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A ONE HEALTH APPROACH TO INVESTIGATING THE OCCURRENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF *ESCHERICHIA COLI* IN INFANTS, ANIMALS, AND THE HOUSEHOLD ENVIRONMENT IN BISHOFTU, ETHIOPIA

MSc THESIS

BY

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BISHOFTU, ETHIOPIA

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A Thesis Submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Veterinary Public Health

By

Redeat Kassahun

June, 2025

Bishoftu, Ethiopia

TABLE OF CONTENTS **Pages**

STATEMENT OF THE AUTHOR

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LIST OF ABBREVIATIONS

A/E	Attaching and Effacing
AHI	Animal Health Institute
AIEC	Adherent invasive *Escherichia coli*
AMR	Antimicrobial resistance
BPW	Buffer Peptone Water
CLSI	Clinical Laboratories and Standard Institute
DAEC	Diffuse-adherent *Escherichia coli*
DEC	Diarrheagenic *Escherichia coli*
DALY	Disability-adjusted life-years
eae	Intimin gene
EAEC	Enteroaggregative *E. coli*
E. coli	*Escherichia coli*
EHEC	Enterohemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EMB	Eosin Methylene Blue Agar
EPEC	Enterohemorrhagic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
HC	Haemorrhagic colitis
HUS	Haemolytic Uremic Syndrome
MALDI/TOF	Matrix-Assisted Laser Desorption/Ionization-Time of Flight
MDR	Multidrug Resistance
PCR	Polymerase Chain Reaction
REDCap	Research Electronic Data Capture
Stx	Shiga toxin
TSB	Tryptone soya broth
WASH	Water, Sanitation and Hygiene

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Pathogenic and antimicrobial-resistant *E. coli* remains a serious public health threat, particularly in household settings where humans and animals share space and access to clean water, hygiene, and sanitation facilities is limited. Data on the occurrence of pathogenic and antimicrobial-resistant (*AMR*) *E. coli* in infants, animals, and the environment within household settings in Bishoftu town is currently lacking. The objective of this study was to assess the occurrence and antimicrobial resistant profiles of *E. coli* and hygienic practices at One Health domain in infants, animals, and the environment in household setting in Bishoftu, Ethiopia. A cross-sectional study was conducted from February 2, 2024 to April 26, 2025. Household-level data on access to clean water, sanitation, and hygiene practices were collected through face-to-face interviews with infant caregivers. For microbiological analysis, four samples-one each from infants, soil, caregivers, and animals-were collected per household, totaling 288 samples from 72 households. Isolation and identification of *E. coli* were performed using standard techniques, including primary and secondary selective enrichment, selective plating, and molecular methods. Confirmation of *E. coli* was done using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry, and conventional PCR was used to detect the intimin (*eae*) gene in positive isolates. Antimicrobial susceptibility of the isolates was assessed using the Kirby-Bauer disk diffusion method. Among the surveyed households, 61.1% did not treat their water, 63.9% used shared latrines, 81.9% lacked handwashing facilities near latrines, and 43.1% left animal feces unmanaged within the compound. Of the 288 samples tested, 268 (69 from infants, 66 from soil, 63 from caregivers, and 70 from animals) yielded positive cultures. From these, 152 presumptive *E. coli* isolates were identified, with 66 confirmed using MALDI-TOF. Based on the distribution of positive isolates per household, 33 samples were selected for further analysis using PCR and antimicrobial susceptibility testing. According to MALDI-TOF results, the highest detection rate of *E. coli* was found in animals (51.3%), followed by soil (40.5%), caregivers (44.4%), and infants (37.5%). Of the 33 isolates tested, only 7 (4 from infants, 1 from caregivers, and 2 from animals) were positive for the *eae* gene. A significant proportion of these isolates showed antimicrobial resistance, with the highest resistance observed in animals (55.6%), followed by infants (52.8%), caregivers (51.4%), and soil (37.5%). The highest resistance was recorded against amoxicillin (100%), tetracycline (78.8%), and amoxicillin-clavulanic acid (72.7%). All isolates were susceptible to

gentamicin. Multidrug resistance was detected in 42.4% of the isolates. In conclusion, the study demonstrates the occurrence and distribution of pathogenic and antimicrobial-resistant *E. coli* across the One Health domains. It also reveals critical sanitation gaps, including reliance on shared facilities and inadequate access to basic hygiene infrastructure, such as soap and water near latrines. These findings highlight the urgent need for integrated interventions to improve access to clean water, sanitation, hygiene, and animal management, using a One Health approach.

Keywords*: E. coli, antimicrobial resistance, One health, Infants, Soil, animals, Bishoftu.*

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1. INTRODUCTION

Diarrheal disease is among the major public health problems, particularly in low- and middle-income countries with high rates of morbidity and mortality in children under five (Liu *et al.*, 2015). According to the 2021 Global Burden of Disease study, diarrheal disease caused an estimated 17 million deaths and 59 million of disability-adjusted life-years (DALYs) in all age groups with 443,832 death and 30·9 million DALYs in children younger than 5 years (Kyu *et al*., 2025). Sub-Saharan Africa and Southeast Asia account for the largest percentage of mortality among children under five (Fatima *et al*., 2024). In Ethiopia, the burden of diarrheal disease is high with incidence rate of 186.7 thousand and death rate of 150.7 per 100, 000 children each year (NDMC, 2021).

Evidence has demonstrated that 88% of diarrhea-related deaths are attributable to unsafe water, poor sanitation, and inadequate hygienic practices (WASH) (Black *et al.*, 2003). WASH related practices are essential to prevent disease transmission, combat antibiotic resistance and improve the wellness of individuals or the community as a whole (Prüss-Ustün *et al*., 2019). In Ethiopia, WASH-related services remain limited, with only 68.7% of the population having access to clean water, 27.5% to improved sanitation, and 38% to handwashing facilities (Desye *et al.,* 2023).

Raising animals in the household premises is an alternative way to meet the family's daily expenditure and provide a ready source of animal protein in urban, peri-urban and rural settings of Ethiopia (Ali and Neka, 2012). Although significant progress has been made in expanding access to WASH services, poor domestic animal husbandry and unhygienic waste disposal continue to undermine these efforts, posing serious public health concerns (Odo and Mekonnen, 2021). The human-animal interaction in the household setting can potentially favor the spread of infectious pathogens that cross species barrier from poorly managed animal feces to humans. (Penakalapati *et al*., 2017).

Zoonotic pathogens can spread easily between humans and animals through direct and indirect contact with animals and contaminated environments (Herrero *et al*., 2013). Fecal-oral route is the predominant pathway in which indirect transmission occurs through exposure to contaminated environmental fomites, foods, water, hands and flies with animal feces while the direct

transmission, occurs by direct contact of humans with the domestic animal and its excreta (Matilla *et al*., 2018; Alebie and Tewachew, 2021). Young children are at higher risk due to their tendency to directly ingest animal feces or contaminated fomites potentially carrying sufficient concentrations of pathogenic bacteria causing diarrheal illness (Pintar *et al.*, 2015). Studies indicated that enteric bacteria potentially carrying antimicrobial resistance (AMR) are the common cause of diarrhea in infants of Ethiopia (GebreSilasie *et al*., 2018; Ashenafi *et al*., 2024; Tilahun *et al.*, 2025).

Bacterial pathogens such as *Escherichia coli* (*E. coli*) is the most predominant etiologic agent that causes moderate-to-severe diarrhea (Havelaar *et al*., 2015). *E. coli* is a gram-negative facultative anaerobic bacterium that belongs to the family Enterobacteriaceae. It commonly inhabits the gastrointestinal tract of humans and animals and abundantly disseminated in fecal contaminated environment (Hogg, 2013). Based on the essential virulence gene, diarrheagenic *Escherichia coli* (DEC) is divided in to seven different pathotypes (Donnenberg, 2013). These are enterohemorrhagic (Shiga toxin-producing) *E. coli* (EHEC/STEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), diffuse-adherent *E. coli* (DAEC), enteroinvasive *E. coli* (EIEC) as well as a new pathotype, adherent invasive *E. coli* (AIEC) (Croxen *et al*., 2013). Many of these pathotypes pose a serious threat to public health, given their low infectious dose and ability to spread through medium like water and food (Koutsoumanis *et al*., 2020). Moreover, *E. coli* is recognized for its capacity to quickly develop antibiotic resistance through multiple ways (Dunn *et al*., 2019).

Antimicrobial resistance (AMR) is the capacity of microbial organisms to endure in the presence of antimicrobials (Muteeb *et al*., 2023). According to the estimate of the global burden of AMR, 1.27 million deaths were directly linked to bacterial AMR (Naghavi *et al*., 2024). *E. coli* is among the leading causes of mortality due to AMR infections (Murray *et al.*, 2022). Due to this fact, it is considered as a model indicator organism for surveillance and monitoring of AMR across the one health domain (Djordjevic *et al*., 2024). AMR *E. coli* has been also documented in humans, in the environment and from different animal species, with numerous strains expressing multi-drug resistance (MDR) (Magiorakos *et al*., 2012).

Similarly, in Ethiopia studies have reported the prevalence and AMR of *E. coli* in humans, animals and the environment in various regions (Gemeda *et al*., 2021; Gutema *et al*., 2021; Fujita *et al*., 2022). However, little is known about the occurrence of AMR *E. coli* in infants, animals, and the environment within household settings where humans and animals share space. Furthermore, there is limited information on access to clean water, sanitation, hygiene services, and animal management practices in these settings.

General objective

- The overall objective of the study was to assess hygienic practices as well as the occurrence and antimicrobial resistant *E. coli* at One Health domain specifically in infants, animals, and the environment in household setting in Bishoftu, Ethiopia.

Specific objectives

- To assess access to clean water, sanitation, hygienic practices, and animal management practices of households,
- To examine the occurrence of *E. coli* in infants, soil, infant caregivers and animals,
- To determine the antimicrobial susceptibility patterns of the identified *E. coli*.

2. LITERATURE REVIEW

2.1. General Overview of *E. coli*

A German physician, Theodore Escherich, characterized a commensal of the gastrointestinal tract isolated from infant stool in 1884, which he named Bacterium coli commune. Then this bacterium was renamed Escherichia in 1919, after which the genus Escherichia was officially recognized in 1958, with *E. coli* being the first species (Farmer III *et al*., 1980). Since its discovery, *Escherichia coli* has been one of the most researched and well characterized microbes, serving as one of the key model systems in microbiology (Foster-Nyarko and Pallen, 2022).

Escherichia coli (*E. coli)* is a Gram-negative, rod-shaped, facultative anaerobic, non-spore-forming, motile bacterium. It is found to be family Enterobacteriaceae (Hogg, 2013). Normally, *E. coli* harmlessly inhabits the gastrointestinal tract of humans and animals. But some strains have evolved to cause diarrhea and a range of extra-intestinal diseases due to their acquisition of specific virulence factors through horizontal gene transfer, mutations, and adaptations (Ramos *et al*., 2020). This infection has been a cause of several outbreaks (Chen and Griffiths, 1999; Davies *et al*., 2005; Bielaszewska *et al*., 2011; Smith *et al.*, 2019; Coulombe *et al.*, 2020;).

Pathogenic *Escherichia coli* strains cause three major clinical syndromes: (i) diarrhoea, (ii) urinary tract infections, and (iii) sepsis or meningitis (Nataro and Kaper, 1998; Riley, 2020). Among these, diarrheagenic *E. coli* (DEC) is further classified into distinct pathotypes based on their characteristic features and essential virulence genes. The main *E. coli* pathotypes include: (i) enteropathogenic *E. coli* (EPEC), associated with diarrhoea in children and various animals; (ii) enterohemorrhagic *E. coli* (EHEC), which includes Shiga toxin-producing strains (STEC); (iii) enterotoxigenic *E. coli* (ETEC), linked to traveller's diarrhoea and diarrhoea in porcine and bovine hosts; (iv) enteroaggregative *E. coli* (EAEC), associated with chronic diarrhoea in humans; (v) enteroinvasive *E. coli* (EIEC), which causes invasive intestinal infections and dysentery in humans and animals; (vi) diffusely adherent *E. coli* (DAEC), related to acute diarrhoea; and (vii) adherent-invasive *E. coli* (AIEC), associated with inflammatory bowel diseases, particularly Crohn's disease (Donnenberg, 2013; Croxen *et al*., 2013).

In addition, the antigenic diversity of *E. coli* is characterized primarily by its somatic (O) antigens, flagellar (H) antigens and capsular (K) antigens, with additional contribution from fimbrial (F) antigens (Pokharel *et al.,* 2023). Depending on this, there are over 180 known somatic, 53 recognized flagellar, and over 80 capsular antigens have been identified (Fratamico *et al.*, 2016).

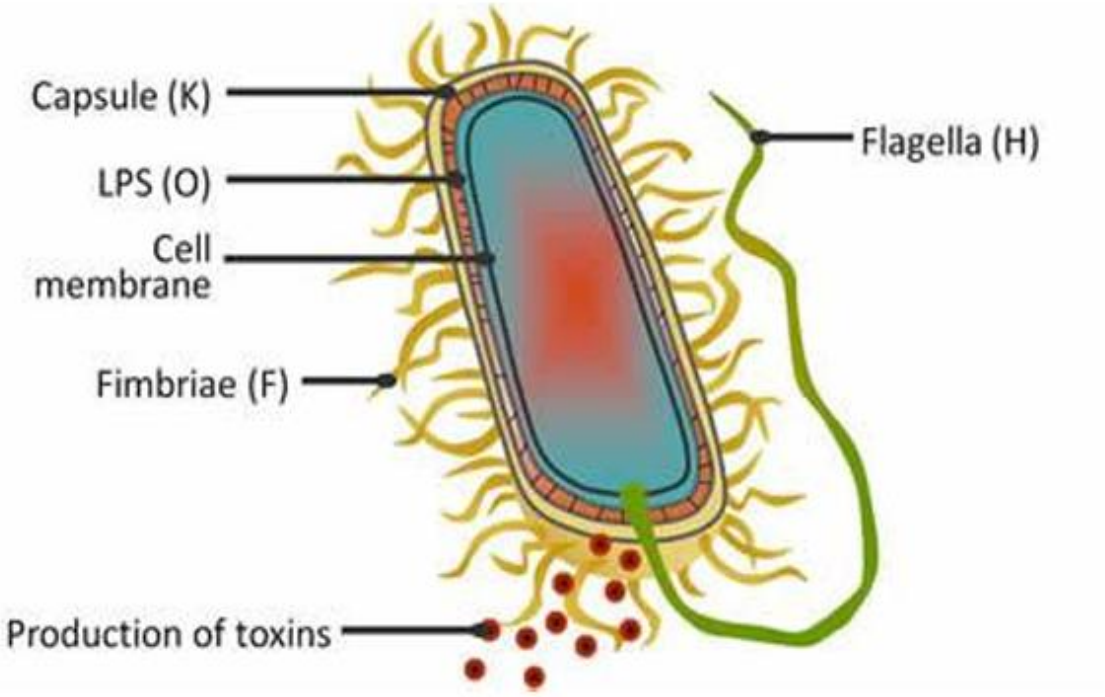

Figure 1: Schematic presentation of *Escherichia coli* surface antigens

Source: (*Escherichia coli* Reference Laboratory, 2004)

2.2. Antigenic Structure

In the 1940s, researchers first presented a serological classification system for *E. coli* based on the immunogenicity of the bacterial surface features (DebRoy *et al*., 2011). Serotyping is a valuable tool in *E. coli* classification for pathogenicity detection and epidemiological studies (Fratamico *et al.*, 2016). Hence, enterohemorrhagic *E. coli* (EHEC) O157:H7 is one of the pathogenic strains of *E. coli* that can be identified by its unique serological markers. It expresses somatic (O) antigen 157 and flagella (H) antigen 7 (Peng *et al.*, 2024).

The O157 is an important *E. coli* O serogroup due to its capability to cause severe illnesses such as hemorrhagic colitis (bloody diarrhea) and hemolytic uremic syndrome (HUS), a potentially fatal condition that can lead to kidney failure, especially in children and the elders (Cramer, 2014). The strain releases strong virulence factors called Shiga toxins (Stx1 and Stx2), which harm host cells and interfere with regular physiological functions (Silva *et al*., 2017). In addition, the low infectious dosage of *E. coli* O157 also makes it highly contagious through contaminated food, water, and close contact with animals or infected individuals. Cattle serve as its main reservoir, and outbreaks are frequently associated with unpasteurized milk, undercooked beef, and contaminated environmental sources (Singha *et al.*, 2023).

On the other hand, H7 antigen denotes to particular type of flagellar protein that defines motility and serotype of the bacterium when paired with an O antigen. However, H7 antigen by itself is not correlated with pathogenicity of *E. coli* (Fratamico *et al*., 2016).

2.3. Virulence Factors and Pathogenicity

E. coli can act as a commensal or an opportunistic pathogen depending on the strain. Pathogenic *E. coli* strains exhibit various virulence factors that enable them to infect humans and animals (Riley, 2020). Virulence factors are certain molecules, mostly proteins produced and secreted by bacteria, fungi, protozoa, and viruses (Brunke *et al.*, 2016). They are encoded by specific genes found on chromosomes or mobile genetic elements (such as plasmids or transposons) in bacterial pathogens. Each *E. coli* pathotype has distinct virulence components expressed by specific gene clusters, as well as unique pathogenicity mechanisms. Genes related to pathogenicity possibly encode traits including motility, adhesion, invasion, attachment, iron acquisition, and toxin activity, among others (Pakbin *et al.*, 2021).

EHEC initially gained recognition in 1982 when it was traced to two outbreaks of hemorrhagic colitis (HC) in the USA (Riley *et al*., 1983). The phage-encoded Shiga toxin, referred to as Vero toxin, is the key virulence factor of EHEC and comes in several forms. Shiga toxin (Stx) is responsible for the pathological effects that result in particular disease symptoms during EHEC infections, including HUS and renal failure. Stx comprises two subgroups stx1 and stx2 as well as various subtypes. Stx2a, stx2c, and stx2d positive EHEC isolates are highly correlated with HC

and HUS compared to other subtypes. Shiga toxin is an AB5 toxin, consisting of an A subunit: the enzymatically active component and B subunits responsible for binding to host cell receptors. Once bound, the toxin is internalized by endocytosis. It is transported retrogradely through the Golgi apparatus to the endoplasmic reticulum. Once inside the cytoplasm, the A subunit is released and cleaves an adenine residue from the 28S rRNA of the 60S ribosomal subunit. This enzymatic activity inhibits protein synthesis. This leads to localized and systemic tissue damage, resulting in symptoms such as diarrhea, HUS, and kidney failure (Melton-Celsa, 2014; Rodríguez-Rubio *et al.*, 2021).

Other elements also contribute for *E. Coli* O157:H7 pathogenicity. PO157-like plasmid is a large plasmid (~90 kb) found in O157 serotype encoding multiple virulence factors that increase pathogenicity. Key factors include EHEC hemolysin (HlyA), which lyses host cells and damages tissue; ToxB and plasmid-encoded pili, which encourage attachment to host epithelial cells; and iron acquisition systems which maintain bacterial viability in nutrient-limited conditions. In addition, catalase-peroxidase helps bacteria to evade immune response by neutralizing reactive oxygen species. These virulence features greatly increase the risk of developing diseases including HUS and HC (Rey *et al.*, 2003: Pilla and Tang, 2018).

Formation of attaching and effacing lesions (A/E lesion) is another unique characteristic of EHEC/EPEC pathogenesis. It is encoded by the LEE pathogenicity island. Adhesion proteins help EHEC serotypes to invade and form biofilm on biotic and abiotic surfaces. Initial attachment, the first stage of EHEC adherence to intestinal epithelial cells, is mediated by the interaction of the pathogen's long polar fimbriae (lfp gene) with extracellular membrane proteins in the host cell, such as laminin, collagen IV, and fibronectin. They profound the attachment, and the A/E effect occur between intimin (eae gene) and the receptor proteins in the host cell membrane interaction, consisting of Translocated intimin receptor (Tir), nuclein, and 1-integrins (Schmidt, 2010; McWilliams and Torres, 2015).

2.4. Epidemiology of *E. coli*

2.4.1. Geographical Distribution of E. coli

The geographical distribution of *E. coli* O157:H7 underlines its significance as a global public health concern, as outbreaks have been documented in both developed and developing countries (Kalule *et al*., 2023; Williams *et al.*, 2023). In high-income nations like the United States and Canada, *E. coli* O157:H7 is commonly associated with undercooked beef, unpasteurized dairy products and contaminated leafy greens (Heiman *et al.*, 2015; John, 2022). Contrarily, in low- and middle-income countries, the infection is linked to inadequate food handling procedures and a lack of access to clean water and hygiene facilities, leading to endemic transmission. (Abebe *et al.*, 2023). Recent outbreaks highlight its global scope, including the waterborne outbreak in Utah, USA, in 2023 (Osborn, 2024), and recurrent incidences in Africa, where accurate reporting is hindered by a lack of surveillance (Musa *et al.*, 2023). Therefore, these geographical variations demonstrate the interplay of environmental, cultural, and infrastructure elements in the persistence of the pathogen and highlight the necessity of implementing One Health strategies to reduce the risk of disease outbreaks and zoonotic transmission.

2.4.2. Transmission of E. coli

E. coli infections can manifest as isolated cases or as significant outbreak (Eppinger and Cebula, 2015). It usually spread through the feco-oral route, cross-contamination, direct contact with infected people, animals, or objects, and most frequently through contaminated food or water. (Mesele and Abunna, 2019). Livestock serve as an important reservoir of *E. coli* O157:H7, with cattle being a primary source and, to a lesser extent, poultry, sheep, and goats.(Persad and Lejeune, 2015). Therefore, *E. coli* can spread through a variety of environmentally mediated pathways in household with domestic animals, endangering human residents, especially vulnerable populations like infants.(Ercumen *et al*., 2017).

Evidences indicate that the possibility of microbial contamination in residential settings, such as soil and water, is greatly increased where humans are living in close proximity to animals (Boehm *et al.*, 2016; Larson *et al.*, 2023). Animal feces mismanagement or its use as fertilizer near home intensifies environmental contamination (Delahoy *et al.,* 2018). In such circumstances babies are at special risk, since they are crawling on the ground and could unintentionally swallow

contaminated stuff (Rosenbaum *et al.,* 2020). Foodborne transmission further compounds this risk, with food from animal origin being a notable source of *E. coli* (Sarba *et al*., 2023). Cross-contamination is also a forefront concern which occur due to improper handling of foods, especially in low-income settings where water, sanitation, and hygiene (WASH) practices are inadequate (Olumide, 2016).

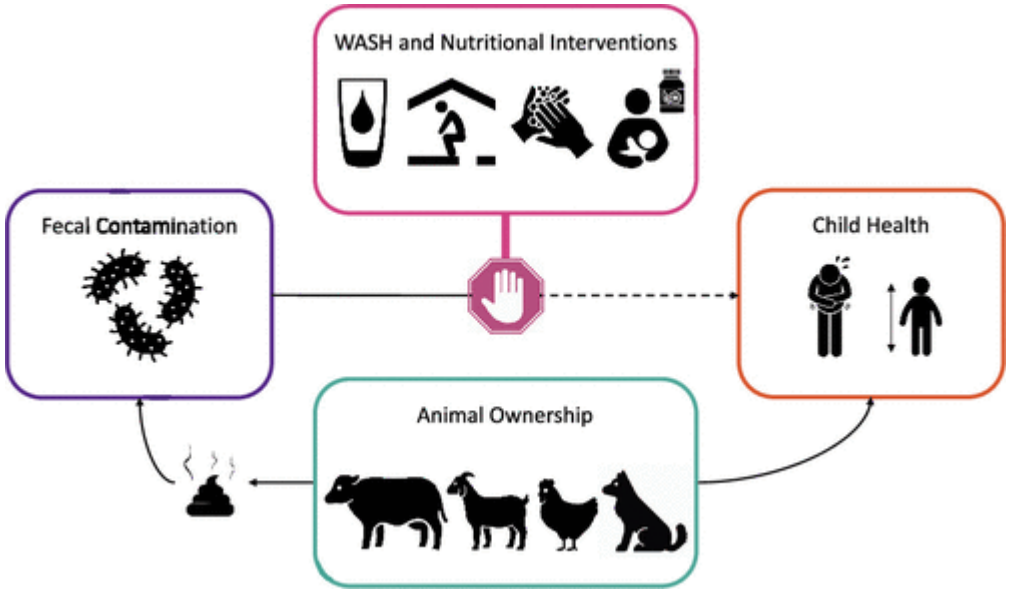

Figure 2: Schematic representation of animal ownership, fecal contamination and WASH intervention for preventing transmission of infection.
Source: (Swarthout *et al.*, 2024)

2.4.3. Clinical aspects of E. coli

E. coli O157:H7 infection vary from person to person but youngsters and old people are more susceptible. Symptoms include mild watery diarrhea, fever, vomiting, severe abdominal cramping and bloody diarrhea. Following bacterial ingestion, symptoms might manifest within hours or days later, with the illness typically lasting five-ten days (Walker *et al*., 2012; Abdissa *et al.*, 2017). The illness generally causes three types of syndromes, such as HC, HUS and thrombocytic thrombocytopenic purpura (TTP). The symptoms of *E. coli* O157:H7-induced HC is characterized by severe cramping in the abdomen, blood in stool, with mild fever (Kropinski *et al*., 2013). HUS, on the other hand, is the primary cause of pediatric acute renal failure. In older adults, TTP is

regarded as a sign of HUS, which manifests as mild renal failure and neurological disorder (Lim *et al*., 2010; Lu and Breidt, 2015).

2.4.4. Growth and environmental survival

The survival and proliferation of *E. coli* O157: H7 in food and the environment is significantly affected by a number of parameters, such as water activity, pH, temperature, and salt (Chauret, 2011). Its optimal growing temperature ranges from 30°C to 42°C, with maximal growth taking place at about 37°C, which is also the temperature of the gastrointestinal tracts of humans and animals (Sutherland *et al*., 1995; Han and Linton, 2004). In spite of this, *E. coli* can tolerate and even grow at refrigeration condition (<4°C) (Duffy and Garvey, 2004). Additionally, the bacterium can thrive in a pH range of 4.0 to 9.0, with optimal growth taking place near neutral pH (6.5 to 7.5). Its resistance to acidic environments, including those in fermented foods or the human stomach, increases its pathogenicity and chances of survival (Suehr *et al.*, 2020). The availability of nutrients, like those in animal excrement or decomposing organic materials, also gives the bacterium resource to develop and grow (Gómez-Brandón *et al.*, 2013). Beyond growth, *E. coli* O157:H7 shows exceptional environmental survival, which adds to its persistence and spread. The bacterium can survive in soil for weeks to months, especially when the soil is moist and nutrient-rich (Chew, 2011).

2.5. Detection of *Escherichia coli*

2.5.1. Bacteriological isolation and biochemical tests

E. coli cells can grow both in a liquid or on a solid growth medium under laboratory setting (Elbing and Brent, 2019). It can be cultured in a basic media that contains glucose as a source of carbon and energy, ammonium salts as a source of nitrogen, trace elements, and other salts (Folsom and Carlson, 2015). Since *E. coli* has simple nutritional needs, it can be readily cultured on a common media including Nutrient agar, Eosin Methylene Blue (EMB) agar, and MacConkey agar. The bacterium thrives at temperatures between 18°C and 44°C on ordinary nutritional media. Then, it forms thick, convex, greyish white, moist, smooth, translucent discs like colonies. A presumptive diagnosis of *E. coli* infection can be established if the majority of the colonies exhibit a green metallic sheen on EMB agar or appear bright pink with a surrounding precipitate on MacConkey

agar. Biochemical tests are also essential approach to identify bacteria based on their metabolic and enzymatic activities (I *et al.,* 2012).

The further identification of the isolated colonies relies on their unique traits, including delayed sorbitol fermentation (taking more than 24 hours) and the lack of β-glucuronidase activity, an enzyme that hydrolyzes the synthetic compound 4-methylumbelliferyl-D-glucuronide (MUG). Sorbitol MacConkey (SMAC) agar supplemented with MUG has thus been utilized to detect *E. coli* O157:H7 (Madic, 2010). Further confirmation of *E. coli* serotypes can be obtained using a commercially available latex agglutination assay (Rocha *et al*., 2014).

2.5.2. Molecular characterization

Molecular characterization methods are the most reliable techniques for differentiating pathogenic strains of *E. coli* by targeting distinct genetic markers (Fleece *et al.*, 2018). The polymerase chain reaction (PCR) is among the most widely employed techniques. PCR techniques for identifying *E. coli* in environmental samples are frequently established using genetic markers such 23S rRNA and lacZ. It has been determined that stx genes and the eae gene encoded on the LEE are potential targets for the detection of EHEC By designing primers that target these genes, it is possible to validate the presence of *E. coli* in different samples (McQuillan and Wilson, 2021). The bacterial load in a sample can be measured in real time using advanced approaches such as quantitative PCR (qPCR), which is particularly helpful in clinical and environmental investigations (Hoorzook and Barnard, 2021).

Another molecular method is loop-mediated isothermal amplification (LAMP), a highly efficient and cost-effective technique that can detect *E. coli* in a shorter time compared to conventional PCR. It is suitable for places with limited resources since it offers high sensitivity and specificity under isothermal circumstances. By targeting stx genes, *eae* gene and the bfp gene and this approach is particularly useful for identifying pathogenic strains of *E. coli* (Yinur *et al*., 2023).

Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry is an emerging powerful tool for diagnosing *E. coli* and other microorganisms at the molecular level (Yoon and Jeong, 2021). Identification of the organisms by MALDI-TOF (MS) relies on assessing protein profiles, namely ribosomal proteins, which are highly conserved indicators for bacterial

identification (Mehramouz *et al.*, 2022). By determining the mass-to-charge ratio of ionized protein molecules, this method offers a unique "fingerprint" of the bacterial species (Haider *et al*., 2023).

Molecular detection has been further transformed by next-generation sequencing (NGS) technology. With the use of whole-genome sequencing (WGS), *E. coli* can be thoroughly characterized, revealing phylogenetic relationship, antibiotic resistance genes, and specific virulence factors. Metagenomic approaches enable detection and analysis of *E. coli* within diverse microbial communities, such as soil, animal, or human microbiomes, without prior culturing (Abernethy *et al.*, 2017; Ko *et al.*, 2022).

2.6. Antimicrobial Resistance

For over a century, the dependence of both animal and public health on antimicrobials is undeniable (Lees *et al.,* 2021). However, the over use and misuse of these drugs have made to become a serious concern to both animal and human health through the occurrence of antimicrobial resistance (AMR). AMR is the capacity of microbial organisms to endure in the presence of antimicrobials, which poses a threat to relapse in the pre-antibiotic era when infectious disease burdens were higher in both human and animal populations (Muteeb *et al*., 2023).

Considering this, antimicrobials are frequently misused for growth promotion, prophylactic and metaphylactic purposes in livestock production (Baptiste and Pokludová, 2020). Moreover, poor hygienic practices play a significant role in the burden of disease and, consequently, in the spread of resistant microorganisms throughout the environment. This situation worsens in low and middle-income countries, where small scale animal production is primarily practiced (Odeyemi and Sani, 2016). Since these farm settings often lack biosecurity resources and water, sanitation and hygiene (WASH) services, antibiotics are used as compensation (Pinto *et al*., 2020).

On the other hand, companion animals play a role in propagating AMR, since antibiotics that are similar to those used in humans are frequently administered to these animals and for their intimate contact with owners (Caddey *et al*., 2025). As a result, the extensive use of antibiotics results in potential pharmaceutical residues entering the environment through animal feces (Li *et al.*, 2013).

Resistant commensal bacteria serve as a source of resistant genes for pathogenic bacteria. Thus, fecal *E. coli* is believed to be a reliable marker of antibiotic-use-induced selection pressure and pathogen resistance (Costa *et al.*, 2008).

2.7. Reported prevalence of *E. coli* in household settings of Ethiopia

The history of animal domestication stretches back to thousands of years in Ethiopia. Since then, Ethiopians have relied on domestic animals and have lived in close proximity to them (Endalew and Ayalew, 2016). Despite this, only a limited number of publications have documented the occurrence of bacterial contamination, mainly focusing on animal, human and environment interfaces in household setting (Table 1). Table 1 reveals studies (Belete *et al.*, 2022; Engda *et al.*, 2023; Tadesse *et al*., 2024) that mainly focused on the occurrence and characteristics of *E. coli* at the animal-human interface. Meanwhile, (Gemeda *et al.,* 2023) reported *E. coli* at the animal-environment interface.

Table 1: Reported prevalence of *E. coli* in animal-rearing households of Ethiopia

Study area	Study unit	Sample type	Prevalence (%)	Detection technique	References
Menz and Yabello district	Cattle	Feces	11.4	MAC, EMB and Omnilog	(Gemeda *et al*., 2023)
	Sheep	Feces	1.4		
	Goat	Feces	20.2		
	Environment	Soil	6.8		
Debre-Markos, Bahirdar, Dessie, Debre-Tabor, and Gondar	Cattle	Recto-anal swab	14.4	SMAC, LAT, PCR	(Engda *et al.*, 2023)
	Human	Stool	11.1		
Bahirdar	Calves	Feces	40	MAC, EMB and PCR	(Belete *et al.*, 2022)
	Human	Stool	37.8		
Addis Ababa, Sululta, and Bishoftu	Cattle	Fecal	76	MAC and EMB	(Tadesse *et al.*, 2024)
	Human	Stool	67		

SMA- Sorbitol MacConkey agar, EMB- Eosin Methylene blue, LAT= Latex Agglutination Test

2.8. Control and Prevention

Actions to prevent and control the spread of *E. coli* should involve vaccination, mass education, clean water access, good sanitation and hygienic practices (Allocati *et al.,* 2013). Although there are no commercially accessible vaccines for humans against *E. coli* at present, considerable advancements have been made in the production of vaccines for cattle (Smith, 2015). The purpose of vaccination is whether to lower the animal's vulnerability to pathogen colonization or to shorten the length of colonization period. By producing antibodies against virulence factors that encode adhesion, a vaccination could hinder the organism's adhesion and cause it to be eliminated from the gastrointestinal tract (Mir *et al*., 2019).

On the other hand, implementing intervention measures across the food continuum, from farm to fork, is necessary for an effective control strategy to significantly decrease *E. coli* infections (Wilson *et al*., 2018). *E. coli* must be controlled in environmental sources like soil, feed, manure and water to terminate the cycle of infection and re-infection of cattle (Ercumen *et al*., 2017). Consumers are also responsible for putting preventive measures in place for the preparation and handling of food. Cutting boards and counters that come into touch with food should be cleaned and sanitized on a regular basis (Kennedy *et al.*, 2011). Handwashing is also one of the most crucial hygiene habits. Hands should be regularly cleaned with soap and water for at least 20 seconds to help get rid of *E. coli* bacteria (Burton *et al*., 2011). Good sanitary facilities, like toilets, help people dispose their waste properly, which improves health. This lowers the chance of illness and avoids environmental contamination (Abney *et al*., 2021).

Generally, One Health approach has been known as major element in preventing *E. coli* infection in domestic environment. One Health is a synergistic approach to improve the health and well-being of animals, people, and their surroundings (Zinsstag *et al*., 2021). It addresses a wide range of concerns, including nutrition, food and water safety, zoonotic disease prevention and antibiotic resistance (Erkyihun and Alemayehu, 2022; Velazquez-Meza *et al*., 2022). Moreover, One Health places a strong emphasis on better sanitation and hygiene conditions in residences with both animals and human lives together. For instance, proper disposal of animal feces to reduce the risk of environmental (Soil, water) contamination (Singh *et al*., 2024). Nowadays, there is also evidence that irrational application of antibiotics in the veterinary sector has resulted in the

development of resistant bacteria that infect people (Naumov *et al*., 2021). Hence, this approach helps to prevent AMR infection through prudent use of antimicrobials and promoting alternate strategies for infection control (Mudenda *et al.*, 2023).

3. MATERIALS AND METHODS

3.1. Study area description

The study was conducted in Bishoftu town, which is located in East Shewa Zone, Oromia regional state of Ethiopia. The town is found 47 kms southeast of Addis Ababa, capital city of Ethiopia. It is positioned at $8^0$45′N latitude and $38^0$59′E longitude, and has maximum altitude of 1850 above sea level (NMSA, 2010). The total human population of the town is estimated to be around 207,383 (City Population, 2022). Bishoftu is considered as livestock corridor, where a large number of commercial and smallholder farms operate, contributing to frequent and close interactions between humans and animals. The area is known for having a dense human population and expanding animal husbandry practices, which enhance the close interaction between animals and humans. The town has 14 kebeles which are small administrative units. Of which, five kebeles that include Ayer hail, Katta, Shibo Gibbi, Lemlem Sefer and Qajima which were considered as rural kebeles has recently been included under city administration. From these, the people's settlement increased in the southwest direction to “Qajima,’ which is currently included in the study.

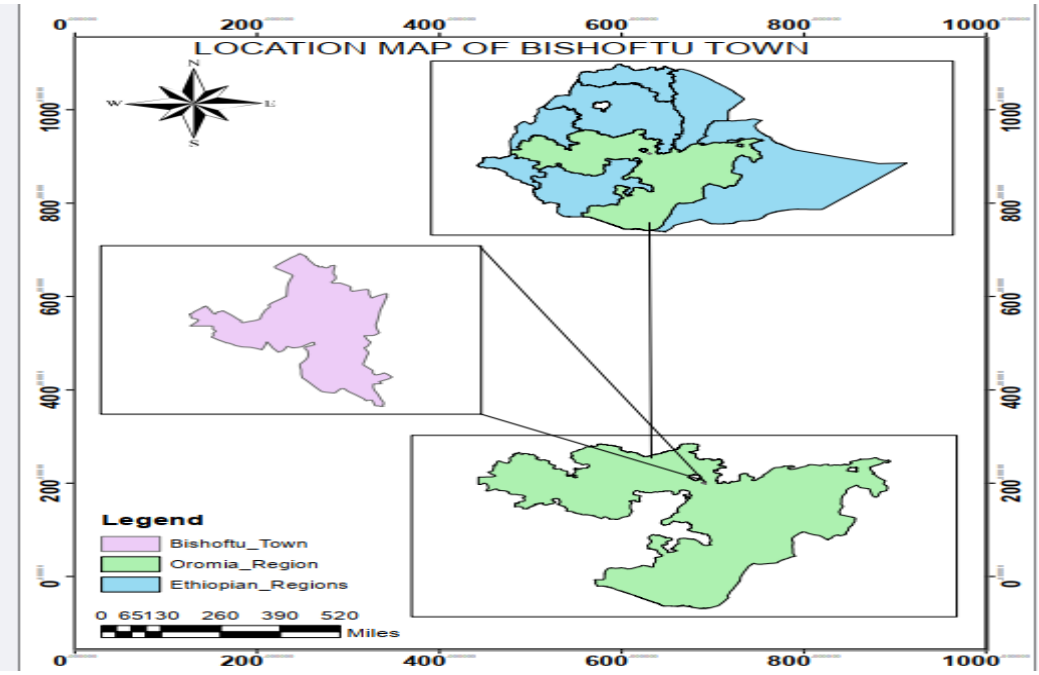

Figure 3: Map of the study area

This map is developed using ArcGIS from Ethiopian shape files.

3.2. Study design and population

A cross-sectional study was conducted from February 2, 2025, to April 26, 2025. Based on the information provided by Bishoftu Health Office, 'Qajima' was purposely chosen for the study because of comparatively high infant population and the widespread practice of animal husbandry in residential compounds. The eligibility criteria were households having infants aged six to twelve months and at least one domestic animal.

3.3. Sample size determination and sampling

The sample size was calculated based on an expected prevalence of 22.5% of diarrhoea in infants aged between six to twelve months, which is the highest prevalence reported among children in Ethiopia by the EDHS (2017), 5% of margin of error and a 95% confidence level using the following formula:

$$n = \frac{Z^2 \times Pexp\ (1\text{-}Pexp)}{d^2}$$

where Z = 1.96 (95% confidence level), d = marginal error to be 0.05, P exp =expected prevalence assumed to be 22.5%, 1- Pexp = proportion of infants not having diarrhea.

n = 266.

However, this study used and reported the preliminary findings on a subset of data from the ongoing research project supported by the Reckitt Global Hygiene Institute. The results reported in this study represent data collected from 72 households having animals. The enrollment of eligible households was performed by community health extension workers, and the households were enrolled on a rolling basis. The sample and data were collected by trained enumerators who have animal and human health professional background. The human subject component of the sample and data collection was handled by public health professionals.

3.4. Data collection tools and methods

3.4.1. Household Survey

A pretested structured questionnaire was used to collect data on infant's potential exposure to *E. coli* and antimicrobial resistance *E. coli*. The questionnaire format was designed based on previous similar studies (Adane *et al.,* 2017; Baker *et al*., 2022; Gizaw *et al.,* 2024) and pretested to customize the questions to collect the desired information. The questionnaire was first developed in English language and then translated to the local dialects (Amharic or Afan Oromo) by a qualified language expert The draft questionnaire was pretested with three caregivers who were excluded from the final study. Finally, the questionnaire was uploaded into Research Electronic Data Capture (REDCap), an online data collection application (REDCap, https://projectredcap.org) that allows offline data collection, and a face-to-face interview with the infant's primary caregiver was performed using a smart tablet. Data was collected from a total of seventy-two households. The structure of the questionnaire consists of four themes or categories: i) social-demographic characteristics of infant caregivers, ii) access to clean water, sanitation and hygiene services iii) animal ownership and hygienic waste management practices, and iv) childcare practices in household. The detailed specific questions under each category are found in the supplementary file (APPENDICE 3). Health extension worker enrolled primary caregiver of the child to participate in the study after explaining the objective and providing written study information about the study.

3.4.2. Sample Collection

Parallel to questionnaire survey, the enumerator visited each participating household in the morning to collect stool samples from infants, soil from household yard, hand rinse of infant caregiver and fresh animal fecal sample in residential compound. All the samples were collected on the same sampling day. A total of 288 samples were collected where four sample types were taken from each animal rearing household.

Infant stool samples were collected, following prior notification and provision of a disposable diaper the preceding evening. On each sample collection date, approximately 2 gram of stool was transferred into a pre-labeled sterile collection container. Soil samples were collected from three to five mapped sites within the household compound. A sterilized spatula was used to collect the

soil at a 45^0 angle to a depth of 5 cm. For cemented surfaces, sterile dry gauze was used to rub the surface horizontally and vertically from three to five sites. The soil or swab samples were then placed in labeled sterile whirl-pack bags. The caregiver hand rinse sample was collected by slowly inserting the caregiver's hands into a whirl-pack bag containing 225 ml Buffer Peptone Water (BPW) until the broth covered the hand up to the wrist and rubbing the hands gently to release any contamination. Animal fecal samples were collected from the ground of one animal species in a selected household. In case the household owns different animal species, the sample was collected from the one most likely to have contact with infants such as poultry and pet animals. Up to 25 gram of freshly voided fecal material was collected and placed in a pre-labeled sterile whirl-pack bag. Finally, all collected samples were transported within 6 hours to the Enteric-pathogens laboratory found in Addis Ababa University, College of Veterinary Medicine and Agriculture using an ice box containing an ice pack and stored at +4 °C until processing within 24 hours.

3.4.3. Isolation of E. coli spp

Isolation and identification of *E. coli* was performed following the recommended techniques that involve primary enrichment, secondary selective enrichment, selective plating and molecular technique (Feng *et al*., 2002; Lupindu, 2017; Saka *et al*., 2019; Baker *et al.*, 2023). The primary enrichment was done by measuring 2 g infant stool sample into 8 ml BPW (Oxoid, UK) and 25 g of animal feces and soil samples into 225 ml BPW. The bag containing soil samples were homogenized by hands before incubation. All the pre-enriched samples including the hand rinse samples were incubated at 37^0c for 24 hr. Then, from the pre-enrichment culture 0.1 aliquot was transferred into a tube containing 10 ml *E. coli* broth (Oxoid, UK), selective secondary enrichment, and incubated for 24 h at 37^0c. The *E. coli* was then observed for its turbidity to check the bacterial growth (APPENDICE 4, B). A loop full of aliquot from the selective enrichment was streaked on Eosin Methylene Blue (EMB) (Oxoid, UK) agar plates and incubated at 37^0c for 24 h. The plates were screened for the growth of *E. coli,* which appeared dark centered and flat with a metallic sheen (APPENDICE 4, C). A typical one colony from each plate was sub-cultured into Tryptic Soya Broth (Oxoid, UK) and incubated at 37°C for 24 h and then preserved at -20°C as glycerol stocks at 1:2 culture and 80% glycerol ratio for future isolation and analysis.

3.4.4. Matrix-assisted laser desorption and ionization technology (MALDI-TOF)

The Extended Direct Transfer (eDT) procedure of MALDI-TOF was conducted at Animal health institute (AHI) for confirming presumptive *E. coli* colonies based on a protocol suggested by Bruker MALDI Biotyper Manufacturer (Bruker, 2015). A presumptive *E. coli* from the glycerol stock was sub-cultured on nutrient agar and incubated at 37°C for 24 h. A single colony from nutrient agar was picked by stick and smeared as a thin layer on a spot on a MALDI target plate. One µl of 70% formic acid was then added to each spot and allowed to dry at room temperature. After drying, 1µl of α-Cyano-4-hydroxycinnamic acid (HCCA matrix solution) was added and again left to air dry.

3.5.5. Antimicrobial susceptibility testing

Antimicrobial susceptibility of the *E. coli* isolates was determined by using disk diffusion method (Kirby-Bauer) according to Clinical and Laboratory Standard Institute (CLSI Supplement M100, 2023). The following nine antimicrobial drugs were used: amoxicillin (2µg), ampicillin (10µg), amoxicillin-clavulanic acid (30 µg), ceftriaxone (30 µg), meropenem (10 µg) from beta lactamase group; gentamycin (10 µg) and streptomycin (10 µg) from aminoglycosides group; tetracycline (30 µg) from tetracyclines group and ciprofloxacin (5 µg) from fluroquinolone group.

A loopful of the preserved *E. coli* culture was sub-cultured on nutrient agar incubated at 37^0c for 12 h to obtain a pure, fresh culture for antimicrobial susceptibility testing. The test broth was adjusted to McFarland 0.5 turbidity to obtain the right bacterial population. The preparation of Mueller-Hinton agar plates (Oxoid, UK) followed the instructions provided by the manufacturer. A sterile cotton swab was soaked in the inoculum suspension then swabbed evenly on the surface of Muller-Hinton agar plates. Once the plates dried, antibiotic disks were positioned on the inoculated plates using antibiotic disk dispenser. Then incubated at 37°C for 18 hours. After incubation, the diameter of the inhibition zone around each disk was measured using a transparent ruler. The results were categorized as either susceptible, intermediate, or resistant based on standardized table provided by CLSI (APPENDICES 3). Isolate that exhibited resistance at least to one antimicrobial from three antimicrobial classes was considered as MDR *E. coli* (Magiorakos *et al*., 2012). Hence, a total of 33 *E. coli* isolates recovered from households with three or more positive samples were subjected to antibiotic susceptibility testing.

3.5.6. Molecular Confirmation

Molecular characterization of 33 *E. coli* isolates, selected based on their distribution within households (i.e., three or more positive samples per household), was conducted at the AHI, Sebeta. A conventional PCR test was performed using the spin column-based method previously described by Dilhari *et al*. (2017) and standard operating procedure provided by AHI to determine whether *E. coli* colonies contained the virulence gene eae that encodes intimin, a protein on outer membrane important to the establishment of A/E lesions. Due to a lack of primers for other important virulent genes used to determine the different pathotypes of pathogenic *E. coli*, they were not determined. A total of 200 µl cell suspension was added in to 2 ml centrifuge tube, followed by 20 µl proteinase-k and 200 µl buffer AL. After this, the mixture was incubated in 70^0c water bath for 20 minutes and centrifuged at 8000rpm for 15 seconds. Then, 200 µl ethanol added and centrifuged at 8000 rpm (15 sec). Next the sample mix placed on mini-spin column tube on to 2 ml collection tube and centrifuged at 8000 rpm for 1 minute and discarded the filtrate. After discarding, 500 µl buffer AW1 added into the spin column tube and centrifuge at 8000 rpm for 1 minute then the mini-spin column tube placed on new 2ml collection tube. Subsequently, 500 µl buffer AW2 added and centrifuge at 14000 rpm for 3minutes to dry the membrane. Lastly, a mini-spin column tube is placed onto a new 1.5 ml microcentrifuge tube, and 100 µl buffer AE is pipetted directly into the membrane, incubated at room temperature for 5 minutes, and then centrifuged at 8000 rpm for 1 minute to elute the DNA.

Amplification of DNA comprise three sequential steps: denaturation of target nucleic acid by heating to 94^0c for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and 30 seconds, extension at 72°C for 1 min, and final extension at 72°C for 7 min. Visualization of the amplified product was done by mixing the 4 µl gel red with loading dye, 10µl PCR products, and 10µl markers (ladder) onto a 1.5% agarose gel. Electrophoresis was conducted at 125 V for 1:20 hours. A 100-1000 base pair molecular weight marker was used to identify the amplified products as a ladder, which was visualized by UV illumination.

Table 2: Primer sequences and master mix used in conventional PCR for amplification of eae (intimin) gene

Reagents	**Volume**
RNase free water	3 µL
Primer EAE1-forward-5pm/µl (5'-AAACAGGTGAAACTGTTGCC-3')	2 µL
Primer EAE2-reverse-5pm/µl (5'-CTCTGCAGATTAACCTCTGC-3')	2 µL
IQ super mix	10 µL
DNA template	3 µL
Total volume	20 µL

3.5. Data management and analysis

The survey data were collected using REDCap, and the laboratory results were entered to Kobo Toolbox, web-based applications used to manage research data. Both datasets were downloaded and exported to a Microsoft Excel spreadsheet and then cleaned and prepared for analysis. Statistical analyses were done using STATA statistical software, version 14. Descriptive statistics such as frequency and percentages were used to summarize the occurrence of *E. coli* and the antimicrobial susceptibility profiles across the sample sources. Frequency and percentage distributions of responses for each question of the survey questionnaire were computed and presented using tables. Given the small sample size and low detection rates of *E. coli* carrying intimin gene in each sample type, regression analyses were not performed to quantify the degree of association between *E. coli* occurrence and potential predictor variables such as hygienic practices, in order to avoid misinterpretation of the results.

3.6. Ethical clearance

Community health extension worker facilitated the enrolment process, and trained enumerators collected survey data and samples after obtaining written informed consent from the child's primary caregiver. A copy of the consent form was provided to the participant for their permanent record. Respondents were informed that their responses would be kept confidential, and they are free to withdraw from the study at any time. The study was conducted only after obtaining informed written consent from all voluntary participants. If a caregiver declined to participate, the

next eligible household was approached following the same protocol. Ethical clearance for the study was obtained from the scientific and ethical review committees of Addis Ababa University, Aklilu Lemma Institute of Pathobiology (Ref. No: ALIPB IRERC/97/2015/23), and the University of Iowa, USA (IRB ID No: 202302098).

4. RESULTS

4.1. Household survey result

4.1.1. Demography and socio-economic characteristics of surveyed households

Of the infants included in this analysis, 39 (54.17%) were females. The majority (87.5%) of infant's primary caregivers were mothers. Only 31.9% respondents had attended higher education and more than half (73.61%) of them were housewives. The monthly income for the majority (55.56%) of the surveyed households is below 5000 ETB. About 66.7% of the caregivers shared a living yard with multiple unrelated families, 20.8% shared with multiple private family households. Approximately one-third (31.9%) of households had a dirt floor covered with carpet or vinyl, while 68.1% had a cemented floor. Most (80.6%) households had grass or dirt in the compound. The detail socio-demographic characteristics of households are summarized in Table 3.

Table 3: Demographic and socio-economic characteristics of caregivers and their infants

Characteristics	Number (%)
Infant sex	
Male	33 (45.8)
Female	39 (54.2)
Relationship with infant	
Mother	63 (87.5)
Father	4 (5.6)
Aunt	5 (6.9)
Marital status of caregiver	
Married	68 (94.4)
Divorced	2 (2.8)
Single	2 (2.8)
Length of year living in this house	
<1 year	6 (8.3)
1-5 years	44 (61.1)
>5 years	22 (30.6)
Education level	
Primary	25 (34.7)
Secondary	24 (33.3)
Higher	23 (31.9)

Occupation	
Housewife	53 (73.6)
Professional	19 (26.4)
Access to media	
No	7 (9.7)
Yes	65 (90.3)
Monthly Income (ETB)	
<5000	40 (55.6)
5001-10000	21 (29.2)
>10000	11 (15.3)
Type of residence	
Free standing house, private yard	9 (12.5)
Compound shared with multiple unrelated families	48 (66.7)
Compound with multiple private family households	15 (20.8)
Residence ownership	
Own	23 (31.9)
Rent	49 (68.1)
Floor type	
Cement	49 (68.1)
Dirt floor covered with carpet or vinyl	23 (31.9)
Material of ground in compound	
Grass or dirt	58 (80.6)
Cement, brick, tile	14 (19.4)
Compound locked by gate	
No	2 (2.8)
Yes	70 (97.2)
Playground in the compound	
No	1 (1.4)
Yes	71 (98.6)

4.1.2. Access to clean water, Sanitation, and Hygiene

One-third of the households (31.9%) used bottled water while 44 of them (61.1%) collected drinking water from tap water, and 6.9% of them, obtain water from communal pipe water supply point. What concerning is that more than (61.1%) half of households did not use any water treatment option. Among all households, 98.6% had access to a latrine, and 46 (63.9%) of them used shared latrines (Table 4).

Table 4: Access to clean Water, Sanitation, and Hygiene practices of the households.

Characteristics	Number (%)
Source of drinking water	
Tap water	44 (61.1)
Bottled water	23 (31.9)
Communal pipe water	5 (6.9)
Water treatment option	
None	44 (61.1)
Boiling	4 (5.6)
Wuha-agar	1 (1.4)
Bottled water	23 (31.9)
Access to latrine	
No	1 (1.4)
Yes	71 (98.6)
Location of latrine	
In the compound, not shared	20 (27.8)
In the compound, shared	43 (59.7)
Community latrine	2 (2.8)
In the household	7 (9.7)
Latrine for single household	
No	46 (63.9)
Yes	26 (36.1)
Water for hand washing near latrine	
No	59 (81.9)
Yes	13 (18.1)
Soap near latrine	
No	60 (83.3)
Yes	12 (16.7)

4.1.3. Animal ownership and waste management

Dogs (34.7%) and chickens (31.9%) were the common animal species. The majority (45.8%) were kept for protection/companionship purposes and 40.3% of the animals were allowed to roam freely. Most (90.3%) caregivers reported that their infants did not interact with the animal. Thirty-one households (43.1%) of the respondents did not employ any animal feces management practices where the animals' feces found in the compound (Table 5).

Table 5: Animal ownership and waste management

Characteristics	Number (%)
Animal species	
Dog	25 (34.7)
Cat	14 (19.4)
Chicken	23 (31.9)
Cow	3 (4.2)
Sheep	4 (5.6)
Goat	3 (4.2)
Reason for keeping animals	
Protection/Companionship	33 (45.8)
Home consumption	20 (27.8)
Income	19 (26.4)
Animal kept during the day	
Inside the compound, free roaming	29 (40.3)
Inside the compound, in a pen	16 (22.2)
Inside the compound, free roaming and outside the compound	26 (36.1)
Inside the house and inside the compound, free roaming	1 (1.4)
Child play with animals	
No	65 (90.3)
Yes	7 (9.7)
Animal feces management	
Waste bin or rubbish pile in compound	25 (34.7)
Latrine pit	7 (9.7)
Drain or rubbish pile outside the compound	9 (12.5)
Nothing is done	31 (43.1)

4.1.4. Infant care practices and health history

The majority (65.3%) of the infants played on cloth or mats in the households and 91.7% infants were fed by their caregivers. Regarding infant health, 75% of them didn't exhibit disease symptoms in the past week and 83.3% of infants did not receive any medication (Table 6).

Table 6: Infant care practices and health history

Characteristics	Number (%)
Place where infants play	
In the household, on a cloth or rug on the floor	47 (65.3)
In the household, on a plastic or reed mat on the floor	11 (15.3)
In the compound, on the floor	2 (2.8)
In the household, on the floor	6 (8.3)
In the household, in crib or bassinet	6 (8.3)
Feed the baby	
Feed by caregiver	66 (91.7)
Let the child feed by himself	6 (8.3)
Infant travel to domestic duties	
No	46 (63.9)
Yes	26 (36.1)
Symptoms in the past 7 days	
None	54 (75)
Vomiting, diarrhea, fever	9 (12.5)
Fever	9(12.5)
Treatment received	
None	60 (83.3)
Oral rehydration Solution	1 (1.4)
Drugs/antimicrobials	11 (15.3)

4.2. Occurrence of *E. coli* across the One Health domain

Among the total of 288 samples tested, 268 (93. 1%) samples were positive for *E. coli* (Table 7). The highest detection rate (97.2%) was observed in animals, followed by infants (95.8%), soil (91.7%) and infant caregivers (87.5%).

Table 7: Culture based detection rate of *E. coli* across the one health domain (n=288)

Sample source		Number of samples tested	Number of positive (%)
Human	Infant	72	69 (95.8)
	Caregivers	72	63 (87.5)
Environment	Soil	72	66 (91.7)
Animal	Cat	14	14 (100)
	Chicken	22	21 (95.5)
	Cow	3	3 (100)
	Dog	25	24 (96)

	Geese	1	1 (100)
	Goat	3	3 (100)
	Sheep	4	4 (100)
Total		288	268 (93.1)

Of the 268 presumptive *E. coli,* only 152 isolates were subjected to MALDI-TOF and 66 isolates were confirmed as *E. coli.* Relatively, higher proportion of presumptive *E. coli* obtained from animals was confirmed positive (Table 8).

Table 8: Number and percentage of confirmed *E. coli* using MALDI-TOF testing

Sample source		Number of samples tested	Number of positive (%)
Human	Infants	40	15 (37.5)
	Infant caregivers	36	16 (44.4)
Environment	Soil	37	15 (40.5)
Animal	Cat	7	4 (57.1)
	Chicken	11	5 (45.5)
	Cow	3	3 (100)
	Dog	14	5 (35.7)
	Goat	2	1 (50)
	Sheep	2	2 (100)

Figure (4) shows the number and distribution of MALDI-TOF confirmed *E. coli* positive isolates across the sample sources per household. In 55% (22/40) of the households, *E. coli* was detected in two and more than two sample sources per household.

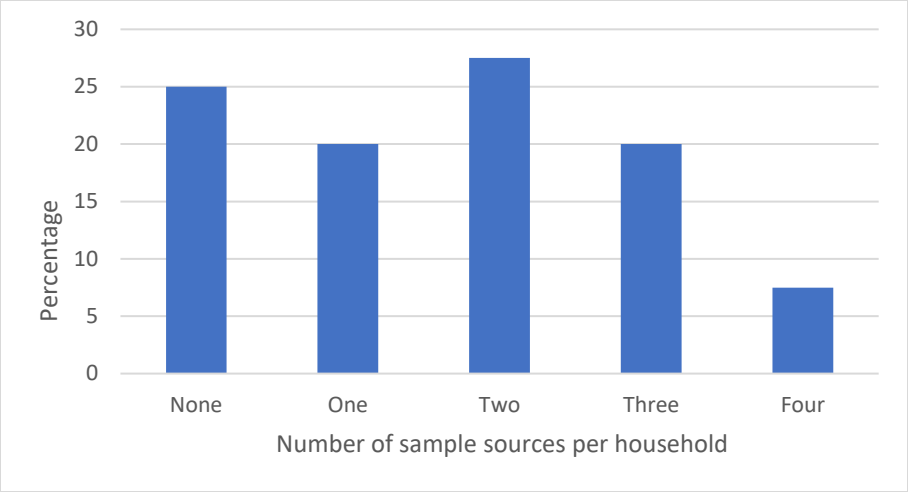

Figure 4: Distribution of MALDI-TOF confirmed *E. coli* across sample sources per household

A total of 33 *E. coli* isolates from infant (8), soil (8), infant caregivers (8), and animals (9) were tested for eae virulent gene and seven of them carried the gene (Table 9, Figures 5 and 6).

Table 9: Percentage of *E. coli* isolates confirmed carrying eae virulent gene

Sample source	**Number of tested samples**	**Number of eae gene positive isolates (%)**
Infant	8	4 (50)
Soil	8	0
Infant caregivers	8	2 (12.5)
Animal	9	2 (22.2)
Total	33	7 (21.2)

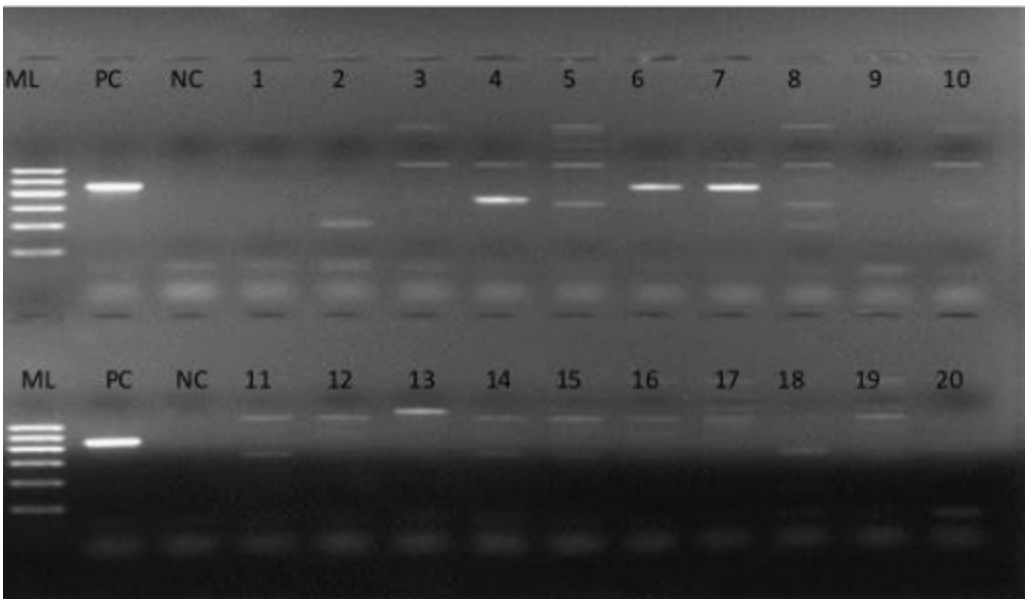

Figure 5: Gel electrophoresis of amplified products of eae genes in *E. coli* samples

Key: Lane ML (DNA ladder), Lane: PC (Positive control), Lane NC (negative control), and Lane 1-20 (PCR products), Lane 4, 6, 7, 13 are positive for eae gene.

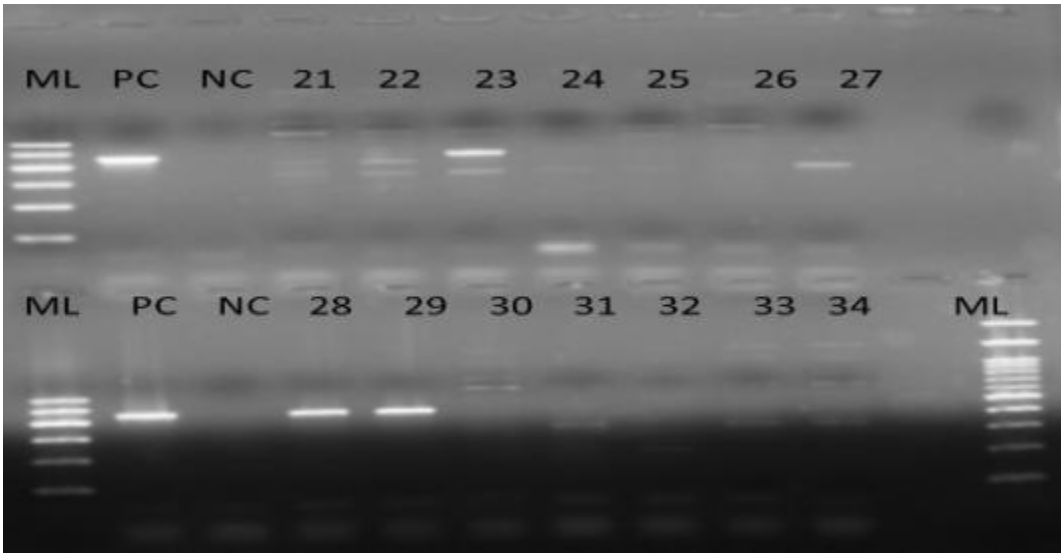

Figure 6: Gel electrophoresis of amplified products of eae genes in *E. coli* samples

Key: Lane ML (DNA ladder), Lane: PC (Positive control), Lane NC (negative control), and Lane 21-33 (PCR products), Lane 23, 28, 29 are positive for eae gene.

4.3. Antimicrobial susceptibility profile of *E. coli*

A total of 33 *E. coli* isolates from infant (8), soil (8), infant caregivers (8), and animals (9) were subjected to an antimicrobial susceptibility test. The overall susceptibility patterns of *E. coli* isolates summarized in Table 10. The maximum resistance was recorded against amoxicillin (100%), followed by tetracycline (78.78%) and amoxicillin-clavulanic acid (72.72%). When evaluated by sample sources, isolates from animals exhibit highest (55.6%) resistance followed by infants (52.8%), infant caregivers (51.4%) and soil (37.5%).

Table 10: Antimicrobial susceptibility pattern of *E. coli* isolates

Antimicrobial tested	Antimicrobial class	Infants			Soil			Caregivers			Animals		
		Number (%)			Number (%)			Number (%)			Number (%)		
		S	I	R	S	I	R	S	I	R	S	I	R
Ceftriaxone	Beta-lactam	6 (75)	1(12.5)	1 (12.5)	7 (87.5)	1 (12.5)	0	6 (75)	2 (25)	0	5 (55.6)	2 (22.2)	2 (22.2)
Meropenem	Beta-lactam	0	4 (50)	4 (50)	1 (12.5)	3 (37.5)	4 (50)	0	4 (50)	4 (50)	0	3 (33.3)	6 (66.7)
AMC	Beta-lactam	1 (12.5)	0	7 (87.5)	1 (12.5)	3 (37.5)	4 (50)	0	2 (25)	6 (75)	0	2 (22.2)	7 (77.8)
Streptomycin	Aminoglycosides	2 (25)	2 (25)	4 (50)	1 (12.5)	5 (62.5)	2 (25)	0	4 (50)	4 (50)	3 (33.3)	3 (33.3)	2 (22.2)
Amoxicillin	Beta-lactam	0	0	8 (100)	0	0	8 (100)	0	0	8 (100)	0	0	9 (100)
Ampicillin	Beta-lactam	1 (12.5)	1 (12.5)	6 (75)	3 (37.5)	1 (12.5)	4 (50)	1 (12.5)	1 (12.5)	6 (75)	1 (11.1)	3 (33.3)	5 (55.6)
Ciprofloxacin	Fluroquinolone	5 (62.5)	1 (12.5)	2 (25)	5 (62.5)	3 (37.5)	0	6 (75)	1 (12.5)	1 (12.5)	6 (66.7)	1 (11.1)	2 (22.2)
Gentamycin	Aminoglycosides	6 (75)	2 (25)	0	7 (87.5)	1 (12.5)	0	8 (100)	0	0	7 (77.8)	2 (22.2)	0
Tetracycline	Tetracycline	2 (25)	0	6 (75)	2 (25)	1 (12.5)	5 (62.5)	0	0	8 (100)	2 (22.2)	0	7 (77.)
Total		23 (31.9)	11 (15.3)	38 (52.8)	27 (37.5)	18 (25)	27 (37.5)	21(29.2)	14 (19.4)	37 (51.4)	24 (33.3)	15 (22.2)	37 (55.6)

S = Susceptible; I= Intermediate; R = Resistant; AMC = Amoxicillin-clavulanic acid.

4.3.1. Multidrug Resistance of E. coli

Among the resistant *E. coli* isolates, 14 (42.4%) isolates were multidrug resistant, resistant to three or more classes of antimicrobials. Of this, 27.3% of the isolates were exhibited resistance to Beta-lactam, Aminoglycosides and, Tetracycline.

Table 11: Pattern of multidrug resistant *E. coli* obtained from various sources

Antimicrobial class	**Sources of MDR isolates**						**Total (%)**
	Infant	Soil	Infant Caregivers	Animal			
				Cat	Chicken	Cow	
Beta-lactam, Aminoglycosides, Tetracycline	2 (6.1)	2 (6.1)	4 (12.1)	1 (3)	0	0	9 (27.3)
Beta-lactam, Fluoroquinolone, Tetracycline	2 (6.1)	0	1 (3)	0	1 (3)	1 (3)	5 (15.2)
Total (%)	4 (12.1)	2(6.1)	5 (15.2)	1 (3)	1 (3)	1 (3)	14 (42.4)

5. DISCUSSION

Animal husbandry within household premises increases human-animal interactions, potentially facilitating the spread of infectious pathogens between animals and humans through contaminated fomites or environmental surfaces (Penakalapati *et al.*, 2017). Children are particularly at high risk due to their tendency to ingest animal feces or come into contact with contaminated fomites, which may carry pathogenic bacteria with significant concentrations causing diseases (Pintar *et al.,* 2015) Understanding the occurrence and resistance profiles of *E. coli* across one health domain helps to identify the possible transmission pathways and contributing factors. The objective of this study was to assess the occurrence of *E. coli*, antimicrobial-resistant strains, and hygienic practices using the One Health framework focusing on infants, animals, and the household environment in household setting.

The study found that most households had access to municipal water and shared sanitation services. However, the majority of households lacked water and soap near toilets for hand washing, indicating a hygiene concern. The findings also revealed a significant detection rate of *E. coli* and antimicrobial-resistant *E. coli* across the One Health domain with significant proportions in infants. The highest detection rate of *E. coli* was observed in animals followed by infant caregivers, soil, and infants, while higher proportion of resistance observed in animals followed by infants, infant caregivers, and soil. The high levels of contamination in the household environment and among infant caregivers with *E. coli* and antimicrobial-resistant *E. coli* suggest that the environment and caregivers specially those who had inadequate hygiene practices may play a mediating role in the transmission of the bacteria from animals to infants. It is important to note that below discussion and comparison of the on the detection rate of *E. coli* was based on the MALDI-TOF results.

Interestingly, as compared to the 68.7% national level (Desye *et a*l., 2023), in this study all the participants had access to clean/improved water services, either directly from the municipal water supply or commercially available bottled water. The observed 100% access to clean or improved water was comparable with the 99% reported from Addis Ababa (Sisay *et al*., 2024) and higher than the 78% access to piped water in Debre Tabor (Geremew Gebremichael *et al*., 2020). The

increased access to piped water coverage in the current study and in other urban areas of the country could be due to the government and NGO efforts toward universal coverage for clean water (Adank *et al*., 2016). Piped water is considered as an improved source/safely managed water because it results in a reduced risk of infection (Winter *et al*., 2021). However, piped water should be complemented with point-of-use water treatment methods to ensure safe drinking water (Sequeira *et al.*, 2019). However, in this study, more than half (61.11%) of the households didn't use any water treatment option. The absence of household-level water treatment has been identified as a risk factor for water borne bacterial diseases. For instance a study showed that households without water treatment had 3.3 times higher odds of having waterborne diseases (Daba *et al*., 2025). Despite the access to improved water, the lack of water treatment in 61.1% of households might harbour pathogens if there is no proper water treatment and regular monitoring of water quality, potentially causing waterborne diseases (Felföldi *et al.*, 2010).

The 36.1% improved sanitation coverage observed in the study area was higher than the national average of 20% and lower than the urban sanitation coverage of 42% (Desye *et al.*, 2023). However, the 63.9% access to unimproved sanitation was higher than the national average of 53%, the urban coverage of 49% (EPHI and ICF, 2021), and the 27.5% access to basic sanitation services (Desye *et al.*, 2023), as well as the 5.2% observed in Shashemene, Oromia Region (Hailu *et al*., 2024). Furthermore, the high proportion (59.7%) of shared sanitation facilities in the study area was lower than the 79.8% access to communal latrine services reported in Addis Ababa (Kefeni and Yallew, 2018). The increasing trend of urban settlement may be a contributing factor to the observed use of shared latrines. Although technically similar to individual latrines, shared facilities are used by multiple households leading to unhygienic use of the latrines linked with the difference in the knowledge on the proper use of latrines and lack of water and soap for handwashing after the latrine use. Epidemiological studies suggest that individuals relying on shared sanitation facilities are at a higher risk of adverse health outcomes, particularly diarrheal diseases (Heijnen *et al*., 2014; Just *et al*., 2018).

The absence of handwashing facilities (water and soap) near latrines in more than 81% of the households was comparable to the 84.2% lack of such facilities reported in Hosanna Town (Aydamo *et al*., 2023). Access to handwashing stations significantly improves hygiene and helps prevent the transmission of enteric pathogens that can cause diarrheal diseases (Khan *et al.*, 2021).

According to the 2016 EDHS, households with soap available near handwashing stations were 5.6 times more likely to have access to basic hygiene services compared to those without. This finding suggests that the presence of a handwashing station equipped with soap encourages proper hand hygiene, particularly after using the latrine (Wolf *et al*., 2019). Therefore, the widespread absence of handwashing facilities observed in the current study could increase exposure to enteric pathogens, especially among households that share latrines, unless strict hygiene practices are consistently followed. Moreover, since most infant caregivers are responsible for feeding the infants, poor hygienic practices particularly inadequate handwashing due to absence of handwashing facilities, especially when feeding infants by hand, can significantly increase the risk of infants contracting enteric infection (Wang *et al*., 2024).

In addition to the lack of handwashing facilities, another concerning hygiene issue is the improper disposal of animal waste. In 43.1% of the households, animal feces were discarded directly into the residential environment without any form of containment. This figure is significantly higher than the 12.5% of households reported to dispose animal feces in the open environment in a previous study (Topp et al., 2009; Alemu and Mezgebu, 2023; Jimenez *et al.*, 2023). Such improper disposal practices increase the risk of environmental contamination with fecal pathogens, which may lead to the exposure of children and other vulnerable individuals to enteric infections (Kaur *et al*., 2017). As evidenced in the current study, some (2.8%) infants often play in outside yard located within household compound where they can be exposed to the pile of fecal materials disposed in the compound. In such situation, infants are at a greater risk of acquiring enteric infections through mouthing animal droppings and soil-contaminated materials with animal feces in residential yards.

The observed high and comparable detection rate of *E. coli* across the One Health domains with predominance in animals and its considerable presence in infants suggests the circulation of the bacterium within the household setting. Moreover, the eae gene was detected in 24.2% of the isolates. Intimin gene-positive isolates are usually linked to potentially fatal infections such hemolytic-uremic syndrome and hemorrhagic colitis (Ramachandran *et al.*, 2003). This circulation potentially facilitates transmission from animals to infants, likely mediated through contaminated soil and caregivers' hands. These findings align with earlier studies (Ercumen *et al.*, 2017; Gizaw *et al.*, 2022), which indicate that animal feces are a frequent source of environmental fecal

contamination due to the close proximity and shared spaces between humans and animals. In this study, *E. coli* was detected in 37.5% of infant stool samples, a proportion similar to those reported by Belete *et al*. (2022) and Natarajan *et al*. (2018). The detection rate in caregivers was proportionally lower than that reported by Kusuma *et al*. (2012). In Ethiopia, mothers and newborns often stay indoors together for several weeks after birth, a practice that may facilitate the transfer of *E. coli* to infants. As shown in previous studies (Ferretti *et al.*, 2018; Abreu-Salinas *et al.*, 2020), daily close contact between mothers and their newborns especially during breastfeeding may increase the infant's exposure to fecal contaminants. Additionally, other studies have documented the presence of *E. coli* in compound soil, further supporting environmental exposure as a transmission route (Ngure *et al.*, 2013; Navab-Daneshmand *et al*., 2018).

Such pattern of occurrence and distribution of the pathogen indicates the possibility of both horizontal zoonotic and reverse zoonotic transmission of enteric pathogens between animals, and humans being mediated by environmental contamination. Other studies conducted in similar settings have reported the occurrence of *E. coli* in humans, animals, and the environment within household environments, highlighting the potential public health risk associated with close human–animal interactions and shared living spaces (Lupindu *et al.,* 2014; Okpara *et al*., 2018; Geuther *et al*., 2023; Beyene *et al.*, 2024). These findings underscore the importance of a One Health approach in understanding the transmission dynamics of enteric pathogens in such settings (Holcomb *et al.*, 2025; Yang *et al*., 2025). However, the current study did not investigate the genetic relatedness of *E. coli* isolates obtained from different sources (infants, animals, soil, and caregivers), which limits the ability to determine the direction or pathways of transmission of pathogenic *E. coli* from animals to humans. Future study, whole-genome sequencing, would elucidate the genetic relatedness among the isolates and determine the epidemiological links to better inform targeted interventions.

Antimicrobial resistance in bacteria has grown to be an urgent public health threat worldwide (Estany-Gestal *et al*., 2024). In the current study, most isolates from different sources in the same households showed a range of drug resistance. The higher *E. coli* resistance to beta-lactam drugs such as amoxicillin (100%), amoxicillin-clavulanic acid (72.7%) and ampicillin (61.3%) was similar to the report in Malawi (Cocker *et al.*, 2023) and in Bangladesh (Flatgard *et al.*, 2024). The observed high levels of resistance might be attributed to the inappropriate use of antibiotics by

both humans and animals, the presence of resistant organisms in the environment, and the high level of interaction at the human-animal-environment interface. The resistance to tetracycline (78.8%). was lower as compared to the 73.7% and 67.3% resistance to tetracycline group reported by Sonola *et al*.(2021) and Hartinger *et al.* (2021), respectively. This result underscores the widespread dissemination of tetracycline resistant *E. coli* within One health domains in the household environment.

In contrary, the study showed that *E. coli* strains were found susceptible to some antimicrobials such as gentamycin that exhibited the highest susceptibility against *E. coli* isolates originated from infants, animals and environment. The finding was nearly in agreement with the study conducted in Ghana (Kichana *et al*., 2022), in Uganda (Muleme *et al*., 2023), and in Thailand (Vannakovida *et al.*, 2021). This may be due to the limited use of gentamicin for treating microbial infections in humans and animals in the study area because of its potential nephrotoxicity and requirement for many daily doses.

The 42.4% of MDR resistant *E. coli* at the one health domain with higher proportions in caregivers (15.2%) and infants (12.1%) implies a significant risk of infant morbidity and mortality linked with *E. coli* infection due to treatment failure. The higher occurrence rates of MDR *E. coli* in the human component could be due to exposure of infants to the resistant *E. coli* due to lack of strict hygienic practices such as handwashing during infant care activities (e.g., changing diaper) and infant food handling and preparation. The presence of MDR *E. coli* isolates in animal feces indicates the irrational use of antimicrobials in animals and this was in agreement with other studies in Tanzania (Sonola *et al.,* 2021)and Peru (Hartinger *et al*., 2021). The 6.1%, detection rate of MDR *E. coli* in soil was close to 10% reported in Tanzania (Sonola *et al.*, 2021). This indicates that household soils are potential reservoirs of MDR *E. coli*, possibly due to poor disposal of animal feces in the household compounds. However, it is crucial to note that this study offers preliminary insight on the magnitude and distribution of AMR *E. coli* in humans, animal and their environment rather than the transmission dynamics.

Limitations

The study has some limitations that needs to be acknowledged. First, due to resource constraints, all presumptive *E. coli* isolates were screened solely for the presence of the *eae* gene, a marker commonly associated with enteropathogenic (*EPEC*) and enterohemorrhagic *E. coli* (*EHEC*) pathotypes. Consequently, the study did not assess the presence of other virulence genes that characterize the full spectrum of diarrheagenic *E. coli* (DEC) pathotypes, such as enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), Entero invasive *E. coli* (EIEC), and others, thereby limiting the understanding of the overall pathogenic diversity within the isolates. Second, the study did not investigate the genetic relatedness or epidemiological connections among *E. coli* isolates recovered from the four household setting sources (infants, domestic animals, caregivers, and soil). This lack of molecular epidemiological data limits the ability to infer transmission dynamics, including the directionality of transmission and the potential mediating role of environmental contamination and caregivers in the dissemination of *E. coli* at the household level. However, this limitation is expected to be addressed during the final phase of the parent project employing whole-genome sequencing, which will provide deeper insights into genomic relatedness, potential transmission events, and the likely interactions across the human-animal-environment interface. Third, antimicrobial resistance profiling in this study was based solely on phenotypic susceptibility testing. The study did not include genotypic characterization of resistance, such as the detection of specific resistance genes or mobile genetic elements, which could offer a more comprehensive understanding of the mechanisms underlying resistance to the tested antimicrobials. Despite these limitations, this study provides valuable insights into the occurrence and antimicrobial resistance of *E. coli* across the one health domain in infants, animals, and the household environment suggesting the need for intervention. The study also highlights the potential role of inadequate access to clean water, sanitation, and hygiene in facilitating transmission between animals and infants.

6. CONCLUSION

This study shows the occurrence and distribution of pathogenic and antimicrobial-resistant *E. coli* across the One Health domains in infants, animals, and the environment within household settings. *E. coli* was most frequently isolated from animals (51.3%), followed by soil (40.5%), infant caregivers (44.4%), and infants (37.5%). At a household level, *E. coli* was detected in two and more than two sample sources in 55% (22/40) per household. A significant proportion of these isolates exhibited antimicrobial resistance (AMR), with the highest resistance observed in animals (55.6%), followed by infants (52.8%), caregivers (51.4%), and soil samples (37.5%). Alarmingly, the study also revealed the presence of multidrug-resistant (MDR) *E. coli* strains across all sampled domains (42.4%), underscoring a serious public health concern. The widespread occurrence of AMR and MDR *E. coli* among various sources suggests a persistent and potentially cyclical exchange of resistant pathogens at the human, animal, environment interface emphasize the interconnectedness of health across animals, humans and environmental reservoirs. In addition to microbiological findings, the study identified several household-level potential exposure factors that may contribute to the transmission and persistence of pathogenic and AMR pathogens. Although most households had access to municipal water, they commonly relied on shared sanitation facilities and often lacked essential hygiene infrastructure, such as soap and water for handwashing near latrines. Furthermore, poor animal waste management practices and the shared use of household environments by animals and humans likely facilitate environmental contamination and cross-species transmission. While the study did not determine the genetic relatedness or transmission dynamics of *E. coli* strains across the sampled sources, the combined evidence points to critical gaps in water, sanitation, and hygiene infrastructure that may enhance the risk of dissemination of pathogenic and drug-resistant bacteria. These findings call for urgent public health interventions aimed at improving household hygiene, strengthening antimicrobial stewardship, and promoting integrated One Health surveillance to mitigate the spread of antimicrobial resistance in similar settings.

Based on the above conclusion, the following recommendations are forwarded:

- There is an urgent need to improve water, sanitation, and hygiene (WASH) infrastructure at the household level. This includes ensuring reliable access to clean water, providing

dedicated handwashing stations equipped with soap near latrines, and improving community-level sanitation to reduce the dependence on shared facilities

- Enhancing household hygiene practices through health education by health extension workers particularly targeting infant caregivers on WASH practices, personal (handwashing) and environmental hygiene. This would help limit the spread of pathogenic and antimicrobial-resistant (AMR) *E. coli* from animals to humans.
- Better management of animal waste is required to minimize the overlap sharing of space between human and animal to reduce environmental contamination and cross-species transmission of enteric pathogens.
- Community based antimicrobial stewardship programs should be introduced to promote the responsible use of antimicrobials in both human healthcare and animal husbandry, including stricter regulations against the non-therapeutic use of antimicrobials in livestock.
- To determine the molecular epidemiology of pathogenic and AMR *E. coli*, further characterization of the isolates using whole genome sequencing is recommended. This approach will enable the identification of specific virulence and resistance genes, as well as the assessment of genetic relatedness among strains from different sources

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8. APPENDICES

APPENDICE 1: Principles and procedures of media preparation, isolation and identification tests

1. Pre-Enrichment and Transportation Media

1.1. Buffered Peptone Water (BPW) (Oxoid, Thermo Scientific, UK)

Preparation

Add 20 gram to 1 litre of distilled water

Mix well and gently heat

Distribute into final containers

Sterilize by autoclaving at 121^0c for 15 minutes.

1.2. Tryptone Soya broth (TSB) (Oxoid, Thermo Scientific, UK)

Preparation

Add 30 gram to 1 litre of distilled water

Mix well and distribute into the final container

Sterilize by autoclaving at 121^0c for 15 minutes

2. Media preparation for Isolation of *Escherichia coli*

A. *E. coli* broth (Oxoid, UK)-for selective enrichment
Dissolve 37 grams in 1 litre distilled water.

Gently heat to completely dissolve the medium.

Dispense into final tubes

Sterilize by autoclaving at 121^0c for 15 minutes.

B. Eosin methylene blue agar (Oxoid, UK)-for selective plating

Suspend 37.5 gram in 1 litre of distilled water

Gently heat to boiling with frequent stirring until the medium is completely dissolved.

Sterilize by autoclaving at 121^0c for 15 minutes.

Cool to 60^0c and shake the medium to oxidize the methylene blue and to suspend the precipitate which is an essential part of the medium

.

C. **Nutrient Agar Preparation (Oxoid, Basingstoke, England)**

Suspended 28 grams in 1000ml distilled water.

Heated to boil to dissolve the medium completely.

Sterilized by autoclaving at 121℃ for 15 minutes.

Mixed well and poured in to sterile Petri dishes.

D. Muller Hinton agar preparations for antimicrobial susceptibility test

38 gm of the medium was suspended in one litter of distilled water.

Mix well and gently heat to boiling with frequent stirring until the medium is completely dissolved.

Autoclaved at 121°C for 15 minutes then cooled to room temperature.

Pour into sterile Petri dishes under aseptic conditions.

Store the plates at 2-8 ºC.

APPENDICE 2: Antibiotic discs used during the antimicrobial susceptibility profile of E. coli with their respective concentrations and its interpretation.

Antimicrobial Discs	**Potency**	**Interpretation Criteria**		
		Susceptible	**Intermediate**	**Resistance**
Ampicillin	10 µg	≥17mm	14-16mm	≤13mm
Amoxicillin	2 µg	≥17mm	14-16mm	≤13mm
Ciprofloxacin	5 µg	≥26mm	22-25mm	≤21mm
Tetracycline	30 µg	≥15mm	12-14mm	≤11mm
Meropenem	10 µg	≥ 23 mm	20-22 mm	≤ 19 mm
Ceftriaxone	30 µg	≥23 mm	20-22 mm	≤19 mm
Gentamicin	10 µg	≥15mm	13-14mm	≤12mm
Streptomycin	10 µg	≥15mm	12-14mm	≤11mm
Amoxicillin-clavulanate	30 µg	≥18mm	14-17mm	≤13mm

Source: (CLSI Supplement M100, 2023)

APPENDICE 3: Questionnaire

Enrollment and Questionnaire Survey

Enumerator ID: _____

Household ID: _____

Date of survey: DD/MM/YYYY

Time of survey: HH:MM

Part I: Infants' demography and **Socio-demographic characteristics of caregivers**

(*Check all details against baseline questionnaire entry before starting*)

Before I begin the interview, could you please bring the infant's Birth Certificate, and Health Card? We will need to refer to those documents. I am going to ask you questions on different topics and afterwards I will ask for a stool sample from the infant and will provide you with instructions on how to collect this. The whole process should not take more than 45 minutes of your time.

1. In what month and year was the infant born? (*Verify with infant's health card, if possible):* MM/YYYY_____
2. Sex of infant
    a. Male
    b. Female
3. What is your relationship to the infant?
    a. Mother
    b. Father
    c. Grandmother
    d. Grandfather
    e. Other guardian (record any further detail/explanation) _____
4. What is your marital status?
    a. Single
    b. Married or in partnership.
    c. Divorced/widowed.
5. Age of the caregiver (years) _____
6. How many adults live in the household? _____

7. How many children between five and 18 years live in the household? _____
8. How many children under five live in the household? _____
9. How long have you been living continuously in this house? _____ Months
10. What is the highest level of school you attended?
    a. None
    b. Primary
    c. Secondary
    d. Higher
    e. Prefer not to answer.
    f. Don't know.
11. What is your occupation?
    a. Professional
    b. Secretarial
    c. Janitorial
    d. Childcare
    e. Market vendor
    f. Other
12. Access to media (TV or radio)
    a. yes
    b. No
13. Household average monthly income (ETB)_____
14. What type of residence is this?
    a. Apartment or room in multi-unit building, no yard
    b. Free standing house, no compound yard
    c. Free standing house, private yard
    d. Free standing house, gated community
    e. Compound with multiple private family households
    f. Compound shared with multiple unrelated families.
15. Does your family own or rent this residence?
    a. Own
    b. Rent

c. Live here for free

16. How many rooms does the house have? _____

17. What is the main material of the floor in the residence? (observe)

a. Uncovered dirt

b. Dirt floor covered with carpet or vinyl.

c. Wood/parquet, marble/granite, cement, mosaic/tile

d. Other: _____

18. What is the main material of the ground in the compound?

e. Grass or dirt

f. Cement, brick, or tile

g. Other: _____

19. Is the compound enclosed by a lockable gate?

a. Yes

b. No

20. Does the compound ever flood?

a. Yes, from rain on the ground inside the home.

b. Yes, from community floodwater.

c. Yes, from wastewater drains.

d. No

21. Is there playground for children in the compound?

a. Yes

b. No

Part III. Access to clean Water, Sanitation and Hygiene facilities

22. Where does your household normally collect water to drink?

a. Tap inside the house.

b. Tap in the compound.

c. Public tap or fountain

d. Protected spring

e. Unprotected spring

f. Collected rainwater.

g. Borehole

h. Bottled or packed water

i. Other: _____

23. How long does it take to go there, get water, and come back?

a. Water source in the yard

b. Less than 30 minutes

c. Greater than 30 minutes

24. Household drinking water treatment options

a. Chlorine

b. Wuha Agar

c. Boiling

d. No usage of treatment

25. Do you have access to a toilet or latrine?

a. No, rely on open defecation/bucket/bag

a. Yes

26. Where is the toilet or latrine? (observe)

a. In the household

b. In the compound, not shared.

c. In the compound, shared with other households.

d. Community latrine

e. Public latrine

27. What type of latrine or sanitation system is it?

b. Flush/pour flush toilet to piped sewer system.

c. Flush/pour flush toilet to underground pit/tank.

d. Flush/pour flush toilet to onsite, above ground, open pit.

e. Pit latrine with concrete slab (not pour flush)

f. Pit latrine without slab (not pour flush)

g. Bucket

h. Bag

i. Other: _____

28. Is the latrine serves single household?

a. Yes

b. No

29. Is there water for hand washing near the latrine?

a. Yes

b. No

30. Is there soap or other detergent for hand washing near the latrine?

a. Yes

b. No

Part IV. Animal ownership and waste management

31. Does your household own any animals?

a. Yes

b. No

32. How many animals of different species do you have?

Animal species	Number
Chicken	❑
Duck	❑
Pig	❑
Cattle	❑
Goat	❑
Sheep	❑
Equines	❑
Dog	❑
Cat	❑
Other(specify) ___	❑

33. What is the main reason of keeping those animals?

Animal species	Income generation	Home consumption (milk, meat etc)	Draught (plowing, Transportation)	Companionship/protection
Chicken	❑	❑	❑	❑
Duck	❑	❑	❑	❑
Pig	❑	❑	❑	❑
Cattle	❑	❑	❑	❑
Goat	❑	❑	❑	❑
Sheep	❑	❑	❑	❑
Equines	❑	❑	❑	❑
Dog	❑	❑	❑	❑
Cat	❑	❑	❑	❑
Other	❑	❑	❑	❑

34. Where are the animals kept during the day? → Mark all that apply

	Inside the house	Inside the compound, free roaming	Inside the compound, in a pen	Outside the compound	Other (specify)
Chicken	❑	❑	❑	❑	_____
Duck	❑	❑	❑	❑	_____
Pig	❑	❑	❑	❑	_____
Cattle	❑	❑	❑	❑	_____
Goat	❑	❑	❑	❑	_____
Sheep	❑	❑	❑	❑	_____
Equines	❑	❑	❑	❑	_____
Dog	❑	❑	❑	❑	_____
Cat	❑	❑	❑	❑	_____
Other___	❑	❑	❑	❑	_____

35. What do you do with animal feces/where does it go (check all that apply)?

a. Nothing is done with animal feces.
b. Latrine pit
c. Waste bin or rubbish pile in compound
d. Used for crop fertilizer.
e. Drain or rubbish pile outside the compound.

36. Presence of animal feces in the compound(observe)

a. Yes
b. No
c. Animal species_____

37. Does the child ever touch or play with any of the household animals?

a. Yes
b. No

Part V. Infant care practices and health history

I am now going to ask you some questions about childcare practices in your household.

38. Is the infant currently breastfed?

a. Yes
b. No
c. Don't know

39. Is the infant exclusively breastfed?

a. Yes
b. No
c. Don't know.
a. Unprotected spring
b. Collected rainwater.
c. Borehole
d. Bottled or packed water
e. Other: _____

40. Who takes care of the infant when you are away? *Select all that apply.*

a. Father
b. Mother-in-law, grandmother, aunt
c. Youth < 18 years
d. Hired help.
e. Neighbor or friend
f. No other caregivers

41. Do you or other caregivers ever take the infant to run errands/travel for your domestic duties?
    a. Yes
    b. No
42. Can you show me where you or other caregivers put the infant down to play or sleep? *Select all that you observe.*
    a. In the household, in crib or bassinet
    b. In the household, on a cloth or rug on the floor
    c. In the household, on a plastic or reed mat on the floor
    d. In the household, on the floor
    e. In the compound, in crib or bassinet
    f. In the compound, on a cloth or rug on the floor
    g. In the compound, on a plastic or reed mat on the floor
    h. In the compound, on the floor
43. Where do you dispose of child feces?
    a. On the ground in the compound
    b. On the ground, drain, or waste dump outside the household
    c. In diapers, bags, or a pot
    d. In the household's latrine
    e. Other _____
44. How do you feed the child?
    a. Let the child feed from utensil.
    b. I feed by myself.
45. How do you wash utensils used for feeding infant?
    a. Boiled water
    b. Non boiled water
    c. Boiled water and soap
    d. Non boiled water and soap
46. How often does the infant defecate?
    a. Every day
    b. Most days
    c. Few times a week

47. Has the infant had any of the following symptoms in the past 7 days? *Check all that apply*.

a. Diarrhea (three or more loose stools per day at any point).
b. Fatigue
c. Vomiting
d. Unusual lack of appetite or willingness to take liquids.
e. Fever
f. Runny nose
g. Cough
h. Rash
i. Others (Specify)_____

48. Has anyone else in the household had the same symptoms in the past month?

a. Yes
b. No

49. Did you seek any health advice or treatment for the infant's symptoms?

a. None
b. Public hospital/clinic
c. Public health post /Community Health extension
d. Private hospital/clinic
e. Private pharmacy
f. Relative/friend
g. Others

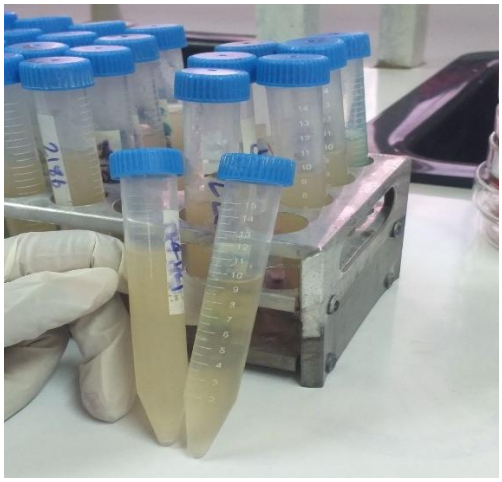
50. During the time the infant had these symptoms, did he/she receive?

a. Hospitalization
b. Fluid made with powdered Oral Rehydration Solution (*name brands*)
c. Pre-packaged liquid Oral Rehydration Solution (*name brands*)
d. Homemade Oral Rehydration Solution
e. Zinc. Yes → Tablet, syrup?
f. Drugs/antimicrobials. Yes → Tablet, syrup, injection?
g. Antimotility (*explain*). Yes → Tablet, syrup?
h. Intravenous (*explain*)
i. Local/herbal medicine. Yes → Name?

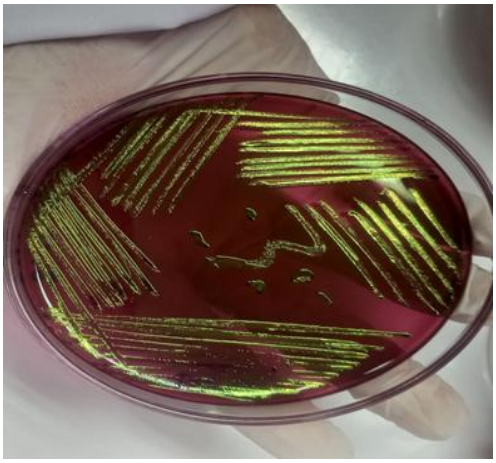
APPENDICE 4: Pictures during data collection, and test results

A, 

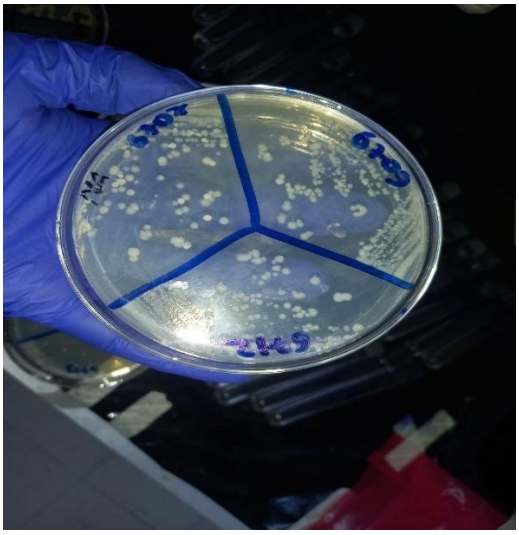
Interviewing an infant caregiver

B. 

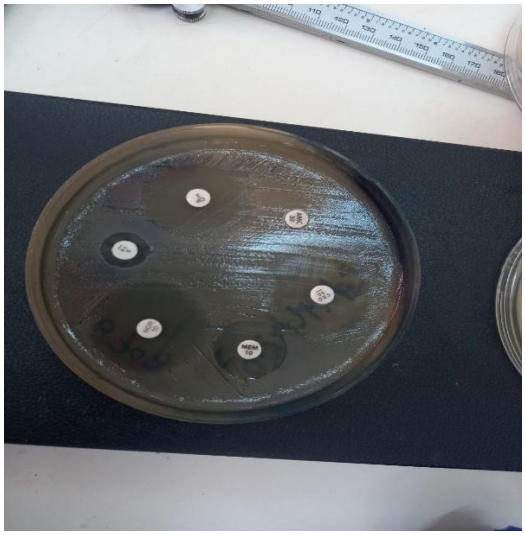
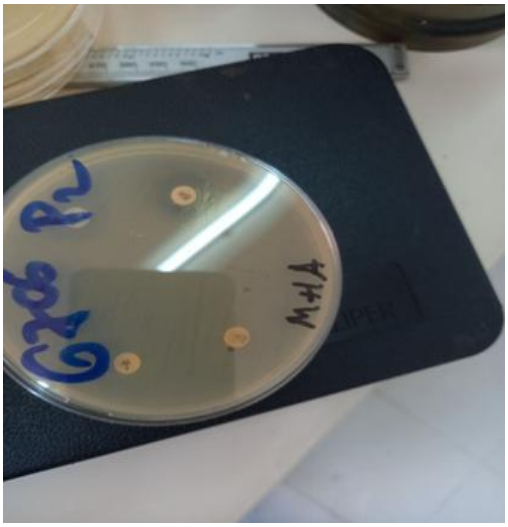
Positive E. coli in E. coli broth

C. 

E. coli metallic green sheen on EMB agar

D. 

E. coli on nutrient agar

E. 


Antimicrobial Susceptibility test result