |
| | Female | 10 (37%) | 4 (33%) |
| Occupation | None | 8 (30%) | 4 (33%) |
| | Student | 9 (33%) | 4 (33%) |
| | Farmer | 7 (26%) | 2 (17%) |
| | Merchant | 2 (7%) | 0 (0%) |
| | Employee | 0 (0%) | 0 (0%) |
| | Others | 1 (4%) | 2 (17%) |
| | Domestic animal ownership | No | 0 (0%) |
| | Yes | 27(100%) | 11 (92%) |
| Source of drinking water | Tap | 11 (41%) | 4 (33%) |
| | Spring | 1 (4%) | 1 (8%) |
| | Well | 14 (52%) | 6 (50%) |
| | River | 1 (4%) | 1 (8%) |
| | Drinking raw milk | No | 18 (67%) |
| | Yes | 9(33%) | 5 (42%) |
| Eating uncooked food | No | 7(26%) | 2 (17%) |
| | Yes | 20 (74%) | 10 (83%) |

5.4. Overall Antimicrobial resistance status of *E. coli* O157:H7 isolates.

The antimicrobial susceptibility of the isolate was evaluated against 15 antimicrobial agents by the Kirby Bauer disk diffusion method. High antimicrobial resistance was observed against Ampicillin [38 (100%)], Amoxicillin with clavulanic acid [34 (89.5%)], tetracycline [22 (57.9%)], Ertapenem [12 (31.6%)], Meropenem [12 (31.6%)]. Moreover, all isolates were sensitive to ciprofloxacin. The overall antimicrobial susceptibility pattern of the isolate is shown in the chart below (Figure 6).

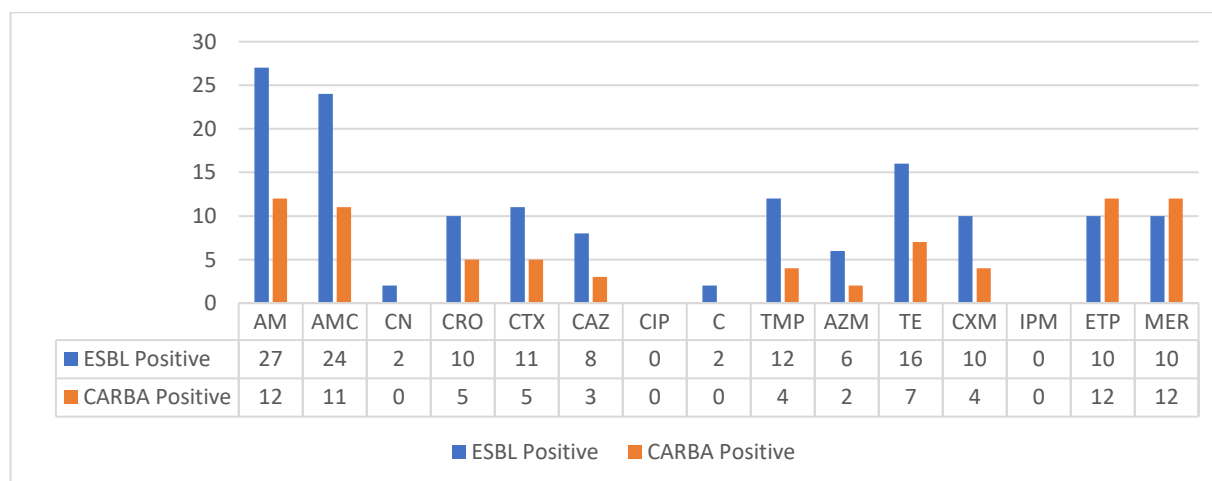


AM= Ampicillin, AMC= Amoxicillin/Clavulanic acid, CN= Gentamycin, CRO= Ceftriaxone, CTX=Cefotaxime, CAZ= Ceftazidime, CIP= Ciprofloxacin, C= Chloramphenicol, TMP= Trimethoprim/sulfamethoxazole, AZM= Azithromycin, TE= Tetracycline, CXM= Cefuroxime, IPM= Imipenem, ETP= Ertapenem, MER= Meropenem

Figure 6. Antimicrobial resistance status of *E. coli* O157:H7 isolates.

5.5. Antimicrobial resistance status of ESBL and carbapenemase-producing isolates.

All ESBL and carbapenemase-producing isolates were resistant to ampicillin but sensitive to ciprofloxacin and imipenem. Varied levels of resistances against the tested antimicrobials between these extremes were observed among these groups ranging from 16/27 (59.25%) against Tetracycline to 2/27 (7.41%) against chloramphenicol and Gentamycin by ESBL-positive isolates, and 11/12 (91.70%) against Amoxicillin/Clavulanic acid and 2/12 (16.70%) against Azithromycin by CARBA-positive isolates (Figure 7).

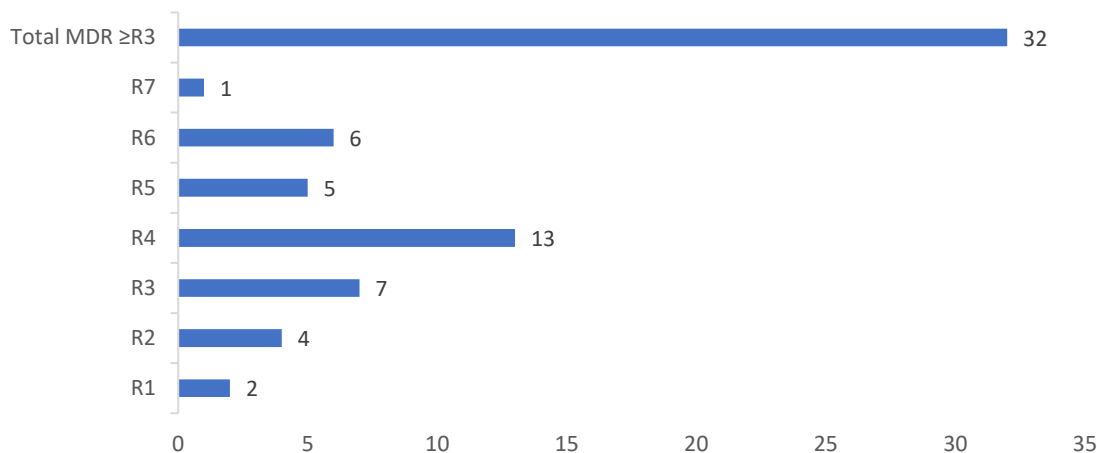


AM= Ampicillin, AMC= Amoxicillin/Clavulanic acid, CN= Gentamycin, CRO= Ceftriaxone, CTX=Cefotaxime, CAZ= Ceftazidime, CIP= Ciprofloxacin, C= Chloramphenicol, TMP= Trimethoprim/sulfamethoxazole, AZM= Azithromycin, TE= Tetracycline, CXM= Cefuroxime, IPM= Imipenem, ETP= Ertapenem, MER= Meropenem

Figure 7. Antimicrobial resistance status of ESBL and carbapenemase-producing isolates.

5.6. Magnitude of Multi-drug Resistance (MDR) isolates.

Resistance to three or more classes of antimicrobial is recorded on 32/38 (84.2%) isolates. Among these, 13 (34.2%) isolates were resistant to 4 classes of antimicrobials while only 1 (2.6%) isolate was resistant to 7 classes of antimicrobials. (Figure 8)



R1=Resistance to one class of antimicrobial, R2=Resistance to two class of antimicrobial, R3=Resistance to three class of antimicrobial, R4=Resistance to four class of antimicrobial, R5=Resistance to five class of antimicrobial, R6=Resistance to six class of antimicrobial, R7=Resistance to seven class of antimicrobial, MDR=resistance to \geq 3 class of antimicrobial

Figure 8. Magnitude of multi-drug resistant isolates

5.7. Molecular characterization of ESBL-producing isolates

There were 27 isolates phenotypically confirmed to produce ESBL and all these isolates were tested for the presence of the three most common genes of ESBL (*bla*CTX-M group, *bla*TEM group, and *bla*SHV group). Of these, 27 ESBL-positive isolates 19 (70.4%) were confirmed to have ESBL genes. From these tested genes, the most abundantly found one was the *bla*TEM group [15 (79%)], followed by the *bla*CTX-M group [12 (63%)], whereas the *bla*SHV group was the least found [2 (10%)]; only one isolate had all three group genes.

5.8. Molecular characterization of Carbapenemase-producing isolates

We have tested for the presence of the two most common carbapenemase encoding genes (*bla*KPC group and *bla*NDM group) from the 12 phenotypically confirmed isolates that produce carbapenemase. Of these isolates, 8 (66.6%) were genotypically confirmed to have the *bla*KPC group gene and none of the isolates were positive for the *bla*NDM group gene (Table 4).

Table 4. Molecular characterization of ESBL and carbapenemase-producing isolates

| Gene type | Tested Genes | Frequency | Percent |
|----------------------|--|-----------|---------|
| ESBL | <i>bla</i> CTX-M group | 3 | 15.8% |
| | <i>bla</i> TEM group | 6 | 31.6% |
| | <i>bla</i> SHV group | 1 | 5.3% |
| | <i>bla</i> CTX-M group and <i>bla</i> TEM group | 8 | 42.1% |
| | <i>bla</i> CTX-M group, <i>bla</i> TEM group, and <i>bla</i> SHV group | 1 | 5.3% |
| Carbapenemase | <i>bla</i> KPC group | 8 | 66.6% |
| | <i>bla</i> NDM group | 0 | 0% |

6. DISCUSSION

6.1. General Finding

This study provides results about the magnitude of *E. coli* O157:H7 and level of ESBL and carbapenemase production as well as molecular characterization of ESBL- and carbapenemase encoding genes among diarrheic patients in Shashemene, Ethiopia. The prevalence of *E. coli* O157:H7 among diarrheic patients was 9%, and there was a significant association between *E. coli* O157:H7 isolation rate with living in an urban area, ownership of domestic animals, and living in a family with ≥ 5 family size. ESBL and carbapenemase production in *E. coli* O157:H7 was not an issue previously, but nowadays there are reports on a small number of articles regarding the spread and *E. coli* O157:H7 becoming positive for these enzymes. These enzymes are detected in different isolates of bacteria from various environments. The ESBL and carbapenemase production rate in our study showed that from the total of 38 *E. coli* O157:H7 isolates, 27 (71.1%) and 12 (31.6%) were phenotypically confirmed ESBL and carbapenemase producers, respectively.

6.2. Magnitude and associated factors

The prevalence of *E. coli* O157:H7 infection found in our study is comparable with the finding of other studies conducted on under-five children in Wolaita Sodo [6.03%; [82]], and Bahirdar [6.8%; [75]]. However, it is much higher than the report from a study in Addis Ababa [4.5%; [74]] and Bishoftu town [2.8%; [20]] but lower than the report from Eastern Ethiopia [15.3%; [42]]; and much lower than studies conducted in other Africa countries (Gabon and Nigeria) [83, 84]. This difference could be the result of cultural, lifestyle, and sanitation status differences. Our study found that both adults and under-five children living in urban areas are more likely to have *E. coli* O157:H7 infection compared to those living in rural areas, a finding which is in disagreement with another study conducted in eastern Ethiopia [42] that shows children living in a rural area were more likely to be infected with *E. coli* O157:H7 than those living in an urban environment. The discrepancy might be due to differences in sanitation practices, hygiene behaviors, and sources of water [85, 86].

6.3. ESBL and Carbapenemase production

From the total of 38 *E. coli* O157:H7 isolates, 27 (71.1%) were phenotypically confirmed ESBL producers which is comparable with the finding from Egypt [63%, [70]] and higher than the finding from Tanzania [9.3%, [71]]. The probable disparity in resistance distribution could be attributed to the overuse of broad-spectrum antibiotics and high rates of patient transfer from private health care facilities where patients received many antimicrobials prior to arriving at the public health care facility

for treatment [87]. To this end, there is no published data, and this work gives an early overview on genotypic characterization of ESBL and carbapenemase genes in *E. coli* O157:H7 causing diarrheal infection at the study site. We have tested the three most common genes of ESBL (*bla*CTX-M group, *bla*TEM group, and *bla*SHV group). A total of 19/27 (70.4%) isolates were positive for at least one of these ESBL coding genes, of which 3 (15.8%) isolates had *bla*CTX-M group, 8 (42.1%) both *bla*CTX-M and *bla*TEM, and 1 (5.3%) all the three tested group genes (*bla*CTX-M, *bla*TEM, and *bla*SHV). From all three genes tested, our study found that *bla*TEM group was the most abundant one, followed by the *bla*CTX-M group. Our finding is in agreement with finding from Egypt [88] which is conducted on general *E. coli* that have found *bla*TEM group gene predominantly and in disagreement with a study conducted in Lebanon [89] where *bla*CTX-M group was the most commonly found gene, and India [90] where it was reported that *bla*SHV was a predominantly detected gene. This finding is in disagreement as well with studies conducted in among general *E. coli* in Portugal [91] and Ethiopia [79] that shows majority of isolates carry *bla*CTX-M group gene.

Twelve (31.6%) isolate from the total of 38 *E. coli* O157:H7 were phenotypically confirmed to produce carbapenemase and were tested for the presence of the two most common genes of carbapenemase (*bla*KPC group and *bla*NDM group). Out of these 12 isolates, 8 (66.6%) were confirmed to have the *bla*KPC group gene which is in agreement with finding from Egypt [88] that is conducted in general *E. coli* and in disagreement with study from India [92] that reported *bla*NDM group predominantly. None of the isolates were positive for the *bla*NDM group gene. Since this is the first study conducted to detect the carbapenemase gene in *E. coli* O157:H7, we couldn't find any study to compare our findings within Ethiopia.

The difference in the distribution of these genes could be due to the difference in geographic location, study population, and variations in antibiotic prescribing practices and usage patterns in the research area may have an impact on the distribution of ESBL genes. Different antibiotic classes' selective pressure can favor the establishment and spread of various ESBL genes. Antibiotic usage, including antibiotic kinds, dosages, and durations, can lead to changes in the prevalence and distribution of ESBL genes in the specific area [93].

6.4. Antimicrobial Susceptibility

The *E. coli* O157:H7 isolates from our study showed high antimicrobial resistance to Ampicillin [38/38 (100%)], Amoxicillin-clavulanic acid [34/38 (89.5%)], Tetracycline [22/38 (57.9%)], and equal rate of 12/38 (31.6%) against Ertapenem, and Meropenem. However, all isolates were sensitive to ciprofloxacin. These findings are more or less similar to the report from a study in Bahirdar [75] which was conducted on under-five children, Ambo Town, [6], Jimma [43], Addis Ababa [40], and central Ethiopia [19] on abattoir and retailer shops which shows high resistance against ampicillin and more than 93% of isolates susceptible to ciprofloxacin. Regarding the MDR rate among our isolates, 32 (84.2%) of them had resistance to three or more classes of antimicrobials, which is in agreement with the study from Bahirdar [75] which reported around 88% multi-drug resistance.

7. CONCLUSION AND RECOMMENDATION

7.1. Conclusion

Upon our laboratory investigation, the magnitude of *E. coli* O157:H7 bacteria in Shashemene town and its surrounding woreda was found to be 9% among diarrheal patients. Isolates exhibited a high level of resistance against Ampicillin 100%, Amoxicillin with clavulanic acid 89.5%, tetracycline 57.9%, Ertapenem 31.6%, and Meropenem. Ciprofloxacin is found to be the drug of choice because all isolates are susceptible to this antibiotic. The magnitude of ESBL and Carbapenemase production among *E. coli* O157:H7 isolates was found to be high 71.1% and 31.6% respectively. From all the three genes tested for ESBL in our study, the *bla*TEM group was the most abundant one (79%), followed by the *bla*CTX-M group (63%). Of the two genes tested for the Carbapenem, the *bla*KPC group gene was the only gene found in our isolates. High resistance of Ampicillin and Amoxicillin/Clavulanic acid were observed among ESBL and carbapenemase-producing isolates, and Ciprofloxacin is found to be the most effective drug for both ESBL producers and non-producers. In addition to the production of ESBL and carbapenemase, an alarmingly high rate of MDR was found in this study. Study participants from urban areas and those who own domestic animals tended to have a high magnitude of ESBL and carbapenemase-producing *E. coli* O157:H7 isolates.

7.2. Recommendation

Based on the findings of our study we have made the following recommendations:

- Routine screening of diarrheal patients for *E. coli* O157:H7 bacteria.
- Capacity building for enabling screening for ESBL and carbapenemase for controlling the spread of this treatment among communities and to implement stringent infection prevention measures.
- Implementing and enforcing antimicrobial stewardship programs to address the high levels of antimicrobial resistance observed in *E. coli* O157:H7 isolates.
- Further large-scale study that promote a One Health approach that integrates human health, animal health, and environmental health perspectives. This will help address the complex nature of *E. coli* O157:H7 infections and antimicrobial resistance, considering factors such as animal reservoirs, agricultural practices, and environmental contamination.

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ANNEXES

Annex I: English version Information Sheet

Title of the study: *Escherichia coli* O157:H7 and their Extended-Spectrum β -Lactamase and Carbapenemase production among diarrheic patients from Shashemene, west Arsi, Ethiopia

Principal Investigator: Shimelis Teshome Ayalneh

Name of the Sponsor: Addis Ababa University and Armauer Hansen research institute

Introduction: Hello. We are conducting a research on the *Escherichia coli* O157:H7 and characterization of their Extended-Spectrum β -Lactamase and Carbapenemase production among diarrheic patients from Shashemene, west Arsi, Ethiopia. This is MSc research work. Please read or has study staff read this text to you and take your time to decide to participate in this study.

Purpose of the study: The purpose of this study is to assess the burden of *Escherichia coli* O157:H7 and characterization of their ESBL and Carbapenemase production among diarrheal patients. The result of this study will help policymakers and hospital managers in planning, implementing, and evaluating various interventions for the management of treatment guidelines that further prevent the possible occurrence of hemorrhagic diarrheal disease due to this bacterium.

Procedure: For this study to be successful we need your participation provided that you are willing to give us permission for the interviewing you, to use stool sample collected from you for this research, and to take clinical information from your medical records which are relevant to the study.

Confidentiality: All personal information you give and data obtained from laboratory analysis Were kept confidential. All the data Were coded with numbers without names. Access to the stored information Were given only to the investigators.

Expected benefits: There is no payment that you Were given for participating in this research. However, the result Were reported to your physician for appropriate treatment and management. The study will have importance in the management of future patients. Moreover, your participation in this study will have a great value on preventive measures in the community.

Risks: There is no risk for participating in this study except that you will spend a maximum of 15 minutes for an interview.

Right to Refusal or Withdraw: Your participation in the study is voluntary; you have the full right to refuse from participating in this research. You can ask any question which is not clear for understanding and skip any or all the questionnaires.

Person to Contact: Thank you for taking your time to read the information sheet/ listen as it is being read to you. If you have questions or problems related to the present study, you can contact the principal investigator at any time using the following address:

Mr. Shimelis Teshome Mobile: +251922645002 E-mail: shimelisteshome35@yahoo.com

Department of Microbiology, Immunology and Parasitology, College of Health Sciences, Addis Ababa University

Annex II: English version Consent form for adults (≥18 years)

I have read the information above, or it has been read to me. I have been allowed to ask questions and my questions have been answered to my satisfaction. **I voluntarily consent that I would participate in this study.**

To collect a stool sample and be a participant in this study and understand that I have the right to withdraw from the study at any time.

Print name of participant, date and signature or thumb impression of participant

_____ /____ /____ (dd/mm/yy) _____

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

_____ /____ /____ (dd/mm/yy) _____

Phone number _____

Print name of researcher, date and signature of researcher

_____ /____ /____ (dd/mm/yy) _____

Annex III: English version Consent form for parents/guardians (for children ≤18 years)

I have read the information above, or it has been read to me. I have been allowed to ask questions and my questions have been answered to my satisfaction. **I voluntarily consent that my child participates** in this study provided he/she gives assent.

To collect her/his stool sample and be a participant in this study and understand that I have the right to withdraw my child from the study at any time.

Print name of participant, date and signature or thumb impression of participant

_____ /____ /____ (dd/mm/yy) _____

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

_____ /____ /____ (dd/mm/yy) _____

Print name of researcher, date and signature of researcher

_____ /____ /____ (dd/mm/yy) _____

Annex IV: English version Assent form for children aged 12-17 years

I have read the information above, or it has been read to me. I have been allowed to ask questions and my questions have been answered to my satisfaction. I voluntarily assent that I would participate in this study provided my parents/guardians give their consent.

To collect my stool sample and be a participant in this study and understand that I have the right to withdraw from the study at any time.

Print name of participant, date and signature or thumb impression of participant

_____ /____ /____ (dd/mm/yy) _____

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

_____ /____ /____ (dd/mm/yy) _____

Phone number (parents/guardians)

Print name of researcher, date and signature of researcher

_____ /____ /____ (dd/mm/yy) _____

Annex V: English version of Questionnaire

QUESTIONNAIRES: Administered for investigation of *Escherichia coli* O157:H7 in patients with diarrhea and characterization of their ESBL and Carbapenemase production. We would be most grateful if you could answer the following questions.

| | | | |
|--|--|---|--|
| Date ____/____/____ | | Study number _____ | |
| 1. Diarrhea | Status <input type="checkbox"/> yes <input type="checkbox"/> no | | |
| 2. Address | Town _____ Kebele _____ | | |
| 3. Age | _____ | | |
| 4. Gender | <input type="checkbox"/> Male <input type="checkbox"/> Female | | |
| 5. Number of household members | Total: /_____/ No. <5 years old: /_____/ | | |
| 6. What is your level of education? | <input type="checkbox"/> Primary | <input type="checkbox"/> Collage | <input type="checkbox"/> None |
| | <input type="checkbox"/> secondary | <input type="checkbox"/> university | |
| 7. What is your occupation? | <input type="checkbox"/> Farmer | <input type="checkbox"/> Merchant | |
| | <input type="checkbox"/> Government worker | <input type="checkbox"/> Other _____ | |
| 8. What is the average monthly net income from you and your spouse's earnings? | <input type="checkbox"/> 1. below 1000 | <input type="checkbox"/> 3.2501 to 4000 | |
| | <input type="checkbox"/> 2.1001 to 2500 | <input type="checkbox"/> 4.> 4001 | |
| 9. Domestic animals in the house | Poultry <input type="checkbox"/> | sheep <input type="checkbox"/> | Donkey <input type="checkbox"/> |
| | Goat <input type="checkbox"/> | cattle <input type="checkbox"/> | Cat <input type="checkbox"/> |
| | Dog <input type="checkbox"/> | Others <input type="checkbox"/> _____ | |
| 10. Drinking water | <input type="checkbox"/> Tap water | <input type="checkbox"/> Springwater | <input type="checkbox"/> Domestic water |
| | <input type="checkbox"/> River water | <input type="checkbox"/> Bottle water | <input type="checkbox"/> others |
| 11. Do you treat your water before use? | <input type="checkbox"/> 1. Yes <input type="checkbox"/> 2. No | | |
| 12. How do you treat the water before drinking or use? | <input type="checkbox"/> boiling | | <input type="checkbox"/> use of chemical |
| | <input type="checkbox"/> filtering | | <input type="checkbox"/> Other _____ |
| 13. Do you drink un-boiled milk and milk products | <input type="checkbox"/> 1. Yes <input type="checkbox"/> 2. No | | |
| 14. Do you eat uncooked or undercooked food | <input type="checkbox"/> Meat <input type="checkbox"/> Salad | | |
| | <input type="checkbox"/> Other _____ | | |

| | | | |
|---|---|---|---------------------------------------|
| 15. Is a toilet available in the home/house? | | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| 16. If available, of what type? | | <input type="checkbox"/> pit latrine | <input type="checkbox"/> flash toilet |
| 17. How often do you wash hands? | <input type="checkbox"/> Always after visiting the toilet | <input type="checkbox"/> After cooking | |
| | <input type="checkbox"/> before cooking | <input type="checkbox"/> others _____ | |
| 18. With what do you wash your hand? | <input type="checkbox"/> with soap and water | <input type="checkbox"/> with water only | |
| Type of diarrhea | | | |
| 1. Duration of diarrhea (days) | | <input type="checkbox"/> < 24h <input type="checkbox"/> other: / _____ / days | |
| 2. No. of episode of diarrhea in the last 24h | | / _____ / per day | |
| 3. No. of household members reported diarrhea within 10 days before the patient's illness | | / _____ / | |
| 4. Have you received antibiotic within 4 weeks before the beginning of the diarrhea | | <input type="checkbox"/> yes | <input type="checkbox"/> no |
| | | <input type="checkbox"/> name of antibiotic: / _____ / | |
| 5. Fever at attendance time (please check the temperature by measuring) | | _____ | |
| 6. Chills during diarrhea | <input type="checkbox"/> yes | <input type="checkbox"/> no | |
| 7. Vomiting during diarrhea | <input type="checkbox"/> yes | <input type="checkbox"/> no | |
| 8. Bloody diarrhea | <input type="checkbox"/> yes | <input type="checkbox"/> no | |
| 9. Mucous diarrhea | <input type="checkbox"/> yes | <input type="checkbox"/> no | |
| 10. Antibiotic therapy for diarrhea | <input type="checkbox"/> yes | <input type="checkbox"/> no | |
| If yes Name of antibiotic: / _____ / | | | |
| Duration of antibiotic therapy: / _____ / days | | | |

| To be filled by laboratory Professional | |
|---|--|
| 11. Microscopic examination | |
| 12. Stool culture results. | <input type="checkbox"/> <i>E. coli</i> O157:H7 <input type="checkbox"/> Non- <i>E. coli</i> O157:H7 <input type="checkbox"/> Other: / _____ / |
| 13. Sensitivity pattern | Drugs listed |
| 1. Sensitive | |
| 2. Resistant | |

Thank you for your cooperation!

Principal Investigator

Date, _____ Time: _____ completed by _____

Annex VI: Amharic version Information Sheet

የጥናቱ ርዕስ:- Escherichia coli O157:H7 and characterization of their Extended-Spectrum β -Lactamase and Carbapenemase production among diarrheic patients from Shashemene, west Arsi, Ethiopia.

ዋና ተመራማሪ:- ሸመልስ ተሾመ

የ እስፖንሰሩ ስም:- አዲስ አበባ ዩኒቨርሲቲ እና አርማወር ሃንሰን ረሰርች ኢንስቲትዩት

ጠቅላላ መረጃ:- ሰላም! እንደምን አለህ/ሽ። እኛ Escherichia coli O157:H7 and their Extended-Spectrum β -Lactamase and Carbapenemase production among diarrheic patients from Shashemene, west Arsi, Ethiopia ላይ ጥናት እያኪያሃድን እንገኛለን። ይህ ለማስተርስ ዲግሪ ማጠናቀቂያ የሚሰራ ጥናት ነው። እባክዎ ይህንን እንብቡና ወይም የጥናቱን መጠይቅ የሚጠይቀውን ባለሙያ እንዲያነቡ ጠይቁት፤ እናም ጊዘዎን ወስደው አስበውበት ጥናቱ ላይ መሳተፍ መፈለግን ወይም አለመፈለግን

የጥናቱ አላማ:- የዚህ ጥናት ዋናው አላማ ለተቅማጥ በሽታ መነሻ የሆነውን Escherichia coli O157:H7 የሚባለውን ተዋሲ ማጥናት እና ESBL እና Carbapenemase ማመንጨቱን መመርመር ነው። የዚህ ጥናት ውጤት ለጤና ፖሊሲ አውጪዎችና ሆስፒታል አስተዳዳሪዎች ኢሄንን ተህዋሲ ለመቆጣጠርን ለማከም ሊረዳ ይችላል።

አኪሃድ:- ይህ ጥናት ስኬታማ እንዲሆን የእርሶን ተሳትፎ ለዚህ ጥናት በፍቃድ ቃለመጠይቅ፣ እንዲያረጉልንና የሰገራ ናሙና፣ እናም ለዚህ ጥናት ጠቃሚ የሆነውን የህክምና መረጃዎን እንዲሰጡን እንፈሊጋለን።

ጥናቱ ላይ በመሳተፍ ሊደረስበት የሚችል አደጋ እና ጥቅም:- በዚህ ጥናት መሳተፍ የሚያስገኘው ቀጥተኛ ጥቅም ባይኖርም ያንተ/ያንቺ በዚህ ጥናት መሳተፍ እና ትክክለኛውን መረጃ መስጠት ለህመማን የሚሰጠውን የህክምና አገልግሎት የማስተካከል እንዲሁም የማሻሻል አቅም አለው። እንዲሁም የጸረ ተዋሲያን መድኃኒቶችን የመቋቋሙትን ተዋሲያን ለመለየት ጥቅሙ የጎላ ነው።
ሚስጥራዊነቱን በተመለከተ:- ስሞት እንዲሁም የእርሶን ማንነት የሚገልጹ ማናቸውም ነገሮች በመጠየቁ ላይ እንደማይመዘገቡ አረጋግጥታለሁ። እንዲሁም የሚሰጡን ማንኛውም መረጃ እና የሰገራ ናሙና በሚጥር የሚያዝ ሲሆን ለጥናቱ አላማ ብቻ የሚውል መሆኑንም ላሳውቆ እወዳለሁ። የዚህ ጥናት ምርመራ ውጤት ለጥናቱ ተሳታፊዎች በሙሉ የሚጠቃለል ሲሆን የጥናቱ ተሳታፊ ግለሰቦችን ማንነት የሚያሳይ አይደለም። መጠይቆቹ የጥናቱ ተሳታፊ ስሞችን በሚስጥራዊ ጽሑፍ (ኮድ) የሚያዙ ሲሆን እና የሰገራ (አይነ-ምድር) ናሙናዎቹ በሙሉ በጥናቱ ዋና ተመራማሪ እጅ ብቻ የሚያዙ ይሆናሉ።

ሙብት:- እዚህ ጥናት ላይ የሚሳተፉት በራሶ ፍቃድ ነው። ልጆች በጥናቱ ላይ እንዲሳተፍ የመምከር ግዴታ የለበትም። በጥናቱ ጊዜ በየትኛውም ሰአት አቋርጠው መውጣት ይችላሉ። በመጠየቅ ውስጥ ያሉ ነገር ግን መመለስ የማይፈልጉት ጥያቄን አለመመለስ ሙብቶ ነው። ጥያቄ ካሎት በየትኛውም ጊዜ መጠየቅ ይችላሉ።

አድራሻ:- በጥናቱ ላይ ወይም በጥናቱ ስነ- ስርዓት /ቅደም ተከተል ጥያቄ ቢኖሮት ከዚህ በታች በተቀመጠው አድራሻ ማግኘት ይችላሉ።

የዋነው ተመራማሪ ስምና እና አድራሻ:- ሸመልስ ተሾመ

ስልክ ቁጥር:- +251922645002 ፤ የኢሜል አድራሻ:- shimelisteshome35@yahoo.com

አዲስ አበባ ዩኒቨርሲቲ፣ የጤና ሳይንስ ኮሌጅ፣ የማይክሮባዮሎጂ፣ ኢሜዩኖሎጂና ፓራሳይቶሎጂ ዲፓርትመንት

Annex VII: Amharic version Consent form for adults (≥18 years)

ይህንን ከላይ ያለውን መረጃ አንብቤአለሁ ወይም ተነባሊኛል፤ እናም ቃለምጠይቅ ለማድረግ ናሙና ለመስጠት ተስማምቻለሁ።
እዚህ ጥናት ላይ ለመሳተፍ ፍቃደኛ ነኝ።

የተሳታፊው ስም፣ ቀንና ፍርሚያ ወይም የጣት አሻራ

_____ / ____ / ____ (dd/mm/yy) _____

የጣት አሻራ ከተወሰደ

የምስክር ፍርሚያ (ቢቻል ምስክሩ ከጥናቱ ጋር ግንኙነት የሌለው ቢሆን ይመረጣል)

_____ / ____ / ____ (dd/mm/yy) _____

የምስክር ስልክ ቁጥር _____

መረጃውን የሰበሰው ባለሙያ

_____ / ____ / ____ (dd/mm/yy) _____

Annex VIII: Amharic version Consent form for parents/guardians (for children ≤18 years)

ይህንን ከላይ ያለውን መረጃ አንብቤአለው ወይም ተነባላችኋል፤ እናም ቃለምጠይቅ ለማድረግ ናሙና ለመስጠት ተስማምቻለው።
እዚህ ጥናት ላይ ልጄ እንዲሳተፍ ወይም እንድሳተፍ ፍቃደኛ ነኝ።

የተሳታፊው ስም፣ ቀንና ፍርሚያ ወይም የጣት አሻራ

_____ / ____ / ____ (dd/mm/yy) _____

የጣት አሻራ ከተወሰደ

የምስክር ፍርሚያ (ቢቻል ምስክሩ ከጥናቱ ጋር ግንኙነት የሌለው ቢሆን ይመረጣል)

_____ / ____ / ____ (dd/mm/yy) _____

የምስክር ስልክ ቁጥር _____

መረጃውን የሰበሰው ባለሙያ

_____ / ____ / ____ (dd/mm/yy) _____

Annex IX: Amharic version Assent form for children aged 12-17 years

ይህንን ከላይ ያለውን መረጃ አንብቤአለው ወይም ተነባቢኛል፤ እናም ቃለምጠይቅ ለማድረግ ናሙና ለመስጠት ተስማምቻለው።
እዚህ ወላጅ/ አሳዳጊዬ ፍቃድ የሰጠብት ጥናት ላይ ለመሳተፍ ፍቃደኛ ነኝ።

የተሳታፊው ስም፣ ቀንና ፍርሚያ ወይም የጣት አሻራ

_____ / ____ / ____ (dd/mm/yy) _____

የጣት አሻራ ከተወሰደ

የምስክር ፍርሚያ (ቢቻል ምስክሩ ከጥናቱ ጋር ግንኙነት የሌለው ቢሆን ይመረጣል)

_____ / ____ / ____ (dd/mm/yy) _____

የወላጅ ወይም አሳዳጊ ስልክ ቁጥር _____

መረጃውን የሰበሰው ባለሙያ

_____ / ____ / ____ (dd/mm/yy) _____

Annex X: Amharic version of Questionnaire

መጠይቅ: Escherichia coli O157:H7 in patients with diarrhea and characterization of their ESBL and Carbapenemase production ለማጥናት የሚጠየቅ. ለተሳትፎ ፍቃደኛ በመሆኖ በጣም እናመሰናለን.

| | | | |
|-------------------------------------|--|--|-----------------------------------|
| ቀን _____ / _____ / _____ | | የሚስጥር ጽሑፍ _____ | |
| 1. ተቅማጥ | ሁኔታ | | |
| | <input type="checkbox"/> አዎ <input type="checkbox"/> አይ | | |
| 2. አድራሻ | ከተማ _____ ቀበሌ _____ | | |
| 3. እድሜ | _____ | | |
| 4. ጾታ | <input type="checkbox"/> ወንድ <input type="checkbox"/> ሴት | | |
| 5. የቤተሰብ አባላት ቁጥር | አጠቃላይ: / _____ / | | |
| | ከአምስት አመት በታች የሆኑ ልጆች ብዛት: / _____ / | | |
| 6. የትምርት ደረጃ | <input type="checkbox"/> አንደኛ ደረጃ | <input type="checkbox"/> ኮሌጅ | <input type="checkbox"/> ሌላ ካለ |
| | <input type="checkbox"/> ሁለተኛ ደረጃ | <input type="checkbox"/> ዩኒቨርሲቲ | |
| 7. ሰራዎት ምንድነው | <input type="checkbox"/> ገበሬ | <input type="checkbox"/> ነጋዴ | |
| | <input type="checkbox"/> የመንግስት ሰራተኛ | <input type="checkbox"/> ሌላ _____ | |
| 8. አማካኝ ወርሃዊ ገቢዎ ሚን ያክል ይሆናል? | <input type="checkbox"/> <1000 | <input type="checkbox"/> 2501—4000 | |
| | <input type="checkbox"/> 1001 – 2500 | <input type="checkbox"/> > 4001 | |
| 9. የቤት ውስጥ እንስሳት አይነት | <input type="checkbox"/> ዶሮ | <input type="checkbox"/> በግ | <input type="checkbox"/> አህያ |
| | <input type="checkbox"/> ፍየል | <input type="checkbox"/> ከብቶች | <input type="checkbox"/> ድመት |
| | <input type="checkbox"/> ውሻ | <input type="checkbox"/> ሌሎች _____ | |
| 10. የመጠጥ ውሃ | <input type="checkbox"/> የባንቧ ውሃ | <input type="checkbox"/> የምንጭ ውሃ | <input type="checkbox"/> የጉርጓድ ውሃ |
| | <input type="checkbox"/> የወንዝ ውሃ | <input type="checkbox"/> የታሸገ ውሃ | <input type="checkbox"/> ሌላ |
| 11. የምትጠቀምበትን ውሃ ከመጠቀም በፊት ታክማለህ ወይ | | <input type="checkbox"/> አዎ <input type="checkbox"/> አላክምም | |
| 12. የምትጠቀምበትን ውሃ እንዴት ታክማለህ | <input type="checkbox"/> በማፍላት <input type="checkbox"/> ኬሚካል በመጠቀም | | |
| | <input type="checkbox"/> በማጥለል <input type="checkbox"/> ሌላ _____ | | |
| 13. ያልተፈለገ ወተት/የወተት ተዋጾ ይጠጣሉ? | <input type="checkbox"/> አዎ <input type="checkbox"/> አይ | | |
| 14. የልቦና/ በደምብ ያልበሰለ ምግብ ይመገባሉ | <input type="checkbox"/> ስጋ <input type="checkbox"/> አትክልት | | |
| | <input type="checkbox"/> ሌላ _____ | | |

| | |
|--|---|
| 15. የመጻዳጃ ቤት አለህ? | <input type="checkbox"/> አዎ <input type="checkbox"/> የለም |
| 16. የመጻዳጃ ቤት ካለህ ምን አይነት | <input type="checkbox"/> የጉድጓድ ሽንት ቤት <input type="checkbox"/> ውሃ የሚደፋበት |
| 17. እጅህን መቼ ትታጠባለህ /ተታጠቢያለሽ | <input type="checkbox"/> የመጻዳጃ ቤት በገባው ጊዜ ሁሉ <input type="checkbox"/> ምግብ ካበሰልኩ በኋላ <input type="checkbox"/> ምግብ ከማብሰል በፊት <input type="checkbox"/> ልላ ካለ ይጥቀሱ: _____ |
| 18. እጅዎን በምንድነው የሚታጠቡት? | <input type="checkbox"/> በሳሙናና ውሃ <input type="checkbox"/> በውሃ ብቻ |
| የተቅማጥ አይነቶች | |
| 1. ተቅማጥ በሽታ ከተያዙ ስንት ጊዜ ይሆናል (በቀናት) | <input type="checkbox"/> < 24ሰዓት <input type="checkbox"/> ሌላ ሰዓት: / _____ / ቀናት |
| 2. ባለፉት 24 ሰዓት ውስጥ ስንት ጊዜ አስቀምጦታል? | / _____ / ጊዜ በቀን ውስጥ |
| 3. ከመታመምህ በፊት በ10 ቀናት ውስጥ ተቅማጥ እንደያዛቸው የተናገሩ የቤተሰብ አባላት ቁጥር | / _____ / |
| 4. ተቅማጡ ከመጀመሩ ከ 4 ሳምንት በፊት የጸረ ተዋሲያን መድሐኒቶችን ወስደሃል? | <input type="checkbox"/> አዎ <input type="checkbox"/> አልወሰድኩም <input type="checkbox"/> አዎን ካሉ ምን ያህል የጸረ ተዋሲያን መድሐኒቶችን ወስደሃል: / _____ / |
| 5. ለህክምና በመጡ ጊዜ ትኩሳት ስለመኖሩ (እባክዎትን ትኩሳት ስለመኖሩ የሙቀት መጠኑን ይለኩ) | _____ |
| 6. በተቅማጡ ጊዜ ቅዝቃዜ | <input type="checkbox"/> አዎ <input type="checkbox"/> የለም |
| 7. በተቅማጡ ጊዜ ትውከት | <input type="checkbox"/> አዎ <input type="checkbox"/> የለም |
| 8. ደም የቀላቀለ ተቅማጥ | <input type="checkbox"/> አዎ <input type="checkbox"/> አልታየኝም |
| 9. ንፍጥ የቀላቀለ ተቅማጥ | <input type="checkbox"/> አዎ <input type="checkbox"/> የለም |
| 10. የክብደት መቀነስ | <input type="checkbox"/> አዎ <input type="checkbox"/> የለም |
| 11. ለተቅማጡ የጸረ ተዋሲያን መድሐኒት ወስደዋል | <input type="checkbox"/> አዎ <input type="checkbox"/> አልወሰደኩም |
| መልሶ አዎ ከሆነ የጸረ ተዋሲያን መድሐኒት ስም ይጥቀሱ/ _____ / | |
| ለምን ያህል ጊዜ የጸረ ተዋሲያን መድሐኒት ወስደዋል: / _____ / በቀን | |

| በ ላቦራቶሪ ባለሙያ የሚሞላ | |
|--------------------------------|--|
| 12. የማይክሮስኮፕ ምርመራ ውጤት | |
| 13. የሰገራ ካልቸር ውጤት | <input type="checkbox"/> <i>E. coli</i> O157:H7 <input type="checkbox"/> Non- <i>E. coli</i> O157:H7 <input type="checkbox"/> Other: / _____ / |
| 14. Sensitivity pattern | Drugs listed |
| 1. Sensitive | |
| 2. Resistant | |

ለትብብርዎ በጣም እናመሰግናለን!

ዋና ተመራማሪው

ቀን, _____ ሰዓት: _____ ፎርምን የሞላው ሰው _____

Annex VI: Declaration form

Declaration of Investigator

I, the undersigned student of Medical Microbiology, declare that this thesis is my original work in partial fulfillment of the requirement for the master’s degree in medical microbiology to my best knowledge.

Principal investigator: Shimelis Teshome Ayalneh

Signature: _____ Date_____

Declaration of Advisors

We, the undersigned Advisors, declare that this thesis is our original work in partial fulfillment of the requirement for the master’s degree of Medical Microbiology for the stated student above to our best knowledge. We confirmed that this thesis is ready for defense with our approval as the university advisor(s).

Date of Submission: _____

| Advisors: | Signatures | Date |
|-------------------------------|-------------------|-------------|
| 1. Dr. Woldaregay Erku Abegaz | _____ | _____ |
| 2. Dr. Solomon Gebreselassie | _____ | _____ |
| 3. Dr. Mekonnen Teferi | _____ | _____ |
| 4. Biruk Yeshitila Beshasha | _____ | _____ |
| 5. Seifegebriel Teshome | _____ | _____ |



Department of Microbiology, Immunology and Parasitology (DMIP)

Department Research Ethics Review Committee (DRERC)

Meeting No: DRERC/002/2022

Date: 20 May 2022

| | |
|---|--|
| Protocol Title: Detection of Escherichia coli O157:H7 and characterization of their Extended-Spectrum β -Lactamase and Carbapenemase production among diarrheic patients from Shashemene, west Arsi, Ethiopia. | |
| Principal Investigator | Shimelis Teshome |
| Institute/Department | CHS-AAU/DMIP |
| Type of review | <input checked="" type="checkbox"/> Initial Review; <input type="checkbox"/> Amendment <input type="checkbox"/> Other (specify): _____ |
| Elements Reviewed | <input type="checkbox"/> Attached <input type="checkbox"/> Not attached |
| Decision of the meeting | <input checked="" type="checkbox"/> Approved <input type="checkbox"/> Approved with Recommendation <input type="checkbox"/> Revision requested <input type="checkbox"/> Disapproved |
| Action Required | <input type="checkbox"/> Send to IRB <input checked="" type="checkbox"/> Authorize Implementaion |

Obligations of the PI:

- i. Should comply with the standard international and national scientific and ethical guidelines
- ii. All amendments and changes made in protocol and consent form needs DREC approval
- iii. The PI should report Serious Adverse Events (SAE) within 10 days of the event
- iv. End of the study, including thesis work and manuscript should be reported to the DREC

Follow up report expected in:

3 Months _____ 6 Months X 9 Months _____ one year _____

Asrat Hailu (Prof)

Chair, DRERC

Signature

Date:

Asrat Hailu
20/05/2022

Department Stamp

