**ADDIS ABABA UNIVERSITY**
**COLLEGE OF HEALTH SCIENCES**
**SCHOOL OF BIOMEDICINE AND LABORATORY SCIENCES**
**DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY, AND PARASITOLOGY**

# MOLECULAR FEATURES, EPIDEMIOLOGY AND ANTIMICROBIAL RESISTANCE PROFILE OF SELECTED FOODBORNE BACTERIAL PATHOGENS AMONG DIARRHEIC PATIENTS IN THREE SELECTED CITIES IN ETHIOPIA

**BY**

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**A DISSERTATION SUBMITTED TO DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY, SCHOOL OF BIOMEDICINE AND LABORATORY SCIENCES, COLLEGE OF HEALTH SCIENCES, ADDIS ABABA UNIVERSITY IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN MEDICAL MICROBIOLOGY.**

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**Addis Ababa University**
**College of Health Sciences**
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# Molecular features, epidemiology, and antimicrobial resistance profile of selected foodborne bacterial pathogens among diarrheic patients in three cities in Ethiopia

By
Amete Mihret Teshale

A thesis submitted to the Department of Microbiology, Immunology and Parasitology; College of Health Sciences; Addis Ababa University in the fulfilment of the requirements for the Degree of Doctor of Philosophy in Medical Microbiology

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**September 19, 2025**

## DISSERTATION DECLARATION

I, Amete Mihret Teshale, hereby declare that this PhD dissertation title: "Molecular features, Epidemiology and Antimicrobial Resistance profile of Selected Foodborne Bacterial Pathogens among Diarrheic Patients in Three Selected Cities in Ethiopia" is my original work and has not been submitted previously in any university or institution for the award of any academic degree, diploma, or certificate. All sources and materials used in this thesis have been appropriately acknowledged and cited.

This thesis is submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy (PhD) in the Department of Microbiology, Immunology and Parasitology, School of Biomedicine and Laboratory Sciences, College of Health Sciences, Addis Ababa University.

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***Declaration from Supervisors***:

I hereby declare that I have advised this PhD work, the dissertation has been prepared under my supervision and has been submitted with my approval.

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## LIST OF ABBREVIATIONS

**AMR:** Antimicrobial Resistance

**ATCC:** American Type Culture Collection

**CDC**: Centre for Disease Control and Prevention

**CLSI:** Clinical and Laboratory Standards Institute

**DALYs:** Disability-adjusted life year

**DNA**: Deoxyribonucleic acid

**ESBL:** Extended spectrum beta-lactamase

**FAO:** Food and Agriculture Organization

**FBD:** Foodborne disease

**FBP:** Foodborne pathogens

**FERG:** Foodborne Disease Epidemiology Reference Group

**HUS**: Hemolytic uremic syndrome

**ISO/TS**: International Organization of Standardization/ Technical Standard

**LMICs**: Low and middle-income countries

**MDR**: Multi-drug resistance

**MIC**: Minimum Inhibitory Concentration

**NTS**: Non-typhoidal *Salmonella*

**PCR**: Polymerase Chain Reaction

**QC**: Quality Control

**REDCap**: Research Electronic Data Capture

**SOP**: Standard Operating Procedures

**STEC**: Shiga-toxin producing *Escherichia coli*

**TSA**: Trypticase Soya Agar

**TSB**: Trypticase Soya Broth

**UNICEF**: United Nations International Children's Emergency Fund

**WASH**: Water, sanitation, and hygiene

**WHO**: World Health Organization

**XDR**: Extensively drug-resistant

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# DEDICATION

This thesis is lovingly and wholeheartedly dedicated to two of the most extraordinary blessings in my life-my beloved mother, Emahoy Tibeyen Adane Reta (Enanaye) and my devoted husband, Mr. Hiwotie Simachew Terefe (Maye).

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# ABSTRACT

**Background:** Foodborne disease is a devastating global health threat, as it may pose risks of serious and life-threatening diseases along with chronic complications. It disproportionately affects low- and middle-income countries like Ethiopia. However, there is fragmented evidence in Ethiopia on specific (mostly single pathogen), restricted age groups (mainly children under five years), limited geographic location, and season of the year**.**

**Objective:** This study aimed to determine the molecular features, epidemiology and antimicrobial resistance profile of selected foodborne bacterial pathogens (FBPs) among diarrheal patients in three selected cities in Ethiopia.

**Method:** A cross-sectional study was conducted from October 2021 to November 2022 in three hospitals in Ethiopia (Addis Ababa, Gondar, and Harar). Information on sociodemographic characteristics and clinical signs and symptoms was collected from diarrheic patients submitting stool samples for the physician requested stool analysis (microscopic stool examination, culture and antibiotic susceptibility testing) to the hospitals' clinical laboratories using a structured questionnaire. Stool samples were tested for Shiga-toxin producing *Escherichia coli* (STEC), non-typhoidal *Salmonella* (NTS), *Shigella* spp., and *Campylobacter* spp. using standardized conventional culture and enzyme immunoassay technique. The stool samples were inoculated on to Selenite-F-broth, and sub-cultured on to xylose deoxycholate agar (XLD), and MacConkey agar (MAC), followed by the biochemical tests (i.e., Triple sugar iron agar, Lysine iron agar, Simmon citrate agar, Urease agar, and Sulfide indole motility agar) for the isolation and identification of *Salmonella* and *Shigella*. The ChromSTEC agar was used for the isolation of STEC characterized by the mauve colony due to tellurite resistance. *Campylobacter* species was detected using an enzyme immunoassay technique where known enzyme labeled antibody was used for the detection of *Campylobacter* antigens in the stool samples. The antibiotic susceptibility profile of the isolates was determined using the Phoenix (M50) method, using the broth microdilution technique where minimum inhibitory concentration of each of the tested antibiotics and their respective interpretation was provided according to the inbuilt Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antibiotic Susceptibility Testing

(EUCAST) guidelines. Molecular characterization of virulence (*stx1*, *stx2*, *eae*, *invA*, *STM4200*, *SEN1392*, *ipaH*, *lacY*, *16S rRNA*, *hipO*, *glyA*), and the antibiotic-resistance (bla-TEM, bla-SHV, bla-CTX-M, bla-OXA-48-like, bla-VIM, bla-KPC, and bla-NDM*)* genes were conducted using the polymerase chain reaction. All statistical analyses were performed using RStudio (version 4.4.3). Univariate analysis (descriptive statistics and graphics) was performed to summarize the distribution of variables. Age distribution was explored using box plots, and summarized as means with standard deviations, and median with interquartile ranges. Categorical variables (socio-demographic, clinical, and environmental factors) were summarized as frequencies and proportions. The prevalence of targeted pathogens was estimated by dividing the total number of positive results for the targeted pathogen by the total sample processed overall, by study sites, and by seasons. Univariable logistic regression was conducted between predictor and the outcome variables, for the crude odds ratios with 95% confidence intervals were calculated. Multivariable logistic regression analyses were used to identify associated factors associated with occurrence of pathogens by controlling the potential confounders, and adjusted odds ratio with 95% confidence intervals were calculated. Variables with $p < 0.20$ in univariable analysis were included, and adjusted odds ratios (AOR) with 95% confidence intervals (CI) were calculated to quantify effect sizes. Multicollinearity among explanatory variables was assessed using the Variance Inflation Factor (VIF) with values < 10 considered acceptable. Variables exhibiting high collinearity would have been considered for exclusion or combination to ensure model stability.

**Results:** A total of 2,331 patients were enrolled in the study, with a median age of 28 years (range: 2 months to 94 years). The majority of the study participants were within the age group of 20-29 years (23.51%) and the lowest was in the age group of 15-19 years (4.59%). All participants from Addis Ababa were urban residents while about a quarter of participants from Harar and Gondar reported living in rural areas. Most participants in all study sites were males (54.78%). The overall prevalence of NTS, STEC, *Shigella* spp. and *Campylobacter* spp. were 1.29% (95%CI:0.91,1.84), 12.56% (95%CI:11.29,13.98), 2.79% (95%CI:2.19,3.54), and 4.46% (95%CI:4.61,8.00), respectively. Moreover, the prevalence of *eae* harboring STEC was found to be 3.43% (95%CI:2.77,4.25). Harar had the highest prevalence of all the pathogens compared to Addis Ababa and Gondar, for STEC ($p$<0.001), NTS ($p$<0.001), *Shigella* spp.

(*p*=0.030), and *Campylobacter* spp. (*p*=0.004). Multidrug resistance was detected in 83.33% (25/30) of NTS, 71.64%of *Shigella* spp., and 48.12% of STEC isolates. Moreover, 2.5% of STEC isolates were extensively drug-resistant, all from Harar and children under 15 years. Virulence profiling of STEC showed *stx1* in 66.89% of isolates, and the *eae* gene in 27.30%. Among ESBL-positive STEC (n=110), bla-CTX-M predominated (97.12% overall; 100% in Gondar, 98.31% in Harar, and 86.67% in Addis Ababa), followed by bla-TEM (75.00%) and bla-SHV (15.38%, highest in Addis Ababa at 26.67%). Moreover, among the carbapenem resistant STEC isolates, 92.59% and 7.41% were found positive for bla-NDM and bla-OXA-48-like genes respectively. In multivariable logistic regression analysis, foodborne pathogen infections were significantly associated with study site, season, age, water source, sanitation, and household animal ownership. All predictors included in the multivariable models had acceptable Variance Inflation Factor (VIF) values (<10), indicating no problematic collinearity. Modifiable factors with the largest effect sizes included unimproved water sources (*Shigella*: AOR:1.95, 95%CI: 1.27,2.74, $p$ = 0.003; *eae*-harboring STEC: AOR:1.06, 95%CI: 1.01,1.10, $p$ = 0.004), unimproved sanitation (*Shigella*: AOR:1.39, 95%CI: 1.27,1.74, $p$ = 0.030; *eae*-harboring STEC: AOR:1.08, 95%CI: 1.01,1.16, $p$ = 0.031), and household animal ownership (NTS: cattle AOR:1.38, 95%CI: 1.18,1.46, $p$ = 0.01; sheep AOR:1.08, 95%CI: 1.02,1.14, $p$ < 0.001). Non-modifiable factors, including age, season, and study site, identified high-risk groups and periods for targeted public health action.

**Conclusion:** The findings of this study reveal alarming levels of virulent and drug-resistant FBPs, particularly in Harar, posing urgent public health threats. Targeted interventions addressing seasonal, environmental, and antimicrobial resistance patterns are critical to control the spread and impact of these infections.

# CHAPTER ONE: INTRODUCTION

## 1.1. Background

Foodborne disease (FBD) arises when individuals consume food that contains harmful substances or organisms. These health risks in food, known as foodborne hazards, are, categorized by experts into three groups: biological (such as bacteria, viruses, and their toxins), chemical (including both naturally occurring and synthetic substances), and physical (Grace et al., 2018). Both high income and low-income countries experience a substantial burden of FBDs, with a rising incidence reported globally. In Africa, the World Health Organization (WHO), through its Foodborne Disease Epidemiology Reference Group (FERG), has produced detailed evaluations highlighting the significant public health impact of these diseases. Findings from Havelaar et al. (2015) indicated that the burden of FBD in the Africa region exceeds that of other major public health threats like HIV/AIDS, malaria, and tuberculosis.

The overwhelming burden of FBD, of which about 98% affects low-income countries, is due to biological hazards, where the latter are responsible for nearly all the cases. In countries within the WHO African Region, which includes Ethiopia, the greatest share of FBD is attributed to agents that cause diarrheal illnesses, among which bacterial infections contribute substantially (Havelaar et al., 2015). Moreover, the WHO (2024) estimates that unsafe food causes 600 million cases of FBD and 420,000 deaths annually, with 30% of these deaths occurring among children under five years old (WHO, 2024). This burden is disproportionately higher in low- and middle-income countries (LMICs), where factors such as unsafe water, poor hygiene, and inadequate food safety measures exacerbate the prevalence of these diseases (WHO, 2015).

The severity of FBD, and frequency of foodborne outbreaks, driven by globalization and the rise of international food trade, highlights the critical importance of coordinated international efforts to ensure food safety. A significant example is the 2011 *E. coli* O104 outbreak in Germany. This strain represented a hybrid pathotype, combining virulence factors from different diarrheagenic *E. coli* groups. Specifically, it possessed enteroaggregative *E. coli* (EAEC) features, including the *aggR* regulator and fimbrial adhesins that enable aggregative adherence and biofilm formation, while simultaneously carrying the Shiga toxin 2 (*stx2*) gene, a hallmark of Shiga toxin–

producing *E. coli* (STEC) (Mellmann et al., 2011; Frank et al., 2011). The coexistence of these traits enhanced both colonization capacity and toxin-mediated pathogenicity, resulting in unusually severe clinical outcomes, including a high incidence of hemolytic uremic syndrome (HUS) (Bielaszewska et al., 2011). The outbreak, traced to contaminated fenugreek sprouts, caused over 3,800 infections and 54 deaths, underscoring the potential public health threat of hybrid *E. coli* pathotypes (Robert Koch Institute, 2011; Scheutz et al., 2011). Although these countries have food safety regulations and control measures in place, the effectiveness of these measures often varies based on their economic status (Lee & Yoon, 2021).

A notable FBD symptom, i.e., diarrheal diseases, often resulting from foodborne pathogens (FBPs), accounts for a significant portion of this burden, causing 550 million illnesses and 230,000 deaths each year (WHO, 2022). The economic impact is also substantial, with LMICs losing over US$100 million annually due to FBDs. The FBDs not only hinder socioeconomic development but also strain healthcare systems and negatively impact national economies, tourism, and trade (WHO, 2022). These statistics underscore the critical need for improved food safety practices and infrastructure in LMICs to reduce the health and economic burdens associated with foodborne pathogens (Grace, 2023).

Diarrhea remains a critical global public health issue, responsible for over 1.6 million deaths annually and ranking among the top ten contributors to global disability-adjusted life years (DALYs) (Troeger et al., 2018). While diarrhea affects all regions and populations, it disproportionately impacts young children and older adults, with death rates among individuals over 70 being three times higher than those for children under five (Srivastava et al., 2022). In Ethiopia, there is insufficient evidence on diarrheal disease incidence and mortality trends at both national and regional levels, despite the existence of contributing factors such as limited access to healthcare, sanitation, and safe water (Gessese et al., 2023).

Bacterial pathogens such as *Salmonella* spp. (i.e, nontyphoidal *Salmonella*), *Shigella* spp., *Campylobacter* spp., and *E. coli* (i.e, Shiga toxin-producing *E. coli*, STEC) are common causes of diarrhea globally, following viral infections (Akhondi et al., 2025). For example, bacterial agents were responsible for 33.7% reported outbreaks in 26 European Union member states in 2015, with *Salmonella* spp. and *Campylobacter* spp.

accounting for 21.8% and 8.9% of cases, respectively (Bintsis, 2017). In sub-Saharan Africa, *Salmonella* spp. can also cause diarrhea and invasive post-diarrheal infections, with a fatality rate of 20-25% (Dougan et al., 2012). Globally, STEC is linked to sporadic infections and outbreaks, leading to HUS in 5-15% of cases (Bruyand et al., 2018; Dougan et al., 2012). Additionally, *Shigella* spp., known for causing aggressive diarrhea, remains a significant contributor to diarrheal mortality in South Asia and sub-Saharan Africa, despite improvements in access to clean water and sanitation (Muzembo et al., 2023). In Ethiopia, a subset of *Salmonella*, known as nontyphoidal *Salmonella* (NTS), and diarrheagenic *Escherichia coli* (DEC), including STEC, have been reported among the most frequently detected bacterial pathogens in children with diarrhea, with a prevalence of around 22.2% (Belina et al., 2024).

These pathogens pose a significant public health threat due to their severe health impacts and diverse transmission pathways. For example, STEC infections, commonly linked to contaminated meat, dairy products, and vegetables, can result in serious complications like HUS, especially in high-risk groups such as children and the elderly (Mussio et al., 2023). Similarly, *Shigella* spp., a leading cause of bacillary dysentery, spreads through contaminated food, water, or direct contact and poses a serious risk in areas with poor sanitation (Gould et al., 2013). In developed regions, centralized food production systems can magnify outbreaks, affecting large populations through widespread distribution networks (Thomas & Daurte-Gardea, 2017). *Campylobacter* spp., particularly *Campylobacter jejuni* (*C. jejuni),* and *Campylobacter coli* (*C. coli)* are major global causes of gastroenteritis, with *C. jejuni* alone responsible for over 90% of cases; severe infections from this specific species can progress to Guillain-Barré syndrome (GBS), a life-threatening neurological condition (Rahman et al., 2021). In developing countries such as Ethiopia, *Campylobacter* spp. has been identified as being among the primary bacterial diarrheal agents in children under five, with prevalence rates between 12.7% to 15.4% (Lengerh et al., 2013; Mulatu et al., 2014). Moreover, while recent studies have reported STEC prevalence to vary widely ranging from 1.6% to 15.3% (Getaneh et al., 2021; Zelelie et al., 2023), the prevalence of *Salmonella* spp. and *Shigella* spp. has also been recorded as 2.5% and 7.0%, respectively (Mulatu et al., 2014).

These FBPs often originate from animal and environmental reservoirs, for which reason factors such as close human-animal interactions, consumption of raw animal products, and limited food safety awareness in Ethiopia exacerbate their public health impact (Tamene et al., 2022). Moreover, environmental factors such as temperature, precipitation, and humidity significantly influence the survival, reproduction, and spread of these pathogens. For instance, climate change, characterized by rising temperatures, fluctuating precipitation patterns, and increased water scarcity, is expected to enhance pathogen resilience and transmission, affecting over half of all infectious diseases globally (Mora et al., 2022). These environmental shifts not only impact endemic diseases but also foster conditions for the emergence of new pathogens and the resurgence of previously suppressed ones (Dietrich et al., 2023). Hence, studying animal contact and environmental exposures including water sources and sanitation facilities is critical for understanding the epidemiology of FBPs in Ethiopia. Such insights are essential to guide evidence-based interventions that integrate the interconnected roles of humans, animals, and the environment in shaping FBD risk. Specifically, climate factors such as ambient temperature, rainfall, and humidity have been linked to the increased incidence of several FBDs, although they varied significantly among different geographic areas (Park et al., 2018). For instance, a 5°C rise in temperature correlated with a 59.4% increase in *Salmonella* infections, and a 10 mm rainfall increase leads to a 14.6% rise in *Salmonella* (Stephen & Barnett, 2016). Precipitation can raise pathogen levels in rainwater tanks and surface waters (Ahmed et al., 2010). Moreover, a peak in *Salmonella* infections was reported during summer (Akil et al., 2014; Yun et al., 2016). This might be different in Ethiopia, probably due to distinct local environmental or climatic conditions, infrastructure, and behavioral factors.

Moreover, the rise of antibiotic resistance among FBPs further heighten the public health threat, particularly in developing countries (Uma et al., 2009). In 2016, annual death attributable to AMR was 700,000, that is half of the deaths attributable to diarrhea and projected to cause 10 million deaths by 2050 (O'Neill, 2016). Antibiotic-resistant strains of major pathogens, including STEC, particularly *E. coli* O157, *Salmonella* spp., and *Campylobacter* spp., have been identified in 73%, 77%, and 57.3% of cases, respectively, in the United States (Hailu et al., 2021). Resistance to commonly used antibiotics, including nalidixic acid, tetracycline, streptomycin, sulfamethoxazole-

trimethoprim (41.6%), kanamycin (23.6%), and low with public health importance resistance to the last resort of antibiotics such as imipenem (1.7%) and meropenem (1.4%) is widespread (Vaez et al., 2020). This increased resistance is largely driven by the inappropriate use of antibiotics across agriculture, environmental applications, human medicine, and veterinary practices, posing significant risks to food security and public health (Caneschi et al., 2023).

## 1.2. Statement of the problem

Every year, nearly one in ten of global population, fall ill from contaminated food, leading to 33 million DALYs and 420,000 deaths (WHO, 2022). FBDs accounts for 7.69% of global morbidity and 7.5% of all annual deaths (Lee & Yoon, 2021). This burden is comparable to malaria, with over 90% occurring in LMICs where critically weak infrastructure, poor sanitation, and informal food supply chains exacerbate the problem (Lee & Yoon, 2021). Informal markets, street vendors, backyard slaughters, unpasteurized milk sales, and wet markets, play a vital role in food access but heighten contamination risks due to lack of refrigeration, inspection, or hygiene (Grace, 2015; FAO, 2018). Economically, FBDs cost LMICs over US$100 million annually (World Bank, 2018). Beyond health and financial losses, FBDs worsen undernutrition by impairing growth and immunity, especially in children (FAO&WHO, 2020), and are closely linked with poor WASH and environmental contamination (Belina et al., 2024).

Globally, diarrhea accounts for over 90% of FBD cases and more than half related deaths and DALYs (Kirk et al., 2015). STEC, *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. are major global bacterial gastroenteritis agents (Uddin et al., 2021). *Campylobacter* alone causes over 95 million cases and 21,000 deaths annually (Havelaar et al., 2015; WHO, 2015). Although the case fatality rate of *Campylobacter* infections is generally lower than that of *Salmonella* spp. or *E. coli* O157, its widespread prevalence contributes substantially to the overall foodborne disease burden, accounting for approximately 5% of foodborne deaths worldwide (WHO, 2015). On the other hand, illnesses from *Salmonella* spp. cost approximately $405 million per year due to premature deaths and healthcare (Park, 2011). *Shigella* spp. caused around 188 million cases in 2010 and 212,438 deaths in 2016, of whom 60,000 were children under five (Khalil et al., 2018; Muzembo et al., 2023).

In Ethiopia, FBDs are worsened by poor sanitation, informal markets, and weak surveillance (Grace, 2015). NTS causes 932,000 cases and 1,340 deaths annually, while *Campylobacter* results in 2.38 million cases and 671 deaths (Havelaar et al., 2022).

While primarily foodborne, pathogens like *C. jejuni,* and *Salmonella* spp. are also linked to contaminated drinking water, attributed to non-food sources. In rural areas, groundwater often serves as the main drinking water source, increasing outbreak risks (de Silva et al., 2021, Mahagamage et al., 2020). Cattle, key reservoirs for these pathogens, contribute to food contamination through faeces (Verstraete et al., 2014). However, other food animals also play critical roles. For instance, poultry is the predominant reservoir of *Campylobacter jejuni* and *Salmonella* spp., two of the leading bacterial causes of foodborne disease globally (EFSA & ECDC, 2021). Poor agricultural practices, such as raw manure application, spread FBPs such as STEC, *Salmonella spp.*, *Shigella spp.*, and *Campylobacter spp.* to soil and vegetables. *Salmonella spp.* and *Campylobacter spp.* often found in livestock, can persist in soil and vegetables sold in markets (Pires et al., 2019).

Ethiopia, there is a notable research gap in pathogen surveillance and food safety risk assessment (Grace, 2015). Moreover, surveillance of key FBPs such as STEC, *Salmonella spp.*, *Shigella spp.*, and *Campylobacter spp.* is practically non-existent. Even those available studies are fragmented, site-specific, or limited to a single pathogen, with insufficient molecular-level confirmation or comprehensive epidemiological profiling (Belina et al., 2023; Feleke et al., 2022; Mama & Alemu, 2016; Mulatu et al., 2014). In addition, other available studies often cover these pathogens, like *Campylobacter spp.*, (Terefe et al., 2020), *Salmonella spp., and Shigella spp.* (Ararsa et al., 2023; Terfassa & Jida, 2018), or *E. coli* O157 (Getaneh et al., 2021). This lack of comprehensive national data limits our understanding of pathogen distribution, seasonality, and transmission dynamics.

The problem of FBD is compounded by the rise of antimicrobial resistance (AMR). AMR is a significant global health threat, particularly due to the spread of drug-resistant Gram-negative bacteria like carbapenem-resistant *Enterobacteriaceae*, linked to approximately 23,000 deaths annually (Heidary et al., 2014; Huttner et al., 2013). Enteric pathogens acquire AMR through horizontal gene transfer, exacerbated by antibiotic misuse in agriculture, which introduces antibiotics into ecosystems and

contaminates water sources (Au et al., 2022; Checcucci et al., 2020) *Salmonella spp.,* and *E. coli* isolates from livestock showed high antimicrobial resistance, contributing to an increased burden of FBD (Assoumy et al., 2021; Manishimwe et al., 2021). Furthermore, AMR-related complications happened due to the production of extended-spectrum beta-lactamases (ESBLs) and carbapenemases, which currently are target key antibiotics (Swedan & Alrub, 2019). In this connection, pathogens like *E. coli*, *Shigella* spp., and *Salmonella* spp. have shown alarming resistance to commonly used antibiotics in Ethiopia, with resistance rates reaching to 86.8% for ampicillin and 85.7% for amoxicillin-clavulanic acid (Adugna et al., 2015; Engda et al., 2023; Melese et al., 2019). Multidrug resistant (MDR) strains, including those producing ESBLs and carbapenemases, have been reported increasingly (Ayalneh et al., 2024; Zelelie et al., 2023), but comprehensive molecular characterization of the responsible genes is still limited.

Moreover, studies rarely integrate both phenotypic resistance profiles and genotypic confirmation (e.g., detection of bla-TEM, bla-CTX-M, or bla-SHV genes), representing a critical limitation in existing AMR research. Integrating phenotypic and genotypic studies provides valuable data that would increase our understanding of the roles and distribution of resistance genes among the FBPs affecting a given locality. For example, some studies indicate significant resistance, with 80% of ESBL-positive *E. coli* carrying beta-lactamase genes like bla-TEM, bla-CTX-M, or bla-SHV (Ayalneh et al., 2025; Muhummed et al., 2024; Zelelie et al., 2023) that can transfer the resistance genes to other pathogens and commensal. With increasing antibiotic use in livestock and the potential for horizontal gene transfer, the link between AMR in FBPs and zoonotic transmission remains poorly understood and inadequately mentioned in the Ethiopian context (Ayalneh et al., 2025; Muhummed et al., 2024; Worku et al., 2022; Zelelie et al., 2023).

In summary, there is insufficient research on the prevalence and AMR profiles of key FBD pathogens across Ethiopia. This limitation hinders a comprehensive understanding of the national burden and distribution of these pathogens. Additionally, there is a lack of consistent data on regional and seasonal pathogen distribution, like Addis Ababa, Gondar, and Harar. Furthermore, there is a critical gap in molecular confirmation and genotyping of virulent factors and resistant strains, which limit the reliability of

epidemiological conclusions and prevents identification of underlying mechanisms of resistance. Characterizing these pathogens through site-specific prevalence studies, AMR testing, and molecular analyses is therefore essential. Such characterization allows researchers and public health authorities to determine which pathogens are circulating, assess their resistance profiles, identify virulence and resistance genes, and understand transmission dynamics. The significance of this characterization lies in its ability to inform evidence-based interventions, strengthen national surveillance systems, guide treatment and antimicrobial stewardship programs, and ultimately reduce the burden of foodborne illnesses.

## 1.3. Hypothesis

We hypothesize (alternative hypothesis) that;

- The prevalence of STEC, *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. differs significantly from that reported in previous studies conducted in the same geographical area of Ethiopia.
- The prevalence of STEC, *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. differs significantly between different cities (Addis Ababa, Gondar, and Harar) and across seasons in Ethiopia.
- The antibiotic susceptibility profile of STEC, *Salmonella* spp., and *Shigella* spp. differ significantly between the study sites.
- The prevalence of STEC, *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. is significantly associated with selected socio-demographic and environmental factors.
- The distribution of virulence and antibiotic-resistant genes differs among the foodborne bacterial pathogens, and between the study sites.

## 1.4. Objectives

### 1.4.1. General objective

- To determine the molecular features, epidemiology and antimicrobial resistance profiles of selected FBPs among diarrheal patients in three selected cities in Ethiopia.

### 1.4.2. Specific objectives

- To determine the prevalence of STEC, *Salmonella* spp., *Shigella* spp., *and Campylobacter* spp., among diarrheic patients in Addis Ababa, Gondar, and Harar in Ethiopia.
- To assess the seasonal and regional distribution of STEC, *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. among diarrheic patients in Addis Ababa, Gondar, and Harar in Ethiopia.
- To determine the antibiotic susceptibility profile of STEC, *Salmonella* spp., and *Shigella* spp., in Addis Ababa, Gondar, and Harar in Ethiopia.
- To identify the presence and distribution of virulence and resistance genes (including ESBL and carbapenemase-producing genes) among the targeted bacterial pathogens in Addis Ababa, Gondar, and Harar.
- To determine the association between selected socio-demographic and environmental risk factors and the occurrence of targeted pathogens among diarrheic patients.

## 1.5. Scope of the study

The study was conducted in three major cities of Ethiopia: Addis Ababa, Gondar, and Harar to capture a broad range of geographic, climatic, and socio-demographic settings across the country. Addis Ababa represents the central urban area, Gondar the northern highlands, and Harar the eastern region, capturing variations in population density, climate, and potential exposure to foodborne pathogens. Patient's flow was assessed using five years of retrospective data on diarrheal diseases from multiple hospitals, and these sites were identified as having the highest number of cases, ensuring an adequate and representative sample for the study. Data was collected from October 2021 to November 2022, encompassing seasonal variations throughout the year. The target population consisted of diarrheal patients visiting selected hospitals: Yekatit-12

Hospital in Addis Ababa, the University of Gondar Comprehensive Specialized Hospital in Gondar, and Hiwot Fana Comprehensive Specialized Hospital in Harar, with a focus on patients requesting stool examinations. A total sample size of 2,331 was estimated to allow comparison across regions and seasons. The study measured the molecular epidemiology of selected FBPs, their antimicrobial resistance and associated factors among patients with diarrhea.

## 1.6. Outcome of the study

This study determined the burden, distribution, and antimicrobial characteristics of key foodborne bacterial pathogens among diarrheal patients in Addis Ababa, Gondar, and Harar, Ethiopia. The dependent variables included the presence of STEC, *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp., the antibiotic susceptibility patterns of these pathogens, and the presence of ESBL and carbapenemase-producing genes. The independent variables comprised socio-demographic factors (age, gender, household income), environmental exposures (water source, types of toilets, and animal contact), study sites, and seasonal categories (dry: October-February; short rain: March-May; long rain: June-September).

Using these variables, the study specifically examined:

- The prevalence of bacterial pathogens i.e., STEC., *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. and associated risk factors, by assessing how the independent variables influenced the likelihood of detecting the targeted pathogen in Addis Ababa, Gondar, and Harar, Ethiopia.
- The regional and seasonal distribution of STEC, *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp, linking pathogen detection to study site and season
- The antibiotic susceptibility profile of STEC, *Salmonella* spp., and *Shigella* spp., in relation to both the pathogen types and demographic characteristics such as study sites.
- The detection and distribution of common virulence genes, ESBL and carbapenemase genes, analyzing associations with pathogen type and study sites.

## 1.7. Significance of the study

This study assessed four major FBPs: STEC, *Salmonella spp.*, *Shigella spp.*, and *Campylobacter spp.,* which are known to pose significant public health risks. By using both conventional microbiological methods for the STEC, *Salmonella spp.*, and *Shigella spp.*, and enzyme immunoassay detection for the *Campylobacter* spp. This was followed by confirmatory molecular techniques the research provides insights into the prevalence and antibiotic susceptibility profile of these pathogens. Conducted in three major Ethiopian cities, the study provides a multi-regional analysis of the geographic distribution, prevalence, and characteristics of four key FBPs. While the findings do not allow for direct national-level generalization, the selection of diverse geographic regions (central, northern, and eastern Ethiopia) and high-patient-flow hospitals offers valuable insights into regional trends, seasonal variations, and associated risk factors. Ethiopia's cultural and dietary diversity contributes to varying FBD burdens, and this provides critical data to inform policymakers and the scientific community on areas with higher diarrheal disease burden and to support the design of targeted surveillance strategies and interventions. Moreover, the study covers a wide age range, including children, adults, and the elderly, which may help in the development of targeted public health strategies. Findings on regional antibiotic resistance profiles are crucial for public health authorities to design effective control strategies by at least omitting antibiotics that are highly resistant in each geographic location and emphasize the need for stronger antimicrobial stewardship programs. The data can be an eye-opener for conducting more extensive surveillance studies, contributing to One Health efforts that address the interconnected health issues of humans, animals, and the environment.

# CHAPTER TWO: LITERATURE REVIEW

Foodborne bacterial infections continue to pose a significant public health burden globally, particularly in LMICs where food safety systems are often limited (Grace, 2023; WHO, 2022). These pathogens are major contributors to diarrheal disease, malnutrition, and even mortality, especially among children and other vulnerable populations (Havelaar et al., 2015; Kirk et al., 2015). Prominent foodborne bacteria such as *Salmonella spp.*, *Campylobacter spp.*, *Shigella spp.*, and STEC are frequently linked to outbreaks and sporadic infections (Lee & Yoon, 2021; Scallan et al., 2015). In addition, the increasing AMR among these organisms complicates treatment and raises concerns about transmission through the food chain (Checcucci et al., 2020; Manishimwe et al., 2021). While detailed molecular analysis, such as whole genome sequencing has become a gold standard for high-resolution molecular epidemiology in high-resource settings, PCR-based molecular techniques remain valuable tools for detecting virulence genes, AMR markers, and understanding the basic epidemiology of these pathogens in resource-constrained environments (Feleke et al., 2022; Mohammed & Tamiru, 2014). This literature review explores the current knowledge on the prevalence, molecular characteristics, and AMR patterns of selected FBP targeted in this study, with a particular focus on the Ethiopian context.

## 2.1. *Salmonella* spp.

### *2.1.1. Historical perspective and classification*

*Salmonella* was first discovered in 1855 by Theobald Smith, who isolated the bacterium from the intestines of pigs infected with classical swine fever (Eng et al., 2015). The bacterium was named after Dr. Daniel Elmer Salmon, an American pathologist who collaborated with Smith. The nomenclature of *Salmonella* has been a subject of debate and continues to evolve today.

*Salmonella* belongs to the Enterobacteriaceae family and is classified into two broad species: *Salmonella enterica* and *Salmonella bongori*, based on variations in surface structures, including lipopolysaccharides (LPS), flagella, and capsular polysaccharides (Ferrari et al., 2019). Currently, over 2,600 serovars of *S. enterica* have been described worldwide, and many of these can cause illness in both humans and animals. *S. enterica*

is further divided into six subspecies, denoted by Roman numerals: I (*S. enterica* subsp. *enterica*); II (*S. enterica* subsp. *Salamae*); III (*S. enterica* subsp. *Arizonae*, including *S. enterica* subsp. *Diarizonae*); IV (*S. enterica* subsp. *Houtenae*); V (*S. enterica* subsp. *Indica*) (Eng et al., 2015).

Among these, *S. enterica* subsp. *enterica* (I) is the most common and is predominantly associated with mammals, accounting for approximately 99% of human and warm-blooded animal *Salmonella* infections. These subspecies can be further categorized into two groups: host-restricted typhoidal *Salmonella* (which primarily affects humans), and the more widely distributed NTS, which can infect a variety of hosts (Neuert et al., 2018). The remaining subspecies of *S. enterica* and *S. bongori* are more rarely associated with humans and are typically found in cold-blooded animals or the environment (Jajere, 2019).

### *2.1.2. Microbiological Characteristics*

*Salmonella* is a Gram-negative, facultative anaerobic, rod-shaped bacterium that belongs to the family Enterobacteriaceae (Jajere, 2019). It is chemo-organotrophic, capable of metabolizing nutrients through both respiratory and fermentative pathways. The bacterium is characterized by the presence of three types of antigens: somatic (O), flagellar (H), and capsular (Vi). The capsular Vi antigen is a polysaccharide layer that surrounds the cell wall and is present in certain serotypes, notably *Salmonella enterica* serovar Typhi, the causative agent of typhoid fever. Other serotypes, such as *Salmonella enterica* serovar Paratyphi C and *Salmonella enterica* serovar Dublin, also express the Vi antigen. However, the Vi antigen is absent in many other *Salmonella* serotypes, including *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Enteritidis. The presence of the Vi antigen enhances the virulence of these serotypes by aiding in immune evasion and promoting systemic infection (Eng et al., 2015). The O-antigen, which is a component of the lipopolysaccharide layer of the bacterial cell wall, consists of repeating sugar units. These O-antigens are stable under heat (able to withstand heating at 100°C for up to 2.5 hours) and in alcohol, making them useful for serological identification. The H-antigens are proteins found on the bacterial flagella and are heat-labile and alcohol-sensitive. *Salmonella* is unique among Enterobacteriaceae in that it exhibits two distinct flagellar antigen phases (Phase I and

Phase II). This diphasic characteristic allows *Salmonella* to express one flagellar protein at a time, with Phase I antigens being specific to individual serovars and Phase II antigens being more generalized across serovars (McQuiston et al., 2008). The Vi antigen, found in certain *Salmonella* serovars like *S.* Typhi, is found over the O-antigen and is crucial for virulence in some strains. The antigenic profile (O) forms the basis for serotyping. Serovars such as *S.* Typhi and *S.* Paratyphi are important clinically because they cause enteric fever, while others like *S.* Typhimurium and *S.* Enteritidis are more commonly associated with gastroenteritis (Chattaway et al., 2021).

Most *Salmonella* serovars produce hydrogen sulfide, with exceptions such as *S.* Paratyphi A and *S.* Choleraesuis. The majority do not ferment lactose. Although they can grow in saline concentrations ranging from 0.4% to 4%, they do not require salt for growth. *Salmonella* can thrive in temperatures ranging from 5°C to 47°C, with an optimal growth temperature between 32°C and 35°C, though some strains can survive even at lower extremes of 2°C to 4°C or higher extremes of 54°C. However, the bacterium is sensitive to very high temperature and typically dies at temperatures above 70°C. Its growth is inhibited at a pH below 3.8 and water activity below 0.94, making it sensitive to drying (Jarvis et al., 2016). The pathogenicity of *Salmonella* is primarily linked to several virulent factors, including the *invA* gene, which is essential for the bacterium's ability to invade host cells (Alarjani et al., 2021).

### *2.1.3. Epidemiology*

The molecular epidemiology of NTS worldwide, in Africa, and in Ethiopia underscores the complexity of transmission dynamics, the emergence of antimicrobial resistance, and the burden of invasive infections. Molecular techniques have proven invaluable in identifying serotype distribution, genetic diversity, and resistance profiles of NTS strains, which is crucial for developing targeted public health strategies. In Africa and Ethiopia, the continued surveillance of NTS and antimicrobial resistance patterns is essential to inform treatment guidelines, control measures, and prevention strategies.

*Salmonella* spp., are among the most important FBPs globally, ranking just follows *Campylobacter* and *Norovirus* as the leading causes of diarrheal diseases (Ferrari et al., 2019). They affect humans, domestic animals, and wildlife, with animals being the primary reservoir. The primary transmission route to humans is through the

consumption of contaminated animal products such as poultry, meat, eggs, and milk. *Salmonella* can also spread through improper food handling, cross-contamination, and poor hygiene practices. In regions where humans and animals live in proximity, the risk of *Salmonella* infection is heightened, particularly when animal products are consumed raw or undercooked. Key epidemiologically important NTS serovars of *Salmonella* include *S.* Typhimurium and *S.* Enteritidis, both of which are being widely distributed and cause a large proportion of human infections globally. *S.* Typhimurium is often described as a broad-host-range serovar due to its extensive presence in livestock and wildlife (Jajere, 2019).

The global burden of NTS disease is estimated to result in up to 129.5 million cases annually, leading to between 100,000 and 1 million deaths, predominantly in immunocompromised individuals in sub-Saharan Africa (Dougan et al., 2012). While most infections are presented as self-limiting gastroenteritis, a significant portion of cases progress to more severe, invasive diseases, particularly in vulnerable populations. Moreover, NTS is a significant cause of gastroenteritis worldwide, with an estimated 93.8 million cases and 155,000 deaths annually (Majowicz et al., 2010; Dougan et al., 2012).

The epidemiology of NTS in Africa is marked by the predominance of S. Typhimurium and S. Enteritidis, accounting for over 70% of human infections, particularly in Uganda, Kenya, and Zambia (Gordon et al., 2008). Studies in Ghana, Gambia, and Mozambique have further underscored the widespread prevalence of these serovars, with notable concerns about antimicrobial resistance and genomic diversity (Andoh et al., 2017; Darboe et al., 2022; Garcia et al., 2018). These studies reported that *S.* Typhimurium and *S.* Enteritidis were identified as the predominant serovars, raising concerns about the emergence of multidrug-resistant strains capable of causing, invasive diseases. The overall burden of NTS in Africa is exacerbated by co-infections with HIV, malaria, and malnutrition, along with limited access to clean water and adequate sanitation (Dougan et al., 2012), positioning invasive NTS as a neglected tropical disease with significant public health implications. In Ethiopia, NTS represents a major public health challenge, contributing significantly to the burden of FBDs. It is estimated to cause approximately 932,000 illnesses, annually, leading to 1,340 deaths in the country (Havelaar et al., 2022). As indicated in the cited references, these infections also result in a substantial

loss of 101,000 DALYs, reflecting the combined impact of premature mortality and years lived with illness or disability.

A review of 20 studies in Ethiopia conducted between 1974 and 2012 revealed pooled prevalence of *Salmonella* in stool samples from different groups: 8.72% in diarrheic children, 5.68% in diarrheic adults, and 1.08% in carriers (Tadesse, 2014). In this review, the prevalence of invasive infections was found to differ significantly by age, where 5.71% was observed among children in contrast to 0.76% among adults ($p$<0.001). The predominant serovars included in the review was *S.* Concord, *S.* Typhi, *S.* Typhimurium, and *S.* Paratyphi, which together represented 82.1% of isolates from patients. Furthermore, NTS accounted for 57.9%, and serogroup D was more frequently identified than serogroups C and B. This high disease burden highlights the urgent need for improved food safety measures, better diagnostic capabilities, enhanced surveillance systems, and targeted public health interventions to prevent and control NTS infections in Ethiopia (Havelaar et al., 2022). Another study in Addis Ababa, showed that the major NTS serovars identified in Ethiopia are *S.* Typhimurium, *S.* Enteritidis, and *S.* Concord where *S.* Typhimurium was the most prevalent serovar, followed by *S.* Enteritidis (Eguale et al., 2015).

### *2.1.4. Pathogenesis and Clinical Features*

The *Salmonella* bacterium invades the intestinal epithelium primarily through M cells, where it is translocated and taken up by macrophages. Inside these macrophages, *Salmonella* survives and replicates by modifying host vesicles, enabling both local and systemic dissemination. The pathogen can also enter through the basolateral side of epithelial cells or be captured by dendritic cells that facilitate its spread throughout the body. Upon release, *Salmonella* induces macrophage apoptosis and inflammation through interleukin-1β (IL-1β) production, contributing to tissue damage and disease progression (Figure 1).

Most cases of *Salmonella* infection are limited to uncomplicated gastroenteritis, which typically resolves without the need for antimicrobial treatment (Chen et al., 2013). The infectious dose for healthy adults' ranges from $10^6$ to $10^8$ *Salmonella* organisms, though as few as 1-10 cells can cause disease in susceptible individuals, such as infants or those with compromised immune systems (Jarvis et al., 2016).

The incubation period ranges from 4 hours to 72 hours after the ingestion of contaminated food or water. Symptoms include acute fever, chills, nausea, vomiting, abdominal cramps, and diarrhea, which may be bloody. Typically, fever subsides within 72 hours, and diarrhea lasts 3-7 days. Though *Salmonella* is excreted in feces for a median of 5 weeks, the excretion period may be prolonged in children. Infections may also lead to bacteremia in 5-10% of cases, with more severe manifestations in immuno-compromised patients (Eng et al., 2015).

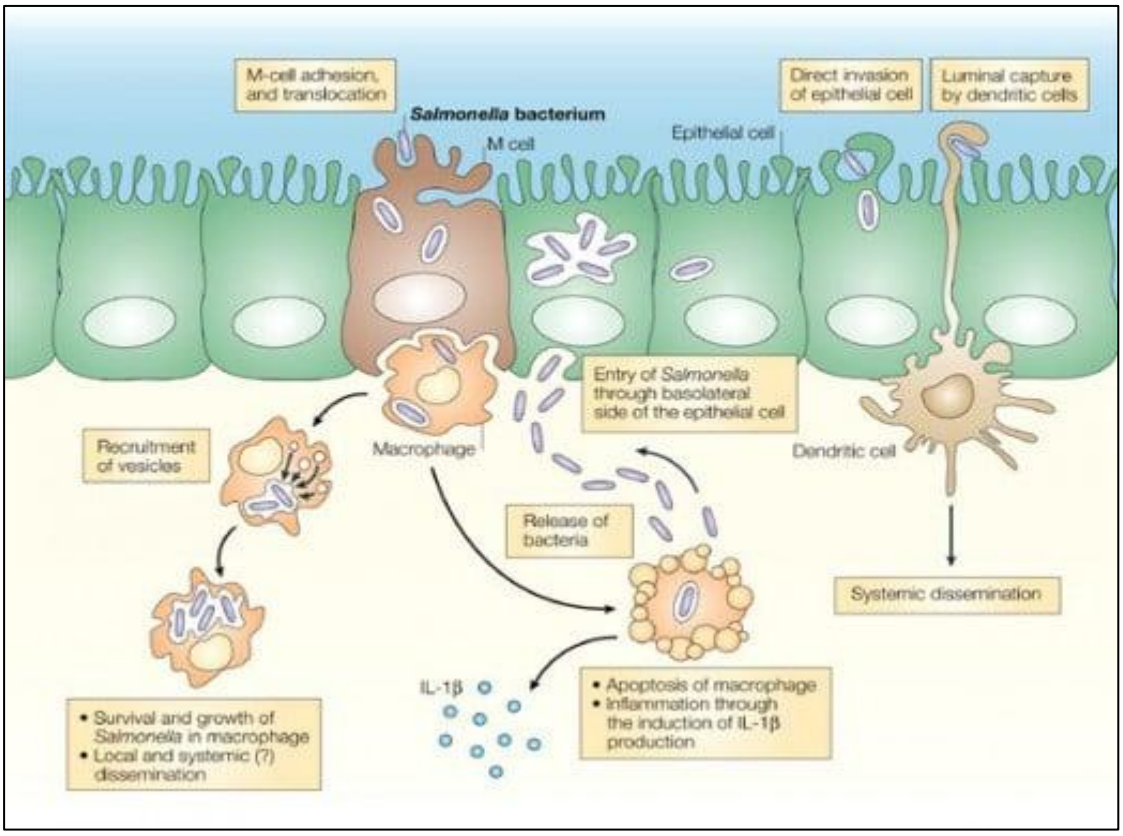


Figure 1. Schematic representation of *Salmonella* pathogenesis. Adapted from Costa et al., 2015.

## 2.1.5. Treatment Options and Prevention Strategies for *Salmonella* spp.

### *2.1.5.1. Treatment Options*

Most NTS infections are self-limiting, presenting as gastroenteritis that are resolved within 4-7 days. Supportive care, including oral or intravenous rehydration and electrolyte replacement, remains the mainstay of treatment (Eng et al., 2015). Antimicrobial therapy is reserved for severe cases, including invasive infections,

bacteremia, or infections in high-risk populations such as infants, elderly individuals, or immunocompromised patients (Crump & Heyderman, 2015). First-line agents include fluoroquinolones (e.g., ciprofloxacin), third-generation cephalosporins (e.g., ceftriaxone), and azithromycin, depending on local resistance patterns (Feasey et al., 2016). Empiric therapy should be guided by antimicrobial susceptibility testing due to the increasing prevalence of multidrug-resistant *Salmonella* strains. Hospitalization may be required for severe dehydration, sepsis, or complications such as reactive arthritis or bacteremia. Novel therapeutics, including bacteriophage therapy and vaccine development, are under investigation, particularly to prevent invasive *Salmonella* infections in endemic areas (Eng et al., 2015; Feasey et al., 2016).

#### *2.1.5.2. Prevention Strategies*

Preventing Salmonella infections focuses on food safety, hygiene, and water sanitation. Key measures include, thorough cooking meat, poultry, and eggs, preventing cross-contamination between raw and cooked foods, proper washing of fruits and vegetables, and avoiding unpasteurized milk and juices (Eng et al., 2015). Hand hygiene, especially after handling animals, raw food, or contact with contaminated environments, is critical to reduce person-to-person transmission. Public education and food handler training are essential to reinforce safe practices (Crump & Heyderman, 2015). Vaccination of poultry and livestock, improved biosecurity, and surveillance systems for antimicrobial resistance and outbreaks are important strategies at the farm and community levels (Feasey et al., 2016). Antimicrobial stewardship and monitoring programs are also critical to manage resistance and inform treatment guidelines.

## 2.2. Shiga-toxin Producing *E. coli*

### *2.2.1. Historical perspective and classification*

In 1885, Theodor Escherichia first reported the isolation and characterization of slender short rods from infant stools, naming them *Bacterium coli commune*. Though this organism was later described under various synonyms, it wasn't formally recognized as *E. coli* until 1954. Over 125 years later, *E. coli* is primarily known as a harmless commensal in the gastrointestinal tracts of warm-blooded animals, becoming a standard organism in laboratory research (Croxen et al., 2013).

However, STEC is one of the most pathogenic subtypes of *E. coli*, linked with severe diarrheal disease and post-diarrheal complications, notably HUS (Moges et al., 2020). A pivotal discovery occurred in 1983 when O'Brien and colleagues identified *E. coli* O157, responsible for an outbreak of hemorrhagic colitis in the United States, as a producer of a toxin like Shiga toxin (Stx), which was later termed Shiga-like toxin (O'Brien et al., 1983). This toxin was shown to be identical to verotoxin (O'Brien et al., 1983). Additionally, this subtype was found to cause attaching/effacing (A/E) lesions that result from the bacteria adhering closely to the enterocyte membrane and effacing. More than 400 serotypes of STEC have been identified, with over 100 of them capable of causing gastrointestinal disease in humans, including severe bloody diarrhea and HUS (Kaper & O'Brien., 2014).

### *2.2.2. Microbiological characteristics*

The STECs are Gram-negative, chemoorganotrophic, and oxidase-negative commensals belonging to the family *Enterobacteriaceae*, typically measuring 1.1-1.5 µm in diameter and 2-6 µm in length. Although STEC can be motile or non-motile, the motile ones use peritrichous flagella. They can be isolated from humans and various animal hosts and are found throughout the gastrointestinal tract. These bacteria thrive in a pH range of 5-9 and can resist brief exposures to pH levels as low as 2, which is crucial for their passage through the acidic stomach environment before intestinal colonization (Hamdallah et al., 2018).

Fecal samples are commonly used for isolation, typically cultured in selective media (Croxen et al., 2013). Notably, *E. coli* O157, a well-known STEC serogroup, can be identified by its inability to ferment sorbitol on sorbitol-MacConkey agar (SMAC) within 24 hours. Other media, like cefixime tellurite-sorbitol MacConkey agar (CT-SMAC) or CHROMagar O157, can also be used to differentiate *E. coli* O157 from others (Zaragoza et al., 2016).

The Stx is a protein encoded by a bacteriophage that integrates its genetic material into the *E. coli* genome, making it STEC. The Stx protein comprises an A subunit and five identical B subunits. The B subunits bind to gut epithelial cells, while the A subunit includes the catalytically active A1 and a stabilizing A2 fragment, forming the AB5 holotoxin structure (Hunt et al., 2010). Two types of Stx are recognized: Stx1 (with

subtypes Stx1a, Stx1c, and Stx1d) and Stx2 (which has subtypes a-g). Stx1 differs from the Shiga toxin produced by *Shigella dysenteriae* serotype 1 by a single amino acid, while Stx2 is more distinct, sharing only around 60% amino acid identity with Stx1. Various sequence variants exist for both Stx1 and Stx2, and a single STEC strain can produce more than one variant (Hunt et al., 2010). Although both Stx1 and Stx2 can cause illness, Stx2 is more commonly associated with severe disease, particularly the subtypes Stx2a, Stx2c, and Stx2d. The detection of Stx genes is crucial to distinguish the virulent strains, as they are key virulence factors of STEC, along with the *eae* gene that encodes intimin, an adherence factor that contributes to disease severity. Although *E. coli* O157 is most well- known, molecular diagnostics have identified other STEC serogroups, including O26, O45, O103, O111, O121, and O145 (Fratamico & Bagi, 2012).

### *2.2.3. Epidemiology*

The transmission of STEC occurs via various routes, including fecal-oral, foodborne, environmental, and person-to-person transmission, the latter being common in childcare settings (Kintz et al., 2017). Globally, STEC infections are a significant public health concern, with the World Health Organization (WHO) estimating over a million foodborne STEC cases in 2010, resulting in approximately 100 deaths (Havelaar et al., 2015). In European Union, STEC was the third most prevalent FBP in 2022, with 8,565 confirmed cases and 71 outbreaks reported across 25 (ECDC, 2022).

Infection mainly arises from consuming improperly processed or contaminated food or water, though direct contact with infected animals, manure, or individuals contaminated with the bacterium. Cattle are the primary reservoir for STEC, with sheep also contributing to human infections, although human infections can also occur through direct contact with these animals or contaminated food and water. Flooding and heavy rainfall often increases zoonotic outbreaks by contaminating crops, with floodwaters carrying elevated pathogen loads (Cunningham et al., 2024). Approximately 10-15% of cases of STEC infections can lead to HUS among vulnerable populations, especially children, characterized by microangiopathic hemolysis, platelet destruction, and renal failure (WHO, 2018). HUS occurs in about 5% of cases within three days of diarrhea onset and has a mortality rate of 3-20%, though this rate is higher in developing countries due to resource limitations (Freedman et al., 2023).

Recent studies highlight the emergence of hybrid pathotypes, particularly Shiga toxin-producing enteroaggregative *E. coli* (Stx-EAEC), which combine virulence factors from STEC and EAEC. For example, the Stx2a-encoding phages identified in Stx-EAEC O86 isolates in Japan were nearly identical to those from the epidemic-related O104:H4 strain that caused a massive outbreak in Europe in 2011, demonstrating the global spread of virulent hybrid strains (Kimata et al., 2020). Such hybrids possess both aggregative adherence and Shiga toxin genes, increasing their potential for severe disease, including HUS, and underscoring the need for enhanced genomic surveillance and monitoring of emerging pathotypes (Figure 2). Further genomic analyses have revealed that hybrid strains of STEC and EAEC exhibit a diverse array of virulence genes, including those encoding enterohemolysin, a key factor in pathogenicity. These findings emphasize the necessity for comprehensive molecular surveillance to detect and characterize emerging hybrid strains, which may not be identified using traditional diagnostic methods (Lee et al., 2024).

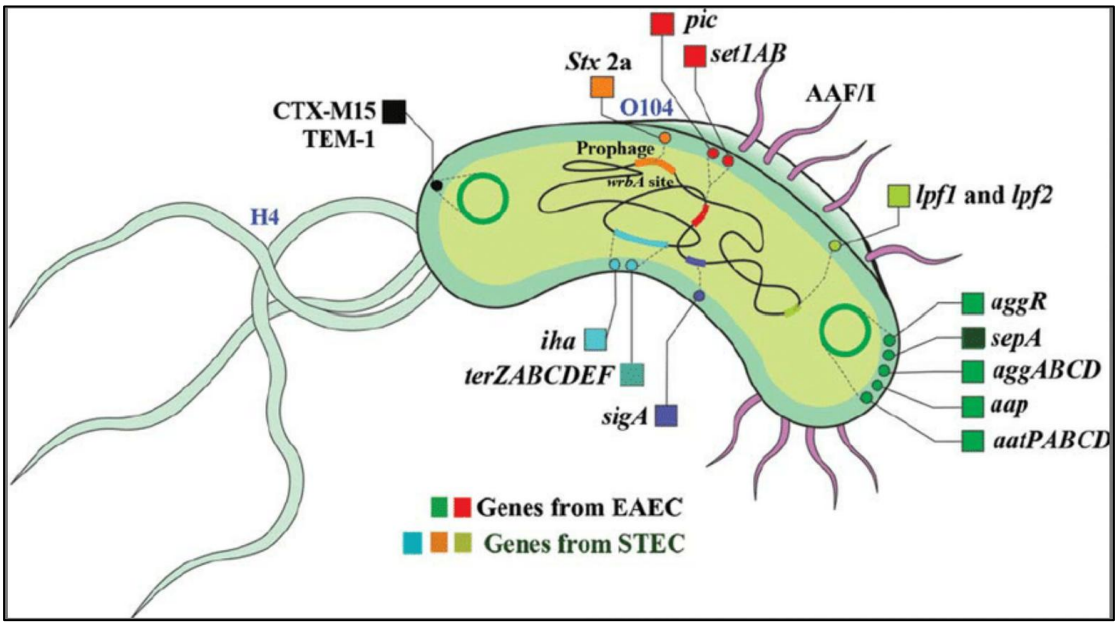


Figure 2. The hybrid characteristics of EAEC (STEC-EAEC) and the virulence and antibiotic resistance genes.

In Ethiopia, the reported burden of STEC is estimated to be approximately 10 times lower than the global burden. This discrepancy is likely not reflective of the actual disease prevalence but instead attributable to limited data availability and underreporting of STEC infections, as is the case across Africa. The lack of robust diagnostic facilities, inadequate surveillance systems, and the underestimation of

asymptomatic carriers or mild cases contribute to the underrepresentation of the true disease burden in national and regional statistics. These challenges emphasize the need for enhanced research, improved diagnostic capabilities, and comprehensive epidemiological studies to better understand the impact of STEC infections across the continent in general and in Ethiopia in particular (Havelaar et al., 2022).

### *2.2.4. Pathogenesis and Clinical Features*

The infectious dose for *E. coli* O157 is estimated to be between 10 and 100 cells, though information on other STEC serotypes is limited (Jinneman et al., 2003). STEC infections are primarily food- or waterborne, often linked to undercooked ground beef, raw milk, cold sandwiches, water, unpasteurized apple juice, and sprouts (USFDA, 2020). After exposure, STEC infections usually have an incubation period of 3 days, and the infection will last for 3.5 to 8.1 days. However, many infections cannot be traced to a specific source, making the timing of ingestion unclear (Awofisayo-Okuyelu et al., 2019). The characteristic feature of STEC is its ability to release Stx, which can kill intestinal cells and spread to other organs like the kidneys and brain (Kaper & O'Brien, 2014) (Figure 3). Compared to other bacterial causes of gastroenteritis, STEC is associated with more severe disease. Infections often present abdominal cramps, vomiting, and diarrhea, which can progress to hemorrhagic colitis (Cunningham et al., 2024). Approximately 30% of cases require hospitalization, and about 10% to 15% of cases progress to HUS, a life-threatening condition with hemolytic anemia, thrombocytopenia, and renal failure (Byrne et al., 2015).

Generally, the pathogenesis of STEC infection indicated that STEC colonizes the intestinal epithelium and releases Shiga toxins, causing mucosal injury and diarrhea that may become bloody (Figure 3). The toxin then crosses the intestinal barrier and enters the bloodstream, where it binds to Gb3 receptors on target cells, particularly in the kidneys. Following endocytosis and retrograde transport through the Golgi and endoplasmic reticulum, the active toxin fragment ($A_1$) is released into the cytosol, where it halts protein synthesis by depurinating the 28S rRNA. This leads to endothelial cell damage, vascular inflammation, and formation of microthrombi, resulting in hemolysis, thrombocytopenia, and renal injury characteristic of HUS.

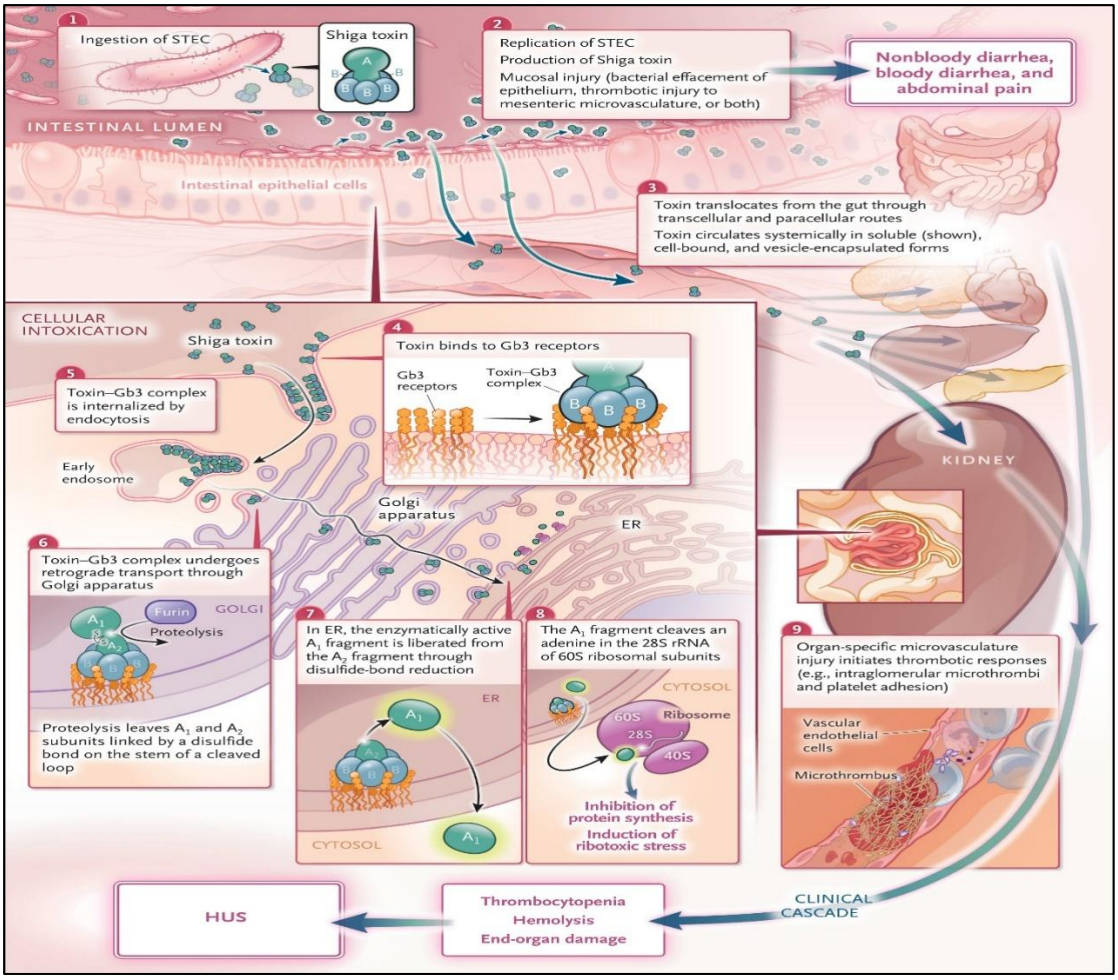


Figure 3. Pathogenesis and progression to the HUS of STEC. Adapted from Freedman et al. (2023).

## 2.2.5. Treatment Options and Prevention Strategies for STEC

### *2.2.5.1. Treatment Options*

Management of STEC infections remains primarily supportive, as no specific antimicrobial therapy has been proven to reduce the risk of complications. Rehydration and electrolyte replacement, administered orally or intravenously, are essential to prevent dehydration, especially in children. The use of antibiotics is generally contraindicated because bacterial lysis may enhance Shiga toxin release, increasing the risk of HUS (Freedman et al., 2016). Antimotility agents, such as loperamide, should also be avoided, as they prolong toxin exposure in the gastrointestinal tract (Mohamed et al., 2020). For patients who develop HUS, hospitalization is required, including interventions such as dialysis for renal failure, blood transfusions, and intensive supportive care. Novel therapeutic approaches under investigation include monoclonal antibodies targeting Shiga toxin, probiotics, and toxin-binding agents, although these

are not yet widely available for routine clinical use (Melton-Celsa, 2014; Hughes et al., 2021).

#### *2.2.5.2. Prevention Strategies*

In the absence of effective curative treatment, prevention is the most reliable approach against STEC. Food safety measures such as thoroughly cooking ground beef, avoiding cross-contamination between raw and cooked foods, washing raw produce, and avoiding unpasteurized milk or juices are strongly recommended (WHO, 2022). Access to safe drinking water, effective wastewater management, and avoidance of contaminated recreational waters also reduce exposure (WHO, 2022). Good hygiene practices, particularly frequent handwashing with soap and water after animal contact, food preparation, and toilet use, significantly reduce person-to-person transmission (Mohamed et al., 2020). Educational interventions in schools, farms, and food-handling environments reinforce these practices (WHO, 2022). Livestock, especially cattle, are the main reservoirs of STEC. Farm-level interventions such as probiotics, vaccination, and bacteriophage therapy are under investigation to reduce colonization and transmission from animals to humans (Hughes et al., 2021). Public health surveillance systems, including those coordinated by EFSA and ECDC in Europe, highlight the importance of robust laboratory capacity and genomic surveillance to detect both classical and hybrid STEC strains, enabling rapid outbreak detection and response (EFSA & ECDC, 2023).

## 2.3. *Shigella* spp

### *2.3.1. Historical perspective and classification*

*Shigella* were first identified by Kiyoshi Shiga during a major outbreak in 1898 in Japan (Halimeh et al., 2021). The closely related to *E. coli*, *Shigella* was formally recognized, and early studies sought to clarify the classification of *Shigella* within the *Enterobacteriaceae* family, based on 192 morphological, biochemical, and phenotypic properties, with Dodd and Jones. (1982) demonstrating that *Shigella* spp. clustered more closely with *Yersinia* and *Proteus* spp., than with *E. coli*. This reinforced the idea of *Shigella* as a separate genus.

However, advancements in molecular techniques have raised questions about the taxonomic status of *Shigella*. Genomic analysis has revealed that *Shigella* spp., and *E.*

*coli* are genetically nearly indistinguishable, with 80-90% similarity in their DNA (Kang et al., 2007). A key difference lies in motility: while *E. coli* is typically motile, *Shigella* spp. are nonmotile. Additionally, most *Shigella* strains are incapable of fermenting lactose (Ragupathi et al., 2018).

### *2.3.2. Microbiological characteristics*

*Shigella* is a genus of highly infectious, Gram-negative, non-motile, non-spore-forming, rod-shaped bacteria within the *Enterobacteriaceae* family. The genus consists of four species and 43 recognized serotypes (Baker et al., 2018). The four species, namely, *S. dysenteriae* (*S. dysentriae*, serogroup A), *S. flexneri* (*S. flexneri*, serogroup B), *S. boydii* (*S. boydii*, serogroup C), and *S. sonnei* (*S. sonnei*, serogroup D) are differentiated based on their O-antigen structure and biochemical properties (Gu., 2024). *Shigella* is known for its acid resistance, salt tolerance, and ability to survive at infective levels in foods, including fruits, vegetables, and vacuum-packed items, with survival enhanced at refrigerated temperatures (Warren et al., 2006).

The molecular characterization of *Shigella* focuses on a virulence-plasmid that encodes genes necessary for its invasive phenotype. A key region of this plasmid, the *ipa/mxi-spa* locus, is responsible for the bacterium's entry into epithelial cells, macrophage apoptosis, and activation of polymorphonuclear cells. This locus encodes a type III secretion system, which functions like a flagellum, delivering *Shigella* effector proteins directly into the target cell. Effector proteins like *IpaH* are crucial to the pathogenesis of *Shigella and* play a key role in promoting dissemination within epithelial cells (Wang et al., 2020).

### *2.3.3. Epidemiology*

*Shigella* is primarily transmitted through the fecal-oral route, either through direct contact with contaminated hands or via food handled by infected individuals or asymptomatic carriers (Baker et al., 2018). Flies breeding on feces also contribute to transmission during outbreaks. Contamination of surface and drinking water is another significant source of infection, especially when the water treatment is inadequate (Moxley et al., 2022). *Shigella* spp., are classified as waterborne and fecal-borne pathogens, with humans serving as the primary natural reservoir and main source of

transmission. In some cases, non-human primates can also be carriers (Pakbin et al., 2023).

*Shigella* can be excreted in large quantities, with infected individuals shedding between $10^5$ and $10^9$ bacteria per gram of feces, while asymptomatic carriers shed smaller amounts ($10^2$ to $10^6$ per gram) (Percival et al., 2013). This asymptomatic shedding increases the risk of transmission through inadequate hand hygiene. Numerous outbreaks have been linked to contaminated food and water, particularly with foods that are manually handled, inadequately heated, or consumed raw. Common sources include ground beef, oysters, raw vegetables, and salads (Pakbin et al., 2022).

Globally, *Shigella* causes approximately 188 million cases annually, resulting in around 164,000 deaths, particularly among children in low-resource settings (Lyngstad et al., 2018). In low-resource countries, the prevalence ranges from 4.9% to 17.8%, with an incidence rate of 31.8 cases per 100 child-years (Lampel et al., 2018). The Centres for Disease Control and Prevention (CDC) report significant outbreaks, often originating from childcare settings. Even in the United States alone, *Shigella* infections continue to pose a major public health threat, with nearly 500,000 cases reported annually (Halimeh et al., 2021).

All four *Shigella* spp., are major causes of bacillary dysentery worldwide, particularly in LMICs. However, S. *flexneri* and *S. sonnei* are the most frequently isolated species. *S. flexneri* dominates in LMICs, while *S. sonnei* is more prevalent in high-income countries due to differences in water, sanitation and hygiene practices between these two economic regions (Kotloff et al., 2018). *S. boydii* is frequently isolated in Southeast Asia, and *S. dysenteriae*, especially serotype 1, was historically responsible for major outbreaks in South Asia and sub-Saharan Africa, though its incidence has declined in recent years (Muzembo et al., 2023). Molecular epidemiological studies reveal high genetic diversity among *Shigella* strains, largely driven by horizontal gene transfer and adaptive evolution. Whole-genome sequencing has identified virulence genes, including *ipaH*, *virB*, and *icsA*, which facilitate epithelial cell invasion and intracellular movement (Connor et al., 2015).

Despite the availability of limited studies in Ethiopia, research on the epidemiology of *Shigella* spp. highlights its significant public health impact, particularly among children

under five and in regions with inadequate water, sanitation, and hygiene (WASH). The recent observation that there were regional trends of rising *Shigella* infections underscore the need for robust surveillance, improved WASH infrastructure, and antimicrobial stewardship programs to combat the burden of *Shigella* in Ethiopia (Ayele et al., 2023).

### *2.3.4. Pathogenesis and Clinical features*

The *Shigella* bacterium invades the intestinal epithelium by first crossing the mucosal barrier through specialized M cells of Peyer's patches. After translocation, it infects macrophages in the underlying tissue, where it triggers apoptosis to evade immune clearance. Using its type III secretion system and effector proteins, Shigella manipulates host cytoskeletal structures to enter epithelial cells from the basolateral side. Once inside, the bacteria spread laterally to neighbouring epithelial cells, causing localized tissue destruction and initiating inflammation, which contributes to the characteristic symptoms of shigellosis (Figure 4).

*Shigella* is a bacterium responsible for causing bacillary dysentery, a severe form of diarrhea (Halimeh et al., 2021). The infectious dose of *Shigella* varies by species, with as few as 10 cells required for infection with *S. dysenteriae*, up to 500 cells for *S. sonnei*, and as many as $10^8$ cells for *S. flexneri* in healthy adults (Bennish and Ahmed, 2020; Kotloff et al., 2018). After ingestion, the incubation period is typically 36 to 72 hours, although symptoms can appear within 12 hours, with severe dysentery often developing within two days (Percival & Williams, 2013).

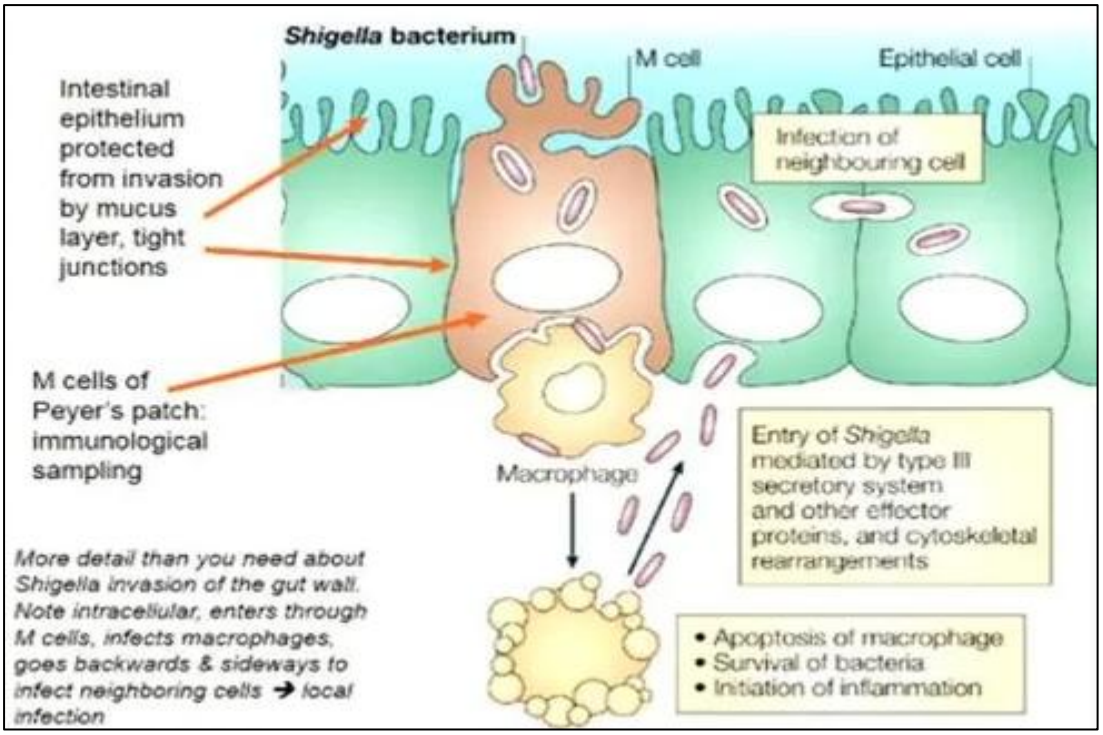


Figure 4. Schematics of *Shigella* pathogenesis. Adapted from Cota et al., 2015.

Shigellosis, or bacillary dysentery, is an acute intestinal disease characterized by the destruction of the colonic epithelium. Symptoms include bloody diarrhea, abdominal pain, mucus in stools, and fever. In individuals with compromised immune systems, such as those who are malnourished or HIV-infected, *Shigella* infections can lead to invasive complications, including meningitis, osteomyelitis, spleen abscess, and sepsis (Halimeh et al., 2021).

Shigellosis manifests with various symptoms, including diarrhea (which can be watery, mucoid, or bloody), fever, abdominal cramps, vomiting, and tenesmus. In severe cases, it can lead to malnutrition, cognitive impairment, stunting, rectal prolapse, encephalopathy, and HUS, which can be fatal (Muzembo et al., 2023). Asymptomatic carriers play a role in the transmission and persistence of *Shigella* within communities.

The severity of shigellosis is influenced by both the host's immune status and the virulence of the infecting *Shigella* strain. Some *Shigella* strains produce Stx, with *S. dysenteriae* type 1 being the most virulent of the two types (Percival & Williams, 2013). *S. sonnei*, on the other hand, is typically associated with milder infections. Disease severity also varies by strain, with *S. flexneri* often causing more severe symptoms. *S. boydii* and *S. dysenteriae* can result in infections of varying intensity, with *S.*

*dysenteriae* frequently causing severe epidemics. Asymptomatic carriers are a key factor in the spread of *Shigella*. *Shigella* infections can range from mild diarrhea, commonly caused by *S. sonnei*, to severe dysentery with bloody stools and vomiting, often caused by *S. dysenteriae* (Kotloff et al., 2018; Bennish, 2020).

## 2.3.5. Treatment Options and Prevention Strategies for *Shigella* spp.

### *2.3.5.1. Treatment Options*

The management of *Shigella* infections primarily focuses on rehydration and supportive care to prevent dehydration, particularly in children and older adults (Kotloff et al., 2018). Most cases are self-limiting, lasting 5-7 days, but antimicrobial therapy is recommended for severe, prolonged, or dysenteric infections to reduce symptom duration and limit transmission (Pérez-Schael et al., 2019). Fluoroquinolones (e.g., ciprofloxacin), azithromycin, and third-generation cephalosporins are commonly used, but increasing antimicrobial resistance poses significant challenges globally (Von Seidlein et al., 2017; Kotloff et al., 2018). Empiric therapy should consider local resistance patterns, and susceptibility testing is encouraged to guide treatment (Guerrant et al., 2021). In severe cases, hospitalization may be required to manage complications such as hemolytic uremic syndrome, sepsis, or severe dehydration. Novel therapeutics, including vaccine candidates and adjunctive therapies, are under investigation to reduce disease burden, especially in high-risk populations (Kotloff et al., 2018; Mani et al., 2020).

### *2.3.5.2. Prevention Strategies*

Prevention of *Shigella* infections relies on improving sanitation, hygiene, and safe food and water practices. Handwashing with soap, proper disposal of human feces, and ensuring access to clean drinking water are critical to interrupting fecal-oral transmission (Guerrant et al., 2021). Educational programs in schools and communities have been shown to reduce incidence significantly (Qadri et al., 2018). Food safety measures, such as proper washing and cooking of food, preventing cross-contamination, and avoiding raw or unpasteurized products, also play an essential role (Kotloff et al., 2018). Vaccination is an emerging preventive strategy. Several vaccine candidates, including live-attenuated and conjugate vaccines, are currently in clinical trials and show promise in protecting children and adults in endemic regions (Mani et al., 2020;

Qadri et al., 2018). Surveillance and antimicrobial stewardship are also vital to monitor resistance trends, detect outbreaks, and guide public health interventions (Von Seidlein et al., 2017).

## 2.4. *Campylobacter* spp.

### *2.4.1. Historical perspective and classification*

The history of *Campylobacter* infection dates to 1913 when McFaydean and Stockman first identified a curved-shaped microorganism in sheep and cattle, which was associated with abortion in these animals (Veron' & Chatelain, 1973). However, the bacterium remained unnamed until 1919, when Smith and Taylor isolated the same microorganism from bovine fetal fluids, naming it *Vibrio fetus* (*V. fetus*). In 1973, Véron and Chatelain reclassified *V. fetus* as *Campylobacter fetus* (*C. fetus*), and subsequently, the genus *Campylobacter* was established. Although *C. fetus* was recognized as a significant veterinary pathogen early on, its relevance as a cause of human infections did not become clear until the early 1980s. This shift in understanding came with the development of selective media that allowed for the isolation of *Campylobacter* from stool samples, leading to its identification as a major cause of human gastrointestinal disease (Man, 2011).

### *2.4.2. Microbiological characteristics*

*Campylobacter* is a Gram-negative bacterium that appears slender, curved, and exhibits a characteristic "gull-wing" shape. It is motile, oxidase-positive, catalase-positive, indole-negative, and does not utilize glucose as an energy source (Salim et al., 2014). *Campylobacter* spp. is known to be a fastidious, micro-aerophilic organism that require specific environmental conditions for growth. They thrive optimally at temperatures between 30$^{o}$C and 42$^{o}$C (Vegge et al., 2012). Depending on the species, *Campylobacter* may possess a single polar flagellum, bipolar flagella, or no flagella at all. These bacteria are non-spore-forming and typically range in size from 0.2 to 0.8 by 0.5 to 5μm. They are chemoorganotrophic, deriving energy from amino acids or intermediates of the tricarboxylic acid cycle. Most species grow under microaerobic conditions and rely on respiratory metabolism. However, certain species, such as *C. concisus*, *C. curvus*, *C.*

*rectus, C. mucosalis, C. showae, C. gracilis,* and *C. hyointestinalis*, require hydrogen or formate as an electron donor for microaerobic growth (Kaakoush et al., 2015).

The genus *Campylobacter* belongs to the family *Campylobacteraceae*, order *Campylobacterales,* class *Epsilonproteobacteria,* and phylum *Proteobacteria* (Kaakoush et al., 2015). Since its initial identification, the genus has expanded to include several pathogens that affect both humans and animals, classified primarily by phylogenetic analysis. The majority of the campylobacteriosis cases in humans are caused by *C. jejuni,* i.e., 89%, 8% by *C. coli,* and 3% by other species (Casalino et al., 2022).

Several virulence factors contribute to *Campylobacter* pathogenicity. These include flagella-mediated motility, regulated by the *flaA* and *flhA* genes; adherence to intestinal epithelial cells via proteins encoded by *cadF* and *docA* genes; invasion and survival within host cells, influenced by markers like *ciaB, iam*, *wlaN*, and *virB11*; and toxin production through the *cdt* genes (Wieczorek et al., 2013). Furthermore, the *hipO* gene, which encodes hippuricase, is crucial for differentiating *C. jejuni* from another *Campylobacter* spp. This enzyme catalyzes the hydrolysis of hippurate into benzoate and glycine, a distinctive feature of *C. jejuni* that is absent in closely related species like *C. coli*. As such, the protein encoded by *hipO* gene serves as a key marker for identifying *C. jejuni* in diagnostic settings (Van et al., 2018).

### *2.4.3. Epidemiology*

The *Campylobacter* spp. is one of the major zoonotic pathogens responsible for foodborne bacterial infections. Farm animals, particularly poultry, serve as the primary reservoirs for *Campylobacter* spp. and consumption of contaminated animal products is the most common route of transmission to humans (Rosner et al., 2017). Globally, *Campylobacter* spp. contributes significantly to bacterial food poisoning, accounting for approximately 8.4% of all global diarrhea cases (Igwaran & Okoh, 2019). While poultry, especially chicken, is a key source of infection, other risk factors include consumption of unpasteurized milk and contaminated water (Igwaran & Okoh, 2019). Contaminated drinking and recreational water, particularly when exposed to animal feces, have also been reported as sources of infection (Hyllestad et al., 2020).

The global prevalence of *Campylobacter* varies geographically. In industrialized countries, campylobacteriosis is a leading cause of bacterial gastroenteritis, primarily linked to contaminated poultry and raw milk consumption. In LMICs, *Campylobacter* infections are hyperendemic, particularly among children under five years, and are associated with poor sanitation and environmental exposure (Mughini-Gras et al., 2021). While infections from *Campylobacter* spp. are usually isolated in sporadic cases, outbreaks linked to unpasteurized milk or contaminated water have been documented. Person-to-person transmission is uncommon, although it can occur in cases of copious diarrhea, with an infective dose as low as 500 *Campylobacter* spp. cells being sufficient to cause illness (Salim et al., 2014).

Among the *Campylobacter* spp., C. *jejuni,* and *C. coli*, are the leading causes of bacterial gastroenteritis worldwide. Molecular studies have revealed that *C. jejuni* accounts for approximately 80-90% of human infections, while *C. coli* contributes to 10-20%, although the latter is often linked to pigs and other livestock (Sheppard, 2015). Other species, such as *C. fetus*, *C. upsaliensis*, and *C. lari*, are less common but may cause opportunistic infections in immunocompromised individuals. Genomic sequencing has identified several clonal complexes, such as *ST-21*, *ST-45*, and *ST-61*, as predominant lineages associated with human infections. These clonal complexes often display host-specific adaptations, with poultry serving as the primary reservoir (Cody et al., 2012). Moreover, the genomic diversity of *Campylobacter* isolates reflects their zoonotic origins, primarily from poultry, cattle, and contaminated water sources. Further characterization of virulence genes, such as *ciaB* (invasion) and *cdtA, cdtB, cdtC* (cytolethal distending toxin), play roles in pathogenesis among diarrheal patients (Wieczorek et al., 2013).

The prevalence of *Campylobacter* varies across African regions. Higher rates are reported in areas with intensive poultry farming and poor hygiene practices (Terefe et al., 2020). Livestock, including cattle and goats, also serve as significant reservoirs. Transmission in these regions is often zoonotic, facilitated by the close interaction between humans and animals. Waterborne outbreaks have also been documented in regions with inadequate water treatment facilities. In Ethiopia, *Campylobacter* spp. is a significant cause of foodborne illness, contributing substantially to the public health burden. It is estimated to cause approximately 2,380,000 illnesses annually, leading to

671 deaths. These infections result in a loss of 61,500 DALYs, a measure that combines the years of healthy life lost due to both premature mortality and the time lived in less than full health due to illness (Havelaar et al., 2022). This high disease burden underscores the importance of targeted interventions to improve food safety, sanitation, and hygiene practices in the country to mitigate the impact of *Campylobacter* spp. infections on public health. Moreover, *Campylobacter* spp. is frequently associated with diarrheal illnesses, particularly in rural settings in Ethiopia where poultry farming is widespread.

### *2.4.4. Pathogenesis and Clinical features*

*Campylobacter* pathogenesis begins with the ingestion of contaminated food or water, followed by bacterial attachment to intestinal epithelial cells using flagella and adhesins. The organism disrupts tight junctions and invades through paracellular and transcellular pathways, triggering inflammation via toll-like receptor activation and cytokine release. Despite host defence such as mucus secretion, antimicrobial peptides, and secretory IgA, *Campylobacter* can persist within cells, leading to intestinal damage, diarrhea, and systemic inflammation, and in some cases, post-infectious complications like Guillain–Barré syndrome (Figure 5).

Campylobacteriosis is characterized by fever, vomiting, and diarrhea, which can be either watery or bloody. The severity of infection varies, ranging from mild to severe, with symptoms typically beginning as watery diarrhea that may progress to bloody stools (Igwaran & Okoh, 2019). While infections caused by *C. coli* and *C. jejuni* tend to be more severe, other species of *Campylobacter* may cause milder symptoms, though cases are less frequently reported (Kaakoush et al., 2015).

The infective dose for *C. jejuni* is relatively low, needing only as few as 500 to 10,000 cells capable of causing illness, depending on the strain and host factors (Igwaran & Okoh, 2019). However, these authors indicated that human infections have also been linked to doses as low as 100 cells. The majority of *C. fetus* are mesophilic species, and are typically invasive, while *C. fetus* strains that are thermotolerant capable of growing at 42°C have also been isolated from infected individuals. However, thermophilic species like *C. jejuni* (which thrive at 42°C) may also invade and cause more severe

conditions such as meningitis, pneumonia, miscarriage, and Guillain-Barré Syndrome (GBS).

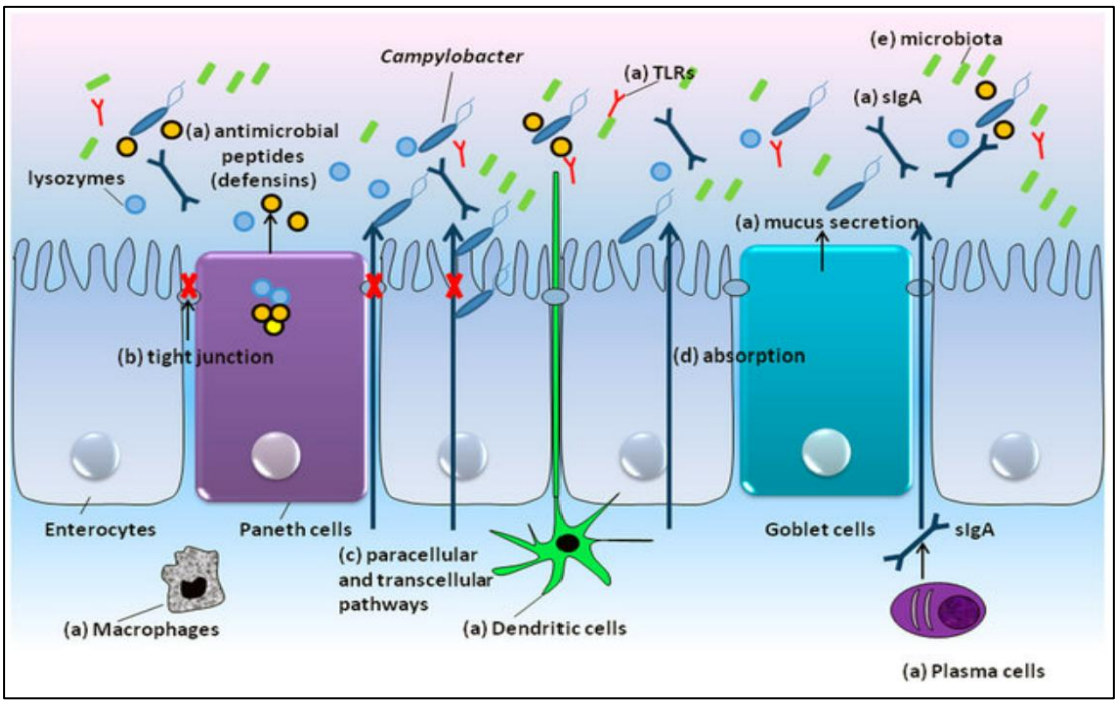


Figure 5. Schematics of pathogenesis of Campylobacter. Adapted from Awad et al., 2018.

Since their recognition as a leading cause of gastroenteritis in 1977, *Campylobacter* spp. has been linked to a range of severe gastrointestinal disorders, including inflammatory bowel disease, periodontitis, and functional gastrointestinal disorders (Kaakoush et al., 2015). Complications arising from *Campylobacter* infection include acute appendicitis, and systemic infections such as septic thrombophlebitis, endocarditis, neonatal sepsis, pneumonia, and bloodstream infections (Lagler et al., 2016). Post-infection complications, particularly GBS and Miller-Fisher Syndrome (MFS), significantly contribute to the overall disease burden (Scallan et al., 2015). Furthermore, *Campylobacter* infections have been associated with long-term gastrointestinal sequelae, such as colorectal cancer and Barrett's esophagus (Igwaran & Okoh, 2019).

## 2.4.5. Treatment Options and Prevention Strategies for *Campylobacter* spp.

### *2.4.5.1. Treatment Options*

Most *Campylobacter* infections are self-limiting, with symptoms resolving within 5-7 days. Supportive care, including hydration and electrolyte replacement, is usually sufficient (Kaakoush et al., 2015). Antimicrobial therapy is generally reserved for severe, prolonged, or systemic infections, such as bacteremia, or in high-risk populations, including immunocompromised individuals (Allos, 2017).

Macrolides, particularly azithromycin, are first-line therapy due to increasing resistance to fluoroquinolones (e.g., ciprofloxacin) in many regions (European Food Safety Authority, 2021). Fluoroquinolones may still be used in adults in areas with low resistance, but antimicrobial susceptibility testing is strongly recommended to guide therapy. Severe cases may require hospitalization for intravenous fluids and monitoring for complications, including Guillain–Barré syndrome, which can rarely follow infection (Kaakoush et al., 2015; European Food Safety Authority, 2021).

### *2.4.5.2. Prevention Strategies*

Preventive measures for *Campylobacter* infections focus on food safety, water sanitation, and personal hygiene. Proper cooking of poultry and meat, avoiding cross-contamination between raw and cooked foods, washing fruits and vegetables, and consuming safe drinking water are critical interventions (European Food Safety Authority, 2021; Kaakoush et al., 2015). Hand hygiene, particularly after handling animals, raw foods, or contact with potentially contaminated environments, reduces person-to-person transmission. Public health education programs targeting food handlers, farmers, and the general population are essential to reinforce these practices (EFSA, 2021). Farm-level interventions, such as improved biosecurity, vaccination of poultry, and the use of probiotics or bacteriophages, are being explored to reduce *Campylobacter* colonization in livestock and subsequent human exposure (Hermans et al., 2018). Surveillance systems and antimicrobial stewardship programs are also important to monitor trends in infection, AMR, and outbreaks (EFSA, 2021).

## 2.5. Risk factors associated with bacterial foodborne illnesses

According to the World Health Organization (WHO) Foodborne Disease Burden Epidemiology Reference Group (FERG), FBDs pose a significant public health burden in LMICs, where nearly 98% of the global FBD burden occurs. This burden is primarily attributed to biological hazards, particularly diarrheal pathogens (Havelaar et al., 2015). The remaining 2% of FBDs occur in high-income countries, where factors such as food safety regulations, water quality, and access to health care reduce the overall burden of these diseases. However, even in high-income countries, outbreaks of FBDs like *Campylobacter* and *Salmonella* continue to occur, though their impact is relatively lower compared to LMICs (WHO, 2015). The WHO African Region (AFR-E), FBDs are a major public health concern, with particularly bacteria contributing significantly to the disease burden, especially in countries with poor sanitation and limited access to clean water, such as Ethiopia (WHO, 2015).

Poor hygiene practices, limited maternal education, and inadequate living standards are linked to increased prevalence of diarrhea. Studies from Ethiopia and Nigeria reveal the complex interplay between environmental sanitation, hygiene behavior, and socioeconomic factors that heighten vulnerability to diarrheal diseases (Bhutta et al., 2008). In Ethiopia, food safety challenges are compounded by inadequate infrastructure, lack of clean water, insufficient washing facilities, weak enforcement of food safety regulations, and few incentives for producers to enhance safety standards (Gazu et al., 2023). Household hygiene and sanitation factors further increase the risk of diarrheal diseases; for example, inadequate nail hygiene, poor hand-washing practices, and lack of food safety knowledge have all been shown to correlate with higher rates of FBD in studies from countries like Indonesia (Lubis et al., 2019). Similar trends are observed in Nigeria, where it was found that poor handwashing before food preparation, blocked drainage, and fly breeding sites were risk factors for diarrhea (Oloruntoba et al., 2014).

Water, sanitation, and hygiene (WASH) factors are critical in LMICs, where an estimated 829,000 diarrhea deaths and 49.8 million DALYs were attributed to WASH-related factors in 2016 alone, with a significant burden on children under five (Pruss-Ustun et al., 2019).

Several studies within Ethiopia also showed how environmental and household factors affect risks of acquiring diarrheal diseases among vulnerable populations. In Addis Ababa, factors like a child's age, maternal education, timing of supplementary feeding, and hand-washing practices after childcare were associated with childhood diarrhea (Ayenew et al., 2019). In East Hararge, inappropriate toilet facilities, improper waste disposal, and unprotected water sources were risk factors for the acquisition of acute diarrhea in children (Gudeta, 2018). Similarly, research in Northern Ethiopia found low maternal education, larger family sizes, and lack of exclusive breastfeeding, to be significantly associated with childhood diarrhea (Asfaha et al., 2018). Moreover, in Southern Ethiopia, children of fathers without formal education were more likely to experience diarrhea, although with poor economic status was further identified for exacerbating this risk (Tarekegn & Enkuselassie, 2012). Children without access to household latrines were observed to have faced a significantly higher likelihood of developing diarrheal diseases compared to those with latrine access in the above Southern Ethiopia study. Furthermore, studies in Ethiopia's nomadic regions indicated that unprotected water sources, inadequate water services, human excreta exposure, and overcrowded living conditions correlated with higher rates of childhood diarrhea (Bitew et al., 2017). These findings align with a report from another research showing that living in overcrowded households, lack of vitamin A supplementation, and low awareness of hygiene and sanitation are also significant factors in diarrheal disease incidence (Gunsa et al., 2018).

## 2.6. Seasonal distribution of Foodborne bacterial pathogens selected for this study

The ongoing impact of climate change such as fluctuations in temperature, rainfall, humidity, storms, floods, and other extreme weather has intensified the spread of FBPs, more specifically those selected for this study (i.e., *Salmonella*, *E. coli*, *Campylobacter* and others). For instance, seasonal and temperature changes play a major role in shaping the prevalence and severity of foodborne illness (Balta et al., 2024). Temperature is a critical factor for *Salmonella* growth throughout the food chain, from contamination in raw food production to transportation and storage. *Salmonella* grows optimally at temperatures between 35°C and 37°C, leading to rapid proliferation at higher

temperatures; in contrast to lower temperatures where its growth is notably hindered by temperatures below 15$^{o}$C (Damtew et al., 2024).

Studies revealed a strong correlation between outdoor temperature fluctuations and *Salmonella* outbreaks. For instance, a 1°C increase in the mean weekly maximum temperature could lead to an 8.8% rise in weekly salmonellosis cases, while a similar increase in the mean weekly minimum temperature could result in a 5.8% rise in salmonellosis cases (Akil et al., 2014). Moreover, a review of the studies conducted from 1960 to 2010 reported that the monthly seasonality index for salmonellosis exhibited a broader curve, spanning from summer to early autumn (June to September) (Lal et al., 2012). The same review also indicated that the US experienced its peak in cases of salmonellosis first in June and July, followed by Canada and Europe in July, and then the UK and Asia in August. After these peaks, disease incidence in these regions declined for the remainder of the year. When adjusted for Northern Hemisphere seasons, the Oceanic region showed a peak in spring (February to March) and a smaller increase in autumn (October to November) (Lal et al., 2012).

In addition, high temperatures also facilitate FBPs' transmission to humans through multiple pathways. For instance, during warmer weather, contaminated foods have a higher concentration of *Salmonella*, especially at outdoor gatherings like barbecues where food handling and refrigeration may be insufficient. Furthermore, elevated temperatures can disrupt the cold chain, impacting food safety. Research has found that drinking water quality, rainfall, and gastroenteritis are interconnected, with positive correlations between temperature, humidity, and salmonellosis incidence (Davis et al., 2022). Increased rainfall can degrade water quality by washing sediment, pollutants, and animal waste into water sources. Severe flooding can also overwhelm waste-water treatment facilities, contaminating human, animal, and agricultural environments by FBP and exacerbating these health impacts (Butsch et al., 2023).

Furthermore, according to the recent report of the CDC, there is a significant association between STEC incidence and climatic factors. Specifically, each 1°C increase in maximum temperature corresponded with an additional three cases of STEC per 10 million people, while each millimeter increase in precipitation was linked to a rise of 1.1 cases per 10 million (CDC, 2024). Moreover, the seasonal distribution of STEC also varies by geographical area. For example, a study conducted in England on STEC

cases reported between 2009 and 2015 indicated that two-thirds of STEC cases were reported during the summer months (May to September), with the peak occurring in August each year (Elson et al., 2018). Although the majority of outbreaks (66.7%) took place in the summer, some outbreaks were also recorded during the winter months. Notably, the largest national outbreaks of STEC in the UK were documented between December 2010 and June 2011. Moreover, another review reported that the overall index indicated a significant peak in July, with a secondary peak in September in the UK (Lal et al., 2012). The UK and Canada peaked first in July, followed by Europe in August. The US also displayed a slight summer peak in July, but its main peak occurred in autumn (September). While North America, Canada, and the UK saw a decrease in incidence during winter, Europe maintained rates above the overall average during the autumn and winter months (October to January). Additionally, STEC was most prevalent during the rainy season in the Gambia and Mozambique and the summer and monsoon (heavy rain) seasons in Bangladesh and India (Chao et al., 2019).

The seasonality of shigellosis significantly varies by temperature and precipitation which in turn varies and months of the year and country. For instance, from 2002 to 2006, the shigellosis incidence exhibited seasonal peaks in spring and winter, with a winter predominance in Korea (Song et al., 2018). Moreover, the incidence positively correlated with temperature and precipitation, showing significant associations; for instance, a 1°C increase in temperature was linked to a 13.6% rise in incidence of shigellosis after two weeks, and a 1 mm increase in precipitation corresponded to a 2.9% increase after five weeks. During spring, temperature increases by 1°C led to a 16.1% rise in the incidence of shigellosis, while 1 mm precipitation increase caused an 8.4% increase (Song et al., 2018). Moreover, *Shigella* was most prevalent during the summer in Bangladesh and the hot/dry season in Mali (Chao et al., 2019). Furthermore, in Vietnam, two incidence peaks have been observed, with a primary peak during the rainy season and a secondary peak occurring either early or late in that season. In China, shigellosis incidence peaked from June to August, while in Zhejiang Province, the peak was recorded between July and October from 2005 to 2010 (Zhao et al., 2022).

Infections caused by *Campylobacter* also generally peak between July and September. The impacts of climate change, including warmer, longer summers, are expected to increase *Campylobacter* infections in humans (Davis et al., 2022). Associations have

been observed not only with temperature (Oberheim et al., 2020) but also with precipitation patterns preceding illness onset (Kuhn et al., 2020). Heavy rain and flooding can heighten exposure to fecal contaminated surface or drinking water, contributing to higher rates of *Campylobacter* infections and enteritis outbreaks (Hyllestad et al., 2020). Conversely, *Campylobacter* cases tend to decline following drought periods (Kuhn et al., 2020; Soneja et al., 2016). Additionally, summer activities such as barbecuing poultry and other meats or swimming in surface waters may indirectly increase infection rates by raising exposure risks (David et al., 2017). Hence, summer was the season where the infections of *Campylobacter* spp., *Salmonella* spp., STEC and other FBPs were significantly high in the study conducted in Canada (John et al., 2022). A systematic review indicated that the monthly seasonality index for campylobacteriosis reached its highest point during the summer months of July and August (Lal et al., 2012). The review indicated that, regionally, North America, the UK, Europe, and Canada all experienced peaks between June and August, with the UK peaking earliest in June and Canada the latest being in August. In the Northern Hemisphere, two peaks were observed: one in May and another in September. Moreover, fluctuations in monthly occurrences of FBPs was reported in Eastern Ethiopia where a peak of 37.1% was documented during the dry season (Gobena et al., 2024).

## 2.7. Antibiotic resistance (AMR) among three of the targeted Foodborne bacterial pathogens

The major public health threat undermining the effectiveness of essential antibiotics for treating infections and complicating critical medical procedures is AMR. The AMR contributes to millions of illnesses and deaths worldwide, with over 2 million infections and 23,000 deaths annually in the U.S., and 426,000 infections and 33,000 deaths in Europe (Yang et al., 2020). Additionally, AMR places a significant burden on healthcare systems, making it crucial to understand its causes and spread. Antibacterial resistance is a key component of AMR, escalating health and economic challenges, and threatening a future where bacterial infections may become untreatable. Major drivers of AMR include improper antibiotic use, poverty, poor sanitation, international travel, and increased healthcare interventions for vulnerable populations. Unlike diseases like malaria and HIV, AMR is not a single disease, but a result of bacterial colonization

rather than active infection. While colonization may or may not cause health issues, drug-resistant infections are the most immediate health threats. Resistance can spread across bacterial species, complicating AMR; this property calls for designing and requiring targeted strategies (Dunachie et al., 2020).

Antibiotic accessibility and regional healthcare policies influence the choice of treatment for diarrhea (Ugboko et al., 2020). Antibiotics for diarrhea are typically reserved for cases with bloody diarrhea or cholera with severe dehydration, yet they are often prescribed unnecessarily (Toner et al., 2015). Current treatment practices, especially early antibiotic administration, have fueled the rise of antibiotic-resistant bacterial serotypes, reducing the effectiveness of interventions. Resistance has been found in bacterial isolates from humans, animals, and environmental sources, driven by the overuse and misuse of antibiotics. This selective pressure facilitates gene transfer between bacterial strains originating from humans, animals, and the environment, making infections harder to treat and increasing reliance on last-line antibiotics, which may be inaccessible in some regions (Hedman et al., 2020).

The burden of AMR varies across different targeted FBPs and geographical regions. For instance, in Australia, a study found that 21.7% of NTS isolates exhibited phenotypic resistance to at least one antibiotic (Sia et al., 2021). The highest resistance rates were for ampicillin (17.3%), followed by tetracycline (14.3%), sulfathiazole (12.1%), and streptomycin (10.3%). Resistance to cefotaxime (6.2%), azithromycin (0.9%), and ciprofloxacin (1.2%) were comparatively low. More importantly, 13.5% of the isolates were MDR, where resistance to three or more classes were observed, with one isolate showing resistance to even meropenem (Sia et al., 2021).

In China, the highest resistance levels of NTS were recorded against cefazolin (86.21%), streptomycin (81.61%), ampicillin (77.01%), the ampicillin-sulbactam combination (74.71%), doxycycline (72.41%), tetracycline (71.26%), and levofloxacin (70.11%) (Chen et al., 2023). In addition, the study also reported that all their NTS isolates were not resistant to cefoxitin and amikacin. Among the serovars, those with higher prevalence, such as *S.* Enteritidis, *S.* Typhimurium, *S.* London, and *S.* Derby, exhibited notable resistance to multiple antibiotics. In total, 97.7% of the isolates showed resistance to at least one antibiotic, 83.91% being MDR as they were resistant to three or more antibiotics. Additionally, 77.01% were resistant to five or more antibiotics

(Chen et al., 2023). Another study in China found that 86.2% of the NTS isolates were resistant to multiple antibiotics, with high resistance rates for ampicillin (73.8%), tetracycline (65.5%), and nalidixic acid (43.2%) (Zeng et al., 2021). Resistance to clinically important antibiotics was concerning, with 21.6% of isolates resistant to ciprofloxacin, 12.9% to cefotaxime, and 5.7% to azithromycin. Notably, 4.0% of isolates, from nine serovars, were resistant to both ciprofloxacin and cefotaxime, with 1.7% also resistant to azithromycin. Decreased susceptibility to ciprofloxacin was seen in 74.9% of isolates, with *S.* Enteritidis as the dominant serovar. Overall, 46.6% of isolates were classified as MDR. Similarly in Taiwan, the majority of NTS isolates showed resistance to ampicillin (92.16%), none were resistant to piperacillin-tazobactam or amoxicillin-clavulanic acid. The cephalosporin resistance was low, with cefepime, ceftriaxone, and ceftazidime showing resistance rates of 13.73%, 19.61%, and 17.65%, respectively. The MDR was observed in 47.06% of strains (Gong et al., 2022).

In Ethiopia, antibiotic resistance among NTS and *Shigella* isolates shows significant variation across different regions. For example, in Addis Ababa, the highest resistance in NTS isolates was observed to streptomycin (74.6%), nitrofurantoin (40.3%), and sulfamethoxazole-trimethoprim (38.8%) (Kebede et al., 2021). In the same study, resistance to two or more antibiotics was found in 64.2% of isolates, and 25.4% displayed resistance to five or more antibiotics. Another study from Addis Ababa reported resistance to sulfamethoxazole-trimethoprim (21.4%), tetracycline (21.4%), and ampicillin (14.3%) among NTS isolates, while all *Salmonella* isolates were susceptible to amikacin, ciprofloxacin, and other antibiotics. In Debre Markos, *Salmonella* isolates showed a high resistance rate (85.7%) to both ampicillin and amoxicillin-clavulanic acid, and *Shigella* isolates also exhibited high resistance to these antibiotics (78.9% and 73.7%, respectively) (Dessale et al., 2023). The same study reported that the overall MDR rate was 88.5% for *Salmonella* and *Shigella* isolates.

In general, the current trend is that the prevalence of antibiotic-resistant *Salmonella* is on the rise, posing significant challenges to public health efforts aimed at controlling outbreaks (Jajere et al., 2019). Specifically, the distribution of NTS serotypes and the rise of MDR strains have significant public health implications. In Ethiopia, as in other African countries, the high prevalence of MDR NTS strains limits treatment options,

leading to prolonged illness and increased risk of invasive disease (Eguale et al., 2015). Factors such as HIV infection, malnutrition, and co-infection with other pathogens further exacerbate the burden of antibiotic resistant NTS infections (Tadesse, 2014) since NTS strains are found to increasingly exhibit resistance to commonly used antibiotics such as ampicillin, tetracycline, sulfamethoxazole-trimethoprim, and even MDR. This trend is of great concern as it is the principal cause of treatment failure that requires the need for seeking for alternative therapeutic options, which are very limited in the country (Tadesse et al., 2014).

In the absence of efficacious alternatives therapeutic options, MDR NTS strains may increase the risk of invasive disease and fatal outcomes (Okoro et al., 2012). Molecular typing methods have uncovered the spread of MDR strains, including ESBL-producing *Salmonella* strains, which are resistant to multiple classes of antibiotics and pose a significant public health threat all over the world. The genetic analysis of these MDR strains is critical for tracking their emergence, persistence, and dissemination in both human and animal populations. High levels of MDR and ESBL production have been reported, with a strong link to plasmid-mediated resistance genes such as bla-CTX-M and *qnr* (Hedman et a., 2020).

On the other hand, the level of antimicrobial resistance, including MDR, by *Shigella* strains are on the rise and higher than *Salmonella* spp., exacerbating treatment challenges. For instance, *Shigella* isolates in Ethiopia consistently demonstrated higher antibiotic resistance rates compared to *Salmonella*. For example, in Addis Ababa, 50% of *Shigella* isolates were resistant to two or more antibiotics, while only 5.4% of *Salmonella* showed similar resistance (Ararsa et al., 2023). The same study reported that *Shigella* also exhibited increased resistance to the tested antibiotics than *Salmonella*, for example, tetracycline (33.3% vs. 5.6%) and amoxicillin-clavulanic acid (20.8% vs. 0%); in addition to having a wider diversity of resistance profiles to more other antibiotics. Another study in Adama reported that the resistance rates of *Shigella* were also high, for example, with 69% of isolates being resistant to both ampicillin and tetracycline, and 61.9% resistant to ciprofloxacin (Teshome et al., 2019). Among the *Shigella* spp., tested, *S. flexneri* demonstrated the highest resistance to multiple antibiotics, followed by *S. dysenteriae*. Overall, these studies indicate that *Shigella*

isolates tend to have higher resistance rates and more diverse resistance patterns than *Salmonella*, and MDR is a significant concern across multiple regions in Ethiopia.

Antibiotic treatment for STEC infections is controversial, as antibiotic-induced stress can increase Stx production. However, common antibiotics such as azithromycin, fosfomycin, and meropenem are being considered for treating early-stage STEC infections, particularly for *E. coli* O157 (Mir et al., 2020). While these antibiotics can prevent Stx release and kidney failure, rising resistance among STEC isolates threatens these treatment options and facilitates the spread of antibiotic resistance genes, especially if they are located on mobile genetic elements like plasmids. In addition, antibiotic exposure of food animals can lead to resistance in STEC strains (Mir et al., 2020), and *E. coli* also acts as both a donor and recipient of resistance genes in the enterobacterial gene pool, which further complicates the situation (Puvaca & Frutos, 2021).

In Ethiopia, various studies have shown different levels of antibiotic resistance among *E. coli* isolates. For example, in one study *E. coli* exhibited significant antibiotic resistance levels to ampicillin (86.8%), tetracycline (76%), and sulfamethoxazole-trimethoprim (76%), while resistance to ciprofloxacin (6.9%) and norfloxacin (9.3%) was comparatively low (Adugna et al., 2015). Another study from Addis Ababa indicated that *E. coli* isolates had 83.6% resistance to ampicillin and amoxacillin-clavulanic acid, and 62.3% to sulfamethoxazole-trimethoprim (Melese et al., 2019). Most recent studies in Addis Ababa reported that all STEC isolates were resistant to ceftazidime and cefotaxime. Moreover, 3.8% and 10% of STEC were MDR and ESBL positive, respectively (Zelelie et al., 2023). Another study in Amhara region of Northern Ethiopia, STEC O157 isolated from diarrheic patients exhibited high levels of antibiotic resistance, with 54.7% resistant to tetracycline, 53.1% to amoxicillin-clavulanic acid, and 43.8% to sulfamethoxazole-trimethoprim (Engda et al., 2023). However, the study also documented lower resistance against chloramphenicol (9.4%), norfloxacin (9.4%), and ciprofloxacin (12.5%). Notably, 11.7% of isolates were susceptible to all the antibiotics tested. In contrast, 43.8% of isolates exhibited MDR. Of these, 3.1% were resistant to six classes, and 0.8% to seven antibiotic classes.

Additionally, a study conducted in Southern Ethiopia, Shashemene, reported that *E. coli* O157 showed high resistance to ampicillin (100%), amoxicillin-clavulanic acid

(89.5%), tetracycline (57.9%), ertapenem (31.6%), and meropenem (31.6%), while all isolates were susceptible to ciprofloxacin (Ayalneh et al., 2024). Resistance levels varied, among the ESBL-positive isolates where 59.25% were resistant to tetracycline, and while the carbapenemase-positive isolates exhibited 91.7% resistance to amoxicillin-clavulanic acid. Overall, 84.2% of the isolates demonstrated resistance to three or more antimicrobial classes, 34.2% of them being resistant to four classes and one isolate resistant to seven classes. Resistance mechanisms in bacteria include the production of ESBLs and carbapenemases. ESBLs are plasmid-mediated enzymes that can hydrolyze a range of beta-lactam antibiotics, including penicillins, cephalosporins, and aztreonam. These enzymes are classified into three main groups: bla-TEM, bla-SHV, and bla-CTX-M. Carbapenemases are a major class of beta-lactamases capable of breaking down penicillins, cephalosporins, monobactams, and carbapenems. The carbapenemase classes include B (e.g., bla-IMP and bla-VIM), D (e.g., bla-OXA-23 to -27), and A (e.g., bla-IMI, bla-KPC, bla-NMC, and bla-SME) (Swedan & Alrub, 2019).

Despite accumulated evidence on the significant contribution of beta-lactamase genes to disease burden, information on their distribution in Ethiopia remains limited. For example, in a study by Zenebe et al. (2023), all phenotypically ESBL positive DEC pathotypes were found to carry at least one beta-lactamase gene. Among the five key beta-lactamase genes tested, bla-CTX-M, bla-TEM, bla-SHV, bla-NDM, and bla-OXA-48, the most prevalent was bla-TEM, detected in 80% of the ESBL-DEC isolates. This was followed by bla-CTX-M in 73% and bla-SHV in 60% of the strains. Alarmingly, 33% of the ESBL-DEC strains harbored a combination of the three primary beta-lactamase genes (bla-TEM, bla-CTX-M, and bla-SHV) (Zenebe et al., 2023). Another very recent study in Ethiopia indicated that 79% of the *E. coli* O157:H7 isolates harbored the bla-TEM gene, 63% carried bla-CTX-M, and 10% possessed bla-SHV (Ayalneh et al., 2025). The same study also indicated that 12 isolates (31.6%) were identified as carbapenemase producers, with 66.6% of these carrying the bla-KPC gene, while none were positive for bla-NDM. These findings highlight a significant presence of MDR *E. coli* O157:H7 strains in the region, posing a considerable public health concern.

In conclusion, as demonstrated by various studies worldwide and in Ethiopia, the complexity and interconnection nature of FBPs, including their virulence and antibiotic

resistance mechanisms through horizontal gene transfer, calls for regular follow-up and large-scale studies. Moreover, previous analyses of risk factors, seasonal distribution, and AMR pattern of FBPs in Ethiopia revealed significant seasonal variations, where incidence of certain pathogens peak during the dry and long rain seasons while other are more favored by heavy precipitation. Key risk factors such as water sources, animal exposure, and hygiene practices play a crucial role in pathogen prevalence and AMR patterns.

# CHAPTER THREE: MATERIALS AND METHODS

## 3.1. Study Area

The study was conducted among diarrheic patients who submitted physician-requested stool samples to the clinical laboratories at three hospitals in Ethiopia: 1) Yekatiti-12 Hospital, a Specialized teaching Hospital in Addis Ababa; 2) University of Gondar Comprehensive Specialized Hospital, a referral and teaching hospital in Gondar; and 3) Hiwot Fana Comprehensive Specialized Hospital, a teaching hospital in Harar. These study sites were selected because they serve a large and diverse population and are representative of the three predominant geographic areas in Ethiopia: central (Addis Ababa), north (Gondar), and south-eastern (Harar).

The catchment area for Yekatit-12 Hospital was defined to be the sub-cities of Gulele, Yeka, and Arada. The catchment area for the University of Gondar Comprehensive Specialized hospital includes Gondar town and surrounding districts woredas (Sabiya Sabina, Diba Defecha, Fenter, Teda town, Azezo Teklehaimanot, Mariam Debir Deresgie, Loza Mariam, Killil Rufaelna ena killil Eysesus, Dabrka, Aba intones, and Anchew Michael). The catchment area for Hiwot Fana Comprehensive Specialized University Hospital covers the town of Harar and the adjacent districts of East Hararge Zone of Oromia including Jarso, Gursum, Kombolcha, Maya (the former name Haramaya), Fedis and Babile (Figure 6).

Yekatit-12 Hospital is a specialized and teaching hospital found in Addis Ababa. The hospital serves an average of 5 million population. University of Gondar College of Medicine and Health Science and Comprehensive Specialized Hospital is located in the north-west of Ethiopia, about 750km from the capital city, Addis Ababa. It is a referral and teaching hospital, serving around 3.5 million people. It is one of the government hospitals in Amhara Regional State. Hiwot Fana Specialized University Hospital was established in 1933 EC (1940 GC), and it was recently structured as Specialized University Teaching Referral Hospital under Haramaya University. Currently, the hospital provides Referral and routine health services for a total of 5-6 million people.

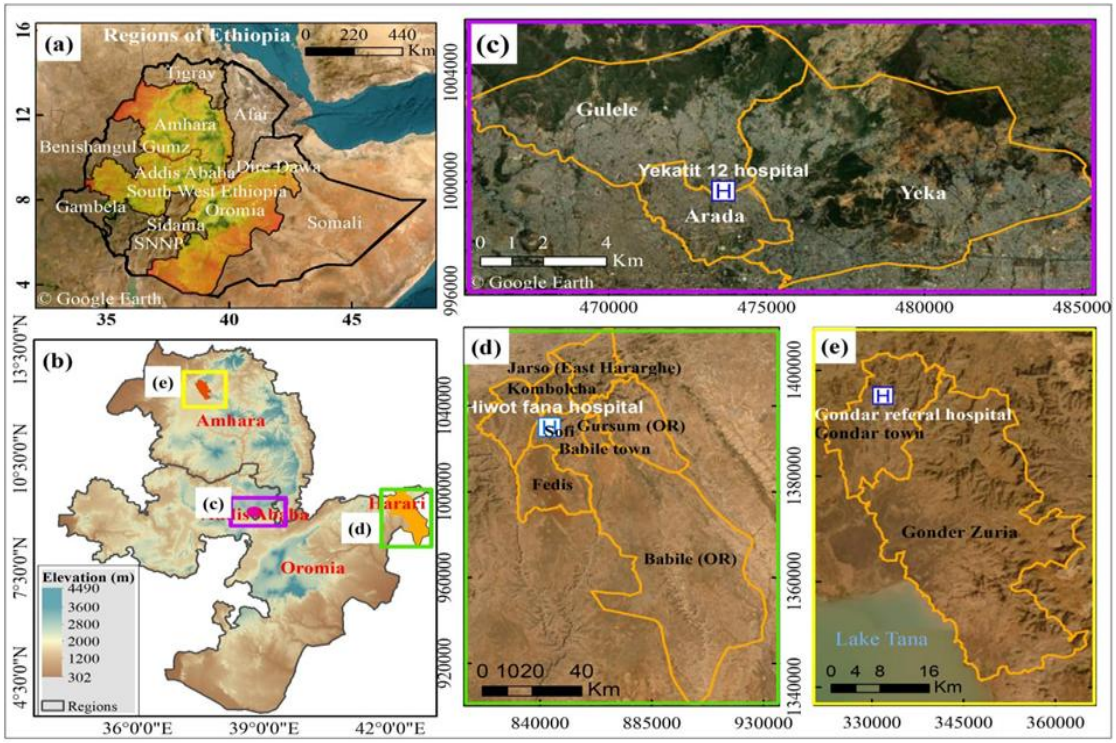


Figure 6. Study area of sample collection in Ethiopia, east Africa (a), from the three target regions (b), and the three hospitals, Yekatit 12 hospital (c), Hiwot Fana hospital (d) and University of Gondar Comprehensive Specialized hospital (e). (Source of the administrative boundaries from the central statistics agency of Ethiopia; background google source data: Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community).

## 3.2. Study Design and Period

This study was parts of the mother project known as TARTARE, a multi-component, risk-based program aimed at assessing and managing risks from *non-typhoidal Salmonella* (NTS), diarrheagenic *Escherichia coli* (DEC), and *Campylobacter* in raw beef and dairy in Ethiopia. The research design employed a combination of epidemiological, microbiological, and intervention-based approaches to generate evidence on burden, transmission, and control strategies. First, descriptive epidemiology was conducted through retrospective analysis of laboratory-confirmed cases from sentinel university laboratories (Addis Ababa University, Haramaya University, and the University of Gondar) to estimate confirmed infections and geographic distribution of the target pathogens. Prospective cross-sectional study was also conducted to assess the prevalence of the targeted pathogens among diarrheal

patients in those targeted hospitals. Furthermore, to address underreporting and capture broader impacts cross-sectional surveys were administered in communities and among healthcare workers to generate multipliers for surveillance data, adjust for care-seeking behaviors, and estimate household and health system level economic costs. Hence, to complement the epidemiological data, microbiological sampling and quantitative risk assessment were performed along beef and dairy value chains to measure contamination levels and characterize exposure. The program further incorporated economic and socio-cultural analyses, explicitly considering gender dimensions and cost-effectiveness of interventions to ensure relevance and sustainability. Finally, a series of stakeholder engagement and participatory risk-ranking exercises were undertaken with Ethiopian policymakers and decision-makers to prioritize risks, evaluate intervention options, and establish a framework for evidence-based, risk-based food safety management in Ethiopia.

As part of this large food safety project in Ethiopia, a prospective cross-sectional study design was conducted within the epidemiological component of TARTARE project. Participants were enrolled from October 2021 to November 2022 to capture seasonal variation and enhance representativeness.

## 3.3. Source Population

- The source population for this prospective cross-sectional study consisted of all patients visiting the three hospitals during the study period. From this population, eligible participants were systematically recruited based on the predefined inclusion and exclusion criteria to ensure representativeness of patients presenting with diarrheal illness for combining the finding with other components of the TARTARE project.

## 3.4. Study Population

- The study population was patients who visited the three hospitals and had signs and symptoms of gastrointestinal illness during the study period.

## 3.5. Study Participants

- The study participants were diarrheic patients from the hospital catchment areas who visited the clinical laboratories of these hospitals to provide stool

samples for the physician requested stool examination (e.g., bacterial culture and antibiotic susceptibility testing, direct wet mount or stool microscopy for parasite detection) during the study period.

## 3.6. Sample Size Determination

The single population proportion formula was used to calculate the sample size with the estimated population proportion of 50%. Although there are fragmented studies focusing on individual pathogens, we did not find any study that simultaneously investigated all the pathogens targeted in our work. To ensure sufficient power for comparing prevalence across geographic locations, seasons, clinical features, and environmental exposures, we used a large sample size.

$n= (Z\alpha/2)^2 * p(1-p)/(e)^2$

Where p=population proportion (50%), $Z\alpha/2=1.96$ at a 95% confidence interval, and E=0.05 (acceptance margin) (Lwanga & Lemeshow; WHO). The 384 per site (three sites), per season (with the assumption of collecting for dry and rainy seasons) gives a total of 384*2= 768 participants per site. The overall sample size becomes 768*3 (number of sites) =2304.

This conservative strategy (p=50%) was chosen for three reasons: (1) multiple primary endpoints, the study targets four pathogen groups with differing prevalence and the least common outcomes (e.g., *Salmonella* isolates) would be underpowered if we used only a single low pooled prevalence for calculation; (2) multivariable modelling, the analysis planned evaluation of ≈11 candidate predictors (age category, household livestock, water source, sex, residence, season, toilet type, etc.); following events-per-variable guidance we planned sample size so that even the less common outcomes are more likely to meet events per variable (EPV) criteria; and (3) AMR/isolate objectives: according to the WHO GLASS manual also emphasize that AMR studies require sufficient sample and isolate numbers to estimate resistance proportions (WHO, 2020). Hence, AMR analysis is conditional on recovered isolates and therefore benefits from a larger screened sample to increase the probability of obtaining sufficient isolates for precise resistance estimates. Full sensitivity calculations (alternative pooled prevalence, EPV scenarios, isolate-based requirements, and design-effect/non-response adjustments) are also provided in the Appendix (see Appendix I).

## 3.7. Inclusion and Exclusion Criteria

### *3.7.1. Inclusion criteria*

- Clinically confirmed diarrheic patients of all ages, and determined by the need the stool examination (i.e., stool microscopy, culture, and antibiotic susceptibility testing) requested by the physicians who came from the catchment area that was selected for a mother project TARTARE, and willing to participate in the study were eligible for inclusion.

### *3.7.2. Exclusion criteria*

- Diarrheic patients who took antibiotics within the last two weeks to avoid false-negative cultures and confounding of antimicrobial resistance patterns, ensuring that detected pathogens accurately reflect the circulating resistance in the population (WHO GLASS manual, 2020).
- Patients who provide solid stool

## 3.8. Recruitment of Study Participants

Eligible patients visiting the clinical laboratory were recruited for the study after providing written informed consent, with parental consent obtained for participants under 18 years of age and assent for children aged 12 to 17 years. All the informed consents, parental consents and assents were translated into the local languages (Amharic and Afan Oromo). Participants completed a pre-tested survey through interviews conducted by trained study team members as part of the TARTARE food safety project, which aimed to assess various aspects of food safety and public health. The survey included questions on socio-demographic characteristics (e.g., age, gender, marital status, education, and monthly income); current diarrheal illness (e.g., symptoms, duration, treatment); and environmental exposures (e.g., water sources, sanitation facilities, availability of domestic animals in the household) (see Appendix II).

## 3.9. Sampling Method and Procedure

Purposive sampling technique was employed in this study. Briefly, patients who met the predefined inclusion criteria were consecutively recruited from the respective study sites throughout the specified study period.

## 3.10. Study variables

### *3.10.1. Dependent variables*

- Prevalence of STEC, *Salmonella spp.*, *Shigella spp.*, and *Campylobacter spp.*, among diarrheal patients.

### 3.10.2. *Independent variables*

- Socio-demographic (e.g., age, gender, household income); months of the year divided into dry (October to February), short rainy (March to May), and long rainy seasons (June to September); study sites, and environmental exposure (e.g., animal, water, types of toilets) were collected.

## 3.11. Data Collection

### *3.11.1. Sample collection and transportation*

All stool specimens were collected before the study participants took any antibiotics. Clear instructions were given for proper specimen collection for study participants. For the participants who could not collect specimens by themselves, such as children, their families or guardians collected their specimens. Care was also taken to prevent specimen contamination with urine. Specimen containers or collection devices were labeled with the patient's full name, medical record number, age, date of collection, and initial of collector.

Stool specimen collected using a clean screw cap container was processed within 2hrs of collection. We have been targeting 4, 5, and 3 sample collection sites at Yekatit-12 hospital, University of Gondar referral hospital, and Hiwot Fana Hospital respectively. Moreover, we used Haramaya University College of Health Sciences microbiology laboratory (found outside the Hiwot Fana Hospital) for analysis of the samples collected from Hiwot Fana hospital. We checked the feasibility of transporting samples from sample collection sites to the microbiology laboratory for analysis, and it was difficult to transport samples within 2 hrs of collection. Hence, the specimen was placed into Cary-Blair transport medium immediately after collection and refrigerated at 2-8$^{\circ}$C until transported to the laboratory for processing. All the specimens were transported to the laboratory two times a day; those samples collected in the morning was transported to the microbiology between 11:30am to 12:30pm, and those samples

collected during the afternoon were transported to the microbiology laboratory between 4:30 to 5:30pm. A cold chain transport system (cold box with an ice pack and a digital thermometer inside) was used to deliver all specimens to the microbiology laboratory. All the leftover stool specimens were transported following standard safety precautions, and a triple package was used for transportation from the collection sites to the site of analysis (Ethiopian Public Health Institute) for further analysis. Additionally, the target bacteria were isolated in the site laboratories; and the isolates were transported using a triple packages transportation system to the Ethiopian Public Health Institute for further analysis and long-term storage. The remaining sample after allocation to the Cary-Blair transport medium was stored in the freezer for any additional research work. This was part of the consent process where participants and or their parents were asked for their permission to store the stool sample (see Appendix III to XI).

### *3.11.2. Bacterial isolation and identification*

Stool samples were obtained according to physician's request and tested for STEC, *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. using standardized protocols that included bacterial culture, biochemical tests, enzyme immunoassay, and polymerase chain reaction (PCR). Submitted stool samples were screened and excluded if the sample was unlabelled or mislabelled; or if placed in a leaking container (see Appendix XII).

#### *3.11.2.1. Isolation and presumptive identification of STEC*

For isolation and identification of STEC, portions of the stool sample were inoculated onto ChromSTEC agar (ChromSTEC, France) using a 10µl plastic inoculating loop and incubated aerobically at 35±2°C overnight. The growth of colonies with mauve color, due to the tellurite resistance of STEC was reported as the presumptive STEC as indicated by the manufacturer's instruction (CHROMagar, 2024) (see Appendix XII).

#### *3.11.2.2. Isolation and presumptive identification of Salmonella and Shigella spp.*

For the the purpose of enriching the scanty *Salmonella* and *Shigella* available in the stool that is dominated by commensal bacteria, portions of stool samples were inoculated into Selenite-F broth using a 10µl plastic inoculating loop and incubated at 35±2°C overnight (Garcia, 2008). For isolation and presumptive identification of these bacteria, the samples from the enriched broth were inoculated onto MacConkey (MAC,

Difco™, USA), and Xylose-Lysine-Deoxycholate (XLD, Difco™, USA) agar plates using the 10µl plastic inoculating loop and then incubated aerobically at 35±2°C overnight. Colonies were examined for *Salmonella* and *Shigella* spp. using such colony characteristics like hydrogen-sulfide production on XLD and non-lactose fermentation on MAC for *Salmonella* spp; reddish, small, and moist on XLD and non-lactose fermentation on MAC for *Shigella* spp. Biochemical analyses were conducted using Triple Sugar Iron Agar (TSIA, Difco™, USA); Lysine Iron Agar (LIA, Difco™, USA); Simmon Citrate Agar (SCA, Difco™, USA); Urea Agar (UA, Difco™, USA); and Sulfide Indole Motility Agar (SIMA, Difco™, USA). Identification was based on standard biochemical characteristics (Garcia, 2008) (see Appendix XII).

#### *3.11.2.3. Detection of Campylobacter spp*

For the detection of *Campylobacter,* stool samples were tested using an enzyme immunoassay technique, *Campylobacter* Quik Chek™ test (*Campylobacter* QUIK CHEK, USA) (TECHLAB, 2024). The *Campylobacter* QUIK CHEK™ test is a rapid membrane enzyme-linked immunosorbent assay for the qualitative detection of a *Campylobacter*-specific antigen in human fecal specimens. The *Campylobacter* QUIK CHEK™ test is designed to detect *C. jejuni, C. coli*, *C. lari,* and *C. upsaliensis* from patients with signs and symptoms of gastroenteritis. The test is intended for use with preserved fecal specimens in transport media and unpreserved fecal specimens. The *Campylobacter* QUIK CHEK™ test uses antibodies that recognize a *Campylobacter*-specific antigen in human fecal samples. The device contains a Reaction Window with two vertical lines of immobilized antibodies. The test line ("T") contains antibodies against a *Campylobacter*-specific antigen. The control line ("C") contains anti-IgG antibodies. The Conjugate consists of antibodies to a *Campylobacter*-specific antigen coupled to horseradish peroxidase. Briefly, diluent (750µL) was added to the test tube; one drop of conjugate was added to each tube containing the diluent; and the stool specimen (25µL) was added into the tube of diluent-conjugate mixture. The diluted specimens were vortexed for 5 to 20 seconds and incubated for 30 minutes at room temperature. The diluent-conjugate-stool mixture (500µL) was then transferred into the sample well of the membrane device and incubated for 15 minutes at room temperature. During the incubation, the *Campylobacter*-specific antigens in the sample bind to the antibody-peroxidase conjugate. The antigen-antibody complexes migrate through a filter pad to a membrane where they are captured by the immobilized anti-

*Campylobacter* antibodies in the line. Wash buffer (300µL), and 2 drops of substrate were added before examining the test result. The appearance of two blue lines (one for the control and one for the test) within 10 minutes was indicative of *Campylobacter* positive results (see Appendix XII).

### *3.11.3. Molecular Detection of Targeted Pathogens*

Molecular confirmation of targeted pathogens was conducted using MIC qPCR targeting the respective virulent genes, *stx1* or *stx2* and *eae* for STEC; *invA* for the genus *Salmonella*, and *SM4200* for *S.* Typhimurium, and *SEN1396* for *S.* Enteritidis; *ipaH and lacY* for *Shigella* spp*; 16S rRNA* for the genus *Campylobacter*, and *hipO* and *glyA* for *C. jejuni and C. coli* respectively by using the pairs of primer and probes listed in Appendix XII-Table 1. (Bio molecular system, 2016) at national reference laboratory of Ethiopian Public Health Institute.

#### *3.11.3.1. DNA Extraction*

DNA was extracted from a pure colony (STEC, NTS, and *Shigella* spp.) using the boiling method (Junior et al., 2016). Briefly, colonies were suspended in sterile nuclease-free water (250µl) and centrifuged at 14,000 rpm for 10 minutes, and the resulting supernatant was discarded while the pellet was maintained for the downstream works. Pellets were resuspended in 250µl nuclease-free water; boiled at 100°C for 10 minutes; cooled for 15 minutes; and centrifuged at 14,000 rpm for 10 minutes. The supernatant was used as DNA templates, and the amount and purity of DNA were measured using a NanoDrop apparatus (Thermo Fisher Scientific, 2024). However, DNA extraction for *Campylobacter* spp., was conducted from frozen stool samples using the Fast Stool DNA Kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocol (Qiagen GmbH, 2024).

#### *3.11.3.2. PCR Protocol for Amplifying Genes of Interest of the Target Pathogens*

The assay followed ISO/TS 13136 guidelines ISO/TS 13136, 2012 guidelines, with a 20µl reaction volume containing primer pairs, dual-fluorescent-labeled probes, and a master mix, with 1µl of DNA template (ISO/TS 13136., 2012). The PCR run condition for STEC was as follows: pre-amplification at 95°C for 2 minutes, followed by 50 cycles of denaturation at 95°C for 10 seconds, and annealing at 60°C for 10 seconds, and extension at 72°C for 15 minutes. The 20µl reaction volume comprised primer pairs

(1µl each), probe (1µl), master mix (12.5µl), nuclease-free water (0.5µl), and template (1µl). *E. coli* O157 (ATCC 35150) serotype served as the positive control, while nuclease-free water was used as the negative control. The presumptive STEC isolates that found positive for *stx1* or *stx2* or both were considered as STEC positive. Confirmation of STEC for the *eae* gene was conducted following the same protocol described above. Then, stool samples positive for *stx1* or *stx2* and *eae* were considered as *eae* harboring STEC positive which is a subset of STEC that is associated with severe illness and complications (FAO/WHO STEC Expert Group, 2019).

The PCR run conditions for the confirmation of *Salmonella* spp., using the targeted gene shown in Appendix XII-Table 1, followed a monoplex PCR protocol adapted from Heymans et al. (2018). The thermal cycling conditions were as follows; an initial denaturation stage at 95˚C for 2 minutes, followed by denaturation at 95˚C for 10 seconds, and annealing/extension at 60˚C for 60 seconds. These reactions were repeated for 50 cycles (Heymans et al., 2018). A reaction volume of 20µl, comprising primer pairs (1µl), probes (1µl), IAC template (1µl), master mix (10µl), nuclease-free water (2µl), and DNA template (1µl). Incorporation of the Internal Amplification Control (IAC) served to identify PCR inhibitors and minimize false-negative results, while *S.* Typhimurium (ATCC®14028) and *S.* Enteritidis (ATCC®13076) serotype was used as positive controls, and nuclease-free water acted as a negative control. The isolates were confirmed as *Salmonella* spp. by detecting the amplified *invA* gene, followed by serotyping to *S.* Typhimurium and *S.* Enteritidis by detecting the amplified *STM4200* and *SEN1392* genes respectively.

The PCR run conditions for *Shigella* spp. were set according to a previously used protocol (Lin et al., 2010) with modifications to optimize detection of *Shigella* spp. Since enteroinvasive *Escherichia coli* (EIEC) also found positive for the *ipaH* gene, a Touchdown PCR approach was implemented to enhance both specificity and sensitivity (Korbie & Mattick, 2008). The 20µl reaction volume comprised primer pairs (1µl), probe (1µl), master mix (12.5µl), nuclease-free water (0.5µl), and template (1µl). The cycling conditions for the PCR began with an initial denaturation step at 95°C for 3 minutes. This was followed by 5 cycles of amplification, each consisting of denaturation at 95°C for 10 seconds, annealing at 58°C for 20 seconds, and extension at 72°C for 15 seconds, then, 40 cycles of denaturation at 94°C for 10 seconds, annealing at 55°C for 20 seconds, and extension at 72°C for 15 seconds. The

Touchdown strategy, with a higher annealing temperature in the first few cycles, reduced non-specific amplification and preferentially amplified the target *Shigella* sequences. For the quality control, *S. dysenteriae* ATCC 13313 was used as positive control, and nuclease-free water as the negative control. The isolates were confirmed as *Shigella* spp. by detecting the *ipaH* gene, followed by the absence of *lacY* gene.

The PCR run conditions for *Campylobacter* spp. were set as follows; an initial denaturation at 95°C for 10 minutes, followed by 50 cycles of 95°C for 15 seconds and 55°C for 60 seconds. The 20µl reaction volume comprised primer pairs (1µl each), probe (1µl), master mix (12.5µl), nuclease-free water (3.5µl), and a template (1µl). *C. jejuni* (ATCC 29411) and *C. coli* (ATCC 43478) served as the positive controls, and nuclease-free water as the negative control. The genus *Campylobacter* was confirmed based on the *Campylobacter* specific 16S rRNA. Then, the same protocol was followed for the confirmation of *C. jejuni* and *C. coli* were conducted by targeting *hipO* and *glyA* genes respectively, employing unique primer pairs and probe sequences as detailed in Appendix XII-Table 1.

## 3.12. Antibiotic Susceptibility Testing

Phenotypic antibiotic susceptibility testing (AST) was conducted for isolates of STEC, *Salmonella* spp., and *Shigella* spp. using the Phoenix M50 machine (Becton Dickinson, USA) according to the manufacturer's instruction (Becton Dickinson, n.d) at the national reference laboratory of Ethiopian Public Health Institute. Briefly, the BD Phoenix AST method is a broth-based micro-dilution test that uses a redox indicator to detect an organism's growth in the presence of an antibiotic agent. Changes to the indicator and bacterial turbidity are continuously measured to determine bacterial growth. In this study, five pure colonies of each isolate were suspended in BD Phoenix AST Broth, and the suspension density was measured using a densitometer (BD PhoenixSpec™ Nephelometer) that was designated with a 0.5 McFarland standard. The standardized inoculum was then used to inoculate BD Phoenix panels, gram-negative AST 501 (NAST/501) and emerge NMIC/474 panels, which were then placed in the machine at 35$^{o}$C, and the machine automatically tested every 20 minutes for up to 16 hours at 35°C. The results were displayed on the minimum inhibitory concentration (MIC) form and interpreted as Susceptible or Resistant based on the BD Phoenix, Software version, V7.21A. Each isolate was tested for ampicillin (0.5-32µg/mL), ciprofloxacin (0.25-4µg/mL), trimethoprim-sulfamethoxazole (0.5/9.5-16/304µg/mL), amikacin (0.5-64µg/mL), gentamicin (0.25-16µg/mL), cefazolin (0.5-32µg/mL), cefuroxime (1-64µg/mL), ceftriaxone (0.5-64µg/mL), ceftazidime (0.5-64µg/mL), cefepime (0.5-64µg/mL), ampicillin-sulbactam (0.5/0.25-32/16µg/mL), ceftazidime-avibactam (0.25-32µg/mL), ceftolozone-tazobactam (0.25-32µg/mL), piperacillin-tazobactam (0.5/4-128/4µg/mL), meropenem (0.125-32µg/mL), azithromycin (0.5-64µg/mL), fosfomycin (16-256µg/mL), and colistin (0.5-4µg/mL).

### *3.12.1. Phenotypic confirmation of ESBL and Carbapenemase production*

ESBL production was conducted using both the BD Phoenix M50 automated microbiology system (Becton Dickinson, USA) and the double disk synergy test (DDST). The Phoenix system provides quantitative MIC-based detection, while the DDST offers phenotypic confirmation by zone synergy, minimizing the risk of false negatives. The Phoenix system automatically interprets ESBL production based on minimum inhibitory concentrations (MICs) of indicator cephalosporins (ceftazidime, cefotaxime, and ceftriaxone) and their respective combinations with clavulanic acid.

According to the manufacturer's and CLSI (2024) guidelines, a ⩾3 twofold dilution reduction in the MIC of any of the indicator cephalosporins tested in combination with clavulanic acid compared with the cephalosporin alone is interpreted as ESBL positive.

Phenotypic confirmation of ESBL was performed using the double disk diffusion method (CLSI, 2024) at the Ethiopian Public Health Institute and Armauer Hansen Research Institute. A combined disk assay was conducted using ceftazidime (30µg) vs ceftazidime-clavulanic acid (30/10µg), and cefotaxime (30µg) vs cefotaxime-clavulanic acid (30/10µg) disks. A bacterial colony of 18-24hrs old from a well-isolated growth on TSA was selected, and a bacterial suspension was prepared in sterile saline to match the turbidity of a 0.5 McFarland standard. Then, the suspension was inoculated onto the Mueller-Hinton agar plate, ensuring that the entire surface is evenly covered. Then, ceftazidime (30µg) and ceftazidime-clavulanic acid (30/10µg), and cefotaxime (30µg) and cefotaxime-clavulanic acid (30/10µg) was placed side by side in the distance of 24mm from the centre of the disks. The plates were incubated at 35 ± 2°C 16-18hrs aerobically.

Following incubation, the zones of inhibition around each antibiotic disk were measured and compared. The production of ESBL was determined by observing the expansion of ≥ 5 mm of the diameters of combined disks (ceftazidime-clavulanic acid and cefotaxime-clavulanic acid) compared to the individual disks (ceftazidime and cefotaxime) alone. *K. pneumoniae* (ATCC 700603) and *E. coli* (ATCC 25922) were used for positive and negative control testing, respectively.

Carbapenemase production was detected using both the BD Phoenix M50 automated system (software version V7.21A) with integrated carbapenemase producing organism detect panels and the modified carbapenemase inactivation method (mCIM). The Phoenix system applied a broth microdilution principle in combination with colorimetric and turbidimetric growth monitoring and determines MICs of carbapenems with and without specific inhibitors (i.e., boronic acid for *KPC*, EDTA for metallo-betalactamases, cloxacillin for *AmpC*-associated enzymes). The carbapenemase-producing bacteria hydrolyze carbapenems, resulting in a high MIC. In the presence of a specific inhibitor that blocks the carbapenemase, the MIC decreases significantly. A reduction of $\underline{\geq}$ 3 two-fold dilutions in MIC in the presence of an inhibitor indicates carbapenemase activity.

The modified carbapenem inactivation method was also conducted as described by the previously designed protocol (Seman et al., 2022). Briefly, a loopful (1µL) of the test isolates, taken from an overnight trypticase soya agar (TSA, Oxoid, UK) culture, was suspended in 2 mL of trypticase soya broth (TSB, Oxoid, UK). A 10 µg meropenem disk was then added to the broth, ensuring it was fully immersed, and the mixture was incubated at 37°C aerobically for approximately 4 hours. After incubation, the meropenem disks were carefully removed using a 10 µL inoculation loop and transferred onto Mueller-Hinton agar plates (MHA, Oxoid, UK), which had been freshly inoculated with a 0.5 McFarland standard suspension of the carbapenem-susceptible *E. coli* ATCC®25922. Following overnight incubation, results were interpreted following CLSI guidelines (CLSI, 2024).

### *3.12.2. Molecular characterization of antimicrobial resistant genes (ESBL and carbapenemase)*

#### *3.12.2.1. DNA extraction*

DNA extraction for the confirmation of the ESBL genes was carried out using the QIAgen kit (Qiagen, n.d.). Briefly, the frozen bacterial culture was sub-cultured onto TSA (Tryptic Soy Agar) plates and incubated at 35°C ± 2°C. A single colony was then inoculated into 5 mL TSB (Tryptic Soy Broth, Oxoid, UK) and incubated overnight at 35°C ± 2°C. Following incubation, 3 mL of the broth culture was centrifuged, and the supernatant was discarded. The resulting sediment was used as the source for DNA extraction, which was performed according to the guidelines provided in the QIAgen protocol (Qiagen, n.d.).

The DNA extraction for the confirmation of the carbapenemase genes were conducted using the thermal/NaOH methods (CDC, 2024). Briefly, the frozen bacterial culture was sub-cultured onto TSA plates and incubated at 35°C ± 2°C. To prepare the samples, 25 µl of molecular-grade water was added to each microcentrifuge tube, and a loopful (1 µl) of colonies was suspended in the molecular-grade water. The tubes were vortexed and 25 µl of 0.1 N NaOH was added, vortexed again, and heated at 95-99°C for 10 minutes. Then, cooled on a pre-cooled block, 18 µl of 0.5 M Tris-HCl (pH 8.0) and 400 µl of nuclease-free cold water was added and gently mixed by inversion. The tubes were centrifuged at 13,200 rpm for 3 minutes, and then 400 µl of the lysate was transferred to a labelled storage tube, after which the lysates were stored at -20°C to -

30°C until use. The amount and purity of DNA were measured using a Nanodrop apparatus (Thermo Fisher Scientific, 2024).

#### *3.12.2.2. PCR protocol*

Confirmation of ESBL production was performed using conventional PCR, a previously used protocol (Seman et al., 2022). First, a master mix was prepared with a total reaction volume of 25 µL, which included the following components: 12.5 µL of Taq Plus 2X Master Mix, 1.5 mM of $MgCl_2$, 5.5 µL of nuclease-free water, 1 µL of forward multiplex primers (bla-CTX-M, bla-TEM, bla-SHV), 1 µL of reverse multiplex primers (bla-CTX-M, bla-TEM, bla-SHV), and 5 µL of DNA template. To prepare the primer multiplex, the initial concentration of the primers (10 µM) was diluted to 2 µM. This was achieved by adding 5 µL each of forward bla-SHV, bla-CTX-M, and bla-TEM primers to a total volume of 85 µL of nuclease-free water. The same dilution was performed for the reverse primers. The thermal cycling conditions were optimized as follows: an initial denaturation step at 95°C for 3 minutes, followed by 40 cycles consisting of denaturation at 95°C for 15 seconds, annealing at 53°C for 1 minute, and extension at 72°C for 2 minutes. A final extension step was conducted at 72°C for 7 minutes. This process was carried out using a thermal cycler (BIO-RAD, California, USA).

Known ATCC strains, *K. pneumoniae* (ATCC 700603), were used as positive controls for the ESBL procedure, while *E. coli* (ATCC 25922) served as the negative control. For gel electrophoresis, 1.5 grams of agarose gel was dissolved in 100 mL of 1X TAE (Tris-acetate-EDTA) buffer, making a 1.5% agarose gel. The solution was boiled until clear, cooled slightly, and then dispensed into the gel casting tray. After the PCR amplification, the products were visualized using gel electrophoresis. The PCR samples were loaded after mixing 10µL of the PCR product with 2 µL of loading dye. The gel electrophoresis was run for 55 minutes. Following electrophoresis, the gel was visualized using a gel imaging system (Geldoc, Cleaver Gel Pro), and a molecular weight ladder (Promega) was used to determine the sizes of the amplified products (see Appendix XII-Table 2). These genes were targeted to identify the types of β-lactamase enzymes potentially involved, as certain variants of these gene families are known contributors to ESBL activity. However, the detection of these genes alone does not confirm ESBL production, since not all variants encode ESBLs. Therefore, the PCR

results in this study should be interpreted as genetic characterization of β-lactamase genes in phenotypically confirmed ESBL isolates.

The molecular characterization of carbapenemase genes was conducted by targeting four common carbapenemase genes: bla-OXA-48-like, bla-VIM, bla-KPC, and bla-NDM, following the recommendations of the CDC and other previous studies (CDC, 2024; Lutgring et al., 2018; Sabour et al., 2024). The characterization was performed using a TaqMan probe-based Real-Time PCR assay on the Applied Biosystems Fast 7500 Real-Time PCR System (Applied Biosystems, Inc., Foster City, CA). Two groups of multiplex PCRs were used during assay development: 1) the bla-OXA-48-like and bla-VIM gene-targeting group, and 2) the bla-KPC and bla-NDM-targeting assay. In both assays, the 16S rRNA gene, a universal bacterial standard, was used as an endogenous control to validate the lysate and amplification in each reaction. The assay simultaneously detected bla-OXA-48-like (including multiple variants) and bla-VIM genes in a single reaction using lysates from Gram-negative bacteria. The sequences of primers and probes were indicated in Appendix XII-Table 3.

For the PCR reaction, a 20 µL volume of reaction solution was prepared in which 2 µL of template DNA was added into 18 µL of master mix containing 10 µL of KiCqStart Probe qPCR Ready Mix, 5 µL of the "Master P (0.5 µL of primers for each target gene (20 µM) and 0.5 µL of each probe (10 µM))", and 3 µL of sterile reagent-grade I water.

The thermal cycling conditions included an enzyme activation step at 95°C for 3 minutes, followed by 35 cycles at 95°C for 3 seconds for denaturation, then an annealing and extension step at 60°C for 30 seconds. The control setup for the bla-VIM and bla-OXA-48-like assay included *P. aeruginosa* AR Bank #0054 as a positive control for the bla-VIM gene and *K. pneumoniae* AR Bank #0039 as a positive control for the bla-OXA-48-like gene. Negative controls included bla-OXA-48-like-negative and bla-VIM-negative *K. pneumoniae* ATCC BAA-1706, along with a No Template Control (NTC), which consisted of sterile reagent-grade water to confirm the absence of contamination. The same procedure was followed for the detection of bla-KPC and bla-NDM genes. The results were interpreted based on Ct values between 10 and 30, and the 16S rRNA gene should be positive all the time to make the reaction valid.

## 3.13. Quality Control and Data Quality Assurance

A three-day training was provided to the data collectors, covering topics like patient screening, consenting, and enrollment procedures, data collection tools (questionnaires), proper collection of stools specimens, specimen labeling, and use of various study logs. Additionally, a one-day training was conducted for porters responsible for transporting specimens from the hospitals (sample collection site) to the microbiology laboratory (sample analysis area). The laboratory team received five days of training on all laboratory-related activities, from media preparation and specimen processing to result reporting and data entry.

Specimen transportation was conducted using a cold chain system through the use of cold box with an ice pack where the internal temperature was also monitored using a thermometer ($2^{o}$C to $8^{o}$C). Any issues encountered with specimens were communicated to the relevant collection and transportation team members upon arrival.

Regular supervision of study sites and related procedures was performed by a designated study supervisor for each region. Issues beyond the supervisors' capacity were addressed by the study team (regional co-investigators). Supervisors monitored the number of specimens collected, transportation conditions, and sample processing progress daily. Urgent issues were communicated directly to the study lead. At the end of each week, supervisors prepared a formal report using the weekly report format for submission to the study lead, who then compiled the reports from all three sites to update the larger study team. This facilitated the assessment of performance across screening, consenting, enrollment, data collection, specimen collection, labeling, and log completion.

All study supplies and reagents were first evaluated with quality control tests, including sterility and performance testing (growth support or inhibition as per manufacturer's recommendations). Quality control procedures included monitoring storage areas for supplies, specimens, and bacterial isolates per laboratory policy. Standard operating procedures (SOPs) were followed rigorously to ensure the media met expiration and quality standards as per CLSI and ISO guidelines, with weekly and daily QC checks as needed. Visual inspections were performed to identify cracks, unequal fills, signs of freezing, bubbles, or contamination. Positive and negative controls were used in all tests,

and standard reference strains from the American Type Culture Collection (ATCC) served as quality checks.

To ensure data quality, seasonal categorization was cross validated using three years of meteorological records from the Ethiopian Meteorological Agency and supported by recent literature. Seasonal classification was based on rainfall distribution in Ethiopia. To account for variations between study sites, we obtained and reviewed three years of meteorological data from the Ethiopian Meteorological Agency for Addis Ababa, Gondar, and Harar. The analysis confirmed that while all sites experienced the long rainy season (June-September) and the dry season (October-February), the short rainy season (March-May) was more distinct in Harar and relatively weak or inconsistent in Addis Ababa and Gondar. To ensure consistency and comparability, we further reviewed recently published literature on Ethiopian rainfall patterns, and adopted a seasonal grouping method (Dry, Short Rain, Long Rain) that is commonly used in similar studies. This approach allowed us to interpret our results considering both empirical meteorological evidence and established scientific practice.

Data on water and sanitation were collected as separate variables based on accessibility and exposure to potential contamination. For comparability, water sources and sanitation facilities were classified as improved or unimproved following the WHO/UNICEF Joint Monitoring Programme (JMP) 2023 guidelines.

- Improved water sources included piped household connections (piped into dwelling), piped yard/compound taps, public standpipes, boreholes, protected dug wells, protected springs, and rainwater collection. These sources are generally less prone to contamination because they are under municipal control or protected by infrastructure.
- Unimproved water sources included unprotected dug wells, unprotected springs, surface water, and tanker truck–supplied water. These sources are often directly exposed to environmental or fecal contamination due to lack of protective structures or uncertain treatment practices.
- Improved sanitation included flush to sewer systems, flush to septic tanks, and pit latrines with covers.
- Unimproved sanitation included pit latrines without covers and open defecation.

For AMR) definitions, results of susceptibility testing were interpreted according to established standards. Multidrug resistance (MDR) was defined as nonsusceptibility to at least one agent in three or more antimicrobial categories. Extensively drug-resistant (XDR) was defined as nonsusceptibility to at least one agent in all but two or fewer antimicrobial categories, meaning isolates remain susceptible to only one or two categories (Basak et al., 2016).

## 3.14. Data Management and Analysis

Data were collected electronically using REDCap (REDCap, 2024) and analysed using R studio version 4.4.3 (R Core Team (2021) (Carroll, 2021)). Descriptive statistics such as frequency and proportion were used to summarize socio-demographic characteristics, symptoms, and laboratory test results. The prevalence of STEC, *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. infection was calculated by dividing the number of positive stool samples for the targeted bacterial pathogens by the total number of stool samples tested. The respective 95% confidence intervals were calculated using the binomial proportion test and logistic regression. Seasonal trends of STEC, *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. were assessed overall by month and by study site using univariable and multivariable logistic regression. Risk factors for a positive laboratory test result were identified for each pathogen and study site separately using univariable and multivariable logistic regression. Potential risk factors associated with foodborne bacterial pathogens such as demographic factors (age, gender), residence (i.e., urban vs rural), and season were assessed using univariable and multivariable logistic regression. In addition, key environmental factors such as types of sanitation facilities, sources of household water, and presence of household animals (e.g., cattle, goats, sheep, chickens, dogs and/or cats) were considered. The univariate analysis of each variable including frequency, proportion, and mean, median, and mode of continuous variables such as age was conducted using the descriptive statistics functions and summary tools. Following the univariate analysis, the univariable logistic regression, that is the pairwise comparisons were performed to assess associations between variables. Variables with a p-value <0.2 in the univariable analysis were then included in the multivariable analysis to control potential confounders and identify independent predictors of the outcome. Multicollinearity among explanatory variables was assessed using the Variance Inflation Factor (VIF) via the car package in R, with

VIF values < 10 considered acceptable. Variables exhibiting high collinearity were considered for exclusion or combination to ensure model stability. Model fit was evaluated using the Hosmer–Lemeshow goodness-of-fit test, and the statistical significance threshold was set at $p < 0.05$. Effect sizes were interpreted based on AOR magnitude, with larger AORs indicating stronger associations and guiding prioritization for potential interventions.

A post hoc power analysis assuming a small-to-moderate effect size (w=0.2) and alpha level of 0.05 showed a power of 99.3% for detecting statistically significant differences in prevalence across the three groups, confirming the sufficiency of the sample size for comparative purposes between the study sites.

## 3.15. Ethical Considerations

Ethical approvals were obtained from all the study sites and collaborative institutes such as, Institutional Review Board of the College of Health Sciences, (Protocol 075/21/DMIP), Addis Ababa University; the Ohio State University (Protocol 2020H0266); International Livestock Research Institute (Protocol IREC2020-47 and amendment IREC2020-47.1); Ethiopian Public Health Institute (Protocol EPHI-IRB-311-2020); Yekatit-12 Hospital Medical College (Protocol 68/21); University of Gondar (Protocol V/P/RCS/05/101/2020); Haramaya University (Protocol IHRERC/020/2021); and George Washington University (Protocol NCR235278). Eligible patients were recruited to participate in the study when they visited the clinical laboratory and provide written informed consent. Parental consent was obtained for children under the age of 18 and assent was also obtained from children aged 12-17 years of age.

Confidentiality of the data was ensured through the following systems: all the collected data were stored in a password-protected data capture system (tablet), and on password-protected computers, accessible only to researchers and authorized oversight authorities. The study utilized REDCap, a software toolset for electronic data collection and management. Data processing and management were centralized at the Ohio State University Center for Clinical and Translational Science (CCTS) Research Informatics Services. REDCap, hosted by OSU Wexner Medical Center IT at the Ackerman Data center, which provided a secure, web-based platform with features like an intuitive data

interface, custom reporting, audit trail functionality, and real-time monitoring. REDCap ensured security by requiring a unique username and password for user identification. Account provisioning and access to specific databases were integrated with the OSUWMC LDAP authentication service. Copies of signed consent or assent forms, or any other research study information, were placed inside the locked shelves.

# CHAPTER FOUR: RESULTS

## 4.1. Socio-demographic and clinical characteristics of the study participants

A total of 2331 patients were enrolled in the study (Table 1), with a median age of 28 years (range: 2 months to 94 years) (Figure 6). The majority of the study participants were within the age group of 20-29 years (23.51%) and the lowest was in the age group of 15-19 years (4.59%). All participants from Addis Ababa were urban residents while about a quarter of participants from Harar and Gondar reported living in rural areas. Most participants in all study sites were male (54.78%). Monthly household income varied by study site. Addis Ababa being the site where highest proportion (25.60%) of the participants earned the lowest incomes category (<2000birr) while the highest proportion of the participants in Gondar (26.60%) and Harar (25.90%) earned relatively better incomes, 8000birr-10,000birr and 6000birr-8000birr respectively. About half of the study participants were enrolled during the dry season. Abdominal pain and abdominal cramps were the most reported symptoms, and watery stool was the most common type of stool consistency.

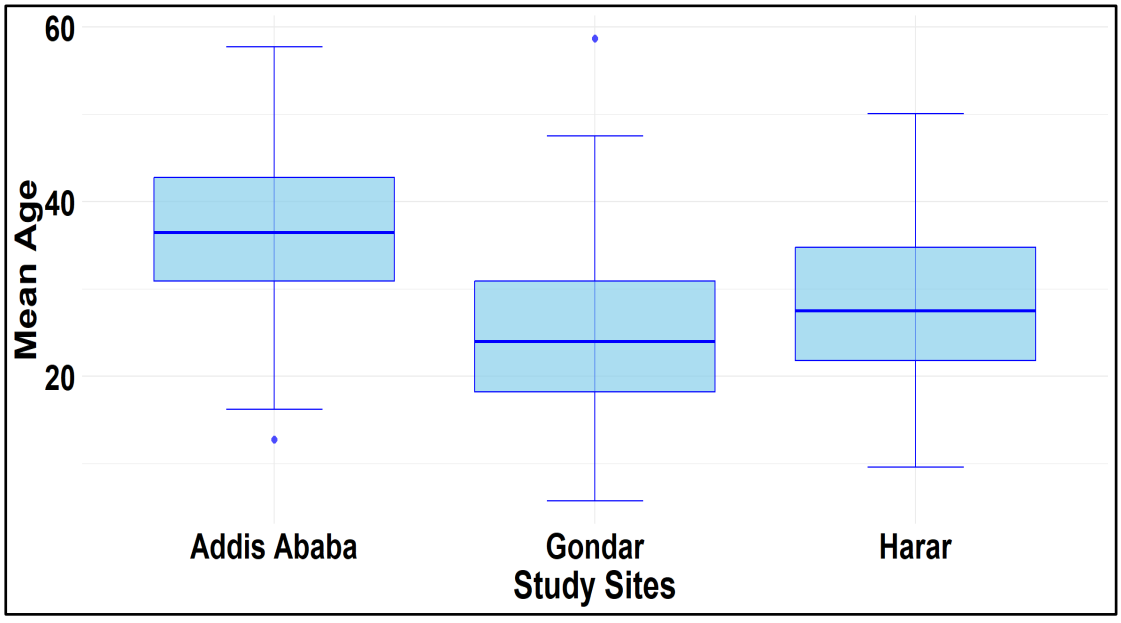


Figure 7. The mean age of patients submitting stool samples per study site.

Table 1. Socio-demographic and clinical characteristics of participants by the study sites.

| **Characteristics** | **Categories** | **Overall**<br><br>**n (%)** | **Addis Ababa**<br><br>**n (%)** | **Gondar**<br><br>**n (%)** | **Harar**<br><br>**n (%)** |
|---|---|---|---|---|---|
| **Total Patients** | | 2331 (100) | 792 (33.98) | 767 (32.90) | 772 (33.12) |
| **Age group** | <5 years | 328 (14.07) | 99 (12.5) | 124 (16.17) | 105 (13.60) |
| | 5-14 years | 228 (9.78) | 53 (6.69) | 96 (12.52) | 79 (10.23) |
| | 15-19 years | 107 (4.59) | 30 (3.79) | 47 (6.13) | 30 (3.87) |
| | 20-29 years | 548 (23.51) | 144 (18.18) | 205 (26.73) | 199 (25.78) |
| | 30-39 years | 412 (17.67) | 123 (15.53) | 125 (16.30) | 164 (21.24) |
| | 40-49 years | 297 (12.74) | 111 (14.02) | 81 (10.56) | 106 (13.73) |
| | 50+ years | 411 (17.63) | 232 (29.30) | 89 (11.60) | 89 (11.53) |
| **Sex** | Male | 1277 (54.78) | 410 (51.80) | 432 (56.30) | 435 (56.30) |
| | Female | 1054 (45.22) | 382 (48.20) | 335 (43.70) | 337 (43.70) |

| | | | | | |
|---|---|---|---|---|---|
| **Residence** | Urban | 1961 (84.13) | 792 (100.00) | 596 (77.70) | 573 (74.20) |
| | Rural | 370 (15.87) | 0 (0.00) | 171 (22.30) | 199 (25.80) |
| **Monthly Household Income (ETB)** | <2000 | 342 (14.67) | 203 (25.60) | 109 (14.20) | 30 (3.90) |
| | 2000-4000 | 352 (15.10) | 195 (24.60) | 49 (6.40) | 108 (14.00) |
| | 4000-6000 | 294 (12.61) | 124 (15.70) | 54 (7.04) | 116 (15.03) |
| | 6000-8000 | 403 (17.29) | 114 (14.40) | 89 (11.60) | 200 (25.90) |
| | 8000-10,000 | 408 (17.50) | 47 (5.93) | 204 (26.60) | 157 (20.34) |
| | >= 10,000 | 166 (7.12) | 20 (2.53) | 114 (14.86) | 32 (4.15) |
| | Unknown | 366 (15.70) | 89 (11.24) | 148 (19.29) | 129 (16.71) |
| **Season** | Dry | 1141 (48.95) | 435 (54.92) | 365 (47.59) | 341 (44.17) |
| | Short Rains | 526 (22.57) | 127 (16.04) | 257 (33.51) | 142 (18.39) |
| | Long Rains | 664 (28.49) | 230 (29.04) | 145 (18.90) | 289 (37.44) |
| | Watery stool | 1622 (69.58) | 370 (46.72) | 628 (81.88) | 624 (80.83) |

| | | | | | |
|---|---|---|---|---|---|
| **Types of stools*** | Bloody stool | 287 (12.31) | 30 (3.79) | 197 (25.70) | 60 (7.80) |
| | Mucoid stool | 430 (18.45) | 61 (7.70) | 269 (35.07) | 100 (12.95) |
| **Signs and Symptoms *** | Abdominal cramps | 1357 (58.22) | 352 (44.44) | 535 (69.75) | 470 (60.88) |
| | Undefined Abdominal pain | 1534 (65.81) | 426 (53.79) | 541 (70.53) | 567 (73.45) |
| | Fever | 812 (34.83) | 166 (20.96) | 345 (44.98) | 301 (38.99) |
| | Nausea | 226 (9.70) | 218 (27.53) | 66 (8.60) | 106 (13.73) |
| | Vomiting | 390 (16.73) | 122 (15.40) | 141 (18.38) | 181 (23.45) |
| | Bloating | 444 (19.05) | 178 (22.47) | 41 (5.35) | 7 (0.91) |
| | None | 25 (1.07) | 11 (1.39) | 9 (1.17) | 8 (1.04) |

***Note**: * Patients could have multiple types of stools and/or symptoms. ETB: Ethiopian birr*

## 4.2. Characteristics of Living Conditions of the Study Participants in the study sites

Access to clean water, sanitation facilities, and domestic animal ownership varied significantly across the study sites (Table 2). Overall, 19.13% of households had access to unimproved water sources with Harar showing the highest proportion (29.66%), and Addis Ababa (7.83%) the lowest. In the cases of sanitation, 37.11% of households used unimproved facilities, the highest being in Harar (44.56%), followed by Gondar (37.03%), and Addis Ababa (29.92%). Although their contact exposure might vary, ownership of domestic animals in the households of the diarrheic patients varied across study sites, with Harar showing the highest proportion, especially for cattle (23.70%), goats (16.32%), and sheep (15.93%), while Addis Ababa showed the lowest ownership rates, except for dogs or cats (13.64%), in which it is the highest of all the study sites.

Table 2. Distribution of selected living conditions of the study participants among the study sites

| **Factors n (%)** | **Category** | **Overall** | **Addis Ababa (n=792) n (%)** | **Gondar (n=767) n (%)** | **Harar (n=772) n (%)** |
|---|---|---|---|---|---|
| **Household water sources** | **Improved** | 1885 (80.87) | 730 (92.17) | 612 (79.79) | 543 (70.34) |
| | **Unimproved** | 446 (19.13) | 62 (7.83) | 155 (20.21) | 229 (29.66) |
| **Sanitation facilities** | **Improved** | 1466 (62.89) | 555 (70.08) | 483 (62.97) | 428(55.44) |
| | **Unimproved** | 865 (37.11) | 237 (29.92) | 284 (37.03) | 344 (44.56) |
| | **Cattle** | 254 (10.90) | 22 (2.78) | 49 (6.39) | 183 (23.70) |

| | | | | | |
|---|---|---|---|---|---|
| **Ownership of domestic animals** | **Sheep** | 107 (4.59) | 14 (1.77) | 30 (3.91) | 63 (8.16) |
| | **Goat** | 145 (6.22) | 8 (1.01) | 11 (1.43) | 126 (16.32) |
| | **Chickens** | 161 (6.91) | 16 (2.02) | 22 (2.87) | 123 (15.93) |
| | **Dogs, and/or Cats** | 236 (10.12) | 108 (13.64) | 72 (9.39) | 56 (7.25) |

## 4.3. Overview of the samples tested and proportion of the target pathogens

Of the total samples tested, 22.05% (514/2331) were presumptive positive for at least one pathogen by conventional culture methods such as using chromSTEC agar for STEC, and XLD and MAC followed by biochemical tests for NTS, and *Shigella* spp., and enzyme immunoassay-based methods of *Campylobacter* spp. Of which, 96.11% (494/514) were confirmed positive using PCR, where there was with variation among the study sites. Among the confirmed isolates, STEC had the highest detection rates, 12.57% (293/2331), followed by *Campylobacter* spp., 4.46% (104/2331), *Shigella* spp., 2.87% (67/2331), while the lowest was *Salmonella* spp., 1.29% (30/2331). Among the study sites, Harar showed the highest positivity rate for all the target pathogens, while Addis Ababa showed the lowest, except for the STEC.

## 4.4. Comprehensive Insights of all the targeted foodborne bacterial pathogens

### *4.4.1. Shiga toxin-producing E. coli*

#### *4.4.1.1. Prevalence of STEC among the study population with respect to different socio-demographic, clinical, and environmental factors*

The overall prevalence of STEC in the study population was 12.57% (95%CI:11.29, 13.98), variation being observed among socio-demographic characteristics, and clinical factors (Figure 7a). Among the study sites, Harar had the highest prevalence at 15.16% (95%CI:12.80,17.86, $p$<0.001), closely followed by Addis Ababa at 14.90% (95%CI:12.59,17.55, $p$<0.001). However, Gondar had the lowest prevalence at 7.56% (95%CI:5.90,9.65, $p$<0.001).

Age group analysis revealed that the highest prevalence of STEC was observed among the age group of 40-49 years, 14.77% (95%CI:11.19,19.24, $p$=0.330) followed by the age group of 15-19 years, 14.02% (95%CI:8.68,21.85, $p$=0.330). There was no substantial difference in the prevalence of STEC between male and female patients, although the latter showed a slightly higher rate, 12.62% (95%CI:10.75,14.76, $p$=0.991). Moreover, urban residents had higher prevalence of 13.00% (95%CI:11.59,14.56, $p$=0.172), than the rural residents, 10.27% (95%CI:7.57,13.78, $p$=0.172).

Regarding the types of diarrhea, the highest prevalence of STEC was found among patients with watery diarrhea at 12.45% (95%CI:10.94,14.15, $p$=0.912), followed by patients with bloody diarrhea, 11.85% (95%CI:8.60,16.10, $p$=0.912), and mucoid diarrhea, 10.70% (95%CI:8.12,13.97, $p$=0.912). In terms of signs and symptoms, the prevalence of STEC was highest among patients who had vomiting at 16.22% (95%CI:13.08,19.93, $p$=0.012) as compared to other signs and symptoms. Moreover, those with the signs and symptoms of undefined abdominal pain, fever, and nausea showed a comparable prevalence. Furthermore, STEC prevalence was slightly higher in households with unimproved water sources at 13.23% (95%CI:10.40,16.69) compared to those who use improved water sources 12.41% (95%CI:11.00,13.98, $p$=0.061) (Figure 7b). Similarly, patients from households with unimproved sanitation facilities showed a higher prevalence, 14.22% (95%CI:11.78,17.08) than improved

sanitation 11.91% (95%CI:10.43,13.55, *p*=0.42). Regarding animal ownership, STEC prevalence was high among patients from households who own cattle, 16.14% (95%CI:12.13, 21.17, *p*=0.167), followed by those who own dogs, and/ or cats, 12.29% (95%CI:8.70,17.09, *p*=0.732), and the lowest prevalence was among those who own sheep, 7.48% (95%CI:3.84,14.06, *p*=0.999). However, these variations in the prevalence of STEC in the context of availability of animals in the household was not statistically significant.

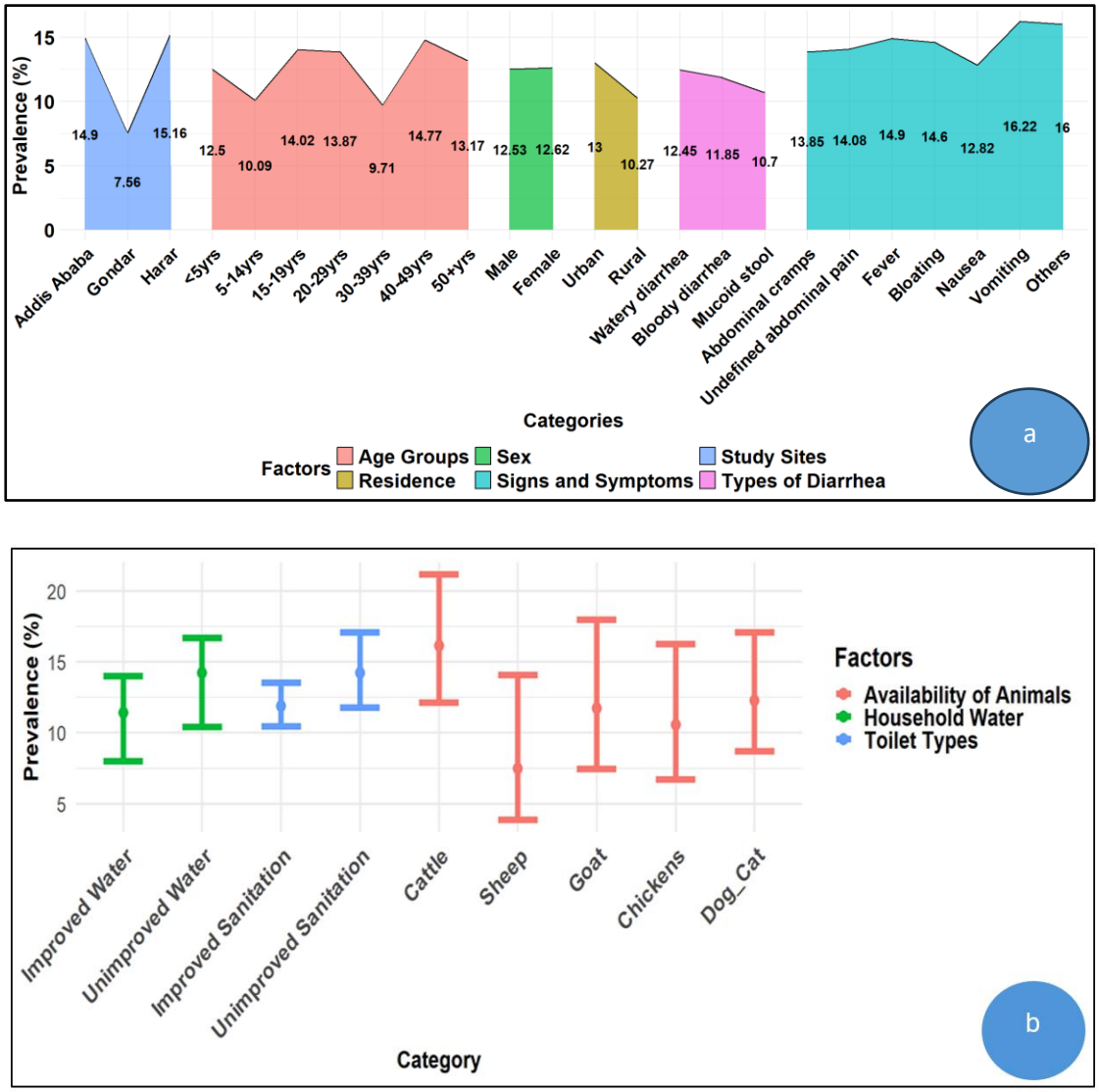


Figure 8. Distribution of STEC by various socio-demographic and clinical (a) and living condition factors (b). Note: yrs: years, Dog_Cat: Dogs, and/or Cats.

#### *4.3.1.2. Seasonal trends of STEC prevalence by the study sites*

Seasonal variations in the overall prevalence of STEC were notable ($p$<0.001), where the highest prevalence at 14.31% (95%CI:11.85, 17.18), was observed during the dry season closely followed by the long rainy seasons, 14.02% (95%CI:12.13,16.16) while the lowest was during the short rainy season at 7.22% (95%CI:5.31,9.76).

The overall monthly trends of the burden of STEC fluctuated significantly, where it peaks in September, October, and November of the dry seasons, and the lowest was during the short rainy seasons (April and May) (Figure 8). Moreover, there was the seasonal variation of STEC prevalence by the study sites. In Addis Ababa, the peak was in October of the dry season, and during the transition month between the dry and short rainy season (March), followed by sudden decline in April and May, and peaks again in June and July of the long rainy seasons. Moreover, in Harar, the peak was observed at the end of the long rainy season (September) and continues to October and November of the dry season. However, in Gondar, the burden was slightly lower throughout the year as compared to other study sites, and gradual increase during the end of the long rainy season, August, September, and the dry season (October and November).

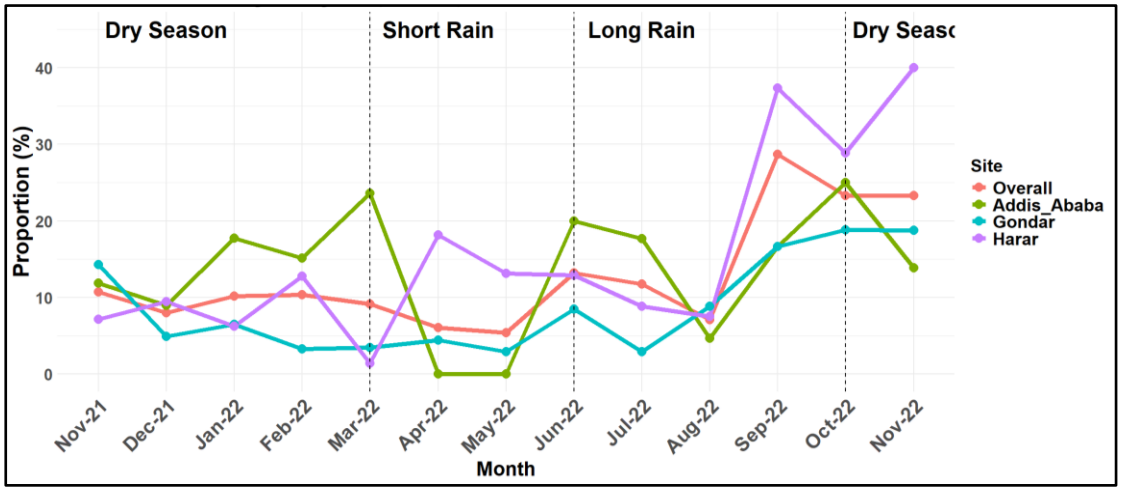


Figure 9. Seasonal distribution of STEC isolates by study sites.

#### *4.3.1.3. Antibiotic susceptibility pattern of STEC isolates*

The antimicrobial resistance profile of STEC isolates showed notable variations across study sites (Table 3). Among the recommended antibiotics for diarrheal illness, STEC isolates exhibited high resistance to ampicillin (63.82%) and sulfamethoxazole-trimethoprim (47.78%), with the highest resistance to the latter observed in isolates

from Addis Ababa (51.69%). Ciprofloxacin resistance was also substantial (28.67%), particularly among isolates from Harar (32.48%), while resistance to ceftriaxone was 25.94%, with no considerable variation by study sites. Resistance to meropenem was observed at the rate of 9.22%, where Gondar showed the highest rate (13.79%). Notably, rate of fosfomycin resistance was shown to be as 5.12%, the highest among the study sites being observed in Harar (6.84%). Azithromycin resistance was also found as 11.60%, but isolates from Addis Ababa showed a relatively more elevated rate (17.80%) as compared with the other two sites. The overall MDR rate of STEC isolates was 48.12%, indicating that nearly half of the isolates were resistant to three or more classes of antibiotics. The MDR rates showed relatively consistent patterns, with only slight variability among the study sites. The highest rate was observed in Addis Ababa, 49.15% (58/118), followed by Harar, 48.72% (57/117), and Gondar, 44.83% (26/58) (Figure 9). Moreover, XDR was reported in 2.56% (3/117) of the STEC isolates in Harar.

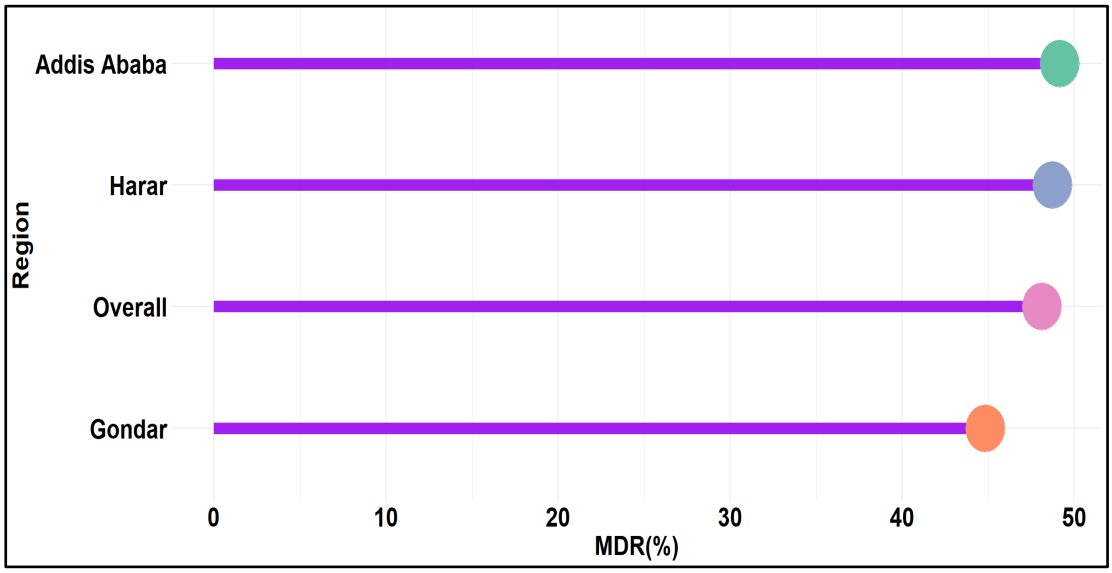


Figure 10. Distribution of MDR among STEC isolates by study sites.

Table 3. Antibiotic susceptibility profile of STEC isolates by study site.

| Antibiotics | Overall (n=293) | | Addis Ababa (n=118) | | Gondar (n=58) | | Harar (n=117) | |
|---|---|---|---|---|---|---|---|---|
| | Susceptible n (%) | Resistant n (%) | Susceptible n (%) | Resistant n (%) | Susceptible n (%) | Resistant n (%) | Susceptible n (%) | Resistant n (%) |
| **AMK** | 271 (92.49) | 22 (7.51) | 113 (95.76) | 5 (4.24) | 54 (93.10) | 4 (6.90) | 104 (88.89) | 13 (11.11) |
| **AMP** | 106 (36.18) | 187 (63.82) | 38 (32.20) | 80 (67.80) | 24 (41.38) | 34 (58.62) | 44 (37.61) | 73 (62.39) |
| **AMS** | 200 (68.26) | 93 (31.74) | 69 (58.47) | 49 (41.53) | 46 (79.31) | 12 (20.69) | 85 (72.65) | 32 (27.35) |
| **AZM** | 259 (88.40) | 34 (11.60) | 97 (82.20) | 21 (17.80) | 53 (91.38) | 5 (8.62) | 109 (93.16) | 8 (6.84) |
| **CN** | 200 (68.26) | 93 (31.74) | 79 (66.95) | 39 (33.05) | 39 (67.24) | 19 (32.76) | 82 (70.09) | 35 (29.91) |
| **FEP** | 236 (80.55) | 57 (19.45) | 92 (77.97) | 26 (22.03) | 47 (81.03) | 11 (18.97) | 97 (82.91) | 20 (17.09) |
| **CAZ** | 222 (75.77) | 71 (24.23) | 92 (77.97) | 26 (22.03) | 44 (75.86) | 14 (24.14) | 86 (73.50) | 31 (26.50) |
| **CZA** | 271 (92.49) | 22 (7.51) | 114 (96.61) | 4 (3.39) | 52 (89.66) | 6 (10.34) | 105 (89.74) | 12 (10.26) |
| **CT** | 272 (92.83) | 21 (7.17) | 115 (97.46) | 3 (2.54) | 52 (89.66) | 6 (10.34) | 105 (89.74) | 12 (10.26) |
| **CRO** | 217 (74.06) | 76 (25.94) | 85 (72.03) | 33 (27.97) | 45 (77.59) | 13 (22.41) | 87 (74.36) | 30 (25.64) |
| **CXM** | 218 (74.40) | 75 (25.60) | 82 (69.49) | 36 (30.51) | 47 (81.03) | 11 (18.97) | 89 (76.07) | 28 (23.93) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **CIP** | 209 (71.33) | 84 (28.67) | 85 (72.03) | 33 (27.97) | 45 (77.59) | 13 (22.41) | 79 (67.52) | 38 (32.48) |
| **CL** | 289 (98.63) | 4 (1.37) | 116 (98.31) | 2 (1.69) | 58 (100.00) | 0 (0.00) | 115 (98.29) | 2 (1.71) |
| **GM** | 261 (89.08) | 32 (10.92) | 103 (87.29) | 15 (12.71) | 54 (93.10) | 4 (6.90) | 104 (88.89) | 13 (11.11) |
| **MEM** | 266 (90.78) | 27 (9.22) | 113 (95.76) | 5 (4.24) | 50 (86.21) | 8 (13.79) | 103 (88.03) | 14 (11.97) |
| **TZP** | 263 (89.76) | 30 (10.24) | 108 (91.53) | 10 (8.47) | 50 (86.21) | 8 (13.79) | 105 (89.74) | 12 (10.26) |
| **TC** | 170 (58.02) | 123 (41.98) | 84 (71.19) | 34 (28.81) | 26 (44.83) | 32 (55.17) | 60 (51.28) | 57 (48.72) |
| **SXT** | 153 (52.22) | 140 (47.78) | 57 (48.31) | 61 (51.69) | 33 (56.90) | 25 (43.10) | 63 (41.18) | 54 (38.57) |
| **FOS** | 278 (94.88) | 15 (5.12) | 114 (96.61) | 4 (3.39) | 55 (94.83) | 3 (5.17) | 109 (93.16) | 8 (6.84) |

***Note:*** *AMK:amikacin, AMP:ampicillin, AMS:ampicillin-sulbactam, AZM:azithromycin, CN:cefazolin, FEP:cefepime, CAZ:ceftazidime, CZA:ceftazidime-avibactam, CT:ceftolozane-tazobactam, CRO:ceftriaxone, CXM:cefuroxime, CIP:ciprofloxacin, CL:colistin, GM:gentamicin, MEM:meropenem, TZP:piperacilllin-tazobactam, TC:tetracycline, SXT:sulfamethoxazole-trimethoprim; FOS:Fosfomycin.*

#### *4.3.1.3. 1. Phenotypic ESBL and Carbapenemase producing STEC isolates*

The overall phenotypic proportion of ESBL-producing isolates among STEC was 37.54% (110/293), with notable regional differences. Gondar and Harar exhibited the highest and almost similar ESBL production rates, at 53.45% (31/58) and 53.85% (63/117), respectively, while isolates from Addis Ababa had significantly lower rate13.56% (16/118)).

The overall rate of phenotypic carbapenem resistance detection was 9.23% (27/293), the highest proportion being from Gondar, 13.79% (8/58), followed by Harar, 11.97 (14/117), while the lowest being from Addis Ababa at 4.23% (5/118). An overall 96.3% (26/27) were positive for the carbapenemase production by the automated detection and the modified carbapenem inactivation method (mCIM). One of the meropenem resistant STEC isolate was not detected as carbapenemase positive by the automated system, yet it was positive by the mCIM, and molecular confirmation.

#### *4.3.1.4. Risk factors associated with STEC: Univariable and Multivariable Analyses*

In the univariable analysis, age, study sites, season, water sources, sanitation facilities, and ownership of domestic animals were significantly associated with STEC infections. For instance, age, particularly, participants aged 20-29 years showed increased odds of STEC infection (COR:1.04, 95%CI:1.00, 1.09, $p$=0.041) compared to the age group of 30-39 years. The $p$=0.041; however, the 95% confidence interval (1.00,1.09) indicates that the lower limit includes the null value, suggesting that the effect may be minimal or uncertain, and needs cautious interpretation. Although mathematically significant, the clinical and practical implications of this association are likely limited and could vary with a larger sample size or in different populations or adjusting a certain confounder. Among the study sites, individuals from Addis Ababa (COR:1.08, 95% CI:1.04,1.11, $p$<0.001), and Harar (COR:1.08, 95%CI:1.04,1.12, $p$<0.001) showed a higher odd of STEC infections compared to Gondar. However, there was no statistically significant difference between Addis Ababa and Harar in this regard. Seasonal variation analysis revealed higher odds during the dry season (COR:1.07, 95%CI:1.03,1.11, $p$<0.001) and long rainy seasons (COR:1.07, 95%CI:1.03,1.12, $p$<0.001) compared with the short rainy season. However, there was no significant difference between STEC infection between dry and long rainy seasons. Additionally, unimproved water sources (COR:1.04, 95%CI:100,1.07, $p$<0.001) and unimproved sanitation (COR:1.03, 95%CI:1.00,1.06, $p$=0.040) were associated with a slightly increased risk of STEC infection. Moreover, households that owned cattle (COR:1.03,95%CI:1.00,1.08, $p$=0.034), and sheep (COR:1.04, 95%CI:1.00,1.08, $p$=0.042) showed a slightly increased risk of STEC infection as compared with those households that did not own these animals.

In the multivariable analysis, Addis Ababa (AOR:1.06, 95%CI:1.02,1.10, $p$<0.001), and Harar (AOR:1.16, 95%CI:1.02,1.39, $p$<0.001) showed higher odds of STEC infection compared with Gondar. However, there was no significant difference in the odds of STEC infection between Harar and Addis Ababa. Additionally, dry season (AOR:1.06, 95%CI:1.02,1.11, $p$<0.001) and long rainy season (AOR:1.05, 95%CI:1.01,1.10, $p$=0.01) continued to be associated with elevated odds compared to the short rainy season. Furthermore, ownership of cattle (AOR:1.38, 95%CI:1.18,1.46, $p$=0.01), and sheep (AOR:1.08, 95%CI:1.02, 1.14, $p$<0.001) was significantly

associated with higher infection rate. However, although age showed borderline statistical significance in the univariable analysis ($p$=0.041), no significant association was observed in the multivariable analysis after adjusting for potential confounders. This indicates that, when accounting for other factors, the effect is likely minimal, and the variable should be interpreted cautiously with respect to its clinical or practical relevance. Other variables such as sex, residence, water sources and sanitation were not significantly associated with STEC infection in the multivariable analysis (Figure 10).

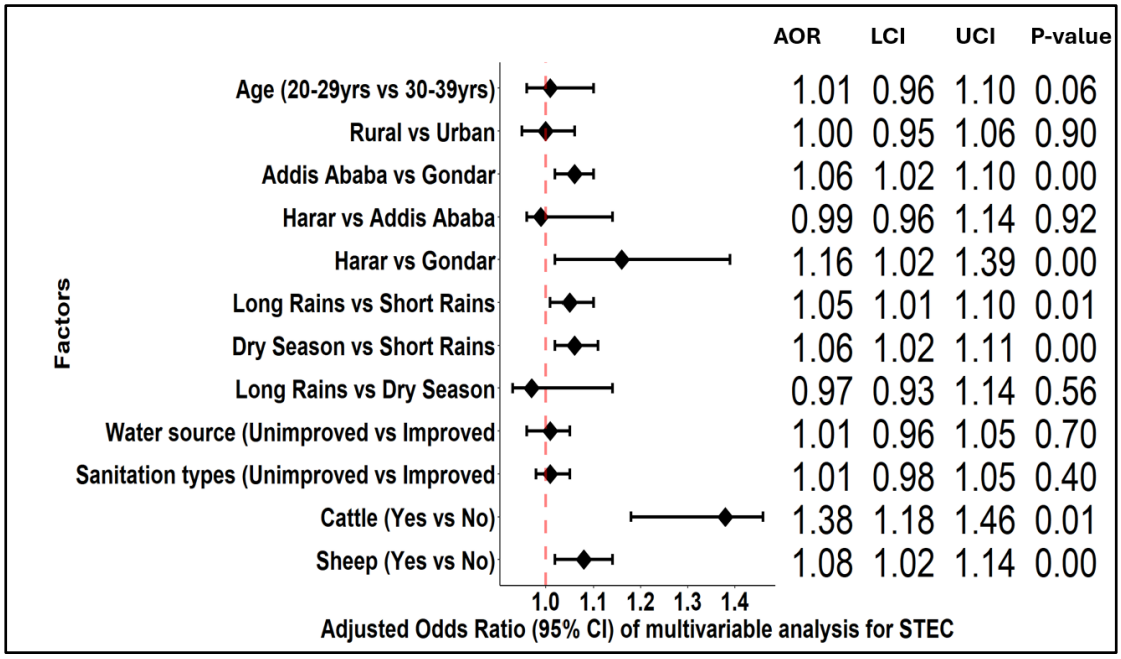


Figure 11. Odds Ratio of multivariable analysis of factors for STEC infection. Note: AOR: Adjusted odds ratio, LCI: Lower confidence interval, UCI: Upper confidence interval. p=0.00: p<0.001. yrs: years.

#### *4.3.1.5. Molecular characterization of STEC*

##### *4.3.1.5.1. Molecular characterization of common virulence genes*

The distribution and co-detection of the targeted common virulence genes of STEC varied by study sites (Table 4). Overall, the *stx1* gene was the most frequently detected virulence factor, present in 66.89% of isolates across all locations, with the highest in Addis Ababa (48.47%) and lowest in Gondar (15.31%). The *stx2* gene was also found in 49.83% of isolates overall. It was most frequently detected in isolates from Addis Ababa (43.84%) and lowest in Gondar (19.18%).

Overall, the combination of *stx* (*stx1* or *stx2)* and *eae* genes was observed in 27.30% of isolates, where the highest (37.90%) was detected from Gondar followed by Harar (25.64%), and Addis Ababa (23.73%) (Table 8). Co-occurrence of both *stx1 + stx2* genes alone was relatively rare, with an overall proportion of 18.43%, most frequently observed in Addis Ababa (33.05%) compared to Harar (8.55%) and Gondar (8.62%). The *stx1 + eae* gene combination was found in 16.38% of cases, with Addis Ababa showing the highest (20.34%) compared to Harar (15.38%) and Gondar (10.34%). The pairing of *stx2 + eae* genes was present in 15.70% of cases, with the highest proportion was from Gondar (25.86%), followed by Harar (15.38%), and Addis Ababa (11.02%). Finally, the combination of the three virulence genes (*stx1 + stx2 + eae)* was less common, detected in only 6.14% of cases with closely similar distribution among the study sites (Table 4).

Table 4. Distribution of virulence genes among STEC isolates from different study sites.

| **Virulence genes** | **n (%)** | | | |
|---|---|---|---|---|
| | **Addis Ababa (n=118)** | **Gondar (n=58)** | **Harar (n=117)** | **Overall (n=293)** |
| ***stx1*** | 95 (80.51%) | 30 (51.72%) | 71 (60.68%) | 196 (66.89%) |
| ***stx2*** | 64 (54.24%) | 28 (48.28%) | 54 (46.15%) | 146 (49.83%) |
| **(*stx1* or *stx2*) and *eae*** | 28 (23.73%) | 22 (37.93%) | 30 (25.64%) | 80 (27.30%) |
| ***stx1 + stx2*** | 39 (33.05%) | 5 (8.62%) | 10 (8.55%) | 54 (18.43%) |
| ***stx1 + eae*** | 24 (20.34%) | 6 (10.34%) | 18 (15.38%) | 48 (16.38%) |
| ***stx1 + stx2 + eae*** | 9 (7.63%) | 3 (5.17%) | 6 (5.13%) | 18 (6.14%) |
| ***stx2 + eae*** | 13 (11.02%) | 15 (25.86%) | 18 (15.38%) | 46 (15.70%) |

***Note****: there is an overlap of the virulence genes, and the overall proportion will not be 100%.*

#### *4.3.1.5.2. Distribution of ESBL genes among STEC isolates and study sites*

Molecular characterization of the ESBL genes bla-CTX-M, bla-TEM, and bla-SHV among STEC isolates revealed the predominance of bla-CTX-M genes across all the study sites. Out of the 110 phenotypically ESBL-producing STEC isolates, 104 (95.45%) were positive for one or more of the targeted ESBL genes. The overall proportion of bacterial isolates positive for bla-CTX-M gene was 97.12% (101/104), with a slight variation between study sites. In Addis Ababa, 86.67% (13/15) of STEC isolates were positive for the bla-CTX-M gene, while all 100.00% (30/30) the ESBL positive isolates of STEC were positive in Gondar. Almost complete bla-CTX-M gene-positivity, 98.31% (58/59) was detected among the ESBL STEC isolates from Harar. The other ESBL gene, bla-TEM, was present in 75.00% (78/104) of STEC isolates, ranging from 66.67% (10/15) in Addis Ababa to 83.05% (49/59) in Harar. On the other hand, the bla-SHV gene was found in a smaller proportion of STEC isolates, with an overall proportion of 15.38% (16/104). However, Addis Ababa showed a higher proportion of STEC isolates with the bla-SHV gene, 26.67% (4/15) compared to Gondar, 10.00% (3/30) and Harar, 15.25% (9/59) (Figure 11a). The detection of these genes indicates the presence of β-lactamase encoding determinants among the STEC isolates; however, molecular detection alone does not differentiate between narrow-spectrum and extended-spectrum variants.

The co-detection of ESBL genes was common among the STEC isolates, with bla-CTX-M and bla-TEM genes being the most frequent combination, found in 72.11% (75/104) of the isolates (Figure 11b). Additionally, 13.46% (14/104) of STEC isolates harbored all three genes, bla-CTX-M, bla-TEM, and bla-SHV. Co-detection of bla-CTX-M and bla-SHV genes and bla-TEM and bla-SHV genes occurred in equal proportions, at 14.42% (15/104) each.

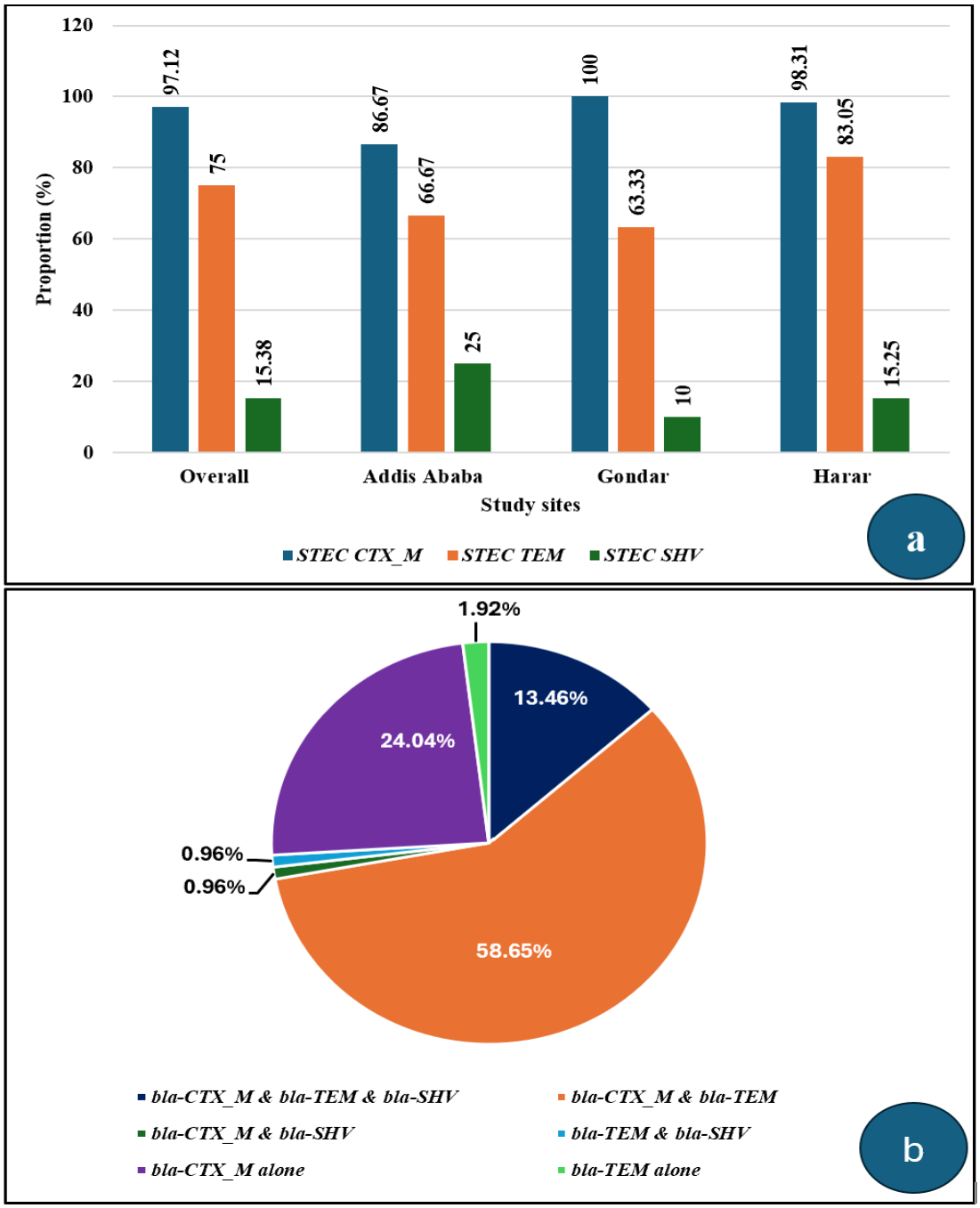


Figure 12. Distribution of the ESBL genes of the STEC isolates by the study sites (a), and co-detection of ESBL genes (b).

#### *4.3.1.5.3. Molecular characterization of carbapenemase genes among STEC isolates by study sites*

The targeted specific genes encoding (bla-NDM and bla-OXA-48-like genes) for carbapenemase production were detected among STEC isolates, although the rate varies between them (Figure 12). The more frequently detected gene was bla-NDM, 92.59% (25/27) with the highest proportion in Addis Ababa at 100% (6/6), followed by Harar at 92.31% (12/13), and Gondar 75.00% (6/8). Conversely, a small proportion (7.41%, 2/27), of bla-OXA-48-like genes were present in the overall STEC isolates, with Gondar and Harar each showing a 12.5% (1/8), and 7.69% (1/13), respectively, but none from Addis Ababa. Moreover, the bla-NDM and bla-OXA-48-like genes of the carbapenemase were not co-detected in any of the tested isolates. None of the STEC isolates were found positive for the bla-VIM and bla-KPC In our study.

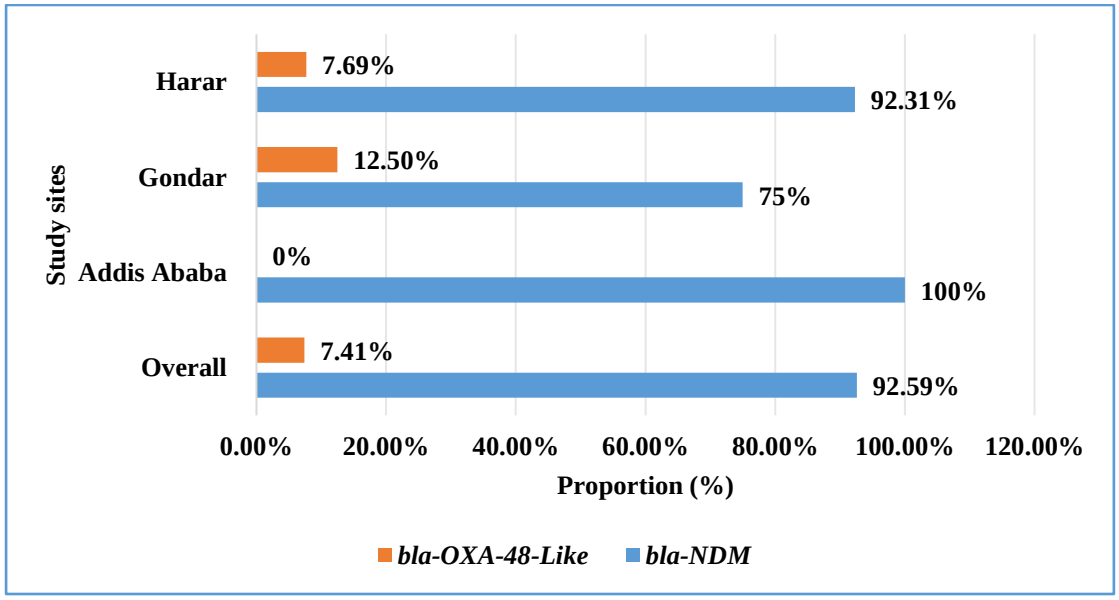


Figure 13. The distribution of Carbapenemase enzyme encoding genes among the STEC isolated by the study sites.

#### *4.3.1.5.4. Sub-typing of STEC using the eae gene*

##### *4.3.1.5.4.1. Prevalence of eae harboring STEC isolates by different socio-demographic, clinical, and environmental factors*

The *eae* harboring STEC were detected at varying rates across the study sites (Table 5). The overall prevalence was 3.43% (95%CI:2.77, 4.25), with the highest from Harar at 4.15% (95%CI:2.95,5.79, *p*=0.372), followed by Addis Ababa at 3.28% (95%CI:2.24,4.77, *p*=0.372) and Gondar at 2.87% (95%CI:1.90,4.30, *p*=0.372). Moreover, among the age groups, the highest prevalence was 4.62% (95%CI: 2.98,7.11, *p*=0.333) among individuals aged 50+ years, followed by 20-29 years at 4.56% (95%CI: 3.11,6.65, *p*=0.333), while the lowest was among children under 5years, 2.44% (95%CI:1.24,4.74, *p*=0.333). Males had a slightly higher prevalence with rates of 3.52% (95%CI:2.64,4.68, *p*=0.877) than females at the rate of 3.32% (95%CI:2.40,4.58, *p*=0.877); and urban residents with rate of 3.62% (95%CI:2.88,4.54, *p*=0.319) showed higher than rural residents with 2.43% (95%CI:1.28,4.56, *p*=0.319). Isolates from patients with bloody diarrhea had the highest prevalence, 4.18% (95%CI:2.41,7.16), among all the diarrhea types, and fever was the most common symptom 4.56% (95%CI:3.32, 6.22, *p*=0.319) associated with *eae* harboring STEC isolates (Table 4.6).

Table 5. Distribution of eae harboring STEC among the socio-demographic and clinical factors.

| **Factors** | **Categories** | **Overall n (%)** | **n (%)** | **Prevalence (95%CI)** |
|---|---|---|---|---|
| **Overall** | | 2331 (100.00) | 80 (3.43) | 3.43 (2.77, 4.25) |
| **Study sites** | Addis Ababa | 792 (33.98) | 26 (3.28) | 3.28 (2.24,4.77) |
| | Gondar | 767 (32.90) | 22 (2.87) | 2.87 (1.90,4.30) |
| | Harar | 772 (33.12) | 32 (4.15) | 4.15 (2.95,5.79) |
| **Age groups** | <5 years | 328 (14.07) | 8 (10.00) | 2.44 (1.24,4.74) |
| | 5-14 years | 228 (9.78) | 7 (8.75) | 3.07 (1.49,6.20) |
| | 15-19 years | 107 (4.59) | 3 (0.38) | 2.80 (0.96,7.92) |

| | | | | |
|---|---|---|---|---|
| | 20-29 years | 548 (23.51) | 25 (31.25) | 4.56 (3.11,6.65) |
| | 30-39 years | 412 (17.67) | 11 (13.75) | 2.67 (1.50,4.72) |
| | 40-49 years | 297 (12.74) | 7 (8.75) | 2.36 (1.15,4.78) |
| | 50+ years | 411 (17.63) | 19 (23.75) | 4.62 (2.98,7.11) |
| **Sex** | Male | 1277 (54.78) | 45 (56.25) | 3.52 (2.64,4.68) |
| | Female | 1054 (45.22) | 35 (43.75) | 3.32 (2.40,4.58) |
| **Residence** | Urban | 1961 (84.13) | 71 (88.75) | 3.62 (2.88,4.54) |
| | Rural | 370 (15.87) | 9 (11.25) | 2.43 (1.28,4.56) |
| **Types of Diarrhea** | Watery diarrhea | 1622 (69.58) | 62 (77.51) | 3.82 (2.99, 4.87) |
| | Bloody diarrhea | 287 (12.31) | 12 (15.00) | 4.18 (2.41,7.16) |
| | Mucoid stool | 430 (18.45) | 17 (21.25) | 3.95 (2.48, 6.23) |
| **Signs and Symptoms** | Abdominal cramps | 1357 (58.22) | 56 (70.00) | 0.04 (0.03,0.05) |
| | Abdominal pain* | 1534 (65.81) | 59 (73.75) | 3.85 (2.99, 4.93) |
| | Fever | 812 (34.83) | 37 (46.25) | 4.56 (3.32, 6.22) |
| | Bloating | 226 (9.70) | 9 (11.25) | 3.98 (2.11,7.39) |
| | Nausea | 390 (16.73) | 11 (13.75) | 2.82 (1.58, 4.98) |
| | Vomiting | 444 (19.05) | 19 (23.75) | 4.28 (2.76, 6.59) |
| | Others | 25 (1.07) | 1 (1.25) | 4.76 (0.85,22.67) |

***Note: *** *Undefined abdominal pain.*

#### *4.3.1.5.4.2. Seasonal trends of eae harboring STEC by study sites*

The analysis of seasonal prevalence of *eae* harboring STEC ($p$=0.178) showed that the highest prevalence was found during the dry season 4.12% (95%CI:3.11,5.43)), followed by the long rainy season, 3.01% (95%CI:1.96, 4.61) and the short rainy seasons, 2.47% (95%CI:1.45, 4.18).

The overall monthly trends of *eae* harboring STEC showed a continuous peak from September to November. However, the low trend was from April to August. There was a variation of trend per study site where Addis Ababa showed a peak in March, with a sudden decline in April to September. In Gondar, the peak was in October, followed by January, while a decline and consistent finding during short rainy season. Moreover, Harar showed a peak in September and November, while the decline in the rest of the months showed slight variation between them (Figure 13).

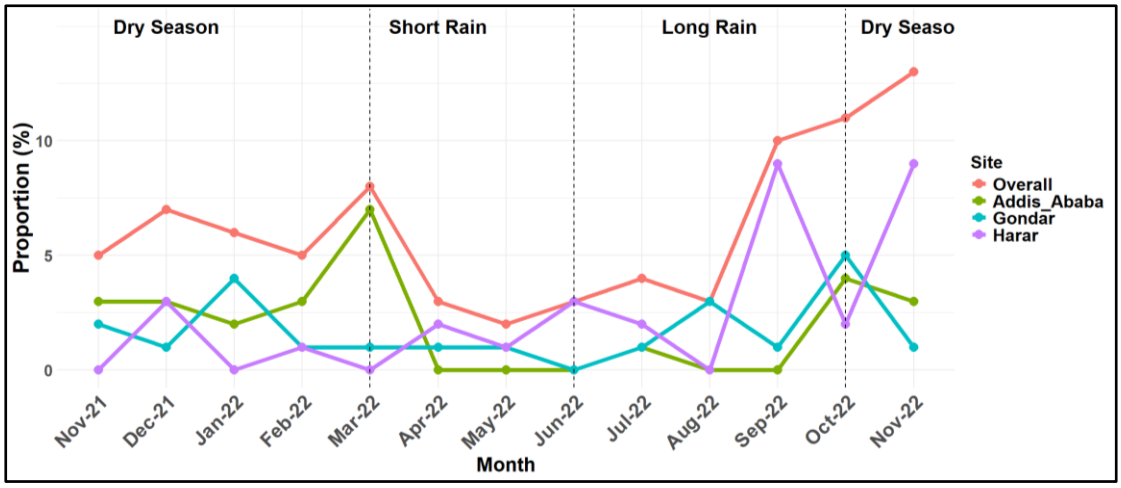


Figure 14. Seasonal distribution of *eae* harbouring STEC isolates by study sites. Note: An overlap is observed between the trends for Gondar and Addis Ababa during June and July.

#### *4.3.1.5.4.3. Risk factors associated with eae harboring STEC: Univariable and Multivariable Analyses*

In the univariate analysis, most of the assessed factors did not show any statistically significant association with the infection of *eae* harboring STEC. However, age groups of 20-29 years showed an increased odd (COR:1.02, 95%CI:1.00,1.05, $p$=0.043), and those with the age of 50+ years also showed higher odds of the *eae* harboring STEC infection (COR:1.02, 95%CI:1.00,1.05, $p$=0.031). Moreover, water sources were

significantly associated with the detection of *eae* harboring STEC among the infected individuals, where those using the unimproved water had higher odds (COR:1.02, 95%CI:1.00,1.04, *p*=0.034), as was the case for those with unimproved sanitation (COR:1.021, 95%CI:1.005,1.038, *p*=0.012).

In the multivariate analysis, age, especially those with the age of 50+ years showed higher odds (AOR:1.22,95%CI:1.02,1.36, *p*=0.040) of infection by the *eae* harboring STEC. Moreover, households with unimproved water sources (AOR:1.06, 95%CI:1.01,1.10, *p*=0.004), and unimproved sanitation (AOR:1.08, 95%CI:1.01,1.16, *p*=0.031) showed higher odds of *eae* harboring STEC (Figure 14). However, sex, residence, or ownership of animals in the households did not show statistical significance for the *eae* harboring STEC infection.

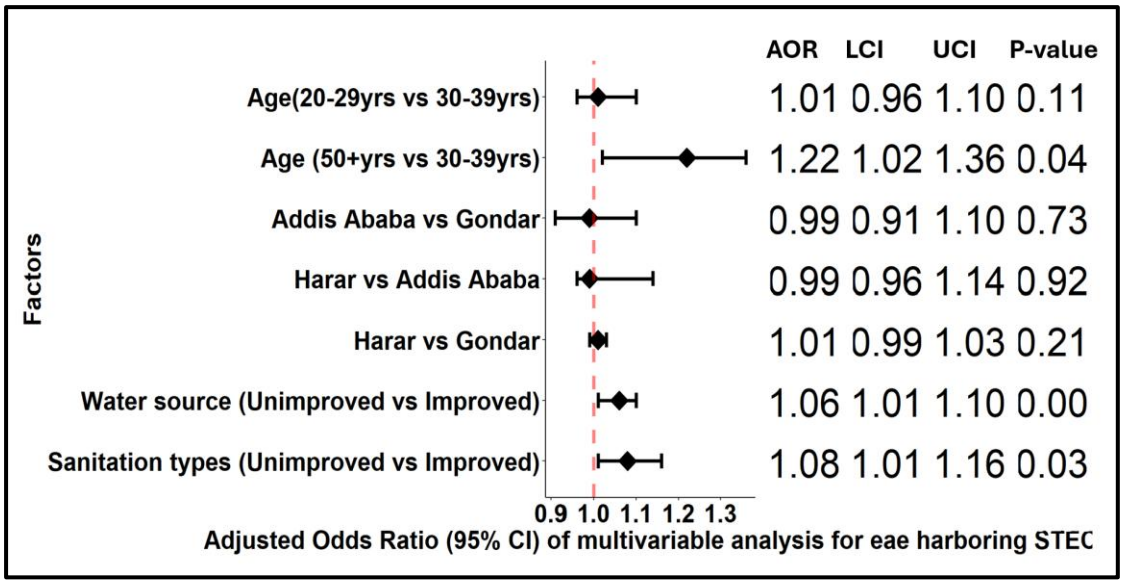


Figure 15. Odds Ratio of multivariable analysis of factors for *eae* harbouring STEC infection. Note: AOR: Adjusted odds ratio, LCI: Lower confidence interval, UCI: Upper confidence interval. p=0.00: p<0.001. yrs: years.

## *4.3.2. Non-typhoidal Salmonella*

### *4.3.2.1. Burden of NTS by different socio-demographic, clinical, and environmental factors*

The overall prevalence of NTS was 1.29% (95%CI:0.90,1.83) with statistically significant variation among the study sites (*p*<0.001). Harar showed the highest rate at 2.85% (95%CI:1.89,4.28), while Gondar and Addis Ababa showing significantly low

prevalence at 0.30% (95%CI:0.07,0.92) and 0.80% (95%CI:0.36,1.70), respectively. The age group 30-39 years had the highest NTS prevalence at the rate of 1.94% (95%CI:0.99, 3.78), followed by children under 5 years,1.83% (95%CI:0.84,3.93), and lower rates among other age groups that ranges from 0.73% (95%CI:0.28,1.86) to 1.22% (95%CI:0.52,2.82) with no statistically significant variation ($p$=0.704). Rural residents exhibited a higher prevalence, 2.43% (95%CI:1.28, 4.56) than urban residents, 1.07% (95%CI:0.70,1.63), where the variation was not statistically significant ($p$=0.060). Among the types of diarrhea, watery diarrhea accounted for most cases, 1.54% (95%CI:1.05,2.27), followed by bloody diarrhea, 1.39% (95%CI:0.54,3.53), and mucoid stool, 0.47% (95%CI:0.13,1.68) (Figure 15a). However, the variation was not statistically significant ($p$=0.255).

Regarding signs and symptoms, patients with fever showed the highest NTS prevalence, 1.97% (95%CI:1.22,3.18, $p$=0.052), followed by abdominal cramps, 1.62% (95%CI:1.07,2.44, $p$=0.133), and undefined abdominal pain, 1.50% (95%Cl:1.00,2.24, $p$=0.285) (Figure 11a). Households with unimproved water sources showed a slightly higher prevalence of NTS, 1.79% (95%CI:0.91, 3.50, $p$=0.411) compared to improved sources 1.17% (95%CI:0.80, 1.76, $p$=0.411). The prevalence of NTS was higher among patients from households with unimproved sanitation facilities, 1.50% (95%CI:0.82,2.73, $p$=0.968), and relatively lower among households with improved sanitation 1.20% (95%CI:0.80,1.85, $p$=0.968). Among animal-owning households, the highest prevalence was observed with chickens, 4.35% (95%CI:2.12,8.70, $p$=0.002), followed by goat, 4.14% (95%CI:1.91,8.73, $p$=0.008) and cattle, 3.94% (95%CI:2.15, 7.09, $p$<0.001) (Figure 15b).

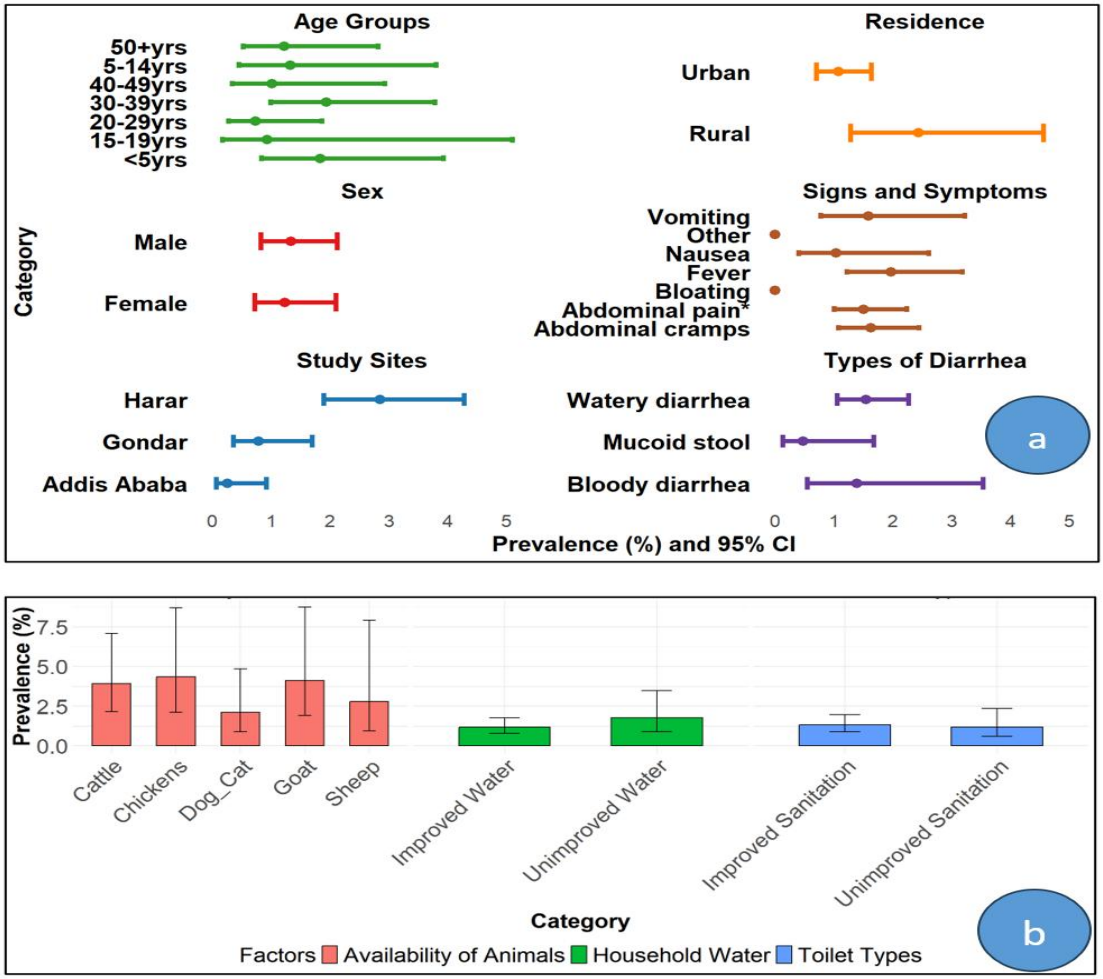


Figure 16. Prevalence of NTS by socio-demographic (a) and living condition factors (b). yrs: years. Dog_Cat: Dogs, and/or Cats. Abdominal pain: Undefined abdominal pain.

#### *4.3.2.2. Seasonal trends of NTS by study sites*

The overall prevalence of NTS was highest in the dry season at 1.40% (95%CI:0.86, 2.27, *p*=0.736), followed by the long rains at 1.36% (95%CI:0.71,2.56, *p*=0.736), and lowest in the short rains at 0.95% (95%CI:0.41,2.21, *p*=0.036).

The overall monthly trend showed peak in October, with slight variation between study sites (Figure 16). Harar showed relatively high peak in August, and a relatively consistent trends among other different months. However, in Gondar, there was no reports of NTS during long rainy seasons with a sudden slight increase in October. Moreover, in Addis Ababa, the only report of NTS was observed in October that overlapped with the trend from Gondar, while all the other months of the year were left with zero reports.

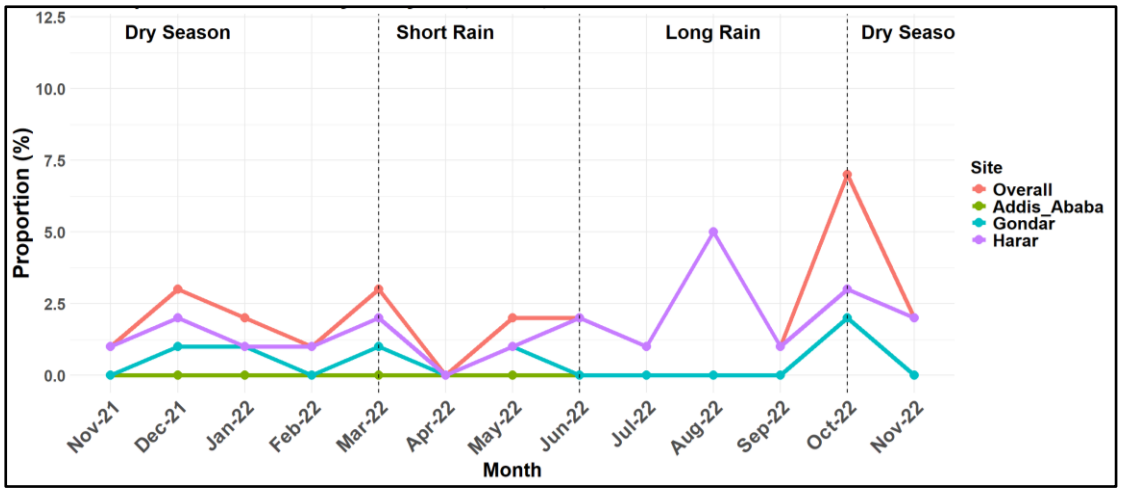


Figure 17. Seasonal trends of NTS by study sites. Note: From June to September 2022, the trends for the overall data and Harar overlapped, resulting in the overall trend line being visually masked due to the color overlap. Additionally, there were no NTS detections reported from Addis Ababa and Gondar during this period. There is also an overlap between Gondar and Addis Ababa from June to November.

#### *4.3.2.3. Antibiotic susceptibility profile of NTS isolates by study sites*

The antimicrobial susceptibility profiles of NTS isolates highlight considerable variability across the study sites (Table 6). High resistance was observed for the antibiotic's ampicillin (73.33%), tetracycline (60.00%), cefazolin and sulfamethoxazole-trimethoprim (46.67% each). Moreover, resistance to ceftriaxone, cefuroxime, ciprofloxacin, ceftazidime-avibactam, ceftazidime, and ampicillin-sulbactam, and amikacin ranged from 20% to 33.33%. Among the study sites, the increased resistance was documented in Harar for ampicillin and, ampicillin-sulbactam (81.82% each), followed by tetracycline (68.18%). Moreover, in Gondar, high resistance (83.33%) was recorded to cefazolin, followed by resistance to ampicillin and, ampicillin-sulbactam (66.67% each), and ceftazidime, and ceftazidime-avibactam (50% each). In Addis Ababa, only two isolates were found susceptible to all the tested antibiotics.

The burden of MDR among NTS was remarkably high at 83.33% (25/30) overall although with significant differences between the study sites. For instance, Gondar and Harar showed resistance rates of 50.0% (3/6), and 81.82% (18/22), respectively while none of the NTS isolates from Addis Ababa were MDR positive. In addition, the number and diversity of antibiotic resistance among NTS varied among the study sites.

Table 6. Antibiotic susceptibility profiles of NTS by study sites.

| Antibiotics | Overall (n=30) | | Addis Ababa (n=2) | | Gondar (n=6) | | Harar (n=22) | |
|---|---|---|---|---|---|---|---|---|
| | Susceptible n (%) | Resistant n (%) | Susceptible n (%) | Resistant n (%) | Susceptible n (%) | Resistant n (%) | Susceptible n (%) | Resistant n (%) |
| **AMK** | 22 (73.33) | 8 (26.67) | 2 (100.00) | 0 (0.00) | 4 (66.67) | 2 (33.33) | 18 (81.82) | 4 (18.18) |
| **AMP** | 8 (26.67) | 22 (73.33) | 2 (100.00) | 0 (0.00) | 2 (33.33) | 4 (66.67) | 4 (18.18) | 18 (81.82) |
| **AMS** | 20 (66.67) | 10 (33.33) | 2 (100.00) | 0 (0.00) | 2 (33.33) | 4 (66.67) | 4 (18.18) | 18 (81.82) |
| **AZM** | 24 (80.00) | 6 (20.00) | 2 (100.00) | 0 (0.00) | 4 (66.67) | 2 (33.33) | 18 (81.82) | 4 (18.18) |
| **CN** | 16 (53.33) | 14 (46.67) | 2 (100.00) | 0 (0.00) | 1 (16.67) | 5 (83.33) | 13 (59.09) | 9 (40.91) |
| **FEP** | 26 (86.67) | 4 (13.33) | 2 (100.00) | 0 (0.00) | 6 (100.00) | 0 (0.00) | 18 (81.82) | 4 (18.18) |
| **CAZ** | 24 (80.00) | 6 (20.00) | 2 (100.00) | 0 (0.00) | 3 (50.00) | 3 (50.00) | 19 (86.36) | 3 (13.64) |
| **CZA** | 23 (76.67) | 7 (23.33) | 2 (100.00) | 0 (0.00) | 3 (50.00) | 3 (50.00) | 18 (81.82) | 4 (18.18) |
| **CT** | 24 (80.00) | 6 (20.00) | 2 (100.00) | 0 (0.00) | 4 (66.67) | 2 (33.33) | 18 (81.82) | 4 (18.18) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **CRO** | 21 (70.0) | 9 (30.0) | 2 (100.00) | 0 (0.00) | 4 (66.67) | 2 (33.33) | 15 (68.18) | 7 (31.82) |
| **CXM** | 23 (76.67) | 7 (23.33) | 2 (100.00) | 0 (0.00) | 4 (66.67) | 2 (33.33) | 17 (77.27) | 5 (22.73) |
| **CIP** | 23 (76.67) | 7 (23.33) | 2 (100.00) | 0 (0.00) | 5 (83.33) | 1 (16.67) | 16 (72.73) | 6 (27.27) |
| **CL** | 30 (100.00) | 0 (0.00) | 2 (100.00) | 0 (0.00) | 6 (100.00) | 0 (0.00) | 22 (100.00) | 0 (0.00) |
| **GM** | 17 (56.67) | 13 (43.33) | 2 (100.00) | 0 (0.00) | 4 (66.67) | 2 (33.33) | 11 (50.00) | 11 (50.00) |
| **MEM** | 30 (100.00) | 0 (0.00) | 2 (100.00) | 0 (0.00) | 6 (100.00) | 0 (0.00) | 22 (100.00) | 0 (0.00) |
| **TZP** | 27 (90.0) | 3 (10.0) | 2 (100.00) | 0 (0.00) | 5 (83.33) | 1 (16.67) | 20 (90.91) | 2 (9.09) |
| **TC** | 12 (40.0) | 18 (60.0) | 2 (100.00) | 0 (0.00) | 4 (66.67) | 2 (33.33) | 7 (31.82) | 15 (68.18) |
| **SXT** | 16 (53.33) | 14 (46.67) | 2 (100.00) | 0 (0.00) | 4 (66.67) | 2 (33.33) | 10 (45.45) | 12 (54.55) |

***Note:*** *AMK: amikacin, AMP: ampicillin, AMS: ampicillin-sulbactam, AZM: azithromycin, CN: cefazolin, FEP: cefepime, CAZ: ceftazidime, CZA: ceftazidime-avibactam, CT: ceftolozane-tazobactam, CRO: ceftriaxone, CXM: cefuroxime, CIP: ciprofloxacin, CL: colistin, GM: gentamicin, MEM: meropenem, TZP: piperacilllin-tazobactam, TC: tetracyclin, SXT: sulfamethoxazole-trimethoprim.*

#### *4.3.2.3.1. Phenotypic ESBL production among NTS isolates*

The phenotypic ESBL production rate among the NTS isolates was found to be 13.33% (4/30), with regional variation. Gondar (2/6), and Harar2/22) shared this equally. However, none of the NTS isolates were found positive for ESBL and carbapenemase production.

#### *4.3.2.4. Risk Factors for NTS: Univariable and Multivariable Analyses*

In the univariable analysis, residence and ownership of domestic animals showed statistically significant association with NTS infection. For instance, rural residence was associated with an increased risk (COR:1.01, 95%CI:1.00,1.03, $p$=0.030) compared to urban settings. Among domestic animals, the presence of cattle (COR:1.03, 95%CI:1.01,1.05, $p$<0.001), sheep (COR:1.01, 95%CI:1.00,1.08, $p$=0.01), goats (COR:1.03, 95%CI:1.01,1.05, $p$<0.001), chickens (COR:1.03, 95%CI:1.01,1.05, $p$=0.00), and dog or cats (COR:1.03, 95%CI:1.00,1.09, $p$=0.022) were significantly associated with increased risk of NTS infection. However, sex, season, water sources, and sanitation facilities did not show significant association.

In multivariable analysis, residence and ownership of domestic animals were associated with high odds of NTS infection. For instance, participants from rural areas showed the higher odds (AOR:1.01, 95%CI:1.00,1.02, $p$=0.031) of NTS infection compared to those in the urban area. Moreover, ownership of cattle, sheep, goats, chickens, and dogs or cats showed higher odds of NTS infection compared to those households that did not own these domestic animals (Figure 17).

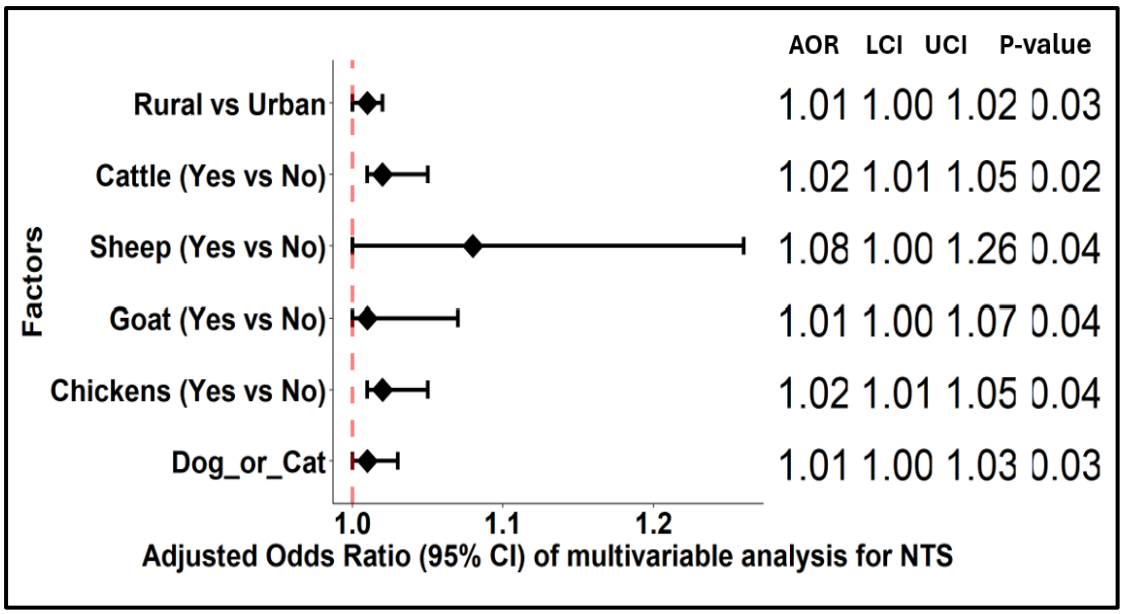


Figure 18. Odds Ratio of multivariable analysis of factors for *Salmonella* spp. Note: AOR: Adjusted odds ratio, LCI: Lower confidence interval, UCI: Upper confidence interval. p=0.00: p<0.001, Dog_Cat: Dogs, and/or Cats.

#### *4.3.2.5. Molecular characterization of NTS isolates*

##### *4.3.2.5.1. Molecular epidemiology of the two key NTS serovars isolated from the study sites*

The molecular characterization revealed that all NTS isolates in our study were identified as either *S.* Enteritidis or *S.* Typhimurium, with their prevalence varying across the different demographic and environmental settings (Table 7). Overall, *S.* Enteritidis, was found among 0.8% (95%CI:0.49,1.22, *p*=0.392) and *S.* Typhimurium among 0.51% (95%CI:0.29,0.90, *p*=0.392) of cases. Regarding the age groups, children under 5 years showed similar prevalence for both serovars, each at the rate of 0.91% (95%CI:0.31,2.65, *p*=063). Harar exhibited the highest prevalence of both serovars, *S.* Enteritidis at 1.55% (95%CI:0.89,2.70, *p*=0.073), and *S.* Typhimurium at 1.29% (95%CI:0.71,2.37 *p*=0.263). However, Gondar and Addis Ababa had low prevalence for *S.* Enteritidis, whereas *S.* Typhimurium was not detected in Addis Ababa. Rural areas showed higher prevalence of *S.* Enteritidis, 1.62% (95%CI:0.75,3.49, *p*=0.041) compared to urban areas 0.61% (95%CI:0.35,1.07, *p*=0.041). Furthermore, seasonal trends indicated that prevalence of both serovars was the highest during the dry season, with *S.* Enteritidis at 0.96% (95%CI:0.54,1.72, *p*=619), and *S.* Typhimurium at 0.44% (95%CI:0.19,1.02, *p*=114).

Table 7. Prevalence of NTS serovars by selected socio-demographic characteristics.

| Factors | Category | *S*. Enteritidis | | *S*. Typhimurium | |
|---|---|---|---|---|---|
| | | **n (%)** | **Prevalence (95%CI)** | **n (%)** | **Prevalence (95%CI)** |
| | Overall (n=2331) | 18 (100) | 0.8 (0.49, 1.22) | 12 (100) | 0.51 (0.29, 0.90) |
| **Age** | <5 years | 3 (16.67) | 0.91 (0.31, 2.65) | 3 (25.0) | 0.91 (0.31, 2.65) |
| | 5-14 years | 2 (11.11) | 0.88 (0.24, 3.14) | 1 8.33) | 0.44 (0.08, 2.44) |
| | 15-19 years | 1 (5.56) | 0.93 (0.17, 5.10) | 0 (0.00) | 0 (0, 00) |
| | 20-29 years | 2 (11.11) | 0.36 (0.10, 1.32) | 2 (16.67) | 0.36 (0.10, 1.32) |
| | 30-39 years | 4 (22.22) | 0.97 (0.38, 2.47) | 4 (33.33) | 0.97 (0.38, 2.47) |
| | 40-49 years | 2 (11.11) | 0.67 (0.18, 2.41) | 1 (8.33) | 0.34 (0.06, 1.88) |
| | 50+ years | 4 (22.22) | 0.97 (0.38, 2.48) | 1 (8.33) | 0.24 (0.04, 1.37) |
| **Study sites** | Addis Ababa | 2 (11.11) | 0.25 (0.07, 0.92) | 0 (0.00) | 0 (0, 00) |

| | | | | | |
|---|---|---|---|---|---|
| | Gondar | 4 (22.22) | 0.52 (0.20, 1.33) | 2 (16.67) | 0.26 (0.07, 0.95) |
| | Harar | 12 (66.67) | 1.55 (0.89, 2.70) | 10 (83.33 | 1.29 (0.71, 2.37) |
| **Sex** | Male | 9 (50.0) | 0.70 (0.37, 1.33) | 8 (66.67) | 0.63 (0.32, 1.23) |
| | Female | 9 (50.0) | 0.85 (0.45, 1.61) | 4 (33.33) | 0.38 (0.15, 0.97) |
| **Residence** | Urban | 12 (66.67) | 0.61 (0.35, 1.07) | 9 (75.0) | 0.46 (0.24, 0.87) |
| | Rural | 6 (33.33) | 1.62 (0.75, 3.49) | 3 (25.0) | 0.81 (0.28, 2.36) |
| **Season** | Dry | 11 (61.11) | 0.96 (0.54, 1.72) | 5(41.67) | 0.44 (0.19, 1.02) |
| | Short Rain | 4 (22.22) | 0.76 (0.30, 1.94) | 1 (8.33) | 0.19 (0.03, 1.07) |
| | Long Rain | 3 (16.67) | 0.45 (0.16, 1.32) | 6 (50.0) | 0.90 (0.41, 1.96) |

#### *4.3.2.5.2. Seasonal trends of S. Typhimurium and S. Enteritidis isolates*

The prevalence of *S.* Enteritidis and *S.* Typhimurium varied across different seasons, and between the two serovars (Figure 18). *S.* Enteritidis was observed as 0.96% (95%CI:0.54,1.72) during the dry season, while *S.* Typhimurium had almost half the *S.* Enteritidis, 0.44% (95%CI:0.19,1.02). During the short rainy season, the prevalence of *S.* Enteritidis decreased to 0.76% (95%CI:0.30,1.94), and *S.* Typhimurium further declined to 0.19% (95%CI:0.03,1.07). However, during the long rainy season, *S.* Enteritidis showed a lower prevalence of 0.45% (95%CI:0.16,1.32), while *S.* Typhimurium exhibited almost double the prevalence of *S.* Enteritidis, at 0.90% (95%CI:0.41,1.96).

The monthly trends showed that, *S.* Enteritidis was peak during in October, followed by December and March (Figure 18). However, consistently lower trends were observed at the end of the short rainy season and the whole long rainy seasons. *S.* Typhimurium showed a peak in August, while no reports in December, and March. The monthly trends of *S.* Typhimurium and *S.* Enteritidis were too low to be considered per study site.

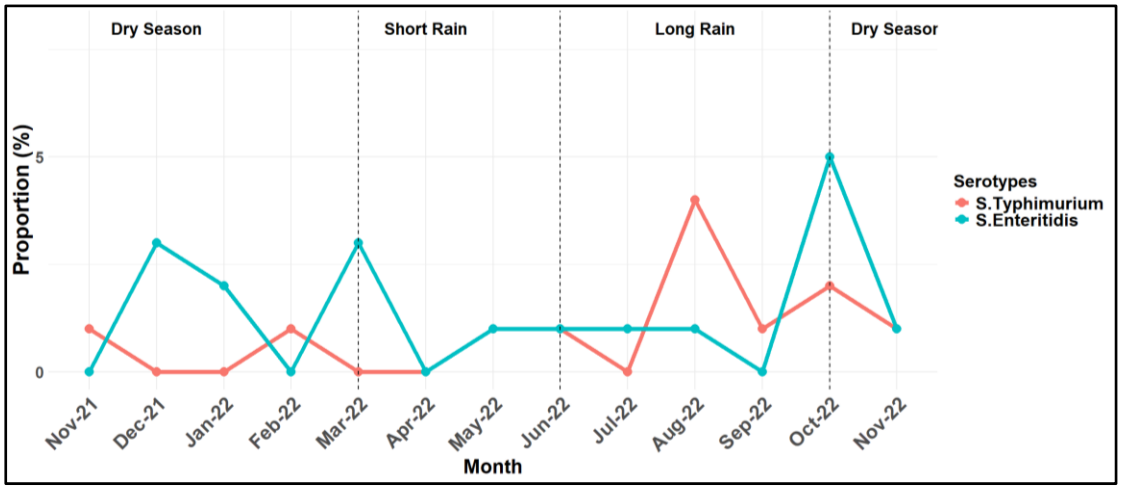


Figure 19. Seasonal trends of the two common NTS serovars.

#### *4.3.2.5.3. Antibiotic susceptibility patterns of S. Typhimurium and S. Enteritidis isolates*

The resistance patterns of *S.* Enteritidis and *S.* Typhimurium revealed notable variations between the two (Table 8). *S.* Enteritidis exhibited high resistance to ampicillin (72.22%), and tetracycline (61.11%), while resistance to amikacin, ampicillin-

sulbactam, azithromycin, and sulfamethoxazole-trimethoprim was observed in 22.22% to 38.89% of the isolates. In contrast, *S.* Typhimurium showed even slightly higher resistance to ampicillin (75.00%) but lower to tetracycline (58.33%). resistance to gentamicin and sulfamethoxazole-trimethoprim (50% each) was markedly higher than that of *S.* Enteritidis.

Table 8. Antibiotic susceptibility profile of *S.* Typhimurium and *S.* Enteritidis by study sites.

| **Antibiotics** | ***S*. Enteritidis (n=18)** | | ***S*. Typhimurium (n=12)** | |
|---|---|---|---|---|
| | **Susceptible** | **Resistant** | **Susceptible** | **Resistant** |
| | **n (%)** | **n (%)** | **n (%)** | **n (%)** |
| **AMK** | 11 (61.11) | 7 (38.89) | 11 (91.67) | 1 (8.33) |
| **AMP** | 5 (27.78) | 13 (72.22) | 3 (25.00) | 9 (75.00) |
| **AMS** | 12 (66.67) | 6 (33.33) | 8 (66.67) | 4 (33.33) |
| **AZM** | 14 (77.78) | 4 (22.22) | 10 (83.33) | 2 (16.67) |
| **CN** | 10 (55.56) | 8 (44.44) | 6 (50.00) | 6 (50.00) |
| **FEP** | 17 (94.44) | 1 (5.56) | 9 (75.00) | 3 (25.00) |
| **CAZ** | 15 (83.33) | 3 (16.67) | 9 (75.00) | 3 (25.00) |
| **CZA** | 14 (77.78) | 4 (22.22) | 9 (75.00) | 3 (25.00) |
| **CT** | 17 (94.44) | 1 (5.56) | 7 (58.33) | 5 (41.67) |
| **CRO** | 13 (72.22) | 5 (27.78) | 8 (66.67) | 4 (33.33) |
| **CXM** | 14 (77.78) | 4 (22.22) | 9 (75.00) | 3 (25.00) |
| **CIP** | 14 (77.78) | 4 (22.22) | 9 (75.00) | 3 (25.00) |
| **CL** | 18 (100.00) | 0 (0.00) | 12 (100.00) | 0 (0.00) |
| **GM** | 11 (61.11) | 7 (38.89) | 6 (50.00) | 6 (50.00) |
| **MEM** | 18 (100.00) | 0 (0.00) | 12 (100.00) | 0 (0.00) |
| **TZP** | 17 (94.44) | 1 (5.56) | 10 (83.33) | 2 (16.67) |
| **TC** | 7 (38.89) | 11 (61.11) | 5 (41.67) | 7 (58.33) |
| **SXT** | 10 (55.56) | 8 (44.44) | 6 (50.00) | 6 (50.00) |

***Note:*** *AMK: amikacin, AMP: ampicillin, AMS: ampicillin-sulbactam, AZM: azithromycin, CN: cefazolin, FEP: cefepime, CAZ: ceftazidime, CZA: ceftazidime-avibactam, CT: ceftolozane-tazobactam, CRO: ceftriaxone, CXM: cefuroxime, CIP: ciprofloxacin, CL: colistin, GM: gentamicin, MEM: meropenem, TZP: piperacilllin-tazobactam, TC: tetracycline, SXT: sulfamethoxazole-trimethoprim.*

#### *4.3.2.5.2. Molecular characterization of ESBL genes in the NTS isolates*

Among the four NTS isolates confirmed as ESBL producers, molecular analysis revealed that the bla-CTX-M gene was present in all the NTS isolates where Gondar and Harar split them equally (50% each). However, neither bla-TEM nor bla-SHV genes were detected in any of the NTS isolates across all sites.

### *4.3.3. Shigella spp.*

#### *4.3.3.1. Burden of Shigella spp. by different socio-demographic, clinical, and environmental factors*

The overall prevalence of *Shigella* spp. in the three sites was 2.79% (95%CI:2.19, 3.54), which varied by socio-demographic factors (Figure 19a). Harar had the highest prevalence at 5.05% (95%CI:3.71,6.83, *p*=0.03), followed by Gondar at 2.48% (95%CI:1.59,3.84, *p*=0.03), and Addis Ababa at 0.88% (95%CI:0.43,1.81, *p*=0.03). Among age groups, those aged 20-29 years had the highest prevalence at 4.20% (95%CI:2.81,6.22, *p*=0.924), while those 50+ years had the lowest at 1.46% (95%CI:0.67,3.15, *p*=0.924). Prevalence was almost similar in urban areas, 2.80% (95%CI:2.16,3.63, *p*=0.999), and in rural areas, 2.70% (95%CI:1.47,4.90, *p*=0.999). Females had a slightly higher prevalence, 3.04% (95%CI:2.16,4.25, *p*=0.790), compared to males, 2.58% (95%CI:1.85,3.61, *p*=0.790). Watery diarrhea was the most common symptom with *Shigella* spp. prevalence, at the rate of 3.39% (95%CI:2.61, 4.39, *p*=0.006).

Prevalence of *Shigella* spp. varied by environmental factors. For example, it is slightly higher in households with unimproved water sources, 2.91% (95%CI:1.71,4.92, *p*=0.013) compared to improved ones, 2.76% (95%CI:2.20,3.72) (Figure 15b). Similarly, prevalence was higher among households with unimproved sanitation, 3.14% (95%CI:2.18,3.81, *p*=0.024) compared with improved sanitation, 2.77% (95%CI:1.83,4.40, *p*=0.024). In addition, 43.08% (28/65) of the *Shigella* spp. were isolated from patients who did not have household animals, while 56.92% (37/65) of the *Shigella* spp. were isolated from patients who have household animals. Moreover, the highest prevalence was observed in households with goats, 4.83% (95%CI:2.36, 9.63, *p*=0.518), followed by cattle, 4.72% (95%CI:2.72, 8.07, *p*=0.242) (Figure 19b).

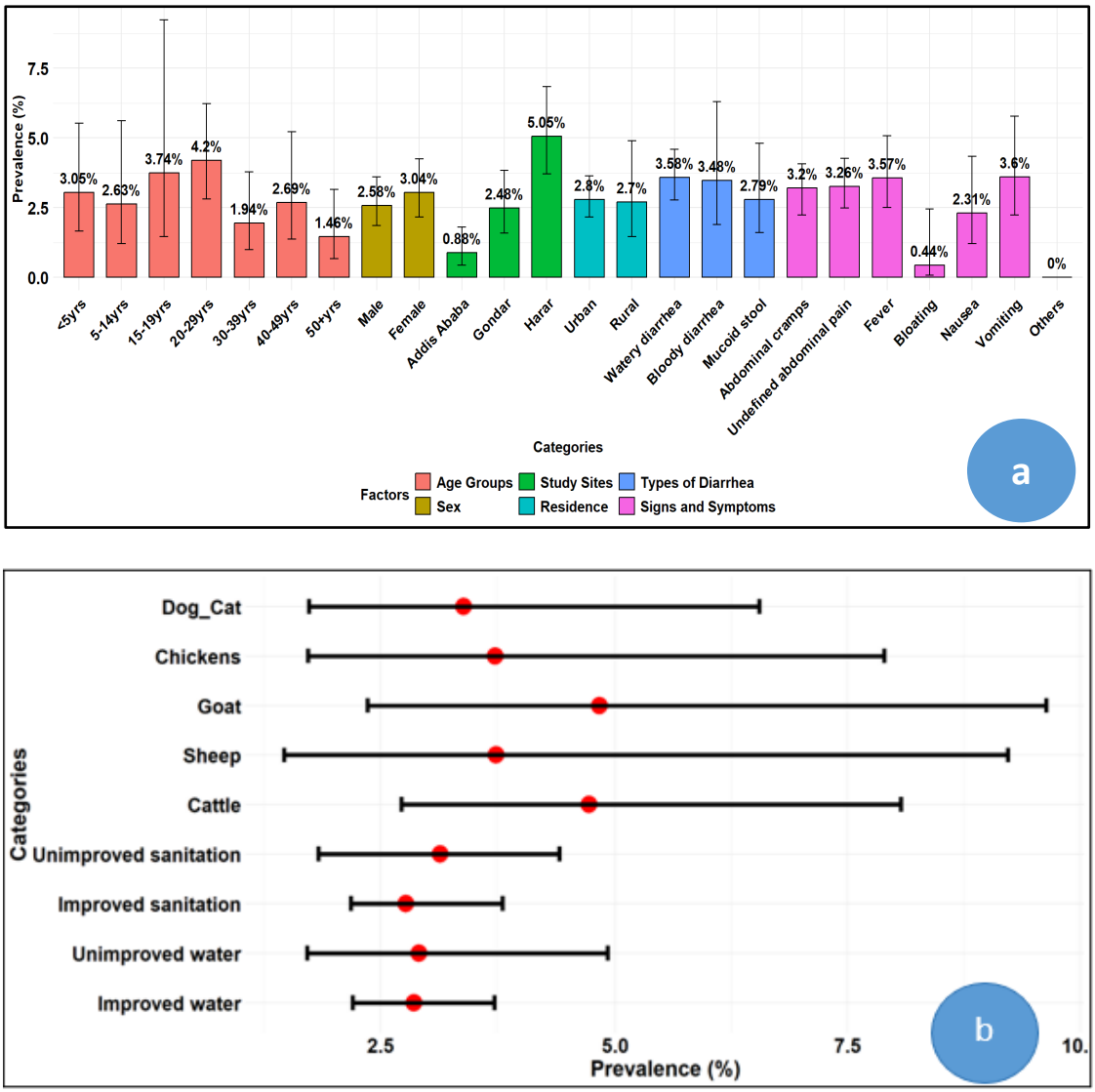


Figure 20. Distribution of *Shigella* spp. by socio-demographic and clinical (a) and living condition factors (b). Note: yrs: years, Dog_Cat: Dogs and, /or Cats.

### *4.3.3.2. Seasonal trends of Shigella spp. by the study sites*

The prevalence of *Shigella* spp. varied across seasons ($p$=0.007) where dry season showed the highest prevalence, at 3.61% (95%CI:2.32,5.57), followed by the short rainy season, at 2.80% (95%CI:1.99,3.93). However, in the long rainy season, the prevalence relatively decreased to 2.41% (95%CI:1.49,3.88).

The overall monthly trends showed that *Shigella* spp. was peaks in December, followed by February and March with notable variations across the study sites (Figure 20). For instance, in Harar, the peak was in December, followed by March, with a continuous decline from April to November. In Gondar, the peak was in May, followed by October,

and the low was from June to August and November to January. In contrast, Addis Ababa showed peak in July and remain declined in the rest of the months of the year (Figure 20).

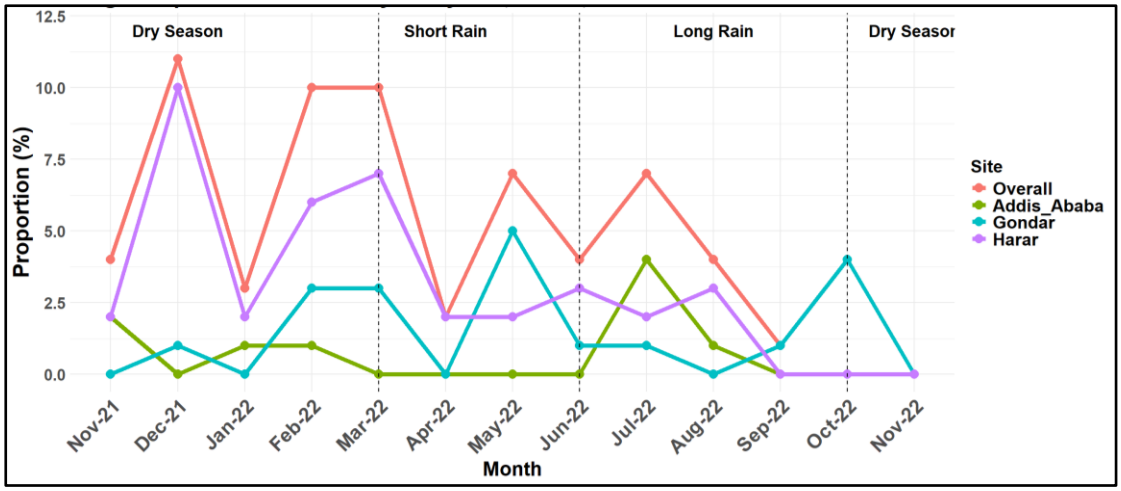


Figure 21. Seasonal trends of *Shigella* spp. by study sites.

### *4.3.3.3. Antibiotic susceptibility patterns of Shigella spp. by the study sites*

The resistance patterns observed for *Shigella* spp. across the study sites demonstrated significant variability among study sites (Table 9). Resistance to ampicillin was high (62.69%), particularly in Harar (66.67%), followed by Gondar (57.89%) and Addis Ababa (55.56%). A similar trend was seen for ampicillin-sulbactam, with a resistance rate of 58.21%, the highest being in Harar (66.67%). The resistance to azithromycin was also notable, with a rate of 31.34%, reaching 33.33% in Harar. The resistance to gentamicin was observed in 23.88% of isolates, with Harar again showing the highest rate (25.64%). Resistance to cefotaxime (43.28%) and ciprofloxacin (41.79%) was most evident in Harar, with rates of 46.15% and 43.59%, respectively. Alarmingly, tetracycline resistance was exceptionally high, with a rate of 80.60%, peaking in Harar at 87.18%. Similarly, resistance to sulfamethoxazole-trimethoprim was 58.21%, with Harar displaying the highest rate (61.54%).

The overall MDR among *Shigella* isolates was significantly high with the rate of 71.64% (48/69). The resistance rates were especially prominent in Harar, 76.92% (30/39), followed by Addis Ababa had the lowest MDR rate of 66.67% (6/9) and Gondar 63.16% (12/19) (Figure 21).

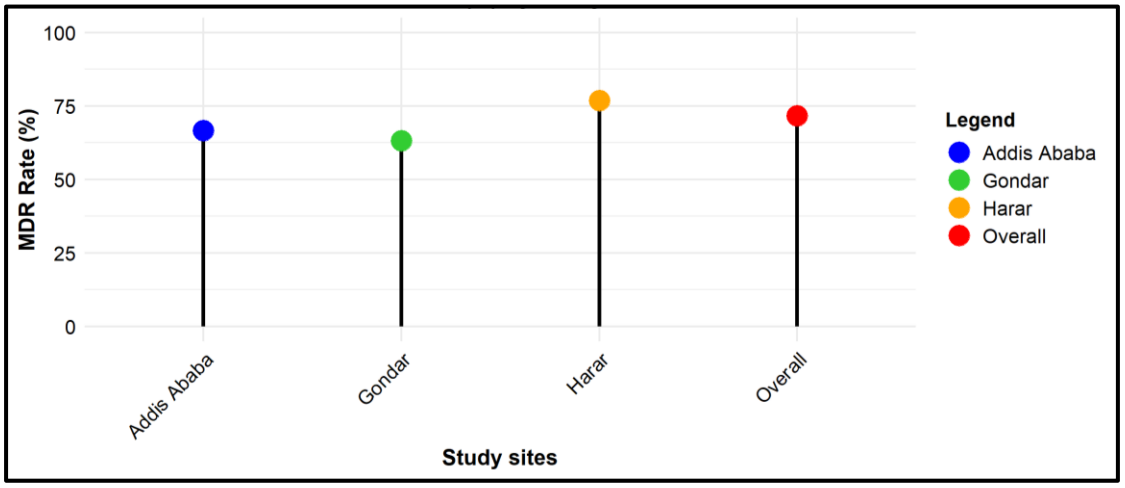


Figure 22. Distribution of MDR among *Shigella* spp. by study sites.

#### *4.3.3.3.1. Phenotypic ESBL production among Shigella Isolates*

Among *Shigella* spp., the phenotypic confirmation of overall rate of ESBL production was 32.84%, with significant regional variation. Harar had the highest rate (35.90%), followed closely by Addis Ababa (33.33%) and Gondar (26.32%).

Table 9. Antibiotic susceptibility profiles of *Shigella* spp. by the study sites.

| Antibiotics | Overall (n=67) | | Addis Ababa (n=9) | | Gondar (n=19) | | Harar (n=39) | |
|---|---|---|---|---|---|---|---|---|
| | Susceptible n (%) | Resistant n (%) | Susceptible n (%) | Resistant n (%) | Susceptible n (%) | Resistant n (%) | Susceptible n (%) | Resistant n (%) |
| **AMK** | 66 (98.51) | 1 (1.49) | 9 (100.00) | 0 (0.00) | 19 (100.00) | 0 (0.00) | 38 (97.44) | 1 (2.56) |
| **AMP** | 25 (37.31) | 42 (62.69) | 4 (44.44) | 5 (55.56) | 8 (42.11) | 11 (57.89) | 13 (33.33) | 26 (66.67) |
| **AMS** | 28 (41.79) | 39 (58.21) | 4 (44.44) | 5 (55.56) | 11 (57.89) | 8 (42.11) | 13 (33.33) | 26 (66.67) |
| **AZM** | 46 (68.66) | 21 (31.34) | 6 (66.67) | 3 (33.33) | 14 (73.68) | 5 (26.32) | 26 (66.67) | 13 (33.33) |
| **CN** | 40 (59.70) | 27 (40.30) | 6 (66.67) | 3 (33.33) | 13 (68.42) | 6 (31.58) | 21 (53.85) | 18 (46.15) |
| **FEP** | 46 (68.66) | 21 (31.34) | 6 (66.67) | 3 (33.33) | 14 (73.68) | 5 (26.32) | 26 (66.67) | 13 (33.33) |
| **CAZ** | 46 (68.66) | 21 (31.34) | 6 (66.67) | 3 (33.33) | 14 (73.68) | 5 (26.32) | 26 (66.67) | 13 (33.33) |
| **CZA** | 65 (97.01) | 2 (2.99) | 9 (100.00) | 0 (0.00) | 19 (100.00) | 0 (0.00) | 37 (94.87) | 2 (5.13) |
| **CT** | 66 (98.51) | 1 (1.49) | 9 (100.00) | 0 (0.00) | 19 (100.00) | 0 (0.00) | 38 (97.44) | 1 (2.56) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **CRO** | 42 (62.69) | 25 (37.31) | 6 (66.67) | 3 (33.33) | 11 (57.89) | 8 (42.11) | 25 (64.10) | 14 (35.90) |
| **CXM** | 38 (56.72) | 29 (43.28) | 4 (44.44) | 5 (55.56) | 13 (68.42) | 6 (31.58) | 21 (53.85) | 18 (46.15) |
| **CIP** | 39 (58.21) | 28 (41.79) | 4 (44.44) | 5 (55.56) | 13 (68.42) | 6 (31.58) | 22 (56.41) | 17 (43.59) |
| **CL** | 66 (98.51) | 1 (1.49) | 9 (100.00) | 0 (0.00) | 18 (94.74) | 1 (5.26) | 39 (100.00) | 0 (0.00) |
| **GM** | 51 (76.12) | 16 (23.88) | 7 (77.78) | 2 (22.22) | 15 (78.95) | 4 (21.05) | 29 (74.36) | 10 (25.64) |
| **MEM** | 65 (97.01) | 2 (2.99) | 9 (100.00) | 0 (0.00) | 18 (94.74) | 1 (5.26) | 38 (97.44) | 1 (2.56) |
| **TZP** | 61 (91.04) | 6 (8.96) | 9 (100.00) | 0 (0.00) | 18 (94.74) | 1 (5.26) | 34 (87.18) | 5 (12.82) |
| **TC** | 13 (19.40) | 54 (80.60) | 3 (33.33) | 6 (66.67) | 5 (26.32) | 14 (73.68) | 5 (12.82) | 34 (87.18) |
| **SXT** | 28 (41.79) | 39 (58.21) | 4 (44.44) | 5 (55.56) | 9 (47.37) | 10 (52.63) | 15 (38.46) | 24 (61.54) |

***Note:*** *AMK: amikacin, AMP: ampicillin, AMS: ampicillin-sulbactam, AZM: azithromycin, CN: cefazolin, FEP: cefepime, CAZ: ceftazidime, CZA: ceftazidime-avibactam, CT: ceftolozane-tazobactam, CRO: ceftriaxone, CXM: cefuroxime, CIP: ciprofloxacin, CL: colistin, GM: gentamicin, MEM: meropenem, TZP: piperacilllin-tazobactam, TC: tetracyclin, SXT: sulfamethoxazole-trimethoprim.*

#### *4.3.3.4. Molecular characterization of antibiotic-resistant genes from Shigella spp. isolates*

The molecular characterization of ESBL genes among the *Shigella* spp. isolates showed a high proportion of bla-CTX-M gene (95.45%). All ESBL producer *Shigella* isolates from Addis Ababa and Harar were bla-CTX-M positive, while 80% of those from Gondar harbored this gene. The bla-TEM gene was found in 59.09% of the isolates with regional rates ranging from 57.14% in Harar to 66.67% in Addis Ababa. On the other hand, the bla-SHV was the least common gene detected among *Shigella* isolates in this study, where it was detected only in one isolate from Harar, (Figure 22a). Co-detection of bla genes among *Shigella* isolates was also prominent, with the bla-CTX-M and bla-TEM gene combination identified in 95.24% of the isolates. One isolate carried all three genes, bla-CTX-M, bla-TEM, and bla-SHV. Co-detection of bla-CTX-M and bla-TEM genes was observed in 57.14% of isolates, each (Figure 22b).

Moreover, two of the *Shigella* spp. isolates were positive for the carbapenemase gene, known as the bla-NDM gene. The regional distribution indicated one isolates from Gondar and Harar each. All the *Shigella* spp. isolates in Addis Ababa were not positive for the carbapenem resistance by phenotypic tests and not targeted for the molecular detection of the carbapenemase genes.

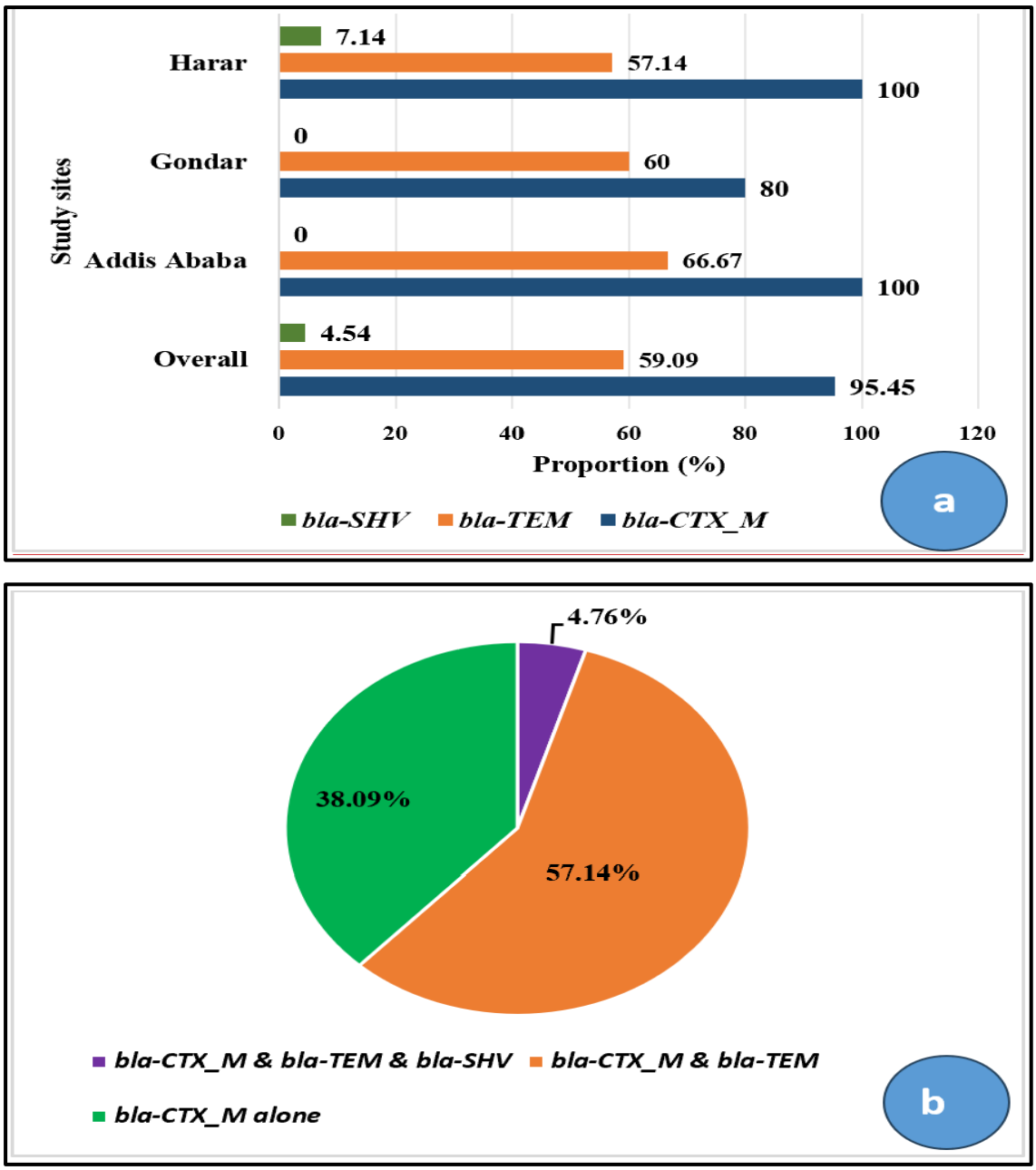


Figure 23. Distribution of the ESBL genes among the *Shigella* spp. by the study sites (a), and co-detection of ESBL genes (b).

### *4.3.3.6. Risk Factors for Shigella spp: Univariable and Multivariable analyses*

The univariable analysis regarding the odds of getting *Shigella* infection by study sites revealed that patients from Harar and Gondar had significantly higher odds (COR:1.04, 95%CI:1.02,1.06, $p$<0.001) and (COR:106, 95%CI:1.02,1.013, $p$=0.003) respectively, compared to Addis Ababa. Moreover, the use of unimproved water sources and unimproved sanitation were associated with higher odds of infection (COR:1.02, 95%CI:1.00,1.08, $p$=0.042), and (COR:1.09, 95%CI:1.03, 1.14, $p$=0.040), respectively.

In multivariable analysis, study sites, household water sources, and household sanitation facilities showed higher odds of *Shigella* infection. For instance, participants from Harar had, higher odds (AOR:1.39, 95%CI:1.02,1.57, $p$<0.001) of *Shigella* infection compared to those from Addis Ababa. Moreover, participants from Harar had higher odds (AOR:1.12, 95%CI:1.01,1.24, $p$<0.001), compared to Gondar. Additionally, participants from Gondar showed higher odds (AOR:1.15, 95%CI:1.09,1.35, $p$=0.040) of *Shigella* infection compared with Addis Ababa. Households with unimproved water sources showed almost double the odds of *Shigella* infection compared to those with improved water sources. Moreover, households with unimproved sanitation showed higher odds (AOR:1.39, 95%CI:1.27,1.74, $p$=0.030) of *Shigella* infection compared to those with improved sanitation (Figure 23).

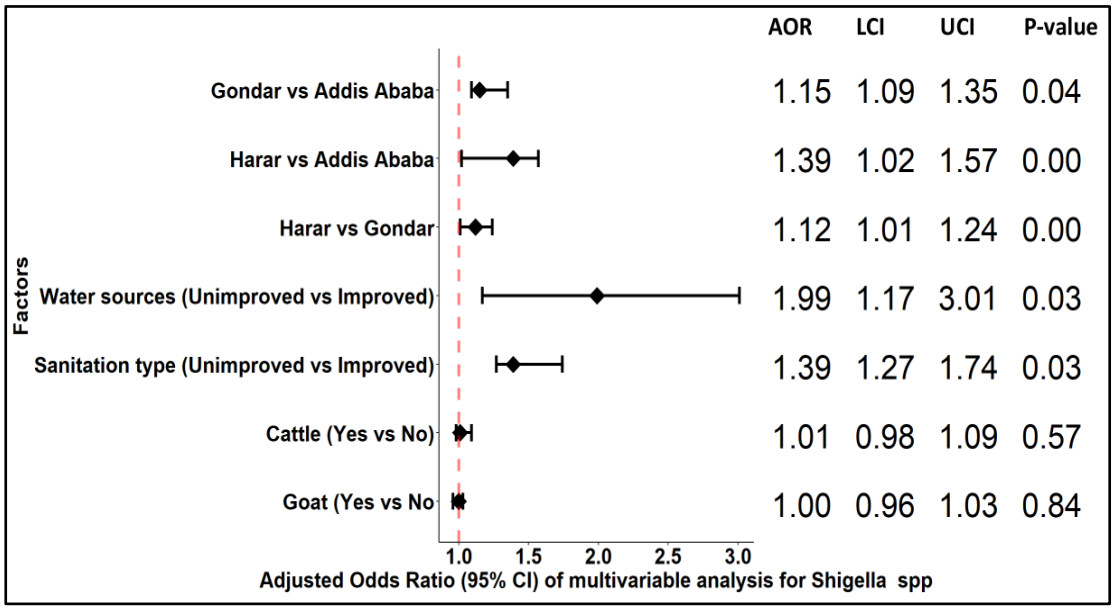


Figure 24. Odds Ratio of multivariable analysis of factors for *Shigella* spp. Note: AOR: Adjusted odds ratio, LCI: Lower confidence interval, UCI: Upper confidence interval. p=0.00: p<0.001.

## *4.3.4. Campylobacter spp.*

### *4.3.4.1. Prevalence of Campylobacter spp. by different socio-demographic, clinical, and environmental factors*

The overall prevalence of *Campylobacter* spp. from the three sites was 4.46% (95%CI:3.70,5.38), showing a significant variation across different demographic factors, clinical symptoms, and seasonal conditions (Figure 24a). For instance, Harar had the highest prevalence at 6.10% (95%CI:4.61,8.00, $p$=0.004) as compared with

other study sites, while the lowest was from Addis Ababa, 2.65% (95%CI:1.74,4.02, *p*=0.004). Children under the age of 5 years had the highest prevalence, 9.15% (95%CI:6.48,12.76, *p*<0.001), followed by those in the 5-14 years group with a prevalence of 12.28% (95%CI:8.63,17.18, *p*<0.001). The prevalence was higher in males, 5.09% (95%CI:4.01,6.43, *p*=0.129) compared to females, 3.70% (95%CI:2.72,5.02, *p*=0.129). Rural residents had a higher prevalence of 6.77% (95%CI:4.62,9.78, *p*=0.028) than urban residents 4.03% (95%CI:3.24,4.99, *p*=0.028). Regarding types of diarrhea in association with *Campylobacter* infection cases, watery diarrhea, 0.06% (95%CI:0.05,0.07, *p*<0.001) and mucoid stool, 0.06% (95%CI:0.04,0.08, *p*<0.001) showed higher prevalence.

*Campylobacter* spp., prevalence was higher in households with unimproved water sources at 6.50% (95%CI:4.57,9.18, *p*=0.028) compared to improved sources, at 3.98% (95%CI:3.19,4.96, *p*=0.028). Unimproved sanitation showed a slightly higher prevalence, 4.63% (95%CI:3.72,5.75) compared to improved sanitation, but did not show statistical significance difference, 4.04% (95%CI:2.79,5.82, *p*=0.609). Among animal-owning households, the highest prevalence was observed among those households with sheep, 11.21% (95%CI:6.53,18.59, *p*=0.001), followed by chickens, 10.56% (95%CI:6.70,16.26, *p*<0.001), goats, 9.66% (95%CI:5.84,15.55, *p*=0.003), cattle, 9.06% (95%CI:6.11,13.22), and the least was among dogs or cat owners, 3.81% (95%CI:2.02,7.09, *p*=0.720) (Figure 24b).

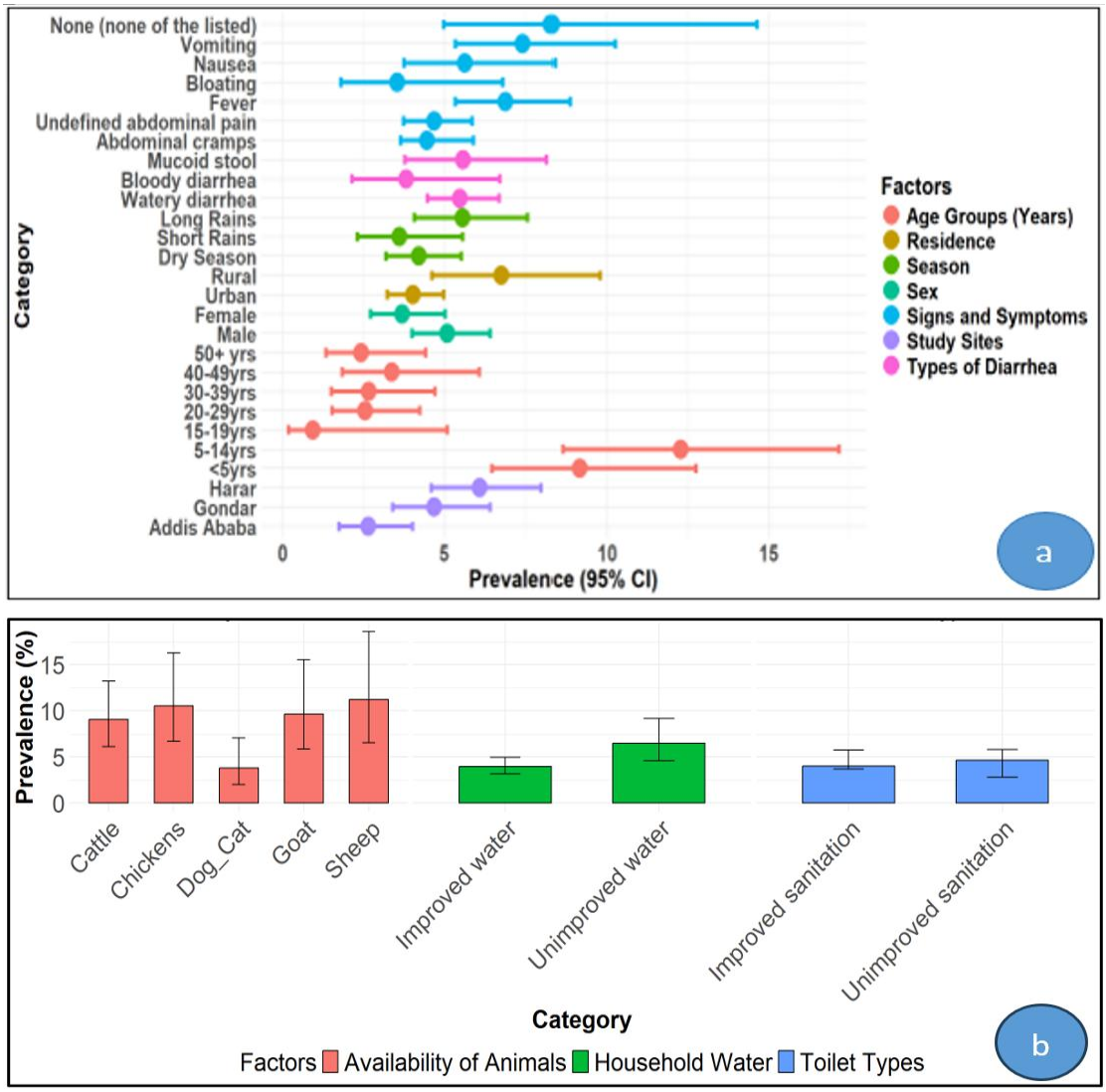


Figure 25. Distribution of *Campylobacter* spp. by socio-demographic and clinical () and living condition factors (b). Note: yrs: years. Dog-Cat: Dogs, and/or Cats.

#### *4.3.4.2. Seasonal trends of Campylobacter spp. infection by study sites*

The long rainy season had the highest prevalence of *Campylobacter* spp. at 5.57% (95%CI:4.07,7.59, $p$=0.224) followed by the dry season at 4.21% (95%CI:3.19,5.53, $p$=0.224), and the short rainy season had the lowest prevalence at 3.61% (95%CI:2.32,5.57, $p$=0.224).

The monthly trend showed peak in August, September, and July, while the decline was from January to April. The monthly trends varied among study sites, for example, Harar showed peak in August followed by July and September, while low trend was in April and June. In Gondar, the trend was relatively peak in May, followed by August, while it declined the rest of the years. In Addis Ababa, low trend was found throughout the

year with zero detection of *Campylobacter* spp. during the short rainy seasons, while a slight increase in February, July, August, and October (Figure 25).

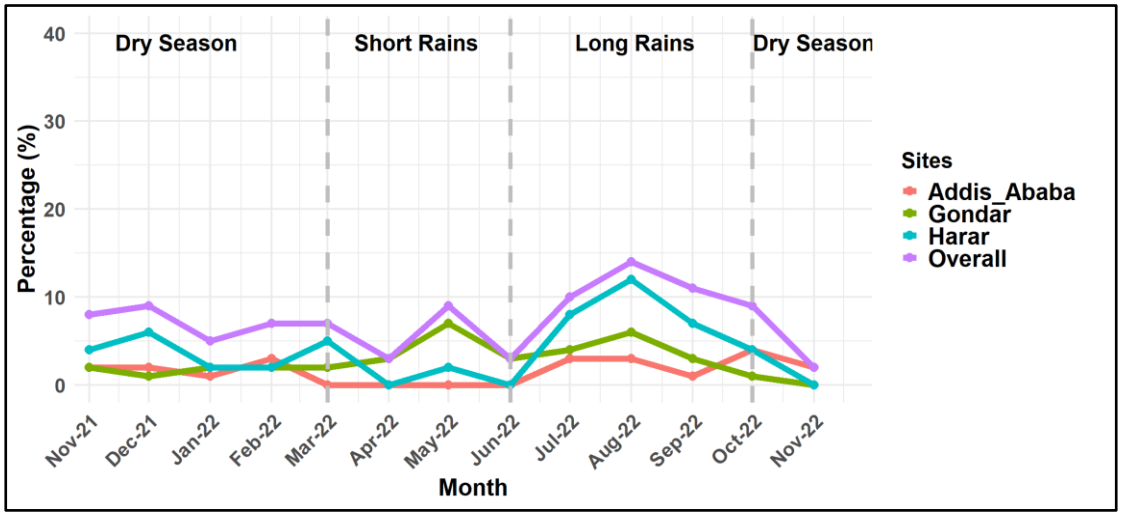


Figure 26. Seasonal trends of *Campylobacter* spp. by study sites.

### *4.3.4.3. Risk Factors for infection with Campylobacter spp.: Univariable and Multivariable analyses*

The univariate analysis on risk factors for *Campylobacter* spp. infection identified significant associations for age, study sites, season, water sources, and ownership of domestic animals. For instance, children under 5 years of age had significantly higher odds of infection compared to those aged 20-29 years (COR:1.07, 95%CI:1.04,1.10, $p$<0.001). Similarly, children aged 5-14 years also showed significantly increased odds of infection (COR:1.10, 95%CI:1.07,1.14, $p$<0.001). Study sites showed mixed results, with participants from Harar showing significantly higher odds of infection (COR:1.03, 95%CI:1.01,1.06, $p$<0.001) compared to Addis Ababa. Participants living in rural areas had higher odds of infection (COR:1.03, 95%CI:1.01,1.05, $p$=0.02) compared to urban residents. Among seasons, the long rainy season showed a statistically significant association with *Campylobacter* infection, (COR:1.02, 95%CI:1.00,1.03, $p$=0.018) and (COR:1.08, 95%CI:1.03,1.17, $p$=0.03) compared to the dry season and short rainy seasons respectively. Regarding environmental factors, households with unimproved water sources were significantly associated with increased *Campylobacter* infection (COR:1.03, 95%CI:1.00,1.05, $p$=0.02) compared to improved sources. Likewise, domestic animal ownership was significantly associations with *Campylobacter* infection, where ownership of cattle (COR:1.05, 95%CI:1.03,1.08, $p$<0.001), sheep (COR:1.07, 95%CI:1.03,1.12, $p$<0.001), goats COR:1.06, 95%CI:1.02,1.09, $p$=0.01),

and chickens (COR:1.07, 95%CI:1.03,1.10, $p$<0.001) were all significantly associated with higher odds of infection.

In multivariable analysis, children under 5 years and 5-14 years showed higher odds of *Campylobacter* infection, (AOR:1.07, 95%CI:1.04,1.10, $p$<0.001) and (AOR:1.10, 95%CI:1.07,1.14, $p$<0.001) respectively, compared to those with ages of 20-29 years. Similarly, residents in rural areas showed higher odds of *Campylobacter* infection (AOR:1.03, 95%CI:1.01,1.05, $p$=0.020) compared to urban residents. Moreover, the long rainy seasons showed higher odds of *Campylobacter* infection at 1.08, and 1.02 compared to short rainy seasons and dry seasons, respectively. Furthermore, ownership of cattle, sheep, goats, and chickens was associated with higher odds of *Campylobacter* infection, that ranging from odds ratio value of 1.05 to 1.09, compared to those who did not own the respective domestic animals (Figure 26).

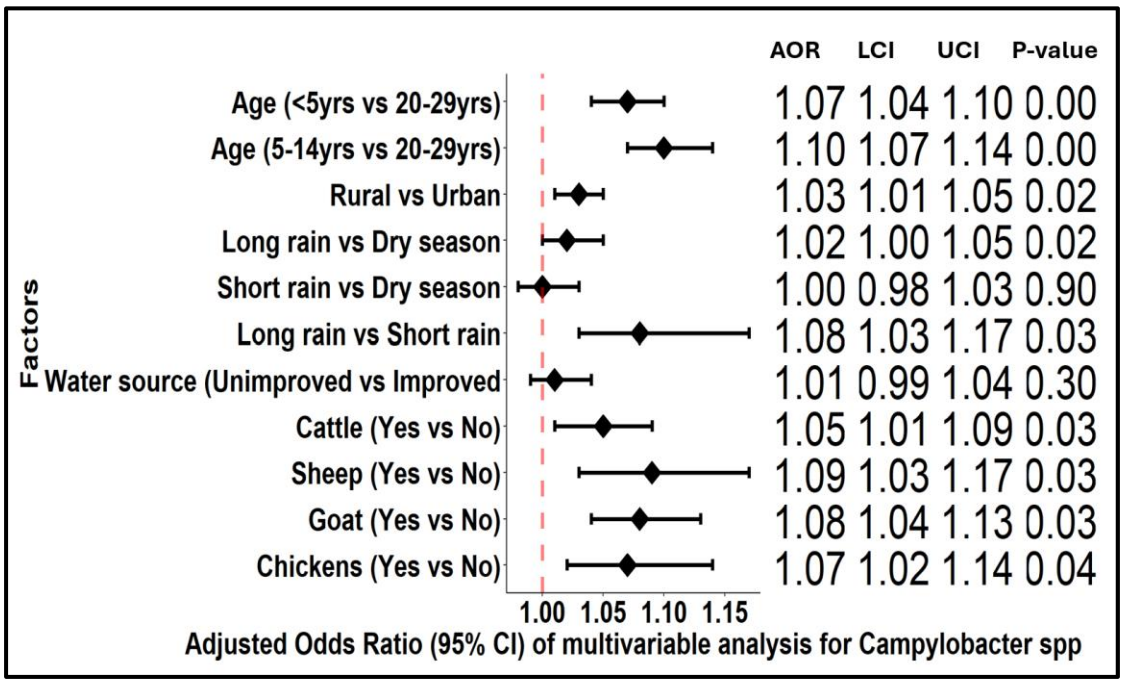


Figure 27. Odds Ratio of multivariable analysis of factors for *Campylobacter* spp.
Note: AOR: Adjusted odds ratio, LCI: Lower confidence interval, UCI: Upper confidence interval. p=0.00: p<0.001. yrs: years.

#### *4.3.4.4. Molecular characterization of the two common Campylobacter spp., using species-specific markers*

##### *4.3.4.4.1. Overall burden of C. jejuni and C. coli*

The prevalence of *C. jejuni* was 3.13% (95%CI:2.50,3.92), the highest rates being among children aged 5-14 years, 9.65% (95%CI:6.46,14.18, $p<0.001$) and under 5 years 6.40% (95%CI:4.23,9.59, $p<0.001$). Of all the three sites, Harar reported the highest prevalence of *C. jejuni* 5.18% (95%CI:3.83,6.98, $p<0.001$), followed by Addis Ababa, 2.27% (95%CI:1.44,3.56, $p<0.001$) and Gondar, 1.96% (95%CI:1.19,3.20, $p<0.001$). Males had slightly higher prevalence of *C. jejuni*, 3.92% (95%CI:2.98,5.12, $p=0.023$) than females, 2.18% (95%CI:1.46,3.25, $p=0.023$). Furthermore, rural areas surpassed urban areas with rate 4.32% (95%CI:2.68,6.91, $p=0.202$) and 2.91% (95%CI:2.25,3.75, $p=0.202$), respectively. On the other hand, the overall prevalence of *C. coli* was 0.90% (95%CI:0.60,1.37), the highest being among in children under 5 years of age, 2.74% (95%CI:1.45,5.13, $p=0.006$) and in Harar, 1.42% (95%CI:0.80,2.53, $p=0.006$). Rural areas, 1.89% (95%CI:0.92,3.85, $p=0.057$) showed higher rates than urban areas, 0.71% (95%CI:0.43,1.19, $p=0.057$) (Table 10). The remaining 10 (9.62%) samples were confirmed as *Campylobacter* spp. other than *C. jejuni* or *C. coli.*

Table 10. Prevalence of *C. jejuni* and *C. coli* among patients with *Campylobacter* spp. (n=104) by different socio-demographic characteristics.

| **Factors** | **Category** | ***C. jejuni*** | | ***C. coli*** | |
|---|---|---|---|---|---|
| | | **n (%)** | **Prevalence (95%CI)** | **n (%)** | **Prevalence (95%CI)** |
| | Overall | 73 (70.19) | 3.13 (2.50,3.92) | 21 (20.19) | 0.90 (0.60,1.37) |
| **Age** | <5 years | 21 (28.77) | 6.40 (4.23,9.59) | 9 (42.86) | 2.74 (1.45,5.13) |
| | 5-14 years | 22 (30.14) | 9.65 (6.46,14.18) | 3 (14.29) | 1.32 (0.45,3.80) |
| | 15-19 years | 1 (1.37) | 0.93 (0.17,5.10) | 0 (0,00) | 0 (0,00) |
| | 20-29 years | 11 (15.07) | 2.00 (1.12,3.56) | 1 (4.76) | 0.18 (0.03,1.03) |
| | 30-39 years | 5 (6.85) | 1.21 (0.52,2.81) | 2 (9.52) | 0.49 (0.13,1.75) |
| | 40-49 years | 6 (8.22) | 2.02 (0.93,4.34) | 3 (14.29) | 1.01 (0.34,2.93) |
| | 50+ years | 7 (7.59) | 1.70 (0.83,3.47) | 3 (14.29) | 0.73 (0.25,2.12) |

| | | | | | |
|---|---|---|---|---|---|
| **Study sites** | Addis Ababa | 18 (24.66) | 2.27 (1.44,3.56) | 3 (14.29) | 0.38 (0.13,1.11) |
| | Gondar | 15 (20.55) | 1.96 (1.19,3.20) | 7 (33.33) | 0.91 (0.44,1.87) |
| | Harar | 40 (54.79) | 5.18 (3.83,6.98) | 11 (52.38) | 1.42 (0.80,2.53) |
| **Sex** | Male | 50 (68.49) | 3.92 (2.98,5.12) | 12 (57.14) | 0.94 (0.54,1.64) |
| | Female | 23 (31.51) | 2.18 (1.46,3.25) | 9 (42.86) | 0.85 (0.45,1.61) |
| **Residence** | Urban | 57 (78.08) | 2.91 (2.25,3.75) | 14 (66.67) | 0.71 (0.43,1.19) |
| | Rural | 16 (21.92) | 4.32 (2.68,6.91) | 7 (33.33) | 1.89 (0.92,3.85) |
| **Season** | Dry | 39 (53.42) | 3.42 (2.51,4.64) | 13 (61.90) | 1.14 (0.67,1.94) |
| | Short Rain | 9 (12.33) | 1.71 (0.90,3.22) | 2 (9.52) | 0.38 (0.10,1.38) |
| | Long Rain | 25 (34.25) | 3.77 (2.56,5.50) | 6 (28.57) | 0.90 (0.41,1.96) |

#### *4.3.4.4.2. Seasonal trends of C. jejuni and C. coli infection by study sites*

The prevalence of *C. jejuni* and *C. coli* varied across the seasons in each study site (Figure 23). For *C. jejuni*, the highest prevalence was observed during the long rainy season at 3.77% (95%CI:2.56,5.50, *p*=0.096), followed by the dry season at 3.42% (95%CI:2.51,4.64, *p*=0.096). The lowest prevalence occurred during the short rainy season, at 1.71% (95%CI:0.90,3.22, *p*=0.096). In contrast, *C. coli* showed its highest prevalence during the dry season at 1.14% (95%CI:0.67,1.94, *p*=0.312), followed by the long rainy season at 0.90% (95%CI:0.41,1.96, *p*=0.312). The lowest prevalence of *C. coli* was shown during the short rainy season, at 0.38% (95%CI:0.10,1.38, *p*=0.312).

The monthly trends of *C. jejuni* showed that the peak was in August and December, with decline in April to June. The trends of *C. jejuni* varied by study sites where in Addis Ababa, peak was in October followed by July, while there was no detection between April and June. In Gondar, the peak was in August, with low trend in the remaining months. In Harar, the peak was in December, followed by September and March, while the decline was in April and June with a continuous peak from July to September (Figure 27a). For *C. coli*, the overall monthly trend was peak in December, followed by September, and the decline was from March to June. In Addis Ababa, the detection was relatively peak in August, followed by February. In Gondar, the peak was in May and September, while no detection between December and March. In Harar, the peak was in December with a decline from January to February, while no detection of *C. coli* from March to August. (Figure 27b).

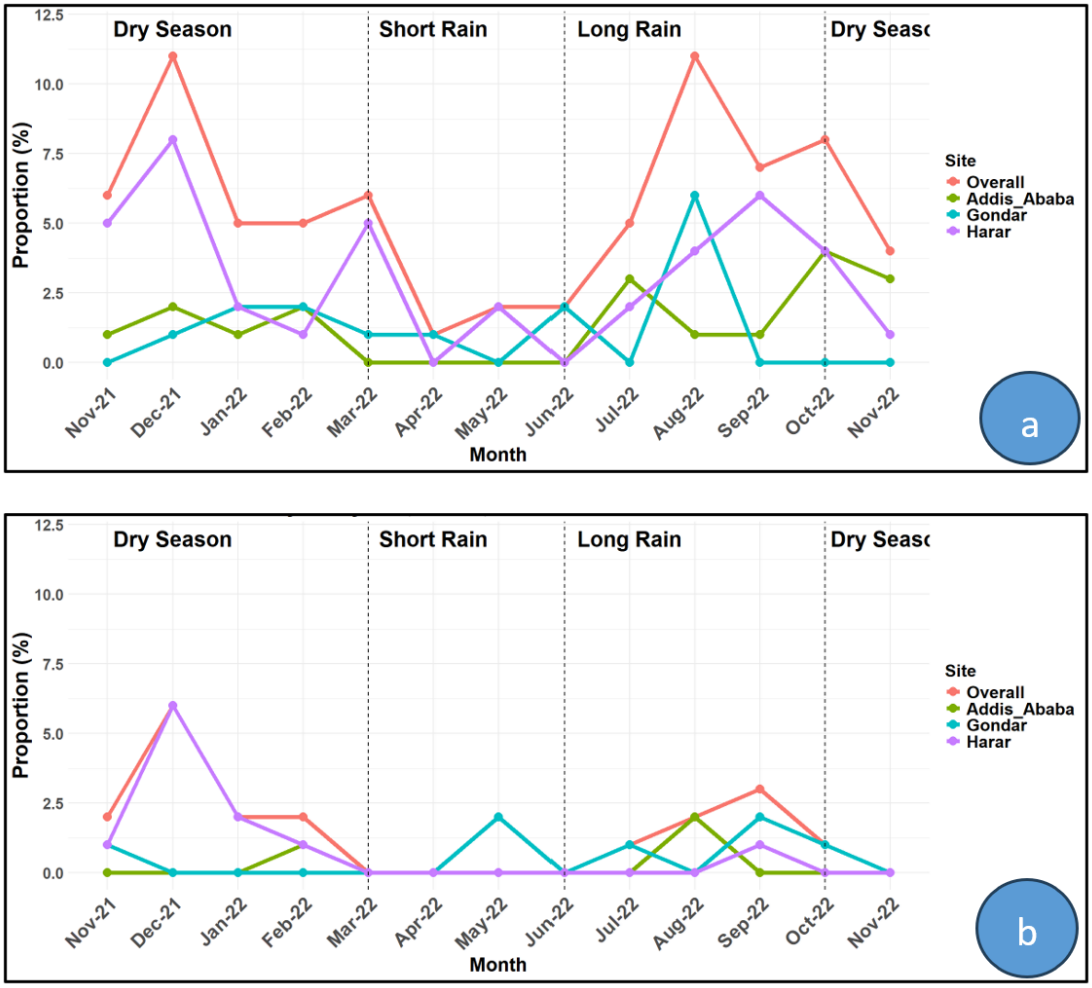


Figure 28. Seasonal distribution of *C. jejuni* (a) and *C. coli* (b) by study sites.

### *4.3.5. Co-detection of the selected foodborne pathogens by study sites*

The co-detection analysis of foodborne pathogens in this study highlighted varying patterns of pathogen pairs across the three study sites (Figure 28). Among the pairs, the co-detection of STEC and *Campylobacter* spp. was the most frequent, observed in 10 cases (0.43%) overall. This co-detection was more common in Harar, where five cases were reported, followed by Gondar with four cases and Addis Ababa with only one case. The combination of STEC and NTS was observed in five cases (0.21%), but no cases were detected in Addis Ababa. Gondar had two cases, while Harar reported three cases. The STEC and *Shigella* spp. co-detection occurred in four cases (0.20%), with a relatively even distribution across the three sites: two cases in Addis Ababa, and one case each in Gondar and Harar. In contrast, the pairing of *Campylobacter* spp. and NTS was observed in three cases (0.13%), all of which were detected in Harar. Similarly, *Campylobacter* and *Shigella* were co-detected in two cases (0.09%), with Harar and

Gondar contributing one case each. Finally, the co-detection of NTS and *Shigella* was the least common, occurring in just one case (0.04%), and it was observed solely in Harar.

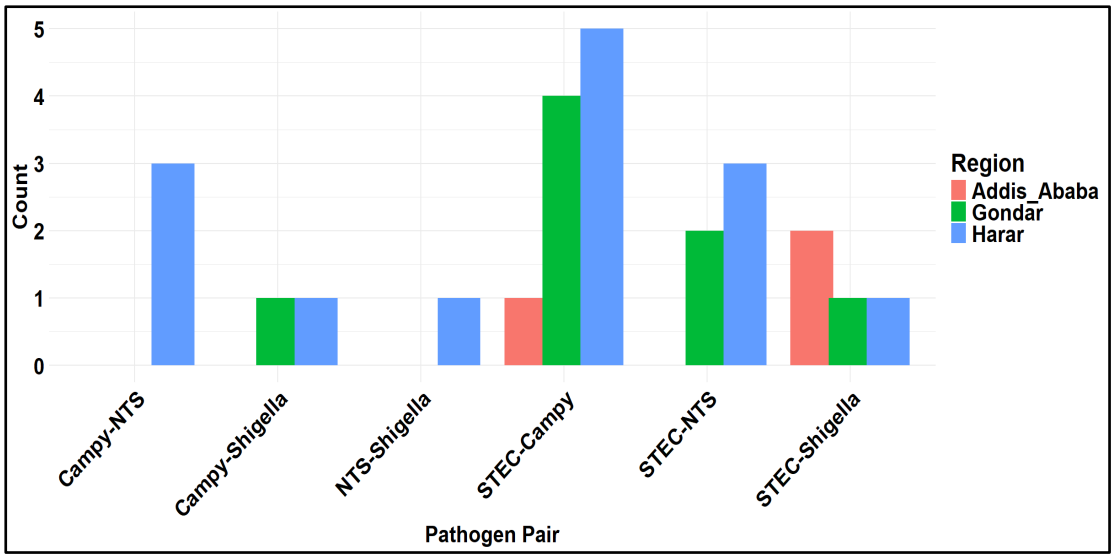


Figure 29. Frequency of foodborne bacterial pathogens co-detection by study sites. Note: Campy-NTS (*Campylobacter* spp. and Non-typhoidal *Salmonella*), Campy-*Shigella* (*Campylobacter* spp. and *Shigella* spp.), NTS-*Shigella* (Non-typhoidal *Salmonella* and *Shigella*), STEC-Campy (Shiga-toxin producing *Escherichia coli* and *Campylobacter* spp.), STEC-NTS (Shiga-toxin producing *Escherichia coli* and Non-typhoidal *Salmonella*), STEC-*Shigella* spp. (Shiga-toxin producing *Escherichia coli* and *Shigella* spp.)

# CHAPTER FIVE: DISCUSSION

## 5.1. Burden of the four targeted Foodborne bacterial pathogens

To the best of our knowledge, this study is the first of its kind in Ethiopia to prospectively estimate the prevalence of STEC, NTS, *Shigella* spp., and *Campylobacter* spp. concurrently in diarrheic patients across different geographic regions, seasons, and age groups. By doing so, this study provides crucial insights into burden of these pathogens and the interplay between them and the factors that may have relevance to their prevalence in diarrheal patients. Thereby helping the concerned healthcare authorities get scientific evidence to make right strategies towards the development of more precise and effective public health interventions.

Overall, the prevalence of the four target pathogens in our study were lower than those reported by most of the previously conducted studies in Ethiopia. One likely reason may be that most of the previously conducted studies used biochemical and serological tests for identification of the targeted pathogens (Kahsay et al., 2023; Sisay et al., 2024). In our study, 9% of the *Salmonella* isolates that were positive by culture tested negative by PCR, which may reflect differences in assay sensitivity, DNA quality, or the presence of non-viable or atypical strains not detected by the molecular assay. Such variations in diagnostic approaches could partly explain the lower prevalence observed in our findings compared to previous reports. Conventional laboratory tests, which generally have lower specificity (Gholami et al., 2019), may overestimate pathogen prevalence, whereas the molecular confirmation methods employed in our study provide more specific and accurate estimates.

Moreover, our study was conducted after the emergence of COVID-19; the control measures imposed during the pandemic could have led to improvements in hygienic practices in the population (UNICEF, 2020). For example, recommendations to reduce consumption of raw meat and dairy products, were part of the COVID-19 control strategy implemented in Ethiopia, which could have promoted a reduction of infections from the targeted pathogens, in the case of STEC and NTS (Swinnen, 2020). In addition, the disruptions to the supply chains in the markets and labour shortage may have caused a decrease in poultry production during COVID-19, conditions that might have

contributed to a reduced exposure to FBP, such as *Campylobacter* spp. (Berhanu et al., 2021).

### *5.1.1. Shiga-toxin producing E. coli*

An important contribution from this study is determination of the estimates of STEC's broader prevalence as most previous studies conducted in Ethiopia investigated only specific subsets of STEC variants such as *E. coli* O157:H7 (Engda et al., 2023; Getaneh et al., 2021). In contrast, our study covered the STEC O157:H7 and non-STEC O157:H7 serotypes, recognizing that the *stx1* and *stx2* genes encode Shiga toxins responsible for bloody diarrhea and sever complications. These include life-threatening conditions such as HUS and central nervous system abnormalities, particularly when the toxins are co-expressed with the *eae* gene, which encodes the adherence factor intimin (*eae*). Our analysis focused on these genes to consider the multitude of STEC serotypes, including the non-O157 strains that are often overlooked (Getaneh et al., 2021; Wolde et al., 2022).

The overall prevalence of STEC from the current study was 12.56%, with Harar showing the highest prevalence of 15.16%, which might be due to limited water supply and poor sanitation facilities observed in this study where 29.66% and 44.56% of the households used unimproved water sources and unimproved sanitation facilities in Harar, respectively. Moreover, limited water supply and poor sanitation such as hand-washing facilities were also reported by other study in Harar (Girma et al., 2024; Sisay et al., 2024). Furthermore, a study conducted by LaPolt et al. (2025) reported that unimproved sanitation facilities were used by 93.6% of diarrheal households in Harar compared to just approximately 50% in the other two cities (LaPolt et al., 2025). Moreover, several environmental and behavioural risk factors were observed to influence the transmission and virulent gene distribution where over 50% of diarrheal cases in Harar occurred in household that owned animals, versus approximately 25% in Gondar and none in Addis Ababa (LaPolt et al., 2025). As expected, the prevalence of STEC was higher than the prevalence of *eae* harboring STEC, as the latter is a subset of the former (Getaneh et al., 2021; Wolde et al., 2022).

In Addis Ababa, our 3.28% estimate for *eae* harboring STEC was slightly higher than previous estimates of 1.6% (Zelelie et al., 2023) and 2.8% (Gutema et al., 2021).

However, Zelelie et al. (2023) only tested stool samples with blood, mucus or pus for *eae* harboring STEC, which may have been missed from patients with other forms of diarrhea unlike ours where all types of diarrheal stools were considered. The problem with consideration of only bloody diarrhea is that certain STEC serotypes can cause watery diarrhea without visible signs of blood or mucus that could lead to underestimating the prevalence of *eae* harboring STEC (Getaneh et al., 2021). In addition, overlapping symptoms such as blood, mucus and pus in the stool can also be a common symptom of other bacterial pathogens such as *Shigella* spp. and *Salmonella* spp. (Croxen et al., 2013), which could dominate the stool samples selected for analysis. Similarly, Gutema et al. (2021) targeted only the STEC O157 serotype unlike ours, which may explain the higher prevalence in our study (Gutema et al., 2021).

Our estimate of 7.56% for STEC from Gondar was like the 7.7% estimate from a previous study from the same geographic area (Mulu et al., 2024). However, our estimate of 2.87% specific for *eae* harboring STEC was substantially lower than another previous estimate of 11.1% reported for the same strain (Engda et al., 2023), where they have considered only children under 5 years who had cattle in their households; the latter conditions might have increased the likelihood of exposure to the pathogen and, hence might, explain the higher estimates observed from that study. Similarly, our estimate of 4.15% prevalence for *eae* harboring STEC from Harar was much lower than the estimate of 15.3% from another prospective study among children under 5 years with diarrhea (Getaneh et al., 2021); however, that study used laboratory methods with lower specificity.

Furthermore, our molecular analysis of STEC revealed variations in the proportion and co-detection of *stx1*, *stx2*, and *eae* genes, consistent with the findings from the very limited other molecular studies in Ethiopia. For instance, in the study by Wolde et. al. (2022), *stx1* (8.8%) was the predominant virulence factor detected, followed by *eae* (7.4%), and *stx2* (5.9%). Across different geographic contexts in this study, *stx1* gene often emerged as the most prevalent virulent factor, it was detected at a higher proportion (66.89% overall) compared to *stx2* (49.83%). Other studies also found similar trend. For instance, a study in Addis Ababa and Hosana also found that *stx1*was detected from two of the 30 diarrheagenic *E. coli* isolates while *stx2* was detected from only one of them (Wolde et al., 2024). The proportion of these virulence factors in our study was higher than from those cited studies, and the possible explanation might be

due to the inclusion of phenotypically confirmed STEC isolates (n = 293), while the previous studies analysed a small subset of diarrheagenic *E. coli* without initial STEC confirmation. Therefore, our approach likely increased the likelihood of detecting *stx*-positive isolates, capturing a wider range of STEC strains circulating in the study population.

### *5.1.2. Non-typhoidal Salmonella*

The prevalence of NTS in our study (1.29%) was notably lower than that reported in previous studies (Kahsay et al., 2023; Abate et al., 2021). A meta-analysis of 34 prospective studies conducted among diarrheal patients in Ethiopia estimated NTS prevalence of 4.8% (Abate et al., 2021), which is more than 3 percentage points higher than our finding. However, this meta-analysis included studies that relied only on biochemical confirmation, a method that may overestimate prevalence due to its limited ability to differentiate between closely related strains (Gholami et al., 2019). Another review of 15 studies conducted from 2010 to 2022 among diarrheal patients in Ethiopia reported NTS prevalence ranging from 1% to 13% (Kahsay et al., 2023). Two of these studies conducted in 2018 and 2020 reported a prevalence of 1%, reflecting a more recent time frame, which may better represent the current state of NTS prevalence.

Our site-specific NTS prevalence estimates were also in general lower than what was reported in other studies (Belina et al., 2023; Reda et al., 2011; Demissie et al., 2014; Teferi et al., 2020; Admassu et al., 2015). In Addis Ababa, our estimate for NTS (0.25%) was significantly lower than the 3.5% to 6.2% prevalence estimates reported in previous studies (Eguale et al., 2015; Demissie et al., 2014; Kebede et al., 2021; Beyene et al., 2011). Additionally, in Northern Ethiopia (i.e., Gondar), our study found a NTS prevalence of 0.8%, which almost aligns with a 1.1% report by a previous study from the area (Demissie et al., 2014) but is lower than the 5.2% (Teferi et al., 2020), and 7.8% (Admassu et al., 2015) reported from other studies. Moreover, the 2.8% prevalence observed from Harar in our study was significantly lower than the previous reports of 6.11% to 14.6% from the same area (Belina et al., 2023; Reda et al., 2011; Demissie et al., 2014). The above site-specific data indicate that the prevalence of NTS varied significantly across the studied regions of Ethiopia. It was lower in Addis Ababa compared to Harar and Gondar, presumably due to the availability of more hygiene infrastructure and public health interventions in the capital of the country. In addition,

all participants in Addis Ababa were urban residents and, therefore, may have increased access to public health and food safety information, and be able to implement prevention measures such as sanitation practices (Girma et al., 2021; Sisay et al., 2024).

The molecular characterization of the two prominent serovars of NTS isolates from this study, *S.* Enteritidis and *S.* Typhimurium, revealed significant variability across demographic and environmental factors. Overall, *S.* Enteritidis was identified from 0.8% of cases, while *S.* Typhimurium from 0.51%. There have been differences among various previous studies regarding the predominant circulating serovar in Addis Ababa; Eguale et al. (2015) reported that the predominant serovar was *S.* Typhimurium, while another recent study in Addis Ababa reported *S.* Kiambu to be the predominant serovar followed by *S.* Saintpaul and *S.* Haifa (Kebede et al., 2021). This variation in circulating serovar might be due to the difference in animal exposure and feeding habit of the people (Hoelzer et al., 2011).

### *5.1.3. Shigella spp.*

In this study, the overall *Shigella* prevalence was found to be 2.79%, which is lower than the rates from meta-analyses that reported 6.6% across 29 studies (Hussen et al., 2019), 6.24% across 43 studies in another review study in Ethiopia (Huruy et al., 2011), and 4.0% (Ayele et al., 2023) among children under five years in Addis Ababa. These discrepancies may have emanated from use of conventional identification methods in those studies included in the meta-analyses, which naturally overestimate prevalence because of their reduced specificity. Site-specific results from this study showed that Addis Ababa exhibited the lowest prevalence (0.88%), likely due to urban residents having improved access to healthcare and food safety information (Girma et al., 2021; Sisay et al., 2024), unlike rural Ethiopians where for example 56% of whom still use unimproved toilet facilities (CSA, 2019). However, another meta-analysis reflecting the findings of both health facility and community studies found a higher (2.2%) prevalence in Addis Ababa (Hussen et al., 2019). Some more prospective studies also reported as high as 5.6% (Ararsa et al., 2023), 8.8% (Ayele et al., 2023) and even 9.1% (Mamuye et al., 2015). Some of the explanations for this disparity may be the seasonality, as the studies by Mamuye et al. (2015) and Ararsa et al. (2023) were conducted during the months known for heightened enteric disease transmission. Mamuye et al. (2015) collected data between August and December, covering the late

long rainy season and early dry season, while Ararsa et al. (2023) conducted their study from April to June, corresponding to the short rainy season and the onset of long rainy season. These seasonal windows are known to influence water quality, sanitation conditions, and pathogen circulation, potentially contributing to the higher *Shigella* prevalence reported in those studies.

Likewise, the findings of this study from Gondar revealed a 2.48% prevalence, which is notably lower than previous reports of 4.57% (Demissie et al., 2014), and 10.7% (Alemu et al., 2019). This variation might be due to the difference in the diagnostic methods where commercially available polyvalent O-antigen antisera, which are prone to cross-reactivity were used in their study (Demissie et al., 2014). Some of the O-antigens associated with *Shigella* strains such as O112ac, O124, and O152 are also present in EIEC (Pavlovic et al., 2011), that might overestimate their prevalence. Moreover, Alemu et al. (2019) used biochemical tests and *Shigella* and EIEC share similar biochemical characteristics, including being Gram-negative, non-motile, non-lactose fermenting, and negative for $H_2S$, urease, and citrate utilization. Because of these similarities, biochemical tests alone cannot reliably differentiate *Shigella* from EIEC, often leading to misidentification of EIEC as *Shigella*. However, this study used PCR confirmation of *Shigella* (*ipaH*$^+$ *lacY*$^-$) which have high sensitivity and specificity for the accurate diagnosis of *Shigella* spp.

In Eastern Ethiopia (Harar), we found a 5.05% prevalence, consistent with a previous report of 5.6% prevalence in Dire Dawa (Mekonnen et al., 2018), and 14.6% in Jigjiga (Mekonnen et al., 2014), but a bit higher than a recent study reporting only 2.5% in Jigjiga (Muse et al., 2024). The notable disparity in the reported prevalence, including between the two studies from Jigjiga may be attributable to several factors. First, Mekonnen et al. (2014) used a biochemical test-based identification, which may detect a broader-ranges of *Shigella* strains and potentially overestimate prevalence. In contrast, more recent studies like ours utilizing molecular diagnostics are generally more specific, which can yield lower prevalence estimates. Secondly, the difference in study design and duration may also contribute to the variation. The study by Muse et al. (2024) covered only a shorter study period and had a smaller sample size, comprising only a quarter of the study period and population compared to ours, which could have influenced the representativeness and the lower observed prevalence in their study.

Moreover, environmental and infrastructure differences, particularly in water access, could influence pathogen transmission. A study by Chekol et al. (2020) reported that 81% of Jigjiga households relied on substandard water sources, a risk factor for fecal-oral transmission of pathogens such as *Shigella* spp. Lastly, seasonal timing of data collection may further explain the differences. For example, the study by Mekonnen et al. (2014) was conducted during the end of the dry season and short rainy season, a period during which our data also showed relatively higher prevalence of *Shigella* spp.

### *5.1.4. Campylobacter spp.*

The overall prevalence of *Campylobacter* spp. in this study was 4.46%, which is about half of the estimates from previous reports. For example, a meta-analysis comprising eight studies conducted between 2000 to 2010 estimated the prevalence of *Campylobacter* spp. in Ethiopia to be 10% (Diriba et al., 2021). The reason for this disparity could be that the studies in the meta-analyses covered areas including Amhara, Oromia, Sidama, and Gambella regions where most of the participants were rural residents, and children under five years. In contrast, our study focused on three major cities and about 70% of the participants were urban residents. Urban areas tend to have lower prevalence of *Campylobacter* spp. due to reduced direct exposure to animals, and increased access to treated water sources while rural residents are more likely to engage in animal-related occupations and have poorer water and sanitation infrastructure, all of which could increase exposure to *Campylobacter* infection (Lengerh et al., 2013).

In line with this reasoning, there were notable differences in this study in regard to the prevalence of *Campylobacter* between the three study sites (2.65% from Addis Ababa; 4.69% from Gondar; and 6.10% from Hara), which may reflect concomitant differences in the status of hygiene practices and public awareness in these places (Girma et al., 2021; Sisay et al, 2024). Moreover, the exposure to reservoir animals such as chickens and cattle, and consumption of unpasteurized milk might be relatively higher in the rural areas compared to the urban areas, which might contribute to the lower prevalence observed in Addis Ababa (Behailu et al., 2022).

Moreover, the overall prevalence of *Campylobacter* infection documented in all the three sites in the current study was lower than the prevalence reported from the ones in the previous studies except for one study from Addis Ababa, where it was 3.2% among children under 5 years of age (Teklu et al., 2018), which is higher than our estimate but

targeted children under 5 years, that is the age group with high prevalence in our study as well. Another study of adults and children in Addis Ababa found the prevalence to be 1.8% (Abebe et al., 2020), which is lower than our estimates. However, the estimates of the latter studies are within the confidence intervals of our result. On the contrary, a prospective study in Northern Ethiopia (i.e., Gondar) among children under 5 years suffering from diarrheal illness estimated the prevalence of *Campylobacter* to be 15.4% (Lengerh et al., 2013) while another study among diarrheal patients of all ages in a different location of Northern Ethiopia estimated 8.0% (Ewunetu et al., 2010). These estimates are much higher than our estimates for Gondar, but both studies relied on conventional culture and biochemical identification methods, including oxidase, catalase, and hippurate hydrolysis tests, which are known to have lower specificity compared to molecular assays such as PCR.

In addition, a recent prospective study among children under 5 years with diarrhea in Eastern Ethiopia (i.e., Harar) estimated prevalence of *Campylobacter* to be 8.4% (Belina et al., 2023), which is slightly higher than the 6.10% estimate from our study for Harar. This difference might be because of the participant's residence, where approximately 50% of the study participants from the study of Belina and coworkers were from rural areas in contrast to the 26% of rural study participants of our study. Moreover, another prospective study among 100 children under 5 years with diarrhea in Harar ranged from 50% by PCR to 88% by meta-total RNA sequencing (MeTRS) (Terefe et al., 2020), which is significantly higher than our estimate; however, it should be noted that the researchers from the previous study used laboratory methods which can detect all actively transcribed microbial RNA in a sample, that is just the detection of the any *Campylobacter* related genes, even the non-pathogenic or without clear clinical relevance.

Of the two *Campylobacter* spp. detected in our study, *C. jejuni* was more prevalent (3.13%) than *C. coli* (0.90%). The highest rate of *C. jejuni* was observed among children aged 5-14 years (9.65%), as in the case of C. *coli*, where the highest prevalence (2.74%) was again seen among children under 5 years. In both of the *Campylobacter* spp. cases, Harar showed the highest prevalence for C. *jejuni* at 5.18% and *C. coli* at 1.42%. In general, variations in *Campylobacter* spp. cases have been observed regarding the distribution of the two species throughout the three study sites and participants' age group, where the highest burden of both species was observed among

children under 5 years, which is consistent with findings from a systematic review that included 12 studies in Ethiopia (Zenebe et al., 2020). The lower prevalence of *C. coli* compared to *C. jejuni* from our findings is also consistent with those from other studies. *C. coli* is generally less prevalent than *C. jejuni* in both developed and developing countries, with most cases being reported in areas with high levels of animal contact or where poultry farming is common (Worku et al., 2024).

## 5.2. Seasonal distribution of the four targeted Foodborne bacterial pathogens

Our findings revealed distinct seasonal patterns in the prevalence of FBPs. These findings are consistent with growing evidence that climate variability, characterized by fluctuations in temperature, humidity, rainfall, storms, and flooding plays a pivotal role in the transmission dynamics of FBPs such as *Salmonella*, *E. coli*, and *Campylobacter* (Balta et al., 2024).

Seasonal changes not only are important in affecting the prevalence of FBPs, but they are also critical in shaping may other aspects of disease burden including transmission dynamics and severity of foodborne illnesses. For example, temperature is a well-established driver of *Salmonella* growth and transmission across the food production and supply chain. Optimal growth occurs between 35$^{o}$C and 37$^{o}$C, while it is significantly delayed at temperatures below 15$^{o}$C (Damtew et al., 2024). A strong correlation has been reported between ambient temperature and salmonellosis incidence. Akil et al. (2014) observed that a 1$^{o}$C rise in the weekly maximum temperature was associated with 8.8% increase in salmonellosis cases, while a similar increase in the minimum temperature resulted in a 5.8% rise.

A review by Lal et al. (2012) demonstrated that the seasonal index for salmonellosis exhibited a broad peak extending from summer into early autumn (June to September). In North America, the earliest peaks occurred in the U.S. (June to July), followed by Canada and Europe in July, and the UK and Asia in August. The Oceanic region, adjusted for Northern Hemisphere seasons, showed a primary peak in spring (February to March) and a smaller secondary peak in autumn (October to November) (Lal et al., 2012). High ambient temperature also elevates human exposure to FBPs via multiple routes. During warmer periods, food contamination risk rises due to improper handling,

particularly during outdoor activities such as barbecuing, where maintaining cold chain storage is challenging. Elevated temperatures may also degrade food safety during transport and storage.

As indicated by Lal et al. (2012) and Akil et al. (2014), temperature, in particular, is a critical environmental factor that drive the growth and dissemination of pathogens such as *Salmonella* along the food supply chain. Warmer conditions favor bacterial proliferation and are consistently associated with increased incidence of foodborne illnesses. Seasonal peaks in salmonellosis have been observed across different regions, typically aligning with higher ambient temperature during summer and early autumn. These patterns suggest that climate variability may influence the timing and intensity of outbreaks. Moreover, high temperatures can compromise food safety through multiple pathways, including poor cold chain maintenance, unsafe food handling during outdoor activities, and inadequate storage conditions. These risks intensify in environments with limited resources, where high temperature amplify vulnerabilities in food handling, transportation, and storage systems, increasing the likelihood of contamination and disease transmission.

Interestingly, high prevalence of *Salmonella* was also observed in October, which is traditionally considered the beginning of the dry season, i.e, mostly characterized by the increased temperature but also characterized by transitional environmental conditions of the year (Bintsis, 2018). For example, in our study the prevalence of NTS and STEC peaked in October. This was in agreement with another study in Eastern Ethiopia where the highest proportions of FBD (37.1%) was recorded during the dry season (Gobena et al., 2024). The high prevalence observed in October and November could also be due to lags between exposure and diagnosis where infections acquired towards the end of the rainy season may only be diagnosed weeks later, often extending into the early dry season (Tornevi et al., 2014). Moreover, residual contamination of the water bodies from prior rain may still be important, especially if they lack consistent water treatment facilities or protective infrastructure (Mushi et al., 2021).

Furthermore, water scarcity and source shifts at the onset of the dry season, which may drive people to rely on unimproved or unprotected sources, resulting in increasing exposure to pathogens from contaminated rivers or shallow wells (Aydamo et al., 2024). Additionally, during the dry season, livestock are often brought closer to households or

communal water points, heightening the risk of zoonotic transmission (Mushi et al., 2021). Furthermore, seasonal behaviors such as increased outdoor activities such as barbecuing undercooked poultry and meats (although not common in Ethiopia), or swimming in contaminated surface, also elevate exposure risks during the dry season (David et al., 2017; Rushton et al., 2019). A study from Canada reported that infections from *Salmonella* spp., STEC, and other FBPs were significantly higher in summer (John et al., 2022).

The seasonality of shigellosis is also significantly shaped by environmental conditions such as temperature change. Our study revealed the highest prevalence (3.61%) during the dry season, followed by the short rainy season (2.80%), and the lowest during the long rainy season (2.41%). This finding is consistent with global patterns that links *Shigella* incidence to temperature and precipitation, though with variations in the direction and strength of association depending on the season. For example, Song et al. (2018) reported that in China, a 1$^{o}$C rise in temperature linked to a 13.6% increase in incidence of *Shigella* after two weeks, and a 1 mm rise in rainfall corresponding to a 2.9% increase in incidence of *Shigella* after five weeks. Seasonal effects were also reported where in spring and autumn, both temperature and precipitation were associated with increased incidence, whereas in winter, precipitation showed a suppressive effect. Similarly, Chao et al. (2019) and Zhao et al. (2022) reported peak *Shigella* infection during warmer or wetter periods in countries such as Bangladesh, Mali, Vietnam and China. In our study, regional and temporal patterns further supported this heterogeneity. For instance, Harar had higher prevalence during dry and short rainy seasons, consistent with the dry or hot season peaks observed in Mali and Bangladesh. Gondar also reported variable peaks, including in May and October, potentially influenced by transitional weather patterns. Conversely Addis Ababa, showed higher prevalence during the long rainy season, with a peak in July, aligning with the summer incidence peaks reported in Zhejiang Province, China (Zhao et al., 2022). This variation suggests that local climatic conditions, urban infrastructure, and behavioural patterns may shape the seasonal dynamics of *Shigella* transmission.

Prevalence of *Campylobacter* infection was high in our study during the long rainy seasons where heavy rainfall is common. Heavy rainfall and flooding can increase human exposure to fecally contaminated drinking or surface water, thereby contributing to higher incidence rates and localized outbreaks of *Campylobacter* infection (Hyllestad

et al., 2020). Additionally, intense precipitation can wash sediments, pathogens, and animal waste into surface waters, while extreme flooding can overwhelm sanitation systems, spreading pathogens across human, animal, and agricultural settings (Butsch et al., 2023; Davis et al., 2022). For example, longitudinal studies in tropical settings show that rainfall events correlate with spikes in fecal indicator bacterial in drinking water during wet periods (Mushi et al., 2021). Furthermore, the Mushi et al. (2021) study also indicated that microbial fecal pollution in Ethiopia rivers is primarily driven by diffuse inputs from livestock grazing, which are mobilized during heavy rains.

On the other hand, summer in other countries is known to be low in rainfall and increased humidity that can be favourable condition for the multiplication of the bacteria pathogens that prefer the high temperature such as *Campylobacter*. For instance, a study conducted in Australia and Japan, *Campylobacter* infection peak during the summer months, particularly between July and September (Davis et al., 2022; Ishihara et al., 2012). A systematic review found that the monthly seasonality index for campylobacteriosis peaked during July and August, with regional variations; the UK showed the earliest peak in June, Canada the latest in August, and Europe and North America between June and August. In Oceania (Northen Hemisphere seasons), two distinct peaks were noted, one in May and another in September (Lal et al., 2012). In general, the influence of climate change such as increasing warmer and prolonged summer is projected to exacerbate the burden of *Campylobacter* infections in humans.

## 5.3. The Burden of Antimicrobial resistance among the targeted food-borne bacterial pathogens

### *5.3.1. Phenotypic Antimicrobial Resistance among Shiga-toxin producing E. coli isolates*

In this study, the STEC isolates showed low resistance to critical antibiotics such as amikacin, ceftazidime-avibactam, ceftolozane-tazobactam, and meropenem in urban areas like Addis Ababa, which offers some hope. In contrast, however, the rising resistance rates of these isolates to first-line antibiotics such as ampicillin and sulfamethoxazole-trimethoprim, as well as the emergence of increased XDR profiles (2.5%) among the STEC isolates were alarming.

This was in alignment with findings from other studies in Ethiopia where the increased resistance patterns of different diarrheagenic *E. coli* pathotypes, including STEC, to the most prescribed antibiotics tested were reported (Zelelie et al., 2023; Engda et al., 2023). The same trend of low resistance of STEC to the critical antibiotics but increased resistance to the commonly prescribe antibiotics such as tetracycline, ampicillin, and sulfamethoxazole-trimethoprim was reported in Irean (Jafari et al., 2021).

The findings of this study revealed an overall proportion of phenotypic ESBL among STEC isolates at 37.54%. Moreover, the overall rate of phenotypic carbapenem resistance detection was 9.23% (27/293), the highest proportion being from Gondar, 13.79% (8/58), followed by Harar, 11.97 (14/117), while the lowest being from Addis Ababa at 4.23% (5/118). These results are consistent with the findings of Ayalneh et al. (2025), who also reported high levels of phenotypic ESBL production among *E. coli* isolates, 30% from diarrheal patients. Additionally, Ayalneh et al. (2025) highlighted the presence of carbapenem-resistant strains, particularly in referral hospital settings, further supports the evidence of emerging resistance trends noted in this study. Furthermore, Mahamat et al. (2024) in Cameroon reported the phenotypic ESBL among *E. coli* isolates as 52.4%, and carbapenemase production as 3.6%, underscore a significant burden of ESBL producing *E. coli* in the region.

The current study also found that nearly half of the STEC isolates (48.12%) were MDR, that is, they were resistant to three or more classes of antibiotics. Slight variability of MDR rates was evident among STEC isolates across the study sites, the highest proportion being from Addis Ababa (49.15%) and relatively lowest from Gondar (44.83%). Yet, these rates were lower than the rates reported from other studies of low- or middle-income countries; for example, a study conducted in Iran (Jafari et al., 2021) reported a 60.9% MDR rate.

The most alarming finding of this study was that both the XDR isolates were detected from Harar and among children of below the age of 15 years. This raises serious public health concerns and highlights a complex interplay of factors driving resistance and infection in this region. Children are particularly vulnerable to STEC infections due to their developing immune systems and increased exposure to contaminated water, food, and surfaces through daily activities. This is in agreement with the WHO-Afro report

(December 2024) where children under five were disproportionately affected by antimicrobial resistance in Ethiopia (WHO-AFRO, 2024).

Furthermore, children are also more likely to be exposed to repeated antibiotic treatments due to frequent infections in early childhood, especially in resource-limited settings. Inappropriate or excessive antibiotic use creates selective pressure that encourages the survival and spread of XDR organisms, especially in regions like Harar, where access to regulated healthcare and rational antimicrobial use may be limited, where self-prescription of antimicrobial agents was 70% and self-prescription of antibiotics was reported as 47% (Hailemichael et al., 2016). Additionally, environmental conditions and socio-economic conditions such as inadequate sanitation facilities, limited access to clean water, and increased ownership of domestic animals might contribute for this alarming resistance in Harar. Several studies from Ethiopia have documented the presence of multi-drug-resistant bacteria from rural water sources, milk, and animal reservoirs, which may also contribute for such resistance.

### *5.3.2. Phenotypic Antimicrobial Resistance among Non-typhoidal Salmonella Isolates*

In this study, NTS isolates showed no resistance to critical and believed to be the last choice of antibiotics such as colistin and meropenem. This is consistent with findings from other studies conducted in sub-Saharan Africa, which have shown that these antibiotics remain effective against many enteric pathogens, including NTS, despite the increasing burden of antimicrobial resistance in the region (Amir et al., 2024). There was considerable variability in the antimicrobial susceptibility for the other tested drugs between the three study sites, in which isolates from Harar showed increasing resistance to most of the commonly prescribed antibiotics such as tetracycline and sulfamethoxazole-trimethoprim, which might be due to the misuse of antibiotics including high burden of self-prescription of antibiotics which has been documented previously to be 47% in Harar (Hailemichael et al., 2016) that is closer to the national antibiotic self-medication, 46.14% (Ayenew et al., 2024) where tetracycline and sulfamethoxazole-trimethoprim are among the most commonly self-prescribed antibiotics.

Furthermore, 13.33% of the NTS isolates, regardless of their sites, were found to be ESBL producing. Moreover, the overall prevalence of MDR among NTS isolates in this study was 83.33%, which is higher than the rate reported from a systematic review in sub-Saharan Africa where the MDR of NTS was reported as 68.5% (Amir et al., 2024). As in the cases of other issues in this study, there was also marked differences in MDR prevalence between the study sites. For instance, Gondar and Harar showed resistance rates of 50.0%, and 81.82%, respectively while none of the NTS isolates from Addis Ababa were MDR positive. The latter case contrasts with a previous report of 40.3% from in Addis Ababa (Eguale at al., 2015), which might be due to the lower NTS isolates detected from Addis Ababa in the current study. Another important finding from this study is that resistance to fluoroquinolones such as ciprofloxacin was >33.77% across all study sites, which is alarming given that these drugs are essential for treating *Salmonella* infections. This increasing resistance to fluoroquinolones could be linked to the widespread use of these antibiotics in both human and veterinary medicine in Ethiopia (Kebede et al., 2021).

### *5.3.3. Phenotypic Antimicrobial Resistance among Shigella spp. Isolates*

*Shigella* is one of the eight WHO priority pathogens due to its resistance burden (Hope et al., 2024). In our study, 98.51% of the *Shigella* isolates were resistant to at least one antibiotic tested, particularly to the first-line treatments like ampicillin and sulfamethoxazole-trimethoprim. These findings underscore a critical therapeutic challenge in Ethiopia, where access to alternative antibiotics is limited. Our results are higher than reports by Hussen et al. (2019) who documented over 50% resistance to commonly used antibiotics. This reinforces the ongoing concern that empiric treatment strategies may increasingly fail due to widespread resistance among key enteric pathogens, including *Shigella*.

Moreover, this study found that resistance to tetracycline in Harar was greater than 87%. This is a significant public health challenge, as tetracycline, despite being an older antibiotic, remains in use both in human medicine and livestock production, often without proper regulation or stewardship. Moreover, a previous study conducted in Harar and adjacent Dire Dawa abattoirs revealed that over 87% of the tested bovine tissue samples contained oxytetracycline residues, with 87.2% of kidney samples in

Dire Dawa and 80.8% in Harar, while an overall 84% of samples tested positive for tetracycline-class residues (Mohammed et al., 2022). This scenario creates a conducive environment for tetracycline-resistant strains to emerge and persist, as these residues in animal products effectively act as low-level antibiotic exposures, fuel the selection and dissemination of resistant bacteria through the food chain and broader environment.

Additionally, 13.43% of the *Shigella* isolates in this study, disregarding the specific sites, were found to be ESBL-producing by phenotypic testing method; and the MDR rate was 71.64%. Harar had the highest ESBL rate (63.64%), showing more than double of that observed in Gondar, and nearly five times higher than that of Addis Ababa. These variations may indicate differences in antibiotic usage practices across the different geographic locations in the country. The systematic review of 29 studies in Ethiopia mentioned previously reported MDR rate of 83.2% from *Shigella* isolates (Hussen et al., 2019), which is relatively higher than the rate detected in our study. The low MRD rate from our study could likely be due to difference in the definitions of MDR, where we classified MDR as resistance to three or more antibiotic groups while the other studies have defined MDR as resistance to one or more groups of antibiotics. Moreover, a meta-analysis of 43 studies reported an MDR of 79.08% (Beyene et al., 2022), which is slightly closer to our findings. Harar showed the highest MDR burden (44.78%), followed by Gondar with 20.69%, and Addis Ababa with 5.08%.

## 5.4. Molecular Detection of ESBL and Carbapenemase genes among the targeted foodborne bacterial pathogens

Extended spectrum beta-lactamase producing Enterobacteriaceae were associated with over 800,000 deaths globally in 2019 alone, and carbapenem-resistant strains are increasingly implicated in untreatable infections across both healthcare and community settings (Murray et al., 2022). Foodborne pathogens such as *E. coli*, and *Salmonella* spp. have emerged as important reservoirs for these resistance genes, often acquired through contaminated food, animal contact, or environmental sources (WHO, 2017). In low- and middle-income countries like Ethiopia, the situation is further exacerbated by the widespread and unregulated use of antibiotics in both human medicine and food animal production. Hence, the molecular characterization of ESBL and carbapenemase genes in foodborne bacterial pathogens is critical to understanding the mechanisms behind the rising prevalence of antimicrobial resistance in Ethiopia. Recent study in

Southern Ethiopia have revealed a worrying level of ESBL and carbapenemase-producing Enterobacteriaceae in commonly vended street foods, where 15.2% and 8.9% of isolates were confirmed as ESBL and carbapenemase producers respectively (Alelign & Kidanewold, 2023).

## *5.4.1. Molecular Detection of Extended spectrum beta-lactamase genes*

### 5.4.1.1. ESBL Genes among Shiga-toxin producing *E. coli* Isolates

The findings of our study revealed a 37.54% overall proportion of ESBL among STEC isolates, which significantly higher than the 8.7% reported in another study conducted on *E. coli* isolates from stool samples of patients attending primary healthcare centers in Addis Ababa and Hosanna (Wolde et al., 2024).

Several factors may explain this discrepancy. First, differences in the study population could pay a critical role. Our study, like the one from Somali region, focused specifically on diarrheal patients, a group more likely to receive empirical antibiotics, particularly children, who are known to have higher rates of ESBL colonization due to increased antibiotics exposure and immature immune system (Mahamat et al., 2019). Second, healthcare facility level and case severity may influence ESBL burden where the referral or tertiary facilities, where patients with more severe or prolonged illness are treated, tend to have higher antibiotic use and consequently, higher resistance rates than the primary healthcare settings (Doyle et al., 2012). Additionally, differences in regional antimicrobial use practices and environmental hygiene may contribute to the variation. In many low-and middle-income settings like Ethiopia, access to antibiotics without prescriptions remains common, and such unregulated use is a key driver of resistance (Ayukekbong et al., 2017). Rural and semi-urban communities, where access to clean water and sanitation may be limited, are also at increased risk for the spread of resistant organism through contaminated food and water (Baker & The, 2018). Hence, the observed variation in ESBL prevalence across regions may reflect the combined effect of population characteristics, healthcare system level, antibiotic prescribing behaviours, and environmental exposure.

Moreover, our finding is further supported by a recent study from Shashemene, Ethiopia, which investigated the genetic characteristics of ESBL and carbapenemase-producing *E. coli* O157:H7 isolates from diarrheal patients (Ayalneh et al., 2024). The

study found a high burden of ESBL-producing isolates, with bla-CTX-M genes being the dominant one, which is consistent with our results. Alarmingly, the Shashemene study also identified the co-existence of carbapenemase genes such as bla-NDM, highlighting the potential emergence of XDR strains. This convergence of high ESBL rates and the detection of carbapenemase underscores a serious public health concern, as treatment options for such infections become increasingly limited.

The molecular characterization of ESBL genes in the current study further identified at least one type of ESBL gene from 95.45% (104/110) of the phenotypically ESBL positive STEC isolates. The bla-CTX-M, bla-TEM, and bla-SHV were the primary ESBL genes detected among the STEC isolates, bla-CTX-M being the most prevalent. While some from studies elsewhere reported the same bla-CTX-M gene dominance, found as 91.82% (Mahamat et al., 2024), others identified bla-TEM to be the predominant genes of ESBL at 84.5% followed by the bla-CTX-M at 69.0% (Jafari et al., 2021). Regional differences in the burden of the bla-CTX-M gene among the STEC isolates from our study were relatively minor, with Gondar (96.77%), Harar (92.06%), and Addis Ababa (81.25%). The bla-TEM, another commonly detected ESBL gene, was found in 70.91% of the STEC isolates overall, the highest (77.78%) proportion being from Harar, and the lowest from Addis Ababa (62.50%). On the other hand, the bla-SHV gene, although still detectable, it was at a very low level (14.55%). Site-specific analysis showed that Addis Ababa reported the highest proportion (25.00%) of this gene followed by Harar (14.29%) and Gondar (9.68%).

Co-detection of multiple ESBL genes was a prominent feature of the STEC isolates in this study, bla-CTX-M and bla-TEM being the most frequent combination with proportion of 72.11%. The high frequency of bla-CTX-M and bla-TEM co-detection seems to be common as it was also found in other studies (Jafari et al., 2021; Mahamat et al., 2024). Furthermore, 13.46% of isolates harboured all the three ESBL genes (bla-CTX-M, bla-TEM, and bla-SHV). The co-detection of bla-CTX-M and bla-SHV genes, and bla-TEM and bla-SHV genes occurred in equal proportions (14.42% each), highlighting the complexity of ESBL gene profiles among STEC isolates in the region. This high co-occurrence underscores the potential for synergistic effects in resistance, making the treatment of infections caused by these strains more challenging. These genes, often found on mobile elements, spread rapidly across species and environments, making infections harder to control and treat. Strengthened antimicrobial stewardship

and global surveillance are critical to mitigating this threat (Gundran et al., 2019; Lubwama et al., 2024).

#### *5.4.1.2. ESBL Gene among Non-typhoidal Salmonella Isolates*

An overall detection rate of ESBL encoding genes among the NTS isolates from all the three sites combined is 13.33%, with noticeable regional variations. While no ESBL-encoding genes were detected from isolates obtained from Addis Ababa, 33.33% and 9.09% of the isolates from Gondar and Harar, respectively, were found to harbour these genes. This regional variability could be attributed to differences in antimicrobial usage practices, healthcare settings, and local environmental factors, which influence the selective pressure driving ESBL gene maintenance and transmission in susceptible individuals. The detection of the bla-CTX-M gene among all ESBL-producing isolates underscores its critical role as the dominant ESBL gene in NTS isolates. Previous studies have similarly identified bla-CTX-M as the predominant ESBL gene among Enterobacteriaceae, reflecting its global dissemination and significance in the development and distribution of AMR (Belina et al., 2024; Mahamat et al., 2024).

The findings from Gondar and Harar, where half of the NTS isolates carried the bla-CTX-M gene suggests localized dynamics of ESBL gene distribution that warrant further exploration. Among the four NTS isolates confirmed as ESBL producers, neither bla-TEM nor bla-SHV was identified in any isolates. The high resistance to ampicillin (75%) observed in NTS isolates may therefore be mediated by blaCTX-M variants capable of hydrolysing penicillin, or by other β-lactamase genes not included in our molecular analysis, such as bla-CMY, bla-DHA, or chromosomal β-lactamases. Additionally, non-enzymatic mechanisms, including efflux pumps or reduced membrane permeability, may contribute to ampicillin resistance. This highlights the importance of comprehensive molecular screening to fully elucidate the genetic basis of β-lactam resistance in NTS.

#### *5.4.1.3. ESBL Genes among Shigella spp. Isolates*

For *Shigella* spp., the overall detection rate of ESBL encoding genes was 32.84%, which may likely lead to a substantial burden of AMR from this pathogen. Presence of ESBL genes from *Shigella* spp. is not unique to our setting; some studies such as 18.1% in Shanghai (Li et al., 2015) and 10.2% in Iran (Sabour et al., 2022) report detection

levels less than the rate from our study, while others such as in the one from Egypt report higher rate at 54.0% (Badr et al., 2024). Such variabilities are not limited between different countries; they are also observed even within the same country. For example, it was observed in our study that there was regional difference, although not as big as shown between countries, where the rate of detection in Harar was 63.64%, in Addis Ababa 13.64%, and in Gondar 22.72%. Variations in healthcare access, antibiotic prescription practices, and environmental exposure to resistant strains may play roles creating variations.

Gene-specific detection assay in our study on isolates of ESBL-producing *Shigella* spp. from all the study sites combined identified bla-CTX-M to be the predominant one with detection rate of 95.45%. This may not be surprising given that the gene has been identified by other studies as the most widespread of ESBL genes among Enterobacteriaceae (Jafari et al., 2021; Mahamat et al., 2024). In fact, other global studies on *Shigella* isolates have already shown how predominant it was among this pathogen as shown in the following examples 50.0% in Egypt (Badr et al., 2024); 65.3% in Iran (Sabour et al., 2022); and 69.2% in Shanghai (Li et al., 2015).

Another ESBL-encoding gene, bla-TEM, was identified in the current study in 59.09% of the *Shigella* isolates, with regional rates varying from 57.14% in Harar to 66.67% in Addis Ababa, apparently less prevalent than bla-CTX-M. Moreover, this finding was slightly lower than findings from the study conducted in Iran, 61.2% (Sabour et al., 2022). Likewise, a third common ESBL gene from our *Shigella* isolates was bla-SHV, which was relatively low with only one isolate from Harar (4.54%). This low finding is in agreement with reports around the world that bla-SHV genes are less commonly associated with *Shigella* spp. compared to other Enterobacteriaceae (Badr et al., 2024; Sabour et al., 2022).

The co-detection of bla-CTX-M and bla-TEM genes among the *Shigella* isolates was found to be 61.90%, while the detection of all three genes (bla-CTX-M, bla-TEM, and bla-SHV) within a single isolate was very rare, which was detected in only 4.76% of the isolates. Having a combination of ESBL genes may exhibit broader resistance profiles, which has the potential of complicating treatment options. The observation of low proportion of bla-SHV co-existing with bla-CTX-M or bla-TEM (4.76% of each combination) in the *Shigella* isolates in this study aligns with trend reported from

existing literature, suggesting that bla-SHV genes are less commonly implicated in *Shigella* resistance, probably because of their limited distribution, compared to other ESBL genes like bla-CTX-M and bla-TEM or their combination (Badr et al., 2024; Beladi et al., 2019; Somda et al., 2025). The cited references showed low proportion of bla-SHV detection, and the possible explanation might be the bla-SHV is typically carried on narrow-host-range plasmids (e.g., *IncFII*, *IncA/C*), which may not be widely disseminated in *Shigella* spp. compared to *E. coli* or *K. pnuemoniae*. In contrast, bla-CTX-M and bla-TEM are often found on broad-host range plasmids (e.g., *Incl1*, *IncF*, and *IncHL2*) which are more easily transmitted amongst and maintained in *Shigella* (Canton et al., 2012; Liakopoulos et al., 2016).

A meta-analysis from across Africa reported bla-CTX, and bla-TEM as the most common ESBL genes (25.9% and 10.8% respectively) (Somda et al., 2025). The dominance of bla-CTX-M and bla-TEM among the ESBL genes in *Shigella* spp. across Africa can be attributable to multiple factors. First, both genes are frequently located on broad-host-range plasmids such as *IncF*, *Incl1*, and *IncHl2* that are highly mobile and capable of transferring efficiently among diverse members of the Enterobacteriaceae, including *Shigella* spp. (Canton et al., 2012). These plasmids often harbour integrons, transposons, and insertion sequences (e.g., *ISEcp1*), which facilitate the acquisition and stable expression of resistance genes like bla-CTX-M. Additionally, selective pressure plays a key role; widespread use of ampicillin and third-generation cephalosporins such as ceftriaxone in both clinical and community settings exert strong pressure favoring strains harboring bla-TEM and bla-CTX-M (Somda et al., 2025).

### *5.3.2. Molecular characterization of carbapenemase genes*

Among the targeted pathogens in this study, all the NTS were found to be phenotypically susceptible for the meropenem. As a result, they were not considered for the further confirmatory testing for carbapenemase genes. In contrast, STEC and *Shigella* spp. were found phenotypically positive for the carbapenemase production and, hence, were subjected for molecular detection assays to detect the relevant genes.

The molecular characterization of carbapenemase production among the STEC isolates in this study revealed the presence of alarmingly high carbapenemase genes, particularly the bla-NDM (92.59%), followed by low level of bla-OXA-48-like (7.41%). The predominance of the bla-NDM-genes is of particular concern as it is associated

with high-level resistance to carbapenems, which are often considered antibiotics of last resort for treating MDR infections. The same dominance of the bla-NDM-genes was also reported in the very recent study in Ethiopia (Zenebe et al., 2023). This widespread occurrence may be partly explained by the ability of bla-NDM to be carried on diverse plasmids, promoting horizontal transfer among Enterobacterales. Moreover, bla-NDM is often associated with mobile genetic elements such as transposons and integrons, which enhances its dissemination across bacterial species and settings (Acman et al., 2022). Furthermore, the selective pressure such as the extensive use of extended spectrum cephalosporins such as ceftriaxone and cefotaxime in human and veterinary medicine, creating strong selection pressure for resistance mechanisms including ESBLs and carbapenemases. Plasmid often co-harbors bla-NDM along with ESBL genes (e.g., bla-CTX-M-15) leading to high potential for horizontal transfer, and retention of multiple resistance determinants (Hayer et al., 2022).

Site-specific analysis of detection clearly showed that all the Carbapenemase producing STEC isolates in Addis Ababa were positive for the bla-NDM-gene, and 92.31%, and 75.0% of the STEC isolates in Harar and Gondar respectively were positive for the bla-NDM-genes. These high regional rates suggest difference in localized dissemination dynamics, consistent with another study in Ethiopia (Seman et al., 2022) where bla-NDM was the sole carbapenemase gene identified among enterobacteriaceae, but bla-KPC and other variants remain rare or undetected.

Furthermore, a recent study in Ethiopia also showed that bla-NDM-1 and bla-NDM-5 were reported as the predominant genes, 5% among Enterobacteriaceae isolates, with a notably higher frequencies, 13% at referral hospitals in Addis Ababa (Legese et al., 2022). The detection of limited bla-OXA-48-like genes as well as the absence of other carbapenemase genes like bla-KPC and bla-VIM, in the current study was in agreement with study report from by Legesse et al. (2022). This might suggest a regional specificity in the distribution of carbapenemase genes among STEC strains. Several factors could explain this; for example, limited use of carbapenems in the Ethiopian healthcare settings may reduce selective pressure for acquiring carbapenemase genes like bla-KPC or bla-VIM. Moreover, STEC is primarily a zoonotic and enteric pathogen, typically transmitted via fecal-oral route outside the hospital environments, limiting its contact with carbapenem-resistant donor strains, such as *K. pneumonieae* or *Pseudomonas* that carry these genes (Chelaru et al., 2024).

The lowest proportion of the carbapenemase genes were found among the *Shigella* spp. 2.99% (2/67) among the bla-NDM gene. This finding aligns with broader surveillance data suggesting that meropenem resistance remains rare among these enteric pathogens; where a meta-analysis across Africa reported carbapenemase gene carriage in only approximately 0.8% of *Shigella* isolates (Somda et al., 2024). The review also indicated that despite the low frequency, the detection of even a modest number of bla-NDM positive *Shigella* isolates is a concerning early warning. Given the high transmissibility nature of *Shigella*, even low-level reservoirs can cause more extensive outbreaks under suitable conditions, reinforcing the necessity of ongoing molecular surveillance to promptly identify shifts in resistance dynamics.

## 5.4. Factors associated with infections by the targeted foodborne bacterial pathogens

### *5.4.1. Age of the study participants*

Among the targeted pathogens, age was significantly associated with infection by *eae* harboring STEC and *Campylobacter* spp. For example, the elderly patients, 50+ years, had showed higher odds (1.22) of infection from *eae* harboring STEC. While many literatures focus on children, elderly can also be vulnerable due to diversified exposures, weak immune system, or chronic health conditions. This was in alignment with another study in Ethiopia (Engda et al., 2023) where elderly was shown to have higher odds of STEC O157:H7 infection. Moreover, results from other countries where ecological analysis revealed higher STEC infection among >65 years, attributed this to susceptibility due to weak immunity and increased interaction with environmental reservoirs (Cleary et al., 2021).

Likewise, the odds of susceptibility to enteric pathogens were increased among young patients. For instance, children under fifteen years were particularly vulnerable to *Campylobacter* infections in our study, where a higher odd (1.07) and (1.10) by *Campylobacter* infection was detected among children of under 5, and 5-14 years of age, respectively. Lengerh et al. (2013) also found that children under one year had higher *Campylobacter* spp., prevalence, compared to older children. Similarly, systematic review and meta-analysis of 8 studies showed that children under the age of five had significantly high-rate infection with *Campylobacter* spp., and the authors

argued that underdeveloped immunity and higher exposure to environmental reservoirs might be key factors in increased susceptibility by children of this age group (Diriba et al., 2021). Another review comprising 25 studies conducted among diarrheal patients found that age was associated with *Campylobacter* spp., prevalence where children under 5 years had the highest risks of *Campylobacter* infection compared to older children with infants and toddlers under two years being particularly at increased risk, showing prevalence rates as high as 30-40% and much higher than older children (Smith et al., 2019). Additionally, our finding was also consistent with recent reviews and filed studies that highlight significantly elevated *Campylobacter* exposure risks in infants in which as young as 1 month were already colonized with *Campylobacter* (Deblais et al., 2023). The study revealed that exposure was strongly linked to household factors such as soil contact, open defecation, proximity to livestock, and shared living environments with colonized household members where infants and children are vulnerable to these listed factors.

### *5.4.2. Geographic variation and residence type (Urban vs Rural)*

Our data showed that geographic and seasonal variations were also apparently linked to infections with these pathogens, with Harar showing higher odds of STEC, NTS, and *Shigella* infections compared to Gondar and Addis Ababa, likely due to limited sanitation, reliance on unimproved water sources, and greater livestock density. The geographic variation was noted in this study where Addis Ababa and Harar showed higher odds of STEC infection 1.06 and 1.16 respectively compared with Gondar. However, there was no significant difference in the STEC infection in Addis Ababa and Harar. These patterns are supported by studies that attribute regional differences between regions to variation with respect to agricultural practices, sanitation, and access to quality water. Moreover, we found that more than half of the households in Harar used unimproved water sources which might contribute to the highest odds of STEC infection as compared with other cities.

Rural residence was associated with slightly higher odds of NTS infection compared to urban residence. This is not surprising, as many other similar studies also showed higher NTS prevalence in rural areas, because of factors such as lower standards of hygiene, limited access to clean water, and increased direct contact with animals in rural settings (Ngogo et al., 2020).

Moreover, participants from Harar showed 1.39- and 1.12-time higher odds of *Shigella* infection compared with Addis Ababa, and Gondar respectively. Additionally, participants from Gondar showed 1.15 times higher odds of *Shigella* infection compared with Addis Ababa. Like the rest of the pathogens studied, this prevalence variation seems to be largely influenced by environmental factors, including water sources and sanitation practice, where populations in question largely rely on unimproved water sources and shared latrines (Muse et al., 2024). For example, in Harar, over half of the households (51.35%) relied on unimproved water sources, which significantly correlates with a high prevalence (5.05%) of *Shigella* infection compared to other sites. Unimproved water sources are associated with higher microbial contamination, increasing the risk of waterborne diseases, including *Shigella* spp., infections, as documented in low-resource settings worldwide (Daly et al., 2021). This study also showed the high proportion of unimproved water sources and unimproved sanitation facilities being used in Harar. Additionally, the high ownership of domestic animals in Harar, such as cattle and goats, contributes to the increased burden as livestock proximity can increase environmental contamination and facilitate the transmission of *Shigella* spp. through fecal-oral routes (Berendes et al., 2023).

Furthermore, this study found that residency types were significantly associated with *Campylobacter* infection. Patients from rural residence showed 1.02 times higher odds, of *Campylobacter* infection compared to urban settings, which also reflects the significant impact of livestock ownership and proximity to animals, which are common in rural environments. Rural residents often live-in close proximity to livestock, which serve as major reservoirs for *Campylobacter*, and frequently rely on traditional sources of drinking water and local food supplies. Such conditions, alongside limited water treatment and food hygiene infrastructure, amplify transmission potential. This is in alignment with other study conducted in Burkina Faso, where rural residents showed twice the odds of *Campylobacter* infection compared to urban residents (Badjo et al., 2024).

### *5.4.3. Seasonality and climate influences*

Seasonality also emerged as a strong predictor, with higher and very closer odds of STEC infection during both dry season and long rains seasons with the odds of 1.06 and 1.05 respectively as compared to the short rainy seasons. Similarly, the observed

increase in odds of infection during the long rainy season, 1.05 compared to short rainy season aligns with evidence suggesting that rainy periods amplify environmental contamination and zoonotic transmission pathways. In Ethiopia, the rainy season has been identified as a high-risk time for waterborne and zoonotic infections, including *Campylobacter*, due to increased pathogen mobility in water systems and poor sanitation (Levesque et al., 2013; Li et al., 2024). The possible explanations for the variation of the prevalence of individual targeted pathogen in relation to and the season have been described above under section 5.2 of the discussion.

### *5.4.4. Water Sources and Sanitation Infrastructure*

The observed association between unimproved water sources and sanitation facilities with infections caused by *eae* harboring STEC and *Shigella* spp. highlights the critical role of environmental and hygiene factors in pathogen transmission. This finding supports the notion that inadequate water and sanitation infrastructure facilitates fecal-oral transmission of enteric pathogens, consistent with previous studies conducted in low-resource settings (Asgedom et al., 2023). Comparable results were reported in rural Nigeria, where Obanor et al. (2022) showed frequent isolation of STEC O157:H7 from wells and boreholes located in areas with poor sanitation. These results suggest that unimproved water sources, particularly unimproved wells and surface waters, are vulnerable to focal contamination from both human and animal sources. Such contamination is critical route for STEC transmission, especially for *eae* harboring STEC, which promotes adherence to intestinal mucosa and enhances colonization and infection severity. Bain et al. (2014) also noted that unimproved water sources in low- and middle-income countries had significantly higher levels of fecal contamination, reinforcing the likelihood of this transmission pathways.

Regard to *Shigella* spp. infection, our finding showed that households using unimproved water sources had nearly double the odds of infection compared to those households using improved water sources. Additionally, unimproved sanitation facilities were associated with a 1.39-fold increase in the odds of *Shigella* infection. This is consistent with global evidence linking poor water and sanitation access to increased risk of shigellosis, primarily due to the fecal-oral route of transmission. A recent study by Mosisa et al. (2021) in Ethiopia found that children in households

without access to improved WASH services had a significantly higher risk of diarrheal disease.

### *5.4.5. Livestock Ownership*

This study highlights the multifactorial nature of zoonotic and waterborne infections, where the interplay between environmental, socio-demographic, and behavioural factors are demonstrated. Statistical computation clearly showed that livestock ownership, particularly of cattle, sheep, goat, chickens, and dogs or cat was a significant risk factor for STEC, NTS, and *Campylobacter* infections, aligning with global evidence linking proximity to animals with higher infection odds (Engda et al., 2023; Pires et al., 2019).

For example, the households owning cattle had higher odds,1.38 of STEC infection, while those with sheep had an odd of 1.08 times increased risk for the STEC infection without cattle. The same conclusion was reached by other studies in Ethiopia (Engda et al., 2023) and elsewhere abroad (Jaros et al., 2013), indicating that primary livestock serve as reservoirs for STEC infection to humans. Cattles are well-known reservoir of STEC, and close contact with them, either through households rearing or exposure to their feces, has been repeatedly linked to increased risk.

Moreover, ownership of cattle, sheep, goats, chickens, and dogs or cats was associated with 1.01 to 1.08 higher odds of NTS infection, which is also consistent with previous studies (Hoelzer et al., 2011) that have linked animal ownership with increased risk of zoonotic infections, including NTS. This association is likely due to direct or indirect contact between humans and animals and shading of the pathogens into the environment. This may also increase the likelihood of environmental contamination, where feces from infected animals potentially contaminating food, water, and surfaces, leading in turn to human exposure (Hoelzer et al., 2011).

Similarly, the odds of *Campylobacter* infection were 2-3% higher among livestock owners, which is consistent with other recent studies including the one from Ethiopia that showed domestic animal's exposure to be linked to higher odds of *Campylobacter* infection (Worku et al., 2024), emphasizing zoonotic transmission in rural communities where animals serve as reservoirs (ECDC, 2022).

## 5.5. Strength and Limitations of The Study

One of the most critical strengths of this study lies in its comprehensive and integrated design, which uniquely positions it as the first of its kind in Ethiopia to concurrently assess the prevalence, antimicrobial resistance, and associated factors of four major foodborne bacterial pathogens, STEC, NTS, *Shigella* spp., and *Campylobacter* spp. This multidimensional approach, which encompasses diverse geographic regions, varying seasonal contexts, and multiple age groups, provide a more holistic understanding of the distribution and drivers of these pathogens. The inclusion of seasonal variation offers an important layer of insight, allowing for the identification of temporal patterns in pathogen prevalence and potential environmental influences such as flooding and water contamination during rainy seasons. Furthermore, the use of molecular diagnostic techniques enhances the specificity and sensitivity of the pathogen detection, ensuring more accurate estimates than conventional methods. This robust and integrative methodology not only strengthens the validity of the finings but also provide valuable evidence to inform targeted public health strategies, AMR containment efforts, and policy development in resource-limited settings like Ethiopia.

However, there are also certain limitations that should be noted. First, the study's conclusions may not be generalizable to other populations and geographic areas in Ethiopia. Specifically, this study only included three cities in a country, yet different regions in Ethiopia may have varying risk factors for infection, healthcare access, and pathogen prevalence, meaning that the results from these areas may not represent the entire country.

Second, since this study was conducted in referral hospitals, the findings may be subject to bias related to healthcare access. Referral hospitals often receive patients who are referred for more severe or complicated cases, while individuals from rural or underserved areas may face barriers to reaching such facilities. Consequently, our data may underrepresent mild or untreated community cases and overrepresent more severe infections, which could influence the observed prevalence and resistance patterns. Third, the use of chromogenic agar may miss the diagnosis of some STEC serotypes and hence underestimates the total prevalence of STEC. Fourth, the use of an immunoassay techniques where we did not recover the *Campylobacter* isolates limited our understanding of the antibiotic susceptibility pattern, the overall virulence genes such

as such as *cdtA*, *cdtB*, *cdtC*, *cadF*, *peb1*, *flaA*, *flaB*, *gyrA*, and *tet(O)*. Despite these limitations, the results of this study provide important new insights into the epidemiology of these pathogens in Ethiopia. Fifth, DNA extraction was performed using the boiling method, which is simple and cost-effective but yields crude DNA that may contain inhibitors and fragmented nucleic acids. This limitation may have reduced PCR sensitivity compared to column-based extraction methods, possibly contributing to under-detection of some target genes. Sixth, a limitation of this study is related to the biosafety conditions under which the phenotypic characterization of STEC isolates was performed. While the analyses were conducted in a biosafety level 2 (BSL-2) laboratory following standard bacteriological procedures, additional containment measures specific for *Enterohemorrhagic E. coli* (EHEC) were not fully implemented. This poses a potential biosafety concern given that some STEC isolates could belong to highly pathogenic EHEC strains.

Hence, given the findings of this study including the varying prevalence across study sites, distinct seasonal patterns over the one-year period, differences in virulence gene distribution (e.g., *stx1*, *stx2*, *eae*), and (e.g., bla-OXA-48-like, bla-KPC, and bla-VIM), furthermore broad investigation is warranted. Hence, future studies should focus on whole genome sequencing to gain a more comprehensive understanding of the resistance and virulence of all the targeted pathogen. This would allow for the detection of rare or novel resistance and virulence genes not captured by PCR and clarify the genetic mechanisms underlying the observed antimicrobial susceptibility patterns. In addition, expanded geographical surveillance involving incorporating hard to reach areas with diversified dietary practices to see comprehensive burden of FBD.

Furthermore, the findings of seasonal variation in our study suggests the need for longitudinal studies that examine how climate factors, sanitation, and water safety influences transmission patterns throughout the year. Additionally, since the distribution of virulence genes varied across regions, future research should explore the association between virulence profiles, clinical outcomes, and environmental exposures, which may help explain differences in disease severity and outcomes.

# CHAPTER 6: CONCLUSION AND RECOMMENDATION

## 6.1. CONCLUSION

This study provides a comprehensive assessment of the prevalence, antimicrobial resistance, and associated risk factors of four key FBPs, such as STEC, NTS, *Shigella* spp., and *Campylobacter* spp. in Ethiopia. To the best of our knowledge, it stands out as the first investigation to concurrently examine these pathogens across diverse geographic regions, seasons, and age groups in the country.

First, although the overall prevalence of these pathogens was different from the prior studies, likely due to the specificity of molecular diagnostic techniques employed, significant geographic and seasonal variations were observed. Harar consistently exhibited significantly high prevalence of STEC, NTS, *Shigella* spp., and *Campylobacter* spp.

Second, the study revealed alarmingly high levels of AMR and MDR, especially among the NTS, *Shigella* spp., and STEC isolates in general, including the detection of ESBL, and carbapenemase, particularly in isolates from Harar and Gondar. Moreover, clustering of XDR among STEC from children under 15 years, all of whom from Harar, is very alarming.

Third, the widespread presence of ESBL-genes, particularly bla-CTX-M, across all major enteric pathogens (STEC, NTS, and *Shigella*) highlights a significant and urgent AMR threat in Ethiopia. Moreover, the overwhelming predominance of the bla-CTX-M gene, detected in over 95% of ESBL producing isolates, alongside high co-detection with bla-TEM, points to the rapid and sustained dissemination of MDR bacterial strains within the population. Equally concerning is the detection of carbapenemase genes, especially bla-NDM, among both the STEC and *Shigella* spp. isolates. This signals a shift toward resistance against last-resort antibiotics, and a possible silent spread of pan-resistant organisms in the absence of appropriate containment.

Fourth, demographic vulnerability was evident, with children under 15 years being particularly affected by *Campylobacter* spp. Moreover, residency, particularly among those who live in the rural areas was also an important contributor for the increased rate of infection by all the pathogens investigated. Furthermore, the finings emphasized the critical

role of zoonotic and waterborne transmission pathways, shaped by environmental, behavioural (e.g., antibiotic use, sanitation practices), and demographic factors.

Fifth, various living conditions were found to significantly contribute to the increased prevalence of FBPs in this study. Notably, limited access to clean water and improved sanitation emerged as key determinants. Living conditions such as the use of unsafe water sources, poor sanitation, and close contact with domestic animals were key drivers for increased prevalence of FBPs. These living conditions varied across the study sites, with Harar showed the highest proportion of using the using unimproved water sources, unimproved sanitation facilities, and ownership of domestic animals. This clustering of risk factors may explain the higher prevalence of all targeted FBPs observed in Harar compared to other sites.

## 6.2. RECOMMENDATION

Based on the findings from the study, the following recommendations are forwarded that may help mitigate the burden of foodborne bacterial pathogens in Ethiopia.

First, given the marked regional variation in the prevalence of foodborne bacterial pathogens identified across the study cities, and diverse socio-demographic characteristics of participants, it is recommended to establish a national integrated surveillance system for FBD in Ethiopia. Such a system would enable coordinated data collection, timely detection of outbreaks, and evidence-based interventions to reduce the burden of FBDs nationwide.

Second, we acknowledge that data collected over a single year are insufficient to formally assess seasonal variation, which typically requires multiple consecutive years. This underscores the need for future studies that collect data over multiple years to confirm whether temporal trends are consistently repeated. Furthermore, additional research is recommended to investigate environmental, socio-demographic, and behavioural factors that may influence these patterns. Conducting longitudinal studies with larger sample sizes across diverse geographic regions would provide a more robust understanding of the temporal and seasonal dynamics of FBPs.

Third, strengthening access to clean water, and sanitation infrastructure is critical, particularly in high-burden areas like Harar. Improved access to clean water and sanitation

facilities can significantly reduce environmental contamination and interrupt the transmission of FBPs. Complementary to infrastructure, public education campaigns on safe food handing, hand hygiene, and environmental cleanliness should be implemented to improve community-level practices and reduce exposure risks.

Fourth, the application of molecular methods allowed the detection of important resistance determinants, including widespread bla-CTX-M, co-detection with bla-TEM, and the emergence of carbapenemase genes such as bla-NDM. These findings would likely have been underestimated using conventional culture-based methods alone. The ability to accurately detect such markers highlights the critical importance of molecular diagnostics in the containment of the spread of AMR among the FBPs in Ethiopia. Fifth, integration of AMR data into national surveillance systems will support evidence-based decision making and facilitate targeted interventions. Furthermore, the clustering of XDR STEC among children in Harar reflects a complex interaction between microbial virulence, host vulnerability, environmental contamination, and antibiotic misuse. Hence, urgent due attention should be given to antimicrobial stewardship, improved sanitation, regulated antibiotic use in both human and veterinary medicine, and enhanced surveillance system to monitor and control the spread of high-risk STEC strains in Ethiopia.

Sixth, in addition to promoting antibiotic stewardship, enforcing regulations on antimicrobial use in both human and veterinary medicine is vital. Misuse and overuse of antibiotics are major drivers of resistance. Policies should ensure rational prescription practices, restrict over-the-counter sales of critical antibiotics, and promote livestock management practices, such as separating animal living areas from the household can reduce zoonotic transmission. Supporting healthcare access in rural and underserved areas will also ensure timely treatment and reduce complications. These multifaceted strategies, when implemented together, will contribute significantly to reducing the burden of foodborne illnesses and AMR in Ethiopia.

Seventh, future studies should expand the geographical scope of surveillance to include both national and rural settings, particularly hard-to-reach areas with diverse dietary and environmental practices, is essential for capturing the broader burden and distribution of FBD. Moreover, the detail molecular characterization such as the use of WGS to obtain a

more comprehensive understanding of the resistance and virulence profiles of FBPs would help for detection of rare or novel resistance and virulence genes. This can offer deeper insights into the genetic mechanisms driving AMR and pathogenicity. In addition, incorporating samples from animals, food, and the environment is essential to map the broader epidemiology of these pathogens and to better understand their transmission dynamics through a One Health approach. This integrative strategy would not only strengthen the scientific evidence base but also offer critical information for policy makers aiming to design effective interventions and surveillance systems.

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# APPENDICES

## Appendix I. Sample Size Calculation

| Laboratory Study | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *ONLY change values in gray boxes below* | | | | | | | "What If Scenario" | | | |
| | | Suggested Sample Size \| e & α | | Expected Proportion | | *Sample Statistic Estimation* | | *Hypothesis Testing* | | |
| **Statistical Parameters** | | **Ntotal** | **NperGroup** | **p** | **p(1-p)** | **Min. e \| α** | **Max. CI% \| e** | **β \| Str: Season** | **β \| Str: Region** | **β \| Custom Grps** |
| e = | 0.05 | 2305 | 384 | 0.5 | 0.25 | 0.069 | 84.11% | 0.00% | 0.00% | 0.00% |
| α = | 0.05 | 2304 | 384 | 0.49 | 0.2499 | 0.069 | 84.12% | 0.00% | 0.00% | 0.00% |
| Zα/2 = | 1.96 | 2301 | 384 | 0.48 | 0.2496 | 0.069 | 84.15% | 0.00% | 0.00% | 0.00% |
| β = | 0.8 | 2297 | 383 | 0.47 | 0.2491 | 0.069 | 84.19% | 0.00% | 0.00% | 0.00% |
| Z1-β = | 1.28 | 2290 | 382 | 0.46 | 0.2484 | 0.069 | 84.25% | 0.00% | 0.00% | 0.00% |
| | | 2282 | 380 | 0.45 | 0.2475 | 0.069 | 84.32% | 0.00% | 0.00% | 0.00% |
| **Design Info** | | 2272 | 379 | 0.44 | 0.2464 | 0.069 | 84.41% | 0.00% | 0.00% | 0.00% |
| Design Effect = | 1 | 2260 | 377 | 0.43 | 0.2451 | 0.069 | 84.52% | 0.00% | 0.00% | 0.00% |
| Completion Rate = | 1 | 2246 | 374 | 0.42 | 0.2436 | 0.068 | 84.65% | 0.00% | 0.00% | 0.00% |
| Str: Seasons = | 2 | 2230 | 372 | 0.41 | 0.2419 | 0.068 | 84.79% | 0.00% | 0.00% | 0.00% |
| Str: Regions = | 3 | 2213 | 369 | 0.4 | 0.24 | 0.068 | 84.95% | 0.00% | 0.00% | 0.00% |
| | | 2193 | 366 | 0.39 | 0.2379 | 0.068 | 85.13% | 0.00% | 0.00% | 0.00% |
| **What if Scenario** | | 2172 | 362 | 0.38 | 0.2356 | 0.067 | 85.33% | 0.00% | 0.00% | 0.00% |
| Specimens per Group = | 200 | 2149 | 358 | 0.37 | 0.2331 | 0.067 | 85.54% | 0.00% | 0.00% | 0.00% |
| Cost per Test = | $75.00 | 2124 | 354 | 0.36 | 0.2304 | 0.067 | 85.77% | 0.00% | 0.00% | 0.00% |
| Cost per Hospital = | $30,000.00 | 2098 | 350 | 0.35 | 0.2275 | 0.066 | 86.02% | 0.00% | 0.00% | 0.00% |
| Total Cost = | $90,000.00 | 2069 | 345 | 0.34 | 0.2244 | 0.066 | 86.29% | 0.00% | 0.00% | 0.00% |
| | | 2039 | 340 | 0.33 | 0.2211 | 0.065 | 86.58% | 0.00% | 0.00% | 0.00% |
| **Hypothesis Testing** | | 2006 | 334 | 0.32 | 0.2176 | 0.065 | 86.89% | 0.00% | 0.00% | 0.00% |
| Total No. Specimens = | 1200 | 1972 | 329 | 0.31 | 0.2139 | 0.064 | 87.21% | 0.00% | 0.00% | 0.00% |
| No. Specs per Season = | 600 | 1936 | 323 | 0.3 | 0.21 | 0.064 | 87.56% | 0.00% | 0.00% | 0.00% |
| No. Specs per Region = | 400 | 1898 | 316 | 0.29 | 0.2059 | 0.063 | 87.93% | 0.00% | 0.00% | 0.00% |
| No. Specs per Hospital = | 400 | 1859 | 310 | 0.28 | 0.2016 | 0.062 | 88.31% | 0.00% | 0.00% | 0.00% |
| Custom No. Groups* = | 5 | 1817 | 303 | 0.27 | 0.1971 | 0.062 | 88.72% | 0.00% | 0.00% | 0.00% |
| No. Specs per Group* = | 240 | 1774 | 296 | 0.26 | 0.1924 | 0.061 | 89.15% | 1.15% | 0.00% | 0.00% |
| **Assumes Groups are Balanced* | | 1729 | 288 | 0.25 | 0.1875 | 0.060 | 89.60% | 3.19% | 0.00% | 0.00% |
| | | 1682 | 280 | 0.24 | 0.1824 | 0.059 | 90.06% | 5.40% | 0.00% | 0.00% |
| | | 1633 | 272 | 0.23 | 0.1771 | 0.058 | 90.55% | 7.79% | 0.00% | 0.00% |
| | | 1582 | 264 | 0.22 | 0.1716 | 0.057 | 91.06% | 10.39% | 0.00% | 0.00% |
| | | 1530 | 255 | 0.21 | 0.1659 | 0.056 | 91.59% | 13.20% | 0.00% | 0.00% |
| | | 1475 | 246 | 0.2 | 0.16 | 0.055 | 92.14% | 16.24% | 0.00% | 0.00% |
| | | 1419 | 236 | 0.19 | 0.1539 | 0.054 | 92.70% | 19.54% | 0.00% | 0.00% |
| | | 1361 | 227 | 0.18 | 0.1476 | 0.053 | 93.28% | 23.13% | 0.00% | 0.00% |
| | | 1301 | 217 | 0.17 | 0.1411 | 0.052 | 93.88% | 27.02% | 0.00% | 0.00% |
| | | 1239 | 207 | 0.16 | 0.1344 | 0.051 | 94.48% | 31.24% | 0.00% | 0.00% |
| | | 1176 | 196 | 0.15 | 0.1275 | 0.049 | 95.10% | 35.82% | 1.62% | 0.00% |
| | | 1110 | 185 | 0.14 | 0.1204 | 0.048 | 95.71% | 40.77% | 6.20% | 0.00% |
| | | 1043 | 174 | 0.13 | 0.1131 | 0.047 | 96.32% | 46.13% | 11.33% | 0.00% |
| | | 974 | 162 | 0.12 | 0.1056 | 0.045 | 96.93% | 51.89% | 17.09% | 0.00% |
| | | 903 | 150 | 0.11 | 0.0979 | 0.043 | 97.51% | 58.05% | 23.56% | 0.00% |
| | | 830 | 138 | 0.1 | 0.09 | 0.042 | 98.06% | 64.56% | 30.84% | 0.00% |
| | | 755 | 126 | 0.09 | 0.0819 | 0.040 | 98.57% | 71.32% | 39.02% | 0.00% |
| | | 679 | 113 | 0.08 | 0.0736 | 0.038 | 99.02% | 78.16% | 48.16% | 4.69% |
| | | 600 | 100 | 0.07 | 0.0651 | 0.035 | 99.39% | 84.80% | 58.24% | 14.79% |
| | | 520 | 87 | 0.06 | 0.0564 | 0.033 | 99.67% | 90.78% | 69.04% | 27.06% |
| | | 438 | 73 | 0.05 | 0.0475 | 0.030 | 99.86% | 95.55% | 80.03% | 41.93% |
| | | 354 | 59 | 0.04 | 0.0384 | 0.027 | 99.96% | 98.58% | 90.00% | 59.55% |
| | | 268 | 45 | 0.03 | 0.0291 | 0.024 | 99.99% | 99.81% | 97.05% | 78.78% |
| | | 181 | 30 | 0.02 | 0.0196 | 0.019 | 100.00% | 100.00% | 99.79% | 94.79% |
| | | 91 | 15 | 0.01 | 0.0099 | 0.014 | 100.00% | 100.00% | 100.00% | 99.95% |

## Appendix II. Questionnaire

**A0.0** Participant ID [Assigned by Team]

**A1.0** Stratum – Region [Assigned by Team]

**A2.0** Stratum – Season [Assigned by Team]

**A3.0** Date of Interview [Insert Date]

**A4.0** Language of Interview [Afaan Oromo, Amharic, English, Other]

**A5.0** Name of enumerator [Select from list]

**A6.0** Name of hospital where recruitment is done [Select from list]

***Obtain Consent and Begin Interview with Respondent***

**Section 1: Respondent Profile**

**C1.0.** Full address of the person who provides stool sample [Text Entry]

**C1.1.** Distance from the hospital [Number entry…Enter 9999 If Unknown]

**C1.2**. What is the name of the individual who is providing the stool sample? [Text Entry] →[Name] will be used to here after

**C1.3**. What is [Name's] age? (Years) [Numeric Entry – 9999 If Unknown/Prefer to Not Respond] [if less than 12 months, enter 0]

**C1.3.1**. How many months old is the child? [Select from 0 to 11]

**C1.4**. Height of [Name] _____ (UNITS)

**C1.5**. Weight of [Name] _____ (UNITS)

**C1.6**. Delivery Type for [Name]

Vaginal Delivery

C-section

**C1.7**. Was [Name] breast feed?

Yes

**C1.7.1.** Was [Name] exclusively breast feed?

Yes

No

**C1.7.2.** When did [Name] start weaning?

Before 6 Months

After 6 Months

No

**C1.8**. Gender? (Answer by observation)

Male

Female

**C1.9**. What is the highest level of education [Name's] have completed? (Applies only If Child>3yr)

Never Attended

Non- Formal

Pre- School

Grades 1- 8

Other

*For the following questions, "Household" is defined as all individuals sleeping in this house for the past 6 months, and at least 4 days per week, sharing the same resources.*

**C2.0**. How many Females age 15 or older are members of [Name]'S household? [Numeric entry – 9999 If Unknown]

**C3.0**. How many Females younger than 15 years old are members of [Name]'S household? [Numeric Entry – 9999 If Unknown]

**C4.0**. How many Males age 15 or older are members of [Name]'S household? [Numeric Entry – 9999 If Unknown]

**C5.0**. How many Males younger than 15 years old are members of [Name]'S household? [Numeric Entry – 9999 If Unknown]

**C6.0**. Which of the following describes the primary Household source(s) of income (this applies to sources coming from one or more individuals belonging to the household)? [Select All That Apply]

Agriculture (Primary Production)

**C6.1**. Which of the following best describes the Household source of Agricultural income?

Crop Agriculture

Animal Agriculture

Mixed Agriculture

Government

**C6.2**. Which of the following best describes the Household source of Government income?

Labor
Administrative
Clerical Technical
Research
Education Executive
Leadership

Non-Government (Incl. Ngo)

**C6.3**. Which of the following best describes the HOUSEHOLD source of Non-Government Income?

Labor
Administrative
Clerical Technical
Research
Education Executive

Leadership

Private Business – As Owner or Employee (including informal-sector businesses, small and medium enterprises)

**C6.4**. Which of the following best describes the Household source of Private Business income (e.g., selling agricultural products, running storefront)?

Labor
Administrative
Clerical Technical
Research
Education Executive
Leadership

Other

**C6.5**. Describe Other sources of Household income? [Text Entry]

**C7.0**. What was last month's Income for the Household? (All sources together)? [Birr] [*in the case of inconsistent monthly income, enumerator to help participants work out the most accurate monthly average*]

Less than 2000
2000 - 4000
4000 - 6000
6000 - 8000
8000 - 10000
Greater than 10000
Prefers Not to Say
Unknown

**Section 2: Stool Sample**

*For the following questions, "EPISODE" is defined as a period of days where an individual experienced the current illness separated from another episode by at least 48 hours.*

**C8.0**. Does [Name] have any of the following chronic diseases? [Select All That Apply]

Hypertension
Asthma
Diabetes
Cancer
Kidney Disease
Cardiovascular Disease
Gastrointestinal Disease
Other [Text Entry]
Don't Know

**C9.0.** Is the person providing the stool sample ill?

Yes
No

IF C9.0 = "No" Then Skip to **C10.0**

**C9.1**. Duration of current illness: _____

**C9.2**. What are the signs and symptoms? [Select All That Apply]

Loose, Watery Stools
Abdominal Cramps
Abdominal Pain
Fever
Blood In the Stool.
Mucus In the Stool.
Bloating
Nausea
Other [Text Entry]

**C9.3**. Was the stool sample ordered due to this illness?

Yes

No

**C9.3.1**. Why was the stool sample ordered? [Text Entry]

**C9.4**. Is [NAME] hospitalized?

Yes

No

**C10.0**. Who ordered the stool sample?

Healthcare Provider

**C10.1.** What type of healthcare provider requested the stool sample? [Select All That Apply]

Government Hospital

Government Clinic

Private Hospital

Private Clinic

Health Center

Health Post

Pharmacist / Druggist

Traditional Healer

Other

Self-Requested

Others

**C11.0**. What tests are being performed on the stool sample?

Direct Stool Microscopy

Parasitology

Wet Mount

Bacterial Culture

Bacterial Culture and Antibiotic Susceptibility Test

Others

**C12.0.** Did [Name] visit a healthcare provider for the current illness episode?

Yes

**C12.1.** What type of healthcare provider did [NAME] visit? [Select All That Apply]

Government Hospital
Government Clinic
Private Hospital
Private Clinic
Health Center
Health Post
Pharmacist / Druggist
Traditional Healer
Other [Text Entry]

**C12.2**. How far is the facility from the household dwelling? [Numeric Entry – 9999 IF Unknown]

**C12.3**. Indicate the unit of the reported distance

Km
Meters
Hours
Minutes
Price Paid
Other

**C11.3.1.** What Unit is the reported distance? [Text Entry]

No

**C12.4.** Which of the following factors (if any) prevented [Name] from visiting a healthcare provider for the current illness episode? [Select All

That Apply]

Personal Choice

Cost of Visit

No Access to Healthcare Provider Other

Unknown

Other

**C12.4.1.** What other factors prevented [Name] from visiting a healthcare provider for the current illness episode? [Text Entry]

Don't Know

**C13.0.** Did [Name] receive or take any treatment for the current illness episode?

Yes

**C13.1.** Which of the following were used to treat [Name]'s current illness episode? [Select All That Apply] (once a participant is allowed to provide his/her answer, interviewer to read aloud options that the person may have missed).

Oral Fluids to Prevent Dehydration

Intravenous Fluids for Rehydration

Medicine To Relieve Pain

Medicine To Stop Diarrhea

Medicine To Stop Fever

Medicine Unknown Purpose

Antibiotics

Antiparasitic/Antiprotozoa

Natural/Traditional Remedies (Rice, Feto, Garlic, Ginger, Mineral Water, etc.)

Don't Know

Other

**C13.2.** How was the medicine obtained? [Select All That Apply]

Healthcare Provider Prescribed

**C13.2.1.** Did [Name] take this medicine for full duration recommended?

Yes

No

Not Applicable (There Is No Recommended Duration)

Don't Know

Other

Prescribed By a Traditional Healer

Self-Prescribed – Obtained from Local Dispensary

Self-Prescribed – Medicine Remaining from Previous Illness Self-Prescribed – Obtained from Another Individual

Self-Prescribed – Made at Home

Don't Know

Other

No

**C13.3.** Which of the following factors (if any) prevented [Name] from treating the current illness episode? [Select All That Apply]

Personal Choice

Cost Of Medicine

Medicine Unavailable at Pharmacy/ Dispensary

Pharmacy Unavailable in the Area

Other

**C13.3.1.** What other factors prevented [Name] from treating the current illness episode? [Text Entry]

Don't Know

**C14.0.** How many separate/unrelated episodes of Diarrhea has [Name] experienced in the past four (4) weeks? [Numeric Entry – 9999 If Unknown]

**C15.0.** How many separate/unrelated episodes of Bloody Stools has [Name] experienced in the past four (4) weeks presented alongside diarrhea?

[Numeric Entry – 9999 If Unknown]

**C16.0.** How many separate/unrelated episodes of Vomiting (of suspected infectious disease origin) have [Name] experienced in the past four (4) weeks?

[Numeric Entry – 9999 If Unknown]

**C17.0.** How many separate/unrelated episodes of Fever (of suspected infectious disease origin) have [Name] experienced in the past four (4) weeks?

[Numeric Entry – 9999 If Unknown]

**C18.0** How many separate/unrelated episodes of Abdominal Pain (of suspected infectious disease origin) have [Name] experienced in the past four (4) weeks? [Numeric Entry – 9999 If Unknown]

**Section 3: Costs**

*For the following questions, "Episode" is defined as a period of days where an individual experienced the current illness separated from another episode by at least 48 hours.*

**C19.0** What was the total out-of-pocket cost of Healthcare Provider Visits for [Name]'s current illness episode? (Excluding cost of medicines)

[Numeric Entry – Birr, 0 If Provided for Free]

**C20.0** What was the total out-of-pocket cost of Travel, Transportation and Lodging to Healthcare Provider for [Name]'s current illness episode?

[Numeric Entry – Birr, 0 If Provided for Free]

**C21.0** What was the total out-of-pocket cost of Medicine and Other Treatment For [Name]'s current illness episode?

[Numeric Entry – Birr, 0 If Provided for Free]

**C22.0.** Were there Any (Other) Out-Of-Pocket Costs For [Name]'S current illness episode?

Yes

**C22.1.** What were other out-of-pocket costs for [Name]'s current illness episode? [Text Entry]

**C22.2.** What was the total cost of other out-of-pocket expenses for [Name]'s current illness? [Numeric Entry – Birr, 0 If Provided for Free]

No

Don't Know

**C23.0.** Does [Name] attend school?

Yes

> **C23.1.** Did [Name] miss any days of school due to [Name]'s current illness episode?
>
> Yes
>
> **C23.1.1.** How many days of school were missed by [Name] as a result of [NAME]'s current illness episode? [Numberic Entry-0 If None, 9999 If Unknown]
>
> No

No

**C24.0** Has [Name] been assisted by a caregiver during the current illness episode?

Yes

**C24.1** Does [Name]'S caregiver have a paid job?

Yes

**C24.1.1.** Did [Name]'s caregiver miss any days of work due to [Name]'s

current illness episode?

Yes

**C24.1.1.2.** How many days of work were missed by [Name]'s caregiver because of [Name]'s current illness episode? [Numeric Entry –0 If None, 9999 If Unknown]

**C24.1.2.** What is [Name]'s caregiver's main occupation? [Text Entry]

**C24.1.3**. Where does [Name] 's caregiver work?

Business House
Office
At home
On street
'Gulit'/open market
Farm/field
Factory
Quarry or mine
Anywhere as found
Where customer available
Construction site
Lakes/rivers/wells
Other: [Text Entry]

**C24.1.4**. What was the major product or service of this organization? [Text Entry]

**C24.1.5**. What was [Name]'s caregiver's employment status?

Employee: Government or NGO
Employee: Private Org.
Employer
Self-Employed
Unpaid Family Worker
Other: [Text Entry]

**C24.1.6.** What is [Name]'s caregiver's typical Monthly Income (all sources together)? [Birr] [in the case of inconsistent monthly income, enumerator to help participants work out the most accurate monthly average]

Less than 2000
2000 - 4000
4000 - 6000
6000 - 8000
8000 - 10000
Greater than 10000
Prefers Not to Say
No Income
Unknown

**C24.1.7**. What is [Name]'s caregiver's typical hourly rate? [Numerical Entry] 999 If Unknown; 998 If Prefer Not to Say

**C24.1.8.** How many days per month does [Name]'s caregiver typically work? [Numerical Entry – 99 If Unknown]

No
Don't Know

**C24.2**. Does [Name]'s caregiver attend school?

Yes

**C24.2.1.** How many days of school were missed by [Name]'s caregiver because of [NAME]'s current illness episode?

[Numeric Entry–0 If None, 9999 If Unknown]

No
Don't Know

No
Don't Know

**Section 4: Environmental Exposures**

*The following questions pertain to the entire household as previously defined.*

[Repeat Questions CX.0-CX.3 For Each Animal]

**C25.** Cattle

**C26**. Goats

**C27.** Sheep

**C28.** Chickens, Ducks, Or Other Poultry

**C29.** Dogs or Cats

[LOOP - START]

**CX.0.** Do you have [Insert Animal] in your household?

Yes

**CX.1.** Where are the [Insert Animal] kept?

Inside House Outside House
Don't Know

**CX.2.** For what purpose is the [Insert Animal] kept?

Income Trade Consumption Savings Traction
Pet
Don't Know
Other [Text Entry]

**CX.3.** Who cares for the [Insert Animal] of the household? [Select All That Apply]

Female Head of Family
Other Female Adults in Household
Other Female Children in Household
Male Head of Family

Other Male Adults in Household

Other Male Children in Household

Not Cared for by Household Members

Don't Know

No

**CX.4.** Have any household members been in contact with [Insert Animal] in the past four (4) weeks Outside of the Household (i.e., due to their occupation, or else)?

Yes

**CX.5.** What household members have been in contact with [Insert Animal]? [Select All That Apply]

Female Head of Family

Other Female Adults in Household Other Female Children in Household Male Head of Family

Other Male Adults in Household Other Male Children in Household

Don't Know

No

[Loop - Finish]

**C30.0.** What is the primary source of water for the household? Select All That Apply (But It Must Be Primary Sources)

Piped Into Dwelling/House

Piped Into Yard Communal Tap Neighbor's House Protected Well

Unprotected Well Protected Spring Unprotected Spring

Surface Water [E.G., River, Lake, Canal] Rainwater

Tanker Truck

Bottled Water

Filtered Water

Other [Text Entry]

Don’t Know

**C31.0.** What type of toilet is present in the household?

Flush To Piped Sewer System

Flush To Septic Tank

Pit Latrine with Cover

Pit Latrine Without Cover

No Latrine Facility / Open Field

Other [Text Entry]

Don’t Know

**SECTION 5: Food Consumption and Preparation Practices**

**C32.0.** Where do you get your Grains and Dry Goods for The Household? [Select All That Apply]

Self-Grown

Farmer Other Than Self

Local Central Market

Small Shop

Self-Service Grocery

Supermarket

Don’t Know

Other [Text Entry]

**C33.0.** Where do you get your Animal Source Products such as eggs, meat, milk, and cheese for the household? [Select All That Apply]

Self-Grown

Farmer Other Than Self

Local Central Market

Small Shop

Self-Service Grocery

Supermarket

Don’t Know

Other [Text Entry]

**C34.0.** Where do you get your Fruit and Vegetables for the household? [Select All That Apply]

Self-Grown
Farmer Other Than Self
Local Central/Street Market (Incl. 'Gulit')
Small Shop
Self-Service Grocery Supermarket
Don't Know
Other [Text Entry]

**C35.0.** Is there a dedicated area in the residence where All Food is prepared?

Yes

**C35.1** Are animals prevented from entering this area?

Yes
No

No

**C36.0.** Who Usually (i.e., Most Commonly) prepares food for the household? [Select All That Apply]

Respondent
Individuals Employed by Household
Female Head of Family
Other Female Adults in Household
Other Female Children in Household
Male Head of Family
Other Male Adults in Household
Other Male Children in Household

**C37.0**. Describe briefly how you regularly prepare a dish containing

Vegetables or Fruits. Please ensure you tell me all the steps you will take from the moment you start cooking. (Select All that are mentioned)

Wash Hands Prior To Handling Food
Wash Hands After Touching Raw Food
Wash Surfaces Prior To Handling Food
Wash Utensils Before Food Preparation
Boil Or Filter Water Used to Prepare Food
Wash Fruits and Vegetables
Disinfected Fruits and Vegetables (i.e., Including Soaking with Salt, Or A Food-Grade Disinfectant)
Prepare Uncooked Meat and Vegetables with Separate Utensils Measure Temperature of Cooked Foods
None Of the Above Apply
Don't Know
Other

**C37.0**. Describe briefly how you regularly prepare a dish containing ANIMAL Source Foods. Please ensure you tell me all the steps you take from the moment you start cooking. (Select All that are mentioned)

Wash Hands Prior To Handling Food
Wash Surfaces Prior To Handling Food
Boil Or Filter Water Used to Prepare Food
Wash Fruits and Vegetables
Disinfected Fruits and Vegetables (I.E. Including Soaking with Salt, Or A Food-Grade Disinfectant)
Prepare Uncooked Meat and Vegetables with Separate Utensils
Measure Temperature of Cooked Foods
None Of the Above Apply
Don't Know
Other

**C38.0.** Which of the following food handling practices are used In

Addition to The Ones Mentioned Above within the household when preparing food? [Select All That Apply] Prompt only the practices from this list that were not mentioned in the previous 2 questions.

Wash Hands Prior To Handling Food
Wash Hands After Touching Raw Food
Wash Surfaces Prior To Handling Food
Boil Or Filter Water Used to Prepare Food
Wash Fruits and Vegetables Before Preparation
Prepare Uncooked Meat and Vegetables with Separate Utensils
Measure Temperature of Cooked Foods
None Of the Above Apply
Unknown

**C39.0**. Do you treat the water you use to wash your hands when cooking?

Yes

**C39.1**. How do you treat the water?

Filtering
Boiling
Chemicals (disinfectants, tablets,)
(…Other To Be Filled in by Enumerators)

No
Don't Know

**C40.0.** Does the household have a refrigerator?

Yes

**Section 6: Knowledge About Foodborne Disease and Food Safety**

**C64.0.** Which of the following statements do you agree with?

Food and water are needed for nourishment and cannot make people sick.

Food and water are needed for nourishment, but they can also make people sick.

*The next questions will talk about the safety of food, which means making food safe to eat so that it will not make us sick when we eat it.*

**C65.0.** Which of the following statements do you agree with?

Washing Hands Before Preparing Food Is Good Practice to Keep Food Safe.

Handwashing Before Preparing Food Has Nothing to Do with Keeping Food Safe.

**C66.0.** Which of the following statements do you agree with? (Refers to vegetables that are normally consumed cooked, not to those that are normally eaten uncooked.)

Eating Uncooked Vegetables Is Safer than Eating Cooked Vegetables.

Eating Uncooked Vegetables Is as Safe as eating cooked food.

Eating Uncooked Vegetables Is Less Safe than eating cooked vegetables.

**C67.0.** Which of the following statements do you agree with?

Boiling Or Cooking Food Normally Makes It Less Safe to Eat.

Boiling Or Cooking Food Normally Has No Impact on Safety.

Boiling Or Cooking Food Normally Makes It More Safe to Eat.

**C68.0.** What resources do you use to learn more about foodborne disease symptoms and treatment? [Select All That Apply]

Healthcare Providers
Government Agency [Including Websites]
Intergovernmental Agency [e.g., Who (Including Websites)]
Nonprofit or Relief Agency [Including Websites]
Internet Sites [Nongovernmental]
Informal Sources (Neighbors, Families, Friends….)
Tv And Radio
Social Media
Posters, Flyers
Other
Don’t Know

## Appendix III. Informed Consent to Participate in Research (English version)

**Study Title:** Molecular features, Epidemiology and Antimicrobial Resistance profiles of Selected Foodborne Bacterial Pathogens Among Diarrheal Patients in Three Cities in Ethiopia.

**Researchers:** Amete Mihret, Dr. Woldaregay Erku, Dr. Alem Abrha, Dr. Eyasu Tigabu

**Sponsor**: Ethiopian Public Health Institute, Bill and Melinda Gates Foundation, UK Department for International Development, Addis Ababa University.

Hello,

My name is_____. I am working on a public health project with

researchers at Addis Ababa University, The Ohio State University and Ethiopian Public Health Institute along with the International Livestock Research Institute, a government agency under the Ministry of Health. This study is working to estimate the burden of foodborne bacterial pathogens among diarrheal patients in Ethiopia. You have been selected to take part in this study is by chance.

If you take part in this study, your stool sample will be tested for 4 different types of bacteria. Additionally, you will be asked:

 Questions about yourself. For example, your age and education you have completed.

 Whether anyone living with you has gotten sick from food in the past month. Also, what symptoms he/she had, how long he/she was sick, if he/she went to a health facility, if he/she received any care and if he/she paid anything for care.

 Questions about the house. For example, where the house gets water from and if you have any animals.

 What you know about food safety and getting sick from food.

**This is a consent form for research participation.** It contains important information about this study. Please take your time and consider the information provided carefully.

Feel free to ask questions before deciding whether you would like to take part in our study or not or at any point during the study.

**Your participation is entirely voluntary.** You can choose whether or not to take part in this study. You can change your mind later even if you agree now. Whether or not you choose to take part in this study, the care you receive at this health facility will continue. If you choose not to take part in this study, you will be offered care commonly offered at this health facility. This will not change.

**You may or may not benefit from taking part in this study.** There may not be any direct benefit for you if you take part in this study. There may not be any immediate benefit to the society, but the data generated may benefit future generations.

**You will be provided with any new information that develops during the study that may affect your decision whether or not to continue.** If you decide to take part, you will be asked to sign this form. You will receive a copy of the form.

**Key Information About This Study**

2. **Why is this study being done?**

   Public health groups are working to make food safer. However, it is not clearly understood how many Ethiopians get sick from eating contaminated food. This study will help gather information and determine how many people in Ethiopia get sick from foodborne bacterial pathogens each year.

3. **How many people will take part in this study?** A total of 2,304 people will be enrolled in the study.

4. **What will happen if I take part in this study?**

   If you take part in this study, your stool sample will be tested for 4 different types of bacteria. Also, we will also like to ask you a few questions about yourself and your eating habits. We would also like to review your medical history regarding your current diarrhea episode (what symptoms you have, how long you've been sick, what healthcare you've received and the costs of care). This short interview may take 20-30 minutes of your time.

**4. Can I stop being in the study?**

You can stop studying at any time. Nothing will change whether you choose to take part or not. Your decision will not affect your future relationship with study staff, the health clinic, or any of the organizations involved in the study.

5. **What risks, side effects or discomforts can I expect from being in the study?**

   It is possible you may experience minor inconvenience and discomfort from giving a stool sample. Our study team will take the necessary precautions to minimize this discomfort.

6. **What benefits can I expect from being in the study?**

   There may not be any direct benefit for you, but the data generated may benefit future generations. If we find bacteria in your stool that could impact your health, we will share that information with your physician and link you with the appropriate treating unit.

7. **What other choices do I have if I do not take part in the study?**

   You may choose not to take part. If you choose not to take part in this study, you will be offered care commonly offered at this health facility. This will not change.

   **Will my study-related information be kept confidential?**

   The information that we collect from this research project will be kept confidential. Personal identifiers will not be used. The electronic information document will also be protected with password and lock-and-key where access is given only to researchers. Efforts will be made to keep your study information confidential. Your records may be reviewed by the following groups:

   - Members of the investigators team
   - Regulatory agencies including but not limited to the Ethiopian Food and Drug Administration (EFDA)
   - The Institutional Review Board or Office of Responsible Research Practices at The Ethiopian National Research Ethics Review Committee (NRERC); Ethiopian Public Health Institute (EPHI), University of Gondar, Haramaya University, Yekatit 12 Hospital, The Ohio State University, the International Livestock Research Institute
   - The sponsor, if any, or agency supporting the study.

   By signing the consent form, you agree to allow the researchers to access and use the information in your medical records for the current study.

8. **Will my de-identified information be used or shared for future research?**

   De-identified study information may be used or shared with other researchers without your additional informed consent. This information will be kept confidential and stored and used for many years.

**Can we keep your stool sample after it has been tested to use in future studies?**

_____You may keep my stool sample and use it for ANY type of future research study.

_____You may NOT keep my stool sample for a future study. My sample will be destroyed after it is tested.

**Can we contact you again to ask for your interest to participate in subsequent related studies?**

_____You may contact me in the future to ask for my interest to be involved in other related studies.

_____You may NOT contact me in the future to ask for my interest to be involved in other related studies.

**Can we access your medical record for this study?**

_____You can access my medical record

_____You can't access my medical record

9. **What are the costs of taking part in this study?**

   There are no costs for you to take part in this study.

10. **Will I be paid for taking part in this study?**

    You will not be paid to take part in this study.

11. **What happens if I am injured because I took part in this study?**

    It is unlikely you will be injured during the study. If you are injured by the study, please inform the study staff immediately and you will be linked to the appropriate care giving unit.

12. **What are my rights if I take part in this study?**

    Whether or not you choose to take part in this study, the care you receive at this health facility will continue. You do not give up any personal legal rights you may have by signing this form.

    You will be provided with new information, if any, that develops during the study that may affect your decision whether or not to continue the study.

    An Institutional Review Board responsible for human subjects research at The Ethiopian National Research Ethics Review Committee (NRERC); Ethiopian Public Health Institute, University of Gondar, Haramaya University, Yekatit 12 Hospital, The Ohio State

University and International Livestock Research Institute reviewed this research project and found it to be acceptable, according to their guidelines, applicable state and federal regulations and University policies designed to protect the rights and welfare of research participants.

**13. Who can answer my questions about the study?**

For questions, concerns, or complaints about the study, or if you feel you have any discomfort because of the study, you may contact Amete Mihret at amete2004@gmail.com or +251-913773888.

For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who part of the research team is not, you may contact

- EPHI's Scientific and Ethics review Committee (SERO) (Tel: +251 118685503/15)

## Appendix IV. Informed Consent to Participate in Research (Amharic version)

**በጥናቱ ውስጥ ለመሳተፍ ስምምነት**

**የጥናቱ ርዕስ:** የተቅማጥ በሽታ የሚያመጡ ከምግብ ጋር ተዛማጅነት ያላቸው የተመረጡ ባክቴሪያዎችን ስርጭትና ለፀረ-ባክቴሪያ መድሃኒቶች መላምድ ሁኔታ ለማውቅ የተቅማጥ በሽታ ካለባቸው ታማሚዎች በተመረጡ ሶስት ከተሞች በኢትዮጵያ ውስጥ የሚደረግ ጥናት::

**ተመራማሪዎች ፡ አመተ ምህረት፣** ዶ/ር ወልዳረጋይ እርቁ፣ ዶ/ር አለም አብርሃ፣ ዶ/ር ኢያሱ ጥጋቡ

**ድጋፍ ሰጪ፡ የኢትዮጵያ ህብረተሰብ ጤና ኢንስቲትዩት ፣** ቢል እና መሊንዳ ጌትስ ፋዉነዳሽን ፣ የእነግሊዝ አለም አቀፍ ልማት መምሪያ።

ስሜ_____ይባላል። የምሰራው በህበረተሰብ ጤና ምርምር ከአዲስ አበባ ዩኒቨርስቲ፣ ከኦሃዮ ስቴትስ ዩኒቨርስቲ፣ ኢንተርናሽናል ላይቭስቶክ ምርምር ተቋም ጋር በመተባበር ነው። ይህ ምርምርም የተቅማጥ በሽታ የሚያመጡ ከምግብ ጋር ተዛማጅነት ያላቸው የተመረጡ ባክቴሪያዎችን ስርጭትና ለፀረ-ባክቴሪያ መድሃኒቶች መላመድ ሁኔታ ለማውቅ የተቅማጥ በሽታ ካለባቸው ታማሚዎች በተመረጡ ሶስት ከተሞች በኢትዮጵያ ውስጥ የሚደረግ ጥናት ነው። ለዚህ ጥናት የተመረጥከው/ሽው በኢጋጣሚ ነው።

**በምርምሩ ለመሳትፍ የስምምነት ፎርም፡**

ይህ ሰነድ ስለምርምሩ ጠቃሚ መረጃዎቹን ያካትታል፣ እባክዎት ጊዜዎትን ወስደዉ በጥንቃቄ ያንብቡት፡፡ በምርምራችን ተሳታፊ ለመሆን ከመዎሰንዎ በፊት ጥያቄ ለመጠየቅ ነጻ ይሁኑ፡፡

**የእርስዎ ተሳትፎ ሙሉ በሙሉ በፈቃደኝነትዎ ላይ ተመሰረተ ነዉ**

በዚህ ምርምር መሳተፍ ወይም አለመሳተፍ የእርስዎ ምርጫ ነው፡፡ አብረውን ለመስራት ከወሰኑ በላም ሀሳብዎን መቀየር ይችላሉ፡፡ በዚህ ምርምር ለመሳተፍ ቢስማሙ ወይም ባይስማሙም በዚህ ጤና ተቋም የሚያገኙት አገልግሎት ላይ ምንም አይነት ተፅእኖ አይፈጥርም፡፡የእርስዎ ዉሳኔ ከተመራማሪዎች ጋር የሚኖርዎትን የወደፊት ግንኙነት ላይ ተፅእኖ አይኖረዉም፡፡

**በዚህ ምርምር ተጠቃሚ ሊሆኑም ላይሆኑም ይችላሉ**

ከዚህ ምርምር በቀጥታ ተጠቃሚ ላይሆኑ ይችላሉ ፣ ማህበረሰቡም ቢሆን በፍጥነት ተጠቃሚ ላይሆን ይችላሉ ነገር ግን ከዚህ ምርምር የሚገኘዉ መረጃ ለወደፊቱ ትዉልድ ይጠቅማል ብለን እናስባለን፡፡

**በምርምሩ ላይ ሃሳብዎን ሊያስቀይሩ የሚችሉ አዳዲስ መረጃዎች ይደርስዎታል፤**

በዚህ ተስማምተዉ የምርምሩ አካል ለመሆን ከወሰኑ እዚህ ፎርም ላይ እንዲፈርሙ ይጠየቃሉ፡፡የፎርሙ ቅጂም ይደርስዎታል፡፡

**1. ጥናቱ ለምን አስፈለገ?**

የህብረተሰብ ጤና ቡድኖች የምግብን ጤናማነት እየሰሩ የሚገኙ ቢሆንም በኢትዮጵያ በምግብ መበከል ምክንያት የሚታመሙ ሰዎች ቁጥር በግልፅ አይታዎቅም።ይህ ጥናት መረጃዎችን ከማሰባሰብ በዘለለ ት ምን ያህል ኢትዮጵያዉያን በምግብ ብክለት ምክንያት እንደሚታመሙ ለማዎቅ ይረዳናል።

**2. ምን ያህል ሰዎች በዚህ ጥናት ላይ ይሳተፋሉ?**

በአጠቃላይ 2304 ሰዎች በዚህ ጥናት ይሳተፋሉ።

**3. በዚህ ጥናት ተሳታፊ ከሆንኩ ምን ይፈጠራል?**

በዚህ ጥናት ተሳታፊ ከሆኑ ለላቦራቶሪ ምርመራ የሰጡት የሰገራ ናሙና ለሶስት የተለያዩ ተህዋስያን ምርመራ ይደረግለታል። በተጨማሪም ስለ ጤና ተቋሙ እነዚህን እና መሰል ጥያቄዎች ይጠየቃሉ፤

- የ ጤና ባለሙያዎች ቁጥር
- የ ላቦራቶሪ እና የ ህክምና ክትትል ልምድ
- ለ ተቅማጥ ነክ በሽታዎች ተቅማጥ ነክ በሽታ ታማሚዎችን ለማከም ዎጭዉ ምን ያክል ነዉ
- የተቅማጥ ነክ በሽታዎች እና የምግብ ደህንነት ላይ ያለዎት ጠቅላላ እዉቀት

**4. በጥናቱ ለመሳተፍ ምን ያህል ጊዜ ይዎሰዳል**?

ይህ መጠይቅ ከ 10 ደቂቃ ያልበለጠ ይዎስዳል.

**5. ጥናቱን ማቋረጥ እችላለሁ?**

በማንኛዉም ሰዓት ጥናቱን ማቆም ይችላሉ ፤ሃሳብዎን ስለቀየሩ ምንም የሚፈተር ነገር የለም ፤ከተመራማሪዎች ጋር የሚኖርዎት ግንኙነት ላይ ተፅእኖ አይኖረዉም።

**6. የዚህ ጥናት ተሳታፊ በመሆኔ ምን አይነት አደጋዎችን ፤ የጎንዮሽ ጉዳቶችን፤ምቾት የሚነሱ ነገሮችን ልጠብቅ?**

የ ጥናቱ ተሳታፊዎች መልስ ከጥናቱ ዉጭ ያሉ ሰዎች ሊያገኙት ይችላሉ ።የዚህን ተፅእኖ ለመቀነስ ሲባል የጥናቱ መረጃ እንደተሰበሰበ ማንነተዎ ከጥናቱ ላይ ይዎጣል።

**7. ምን አይነት ጥቅሞችን ከዚህ ጥናት ላገኝ እችላለሁ?**

በቀጥታ ከጥናቱ ተጠቃሚ ላይሆኑ ይችላሉ ፤ ነገርግን የሚሰበሰበዉ መረጃ ለመጪዉ ትዉልድ ጠቃሚ ይሆናል።

**8.በዚህ ጥናት ተሳታፊ ባለሆን ሌሎች ምን ምንርጫዎች ይኖሩኛል?**

በዚህ ጥናት ላለመሳተፍ መምረጥ ይችላሉ ፤ በዚህም ምንም አይነት ቅጣትም ይሁን ጥቅምዎ አይነካም።

**9. በዚህ ጥናት ላይ ያሉ ግላዊ መረጃዎች ምንያህል የተጠበቀ ነዉ?**

ከዚህ ጥናት የሚሰበሰቡት መረጃዎች በጥንቃቄ ይያዛሉ፤ ግላዊ መለያዎችን አይጠቀምም፤የዲጅታል መረጃዎች በይለፍ ቃል ይቆለፋል ፤ መረጃዎች የሚቀመጡበት ቦታ የሚቆለፍ ሲሆን ቁልፉም ለተመራማሪዎች ብቻ

ይሰጣል፡፡ የጥናቱ ተሳታፊዎችን መረጃ ለመጠበቅ በተቻለን አቅም እንጥራለን፡፡ የተሳታፊዉ መረጃ በሚከተሉት አካላት ሊታዩ ይችላሉ

- የምርምር ቡድኑ አባላት
- በተቆጣጣሪ ኤጀንሲዎች ፤ እንደ ኢትዮጵያ ምግብ እና መድሀኒት አስተዳደር እና ልሎችም
- የተgሙ ግምገማ ቦርድ ፣በ ኢትዮጵያ ብሄራዊ የምርምር ግምገማ ኮሚቴ፣የኢትዮጵያ ማህበረሰብ ጤና ኢንስቲትዩት፣ንንደር ዩኒsTWüy ፣ሃረማያ ዩኒቨረሲቲ፣የካቲት 12 ሆስፒታል፣ ኦሃዮ ስቴት ዩኒቨረሲቲ፣አለም አቀፍ የእንስሳት ምርምር ኢንስቲትዩት
- ጥናቱን የሚደግፉ አካላት

**10. በዚህ ጥናት የተሰበሰበዉ መረጃ በሌሎች ጥናቶች ጥቅም ላይ ይዉላል?**

በዚህ ጥናት የተሰበሰበዉ ማንነት የማይገልፅ መረጃ ለሌሎች ምርመሮች ጥቅም ላይ ካለ ተጨማሪ ስምምነት ሊዉል ይችላል ፤ይህም መረጃ ለአመታት በጥንቃቄ በማስቀመጥ ጥቅም ላይ ይዉላል፡፡

**የሰገራ ናሙናዎትን ለዚህ ምርመራ ከተጠቀምንበት በኋላ ለወደፊት ተጨማሪ ጥናት እንዲቀመጥ ፈቃደኛ ነዎት?**

_____የሰገራ ናሙናዬን ማስቀመጥና ወደፊት ለሌላ ለማንኛውንም አይነት ጥናት መጠቀም ትችላላችሁ

_____የሰገራ ናሙናዬን ማስቀመጥና ለሌላ ጥናት መጠቀም አትችሉም፡፡ ናሙናው መወገድ አለበት

**ወደፊት በተመሳሳይ ጥናቶች ላይ በመካፈል ፍላጎትዎን ለመጠየቅ እንደገና እንነጋገርዎ እንችላለን?**

_____ ወደፊት በተመሳሳይ ጥናቶች ላይ ለመካፈል ፍላጎት እንደሌኝ ለመጠየቅ ማነጋገር ይችላሉ።

_____ ወደፊት በተመሳሳይ ጥናቶች ላይ ለመካፈል ፍላጎት እንደሌኝ ለመጠየቅ መነጋገር አትችሉም።

**ለዚህ ጥናት የጤና መዝገብዎን ማግኘት እንችላለን?**

_____ የጤና መዝገብዬን ማግኘት ትችላላችሁ።

_____ የጤና መዝገብዬን ማግኘት አትችሉም።

**11. በዚህ ጥናት ለመሳተፍ ምን ያህል ወጪ ያስፈልጋል?**

በዘህ ጥናት ለመሳተፍ ምንም አይነት ወጪ አያስፈለግም

**12. በዚህ ጥናት ለሚሳተፍ ክፍያ ይኖረዋል?**

በዚህ ጥናት ለሚሳተፉ ምንም አይነት ክፍያ አይኖረዉም

**13.በዚህ ጥናት ተሳታፊ ስለሆነኩ ጉዳት ቢደርስብኝስ?**

በዚህ ጥናት ላይ ሆነዉ ጉዳት ይደረስብዎታል ብለን አናስብም ሆኖም ግን በጥናቱ ላይ ሆነዉ ጉዳት ከደረሰብዎ ለሚመለከታቸዉ የጥናቱ አባላት በመንገር ተገቢዉ ህክምና እነዲሰጥዎት ይደረጋል፡፡

**14. በዚህ ጥናት ተሳተፊ ከሆነኩ መብቶቼ ምን ምን ናቼዉ?**

በጥናቱ ለመሳተፍ ከዎስኑ በኃላ ሃሳብዎን መቀየር ይችላሉ፤በዚህ ጥናት ለመሳተፍ በመወሰነዎ ማንኛዉም መብትዎ አይነካም፤በምርምሩ ላይ ሃሳብዎን ሊያስቀይሩ የሚችሉ አዳዲስ መረጃዎች ይደርስዎታል፤

በ ኢትዮጵያ ብሄራዊ የምርምር ስራዎች ግምገማ ኮሚቴ ዉሰጥ ለምርምሩ አባላት ተጠሪ የሆነዉ የተgሙ የግምገማ ቦርድ ፣የኢትዮጵያ ማህበረሰብ ጤና ኢንስቲትዩት ፣ጎንደር ዩኒቨርስቲ ፣ሃረማያ ዩኒቨርሲቲ፣የካቲት 12 ሆስፒታል፣ አሃዮ ስቴት ዩኒቨረሲቲ እና ዓለም ዓቀፍ የእንስሳት ምርምር ተgም ይህን ምርምር በተgሞቹ መመሪያ ፣ለሚመለከታቸዉ ግዛት እና ፌደራል ህጎች እንዲሁም የ ምርመር ተሳታፊዎች መብት እና ደህንነት ለመጠበቅ በዩኒቨርሲቲዎች የወጡ ደንቦች መሠረት ገምግመዉ ተቀባይነቱን አረጋግጠዋል፡፡

**15.ጥናቱን የሚመለከቱ ጥያቄዎችን መልስ መስጠት የሚችል ማነው?**

በጥናቱ ላይ ለሚነሱ ጥያቄዎች፣ስጋቶች፣አቤቱታዎች ወይም በጥናቱ ምክንያት ማንኛዉም አይነት ቅሬታዎች ካላችሁ ፤

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- የኢ.ህ.ጤ.ኢ ሳይንሳዊ እና የስነ-ምግባር ግምገማ ኮሚቴ (Tel: +251118685503/15)

## Appendix V. Informed Consent to Participate in Research (Afan Oromo version)

**Mata Duree Qorannoo:** Qorannoo baakteriyaa qoricha damdamatan, kan karaa nyaataa daddarban dhukkubsattoota dhibee garaa-kaasaa wal qabatee irratti magaalotaa Ethiopia sadii keessatti geggeeffamu

**Qorattoota:** Amete Mihret, Dr. Woldaregay Erku, Dr. Alem Abrha, Dr. Eyasu Tigabu

**Deeggertoota yookiin Ispoonsara qorannichaa:** Instiitiyuutii Fayyaa Hawaasummaa Itoophiyaa (EPHI), Bill fi Melinda Gates Foundation, Waajjira Hojii Misooma Idil-Addunyaa UK, Yuunivarsiitii Finfinneeti.

Akkam jirtu?

Maqaan koo _____ jedhama. Ani projektii fayyaa hawaasummaa keessatti qorattoota waliin Yunivarsiitii Finfinnee irraa, akkasumas Ohio State University, fi Instiitiyuutii Fayyaa Hawaasummaa Itoophiyaa keessatti kan argamu; dabalataanis Dhaabbata Qorannoo beeyiladaa Addunyaa (ILRI) kan eejensii mootummaa ta'e fi Ministira Fayyaa jala jiru waliin hojjachaan jira. Qorannichi baay'ina dhukkuboota baakteeriyaa nyaataan daddarban kan sababa dhukkuba garaachaa (dhibee garaa-kaasaa) ta'an Itoophiyaa keessatti hangam akka ta'e tilmaamuuf hojjetama. Kanaafuu Isin qorannoo kana keessatti akka hirmaattaniif kan filatamtan akka carraa ta'eeti yookaan akka tasaati.

Qorannoo kana keessatti kan hirmaattan yoo ta'e, saamuda sagaraa keessanii gosa baakteeriyaa adda addaa afur 4 irratti ni qoratama. Dabalataanis, gaaffilee tokko tokko isin in gaafanna. Isaanis?

- Gaaffii waa'ee mataa keessaniin wal qabatu; fakkeenyaaf umrii fi sadarkaa barnootaa isin xumuurtan
- Ji'a darbe keessatti namni isin waliin jiraatuu keessaa namni dhibee garaachaa yookiin dhibee garaa-kaasaa nyaataan daddarban kanaan dhibame jiraachuu fi jiraachuu dhabuu isaa. Namni dhukkubsate yoo jiraate, mallattoo akkamii agarsiisee?, yeroo hangamiif dhukkubsatanii?, namni dhukkubsate kun yaalaaf

gara buufata fayyaa deemaniiru? Yoo deeman ta’e yaala argataniiru? Yaala argataniiru yoo ta’e yaala tolaa moo kaffaltiin?

- Gaaffii waa’ee qabiinsa manaa fakkeenyaaf bishaan eessa irraa argattu?, horii manaa kamuu yoo qabaattan?
- Waa’ee nageenya nyaataa fi dhukkuboota nyaata irraa nama qabanii beektuu?

**Kun unka waliigaltee hirmaannaa qorannoo ti; Unki** kun odeefannoo barbaachisaa qorannoo kanaaf tajaajilan of keessaa qaba. Kanaaf yeroo keessan fudhaatii odeefannoo kenname sirriitti ilaala. Qorannoo keenya irratti hirmaachuuf fedha qabachuu fi dhiisuu keessanis ta’e yeroo qorannoo irra jirtanittis ta’e yeroo kamitti iyyuu murteessuu keessaniin dura gaaffii kamuu gaafachuuf bililisa ta’aa gaafadhaa!.

**Hirmaannaan keessan guutummaatti fedhii ofii keessaniin kan ta’uudha.** Hirmaachuuf fedhii qabaattanis ta’e hin qabaanne filachuu dandeessu. Amma yoo waliigaltee keessan kennitanis, boodarra yaada keessan jijjiiruu ni dandeessu. Hirmaachuu dhiiftaniyyuu tajaajila buufata fayyaa kana irraa ni argattu. Kun kan hin jijjiiramneedha.

**Qorannoo kana irratti hirmaachuu keessaniin fayyadamaa ta’uus dhiisuus dandeessu**. Qorannoo kana irratti hirmachuu keessaniif faayidaan kallattiin isiniif dhufuu dhiisuu mala. Faayidaan battalaa hawasaaf ta’us jiraachuu dhiisuu danda’a, garuu daataan maddisiifamu dhaloota egereef bu’aa guddaa buusa.

Odeeffannoo haaraan yeroo qorannochaa uumamu kamiyyuu kan murtoo itti fufuu yookiin dhiisuu keessan irratti dhiibbaa uumuu danda’u yoo jiraate isiniif kennama. Yoo hirmaachuuf murteessitan, unka kana akka mallatteessitan isin gaafanna. Mallattoo keessan booda, copy unka kanaa ni argattu.

**Odeeffannoo Ijoo waa’ee Qorannoo Kanaa Irratti**

1. **Qorannichi maaliif geggeeffamaa jira?**

Qaamoleen fayyaa hawaasummaa fayyummaa nyaataa eegsisuuf hojjachaa jira. Haa ta’u malee, namoota Itoophiyaa keessaa hangamtu nyaata faalama qabuun akka dhukkubsatu hin beekamne. Kanaafuu Qorannichi odeeffannoo barbaachisoo ta’an walitti guuruun waggaa keessatti namoota Itoophiyaa keessa dhibee baakteeriyaa karaa nyaata faalameen dhufan tilmaamuuf kan qophaa’edha

2. **Namoonni meeqa qorannoo kana keessatti ni hirmaatu?**

Walii galatti namoonni 2,304 ta’an qorannoo kana keessatti hirmaatu.

3. **Qorannoo kana keessatti yoon hirmaadhe maaltu ta’a?**

Saampliin yookiin samuudi keessan baakteeriyoota nyaataan daddarban afur irratti qoratama. Akkasumas seenaa yaalaa keessan kan garaacha yookiin garaa-kaasaa kan ammaa ilaalchisee (mallattoo akkamii akka qabdan, yeroo hammamii akka dhukkubsattan, kunuunsa fayyaa akkamii akka argattan fi baasii kunuunsaa yookiin yaalaaf baastan) ilaaluu barbaanna. Gaaffii fi deebii gabaabaan kun yeroo keessan daqiiqaa 20-30 fudhachuu danda'a.

4. **Qorannoo keessaa bahuun yookiin adda kutuun ni danda’amaa?**

Yeroo barbaaddanitti qorannocha adda kutuu yookiin dhiisuu ni dandeessu. Hirmaachuu filattanis filachuu baattanis wanti jijjiiramu hin jiru. Murtoon keessan hariiroo gara fuula duraa hojjettoota qorannichaa, kilinika fayyaa, ykn dhaabbilee qorannoo kana keessatti hirmaatan kamiyyuu waliin qabdu irratti dhiibbaa hin geessisu.

5. **Qorannicha keessatti argamuun balaa, miidhaa cinaa ykn miira namatti hin tolle akkamii narratti uumuu danda’a?**

Saamuda sagaraa kennuudhaan rakkina xixiqqoo fi miira tasgabbii dhabuu isin mudachuu danda’a. Gareen qorannoo keenyaa miira namatti hin tolle kana xiqqeessuuf of eeggannoo barbaachisaa ta’e ni taasisa.

6. **Qorannaarraa kana irratti hirmaachuu kootiin faayidaa akkamiin irraa eeguu danda’a**?

Faayidaan kallattiin isiniif ta'u jiraachuu dhiisuu danda'a, garuu daataan maddisiifamu dhaloota dhufuuf faayidaa qabaachuu danda'a. Sagaraa keessan keessatti baakteeriyaa fayyaa keessan irratti dhiibbaa uumuu danda’u yoo arganne, odeeffannoo sana hakiima keessaniif qoodnee kutaa wal’aansaa barbaachisaa ta’e waliin isin wal qunnamsiisa.

7. **Qorannaa kanarratti yoon hin hirmaanne filannoowwan biroo akkamiin qaba?**

Hirmaachuu dhiisuu filachuu ni dandeessu. Yoo qorannoo kana irratti hirmaachuu dhiisuu filatte, kunuunsi yeroo dhaabbata fayyaa kana keessatti kennamu isiniif kennama. Kun kan hin jijjiiramneedha.

8. **Odeeffannoon koo qorannaa kana wajjin wal qabate iccitii ta'ee naaf ni eegamaa?**

Odeeffannoon pirojektii qorannoo kana irraa walitti qabnu iccitii ta'ee ni eegama. Adda baasttota dhuunfaa hin fayyadamnu. Sanadni odeeffannoo elektirooniksii kun bakka qorattoota qofaaf qaqqabummaan kennamutti jecha icciitii fi qulfii fi furtuunis ni eegama. Odeeffannoon qo'annaa keessan iccitii akka ta'u gochuuf carraaqqiin ni taasifama. Galmeen keessan gareewwan armaan gadiitiin ilaalamuu danda'a:

- Miseensota garee qorattootaa
- Bulchiinsa Nyaataa fi Qoricha Itiyoophiyaa (EFDA) dabalatee manneen hojii to'atan .
- Boordii Gamaaggama Dhaabbilee ykn Waajjira Hojii Qorannoo Itti Gaafatamummaa Koree Gamaaggama Naamusa Qorannoo Biyyaalessaa Itoophiyaa (NRERC); Dhaabbata Fayyaa Hawaasaa Itiyoophiyaa (EPHI), Yuunivarsiitii Gondar, Yuunivarsiitii Haramaya, Hospitaala Yekaatit 12, Yuunivarsiitii Ohaayo, Inistiitiyuutii Qorannoo Beeyladaa Idil-addunyaa
- Ispoonsara yoo jiraate ykn ejensiin qorannicha deeggaru.

Unka hayyamaa mallatteessuudhaan, qorattoonni odeeffannoo galmee yaalaa keessan keessa jiru qorannoo ammaa kanaaf akka argatan fi akka itti fayyadaman hayyamuuf walii galtee irra geessanittu.

9. **Odeeffannoon koo kan adda baafame qorannoo gara fuula duraatiif qoodamu yookiin immoo fayyadamuu ni danda'uu?**

Odeeffannoon qorannoon adda baafame hayyama dabalataa odeeffannoo qabu malee qorattoota biroo wajjin fayyadamuu ykn qoodamuu ni danda'a. Odeeffannoon kun iccitii ta'ee kuufamee waggoota dheeraaf kan itti fayyadamu ta'a.

**Saamuda sagaraa keessan qorannoo gara fuula duraatti fayyadamuuf erga qoratamee booda ol kaa'uun qabachuu dandeenyaa?**

_____Saamuda sagaraa koo qabattanii gosa qorannoo gara fuula duraa KAMIYYUUf itti fayyadamuu dandeessa.

_____ Qo'annoo gara fuula duraatiif saamuda sagaraa koo kaa'uu HIN dandeessan. Saamuda koo erga qoratamee booda haa gatamu.

**Qorannoowwan kanaan walqabatan itti aanan irratti hirmaachuuf fedhii akka qabdan gaafachuuf ammas isin qunnamuu dandeenyaa?**

_____ Gara fuulduraatti fedhii koo qorannoowwan kanaan walqabatan biroo irratti akkan hirmaadhuuf na qunnamuu dandeessu.

_____ Fuulduratti fedhii koo qorannoowwan kanaan walqabatan biroo irratti hirmaachuuf na gaafachuuf na qunnamuu HIN DANDEESSAN.

**Qorannoo kanaaf galmee yaalaa keessan argachuu dandeenyaa?**

_____ Galmee yaalaa koo argachuu dandeessu

_____ Galmee yaalaa koo argachuu hin dandeessan

**10. Qorannoo kana irratti hirmaachuun koo baasii maali na baassisa?**

Qo'annoo kana irratti hirmaachuuf baasiin isisiin baasstan hin jiru.

**11. Qo'annoo kana irratti hirmaachuu kootiif kaffaltiin naaf kaffalamaa?**

Qo'annoo kana irratti hirmaachuuf keessaniif kaffaltiin isisiif hin kaffalamu.

**12. Qo'annoo kana irratti hirmaadheef yoon miidhame maaltu ta'a?**

Yeroo qorannichaa miidhamuun keessan hin oolu. Yoo qorannichaan miidhamtan, maaloo hatattamaan hojjettoota qorannichaa beeksisaa kutaa kunuunsa barbaachisaa ta'e waliin wal isin quunnamsiisna.

**13. Qorannoo kana irratti yoon hirmaadhe mirgi koo maali?**

Qo'annoo kana irratti hirmaachuu filattanis ta'e filachuu baattanis, kunuunsi dhaabbata fayyaa kana keessatti argattan itti fufa. Unka kana mallatteessuudhaan mirga seeraa dhuunfaa qabaachuu dandeessu kamiyyuu hin kennitu.

Odeeffannoon haaraan, yoo jiraate, kan yeroo qorannichaa guddatu kan murtoo qorannicha itti fufuu fi dhiisuu kee irratti dhiibbaa uumuu danda'u isiniif kennama.

Boordii Gamaaggama Dhaabbilee Koree Gamaaggama Naamusa Qorannoo Biyyaalessaa Itoophiyaa (NRERC) keessatti qorannoo dhimmoota namootaa irratti itti gaafatamummaa qabu; Inistiitiyuutiin Fayyaa Hawaasaa Itiyoophiyaa, Yuunivarsiitii Gondar, Yuunivarsiitii Haramaya, Hospitaala Yekaatit 12, Yuunivarsiitiin Naannoo Ohaayo fi Dhaabbanni Qorannoo Beeyladaa Idil-addunyaa pirojektii qorannoo kana gamaaggamuun fudhatama kan qabu ta'uu isaa, akkaataa qajeelfama isaaniin, dambii naannoo fi federaalaa hojiirra jiruu fi imaammata Yuunivarsiitii mirgaa fi nageenya hirmaattota qorannichaa eeguuf qophaa'een fudhatama akka qabu argataniiru.

### 14. Gaaffiiwwan qorannoo ani dhiheessu ilaalchisee eenyutu deebii kennuu danda'a?

Gaaffii, waan yaaddoo isinitti ta'u, ykn komii waa'ee qorannichaa yoo qabaattan, ykn sababa qorannichaatiin miira namatti hin tolle yoo isinitti dhaga'ame, **Amete Mihret**, **amate2004@gmail.com** ykn **+251-913773888** qunnamuu dandeessu.

Gaaffii waa'ee mirga keessan akka hirmaataa qorannoo kanaatti yookiin nama qaama garee qorannoo hin taane waliin yaaddoowwan ykn komii qorannoon walqabatan biroo irratti mari'achuuf, qunnamuu dandeessu.

- Koree gamaaggama saayinsii fi naamusaa EPHI (SERO) (Bilbila: +251 118685503/15)

## Appendix VI. Parental Permission for Child's Participation in Research (English version)

**Study Title:** Molecular features, Epidemiology and Antimicrobial Resistance profile of Selected Foodborne Bacterial Pathogens Among Diarrheic Patients in Three Cities in Ethiopia

**Researchers:** Amete Mihret, Dr. Woldaregay Erku, Dr. Alem Abrha, Dr. Eyasu Tigabu

**Sponsor**: Bill and Melinda Gates Foundation, UK Department for International Development,

Addis Ababa University

Hello,

My name is_____. I am working on a public health project with

researchers at Addis Ababa University, The Ohio State University and Ethiopian Public Health Institute along with the International Livestock Research Institute, a government agency under the Ministry of Health. This study is working to estimate the burden of foodborne bacterial pathogens among diarrheal patients in Ethiopia. You have been selected to take part in this study is by chance.

If you take part in this study, your stool sample will be tested for 4 different types of bacteria. Additionally, you will be asked:

 Questions about yourself. For example, your age and education you have completed.

 Whether anyone living with you has gotten sick from food in the past month. Also, what symptoms he/she had, how long he/she was sick, if he/she went to a health facility, if he/she received any care and if he/she paid anything for care.

 Questions about the house. For example, where the house gets water from and if you have any animals.

 What you know about food safety and getting sick from food.

**This is a parental permission form for research participation.** It contains important information about this study. Please take your time and consider the information

provided carefully. Feel free to ask questions before deciding whether or not to permit your child to take part or at any point during the study.

**Your child's participation is entirely voluntary.** You or your child can choose whether or not to take part in this study. You or your child can change your mind later even if you agree now. Whether or not you permit your child to take part in this study, the care he/she receives at this health facility will continue. If you choose to not permit your child to take part in this study, your child will be offered care commonly offered at this health facility. This will not change.

**Your child may or may not benefit from taking part in this study.** There may not be any direct benefit for your child if he/she takes part in this study. There may not be any immediate benefit to the society, but the data generated may benefit future generations.

**You and your child will be provided with any new information that develops during the study that may affect your decision whether or not to continue.** If you permit your child to take part, you will be asked to sign this form. You will receive a copy of the form.

**Key Information About This Study**

**1. Why is this study being done?**

Public health groups are working to make food safer. However, it is not clearly understood how many Ethiopians get sick from eating contaminated food. This study will help gather information and determine how many people in Ethiopia get sick from foodborne bacterial pathogens each year.

**2. How many people will take part in this study?**

A total of 2,304 people will be enrolled in this study.

**3. What will happen if my child takes part in this study?**

If your child take part in this study, their stool sample will be tested for 4 different types of bacteria. Also, we will also like to ask a few questions about your child and his/her eating habits. We would also like to review medical history regarding the child's current diarrhea episode (what symptoms you have, how long you've been sick, what healthcare you've received and the costs of care). This short interview may take 20-30 minutes.

**4. Can my child stop be being in the study?**

You or your child can stop the study at any time. Nothing will change whether you permit your child to take part or not. Your decision will not affect your future relationship with study staff, the health clinic, or any of the organizations involved in the study

***5.*** **What risks, side effects or discomforts can my child expect from being in the study?**

It is possible your child may experience minor inconvenience and discomfort from giving a stool sample. Our study team will take the necessary precautions to minimize this discomfort.

**6. What benefits can my child expect from being in the study?**

There may not be any direct benefit for your child, but the data generated may benefit future generations. If we find bacteria in your child's stool that could impact his/her health, we will share that information with his/her physician and link your child with the appropriate treating unit.

**7. What other choices does my child have if they do not take part in the study?**

Your child may choose not to take part. If your child chooses not to take part in this study, he/she will be offered care commonly offered at this health facility. This will not change

**8. Will my child's study-related information be kept private?**

The information that we collect from this research project will be kept confidential. Personal identifiers will not be used. The electronic information document will also be protected with password and lock-and-key where access is given only to researchers. Efforts will be made to keep your child's study information confidential. However, there may be instances where this information must be released. For example, records may be reviewed by the following groups:

- Members of the investigators team
- Regulatory agencies including but not limited to the Ethiopian Food and Drug Administration (EFDA)

- The Institutional Review Board or Office of Responsible Research Practices at The Ethiopian National Research Ethics Review Committee (NRERC); Ethiopian Public Health Institute (EPHI), University of Gondar, Haramaya University, Yekatit 12 Hospital, The Ohio State University, the International Livestock Research Institute
- The sponsor, if any, or agency supporting the study.

By signing the consent form, you agree to allow the researchers to access and use the information your child is providing and his/her medical records for the current study.

**9. Will my child's de-identified information be used or shared for future research?**

De-identified study information may be used or shared with other researchers without additional permission. This information will be kept confidential and stored and used for many years.

**Can we keep your child's stool sample after it has been tested to use in future studies?**

- _____ You may keep my child's stool sample and use it for ANY type of future research study.
- _____ You may NOT keep my child's stool sample for a future study. My child's sample will be destroyed after it is tested.

**Can we contact you again to ask for your interest to participate in subsequent related studies?**

- _____ You may contact me in the future to ask for my interest to be involved in other related studies.
- _____ You may NOT contact me in the future to ask for my interest to be involved in other related studies.

**Can we access your child's medical record for this study?**

- _____ You can access my child's medical record
- _____ You can't access my child's medical record

**10. What are the costs of taking part in this study?**

There are no costs for your child to take part in this study.

**11. Will I or my child be paid for taking part in this study?**

You and your child will not be paid to take part in this study.

**12. What happens if my child is injured because they took part in this study?**

It is unlikely your child will be injured during the study. If your child is injured by the study, please inform the study staff immediately and you will be linked to the appropriate care giving unit.

**13. What are my child's rights if they take part in this study?**

Your child can change his/her mind later and stop the study even if he/she agrees now. You do not give up any personal legal rights you may have by signing this form. You and your child will be provided with new information, if any, that develops during the study that may affect your decision whether or not to continue the study.

An Institutional Review Board responsible for human subjects research at The Ethiopian National Research Ethics Review Committee (NRERC); Ethiopian Public Health Institute, University of Gondar, Haramaya University, Yekatit 12 Hospital, The Ohio State University and International Livestock Research Institute reviewed this research project and found it to be acceptable, according to their guidelines, applicable state and federal regulations and University policies designed to protect the rights and welfare of research participants.

**14. Who can answer my questions about the study?**

For questions, concerns, or complaints about the study, or if you feel your child has any discomfort as a result of study participation, you may contact Amete Mihret, at amete2004@gmail.com, and 0913773888.

For questions about your child's rights as a participant in this study or to discuss other study-related concerns or complaints with someone who part of the research team is not, you may contact the following.

- EPHI's Scientific and Ethics review Committee (SERO) (Dr. Getachew Addis, Tel address: 0912100756)

**Signing the permission form**

I have read (or someone has read to me) this form. I am aware that I am being asked to provide permission for my child to participate in a research study. I have had the

opportunity to ask questions and have had them answered to my satisfaction. I voluntarily agree to permit my child to participate in this study.

I am not giving up any legal rights by signing this form. I will be given a copy of this form**.**

**Printed name of participant**

**Printed name of parent/guardian** **Signature/Fingerprint of parent/guardian**

**AM/PM**

**Relationship to the participant** **Date and time**

**<u>Investigator/Research Staff</u>**

I have explained the research to the participant or his/her representative before requesting the signature(s) above. There are no blanks in this document. A copy of this form has been given to the participant or his/her representative.

**Printed name of person obtaining permission** **Signature of person obtaining permission**

**AM/PM**

**Date and time**

## Appendix VII. Parental Permission for Child's Participation in Research (Amharic version)

**በጥናቱ ውስጥ ለመሳተፍ ስምምነት**

**የጥናቱ ርዕስ:** የተቅማጥ በሽታ የሚያመጡ ከምግብ ጋር ተዛማጅነት ያላቸው የተመረጡ ባክቴሪያዎችን ስርጭትና ለፀረ-ባክቴሪያ መድሃኒቶች መላምድ ሁኔታ ለማውቅ የተቅማጥ በሽታ ካለባቸው ታማሚዎች በተመረጡ ሶስት ከተሞች በኢትዮጵያ ውስጥ የሚደረግ ጥናት::

**ተመራማሪዎች ፡ አመተ ምህረት፣** ዶ/ር ወልዳረጋይ እርቁ፣ ዶ/ር አለም አብርሃ፣ ዶ/ር ኢያሱ ጥጋቡ

**ድጋፍ ሰጪ፡ የኢትዮጵያ ህብረተሰብ ጤና ኢንስቲትዩት ፣** ቢል እና መሊንዳ ጌትስ ፋዉነዳሽን ፣ የእነግሊዝ አለም አቀፍ ልማት መምሪያ።

ስሜ_____ይባላል። የምሰራው በህበረተሰብ ጤና ምርምር ከአዲስ አበባ ዩኒቨርስቲ፣ ከአሃዮ ስቴትስ ዩኒቨርስቲ፣ ኢንተርናሽናል ላይቭስቶክ ምርምር ተቋም ጋር በመተባበር ነው። ይህ ምርምርም የተቅማጥ በሽታ የሚያመጡ ከምግብ ጋር ተዛማጅነት ያላቸው የተመረጡ ባክቴሪያዎችን ስርጭትና ለፀረ-ባክቴሪያ መድሃኒቶች መላመድ ሁኔታ ለማውቅ የተቅማጥ በሽታ ካለባቸው ታማሚዎች በተመረጡ ሶስት ከተሞች በኢትዮጵያ ውስጥ የሚደረግ ጥናት ነው። ለዚህ ጥናት የተመረጥከው/ሽው በኣጋጣሚ ነው።

**በምርምሩ ለመሳትፍ የስምምነት ፎርም፡**

ይህ ሰነድ ስለምርምሩ ጠቃሚ መረጃዎቺን ያካትታል፣ እባክዎት ጊዜዎትን ወስደዉ በጥንቃቄ ያንብቡት:: በምርምራችን ተሳታፊ ለመሆን ከመዎሰንዎ በፊት ጥያቄ ለመጠየቅ ነጻ ይሁኑ::

**የእርስዎ ተሳትፎ ሙሉ በሙሉ በፈቃደኝነትዎ ላይ ተመስረተ ነዉ**

በዚህ ምርምር መሳተፍ ወይም አለመሳተፍ የእርስዎ ምርጫ ነው:: አብረውን ለመስራት ከወሰኑ በላም ሀሳብዎን መቀየር ይችላሉ:: በዚህ ምርምር ለመሳተፍ ቢስማሙ ወይም ባይስማሙም በዚህ ጤና ተቋም የሚያገኙት አገልግሎት ላይ ምንም አይነት ተፅእኖ አይፈጥርም::የእርስዎ ዉሳኔ ከተመራማሪዎች ጋር የሚኖርዎትን የወደፊት ግንኙነት ላይ ተፅእኖ አይኖረዉም::

**በዚህ ምርምር ተጠቃሚ ሊሆኑም ላይሆኑም ይችላሉ**

ከዚህ ምርምር በቀጥታ ተጠቃሚ ላይሆኑ ይችላሉ ፣ ማህበረሰቡም ቢሆን በፍጥነት ተጠቃሚ ላይሆን ይችላሉ ነገር ግን ከዚህ ምርምር የሚገኘዉ መረጃ ለወደፊቱ ትዉልድ ይጠቅማል ብለን እናስባለን::

**በምርምሩ ላይ ሃሳብዎን ሊያስቀይሩ የሚችሉ አዳዲስ መረጃዎች ይደርስዎታል፤**

በዚህ ተስማምተዉ የምርምሩ አካል ለመሆን ከወሰኑ እዚህ ፎርም ላይ እንዲፈርሙ ይጠየቃሉ::የፎርሙ ቅጂም ይደርስዎታል::

**1. ጥናቱ ለምን አስፈለገ?**

የህብረተሰብ ጤና ቡድኖች የምግብን ጤናማነት እየሰሩ የሚገኙ ቢሆንም በኢትዮጵያ በምግብ መበከል ምክንያት የሚታመሙ ሰዎች ቁጥር በግልፅ አይታዎቅም።ይህ ጥናት መረጃዎችን ከማሰባሰብ በዘለለ ት ምን ያህል ኢትዮጵያዉያን በምግብ ብክለት ምክንያት እንደሚታመሙ ለማዎቅ ይረዳናል።

**2. ምን ያህል ሰዎች በዚህ ጥናት ላይ ይሳተፋሉ?**

በአጠቃላይ 2304 ሰዎች በዚህ ጥናት ይሳተፋሉ።

**3. በዚህ ጥናት ተሳታፊ ከሆንኩ ምን ይፈጠራል?**

በዚህ ጥናት ተሳታፊ ከሆኑ ለላቦራቶሪ ምርመራ የሰጡት የሰገራ ናሙና ለሶስት የተለያዩ ተህዋስያን ምርመራ ይደረግለታል። በተጨማሪም ስለ ጤና ተቋሙ እነዚህን እና መሰል ጥያቄዎች ይጠየቃሉ፤

- የ ጤና ባለሙያዎች ቁጥር
- የ ላቦራቶሪ እና የ ህክምና ክትትል ልምድ
- ለ ተቅማጥ ነክ በሽታዎች ተቅማጥ ነክ በሽታ ታማሚዎችን ለማከም ዎጭዉ ምን ያክል ነዉ
- የተቅማጥ ነክ በሽታዎች እና የምግብ ደህንነት ላይ ያለዎት ጠቅላላ እዉቀት

**4. በጥናቱ ለመሳተፍ ምን ያህል ጊዜ ይዎሰዳል**?

ይህ መጠይቅ ከ 10 ደቂቃ ያልበለጠ ይዎስዳል.

**5. ጥናቱን ማቋረጥ እችላለሁ?**

በማንኛዉም ሰዓት ጥናቱን ማቆም ይችላሉ ፤ሃሳብዎን ስለቀየሩ ምንም የሚፈተር ነገር የለም ፤ከተመራማሪዎች ጋር የሚኖርዎት ግንኙነት ላይ ተፅእኖ አይኖረዉም።

**6. የዚህ ጥናት ተሳታፊ በመሆኔ ምን አይነት አደጋዎችን ፤ የጎንዮሽ ጉዳቶችን፤ምቾት የሚነሱ ነገሮችን ልጠብቅ?**

የ ጥናቱ ተሳታፊዎች መልስ ከጥናቱ ዉጭ ያሉ ሰዎች ሊያገኙት ይችላሉ ።የዚህን ተፅእኖ ለመቀነስ ሲባል የጥናቱ መረጃ እንደተሰበሰበ ማንነተዎ ከጥናቱ ላይ ይዎጣል።

**7. ምን አይነት ጥቅሞችን ከዚህ ጥናት ልጂ ሊያገኝ ይችላል?**

በቀጥታ ከጥናቱ ተጠቃሚ ላይሆኑ ይችላሉ ፤ ነገርግን የሚሰበሰበዉ መረጃ ለመጪዉ ትዉልድ ጠቃሚ ይሆናል።

**8.በዚህ ጥናት ተሳታፊ ባለሆን ሌሎች ምን ምንርጫዎች ይኖሩኛል?**

በዚህ ጥናት ላለመሳተፍ መምረጥ ይችላሉ ፤ በዚህም ምንም አይነት ቅጣትም ይሁን ጥቅምዎ አይነካም።

**9. በዚህ ጥናት ላይ ያሉ ግላዊ መረጃዎች ምንያህል የተጠበቀ ነዉ?**

ከዚህ ጥናት የሚሰበሰቡት መረጃዎች በጥንቃቄ ይያዛሉ፤ ግላዊ መለያዎችን አይጠቀምም፤የዲጅታል መረጃዎች በይለፍ ቃል ይቆለፋል ፤ መረጃዎች የሚቀመጡበት ቦታ የሚቆለፍ ሲሆን ቁልፉም ለተመራማሪዎች ብቻ

ይሰጣል፡፡ የጥናቱ ተሳታፊዎችን መረጃ ለመጠበቅ በተቻለን አቅም እንጥራለን፡፡ የተሳታፊዉ መረጃ በሚከተሉት አካላት ሊታዩ ይችላሉ

- የምርምር ቡድኑ አባላት
- በተቆጣጣሪ ኤጀንሲዎች ፤ እንደ ኢትዮጵያ ምግብ እና መድሀኒት አስተዳደር እና ልሎችም
- የተgሙ ግምገማ ቦርድ ፣በ ኢትዮጵያ ብሄራዊ የምርምር ግምገማ ኮሚቴ፣የኢትዮጵያ ማህበረሰብ ጤና ኢንስቲትዩት፣ንንደር ዩኒsTWüy ፣ሃረማያ ዩኒቨረሲቲ፣የካቲት 12 ሆስፒታል፣ አሃዮ ስቴት ዩኒቨረሲቲ፣አለም አቀፍ የእንስሳት ምርምር ኢንስቲትዩት
- ጥናቱን የሚደግፉ አካላት

**10. በዚህ ጥናት የተሰበሰበዉ መረጃ በሌሎች ጥናቶች ጥቅም ላይ ይዉላል?**

በዚህ ጥናት የተሰበሰበዉ ማንነት የማይገልፅ መረጃ ለሌሎች ምርመሮች ጥቅም ላይ ካለ ተጨማሪ ስምምነት ሊዉል ይችላል ፤ይህም መረጃ ለአመታት በጥንቃቄ በማስቀመጥ ጥቅም ላይ ይዉላል፡፡

**የሰገራ ናሙናዎትን ለዚህ ምርመራ ከተጠቀምንበት በኋላ ለወደፊት ተጨማሪ ጥናት እንዲቀመጥ ፈቃደኛ ነዎት?**

_____የልጄን የሰገራ ናሙናን ማስቀመጥና ወደፊት ለሌላ ለማንኛውንም አይነት ጥናት መጠቀም ትችላላችሁ

_____የልጄን የሰገራ ናሙናን ማስቀመጥና ለሌላ ጥናት መጠቀም አትችሉም፡፡ ናሙናው መወገድ አለበት

**ወደፊት በተመሳሳይ ጥናቶች ላይ በመካፈል ፍላጎትዎን ለመጠየቅ እንደገና እንነጋገርዎ እንችላለን?**

_____ ወደፊት በተመሳሳይ ጥናቶች ላይ የልጄን የመካፈል ፍላጎት እንደሌኝ ለመጠየቅ ማነጋገር ይችላሉ።

_____ ወደፊት በተመሳሳይ ጥናቶች ላይ የልጄን የመካፈል ፍላጎት እንደሌኝ ለመጠየቅ መነጋገር አትችሉም።

**ለዚህ ጥናት የጤና መዝገብዎን ማግኘት እንችላለን?**

_____ የልጄን የጤና መዝገቤን ማግኘት ትችላላችሁ።

_____ የልጄን የጤና መዝገቤን ማግኘት አትችሉም።

**11. በዚህ ጥናት ለመሳተፍ ምን ያህል ወጪ ያስፈልጋል?**

በዘህ ጥናት ለመሳተፍ ምንም አይነት ወጪ አያሰፈለግም

**12. በዚህ ጥናት ለሚሳተፍ ክፍያ ይኖረዋል?**

በዚህ ጥናት ለሚሳተፉ ምንም አይነት ክፍያ አይኖረዉም

**13.በዚህ ጥናት ተሳታፊ ስለሆነኩ ጉዳት ቢደርስብኝስ?**

በዚህ ጥናት ላይ ሆነዉ ጉዳት ይደረስብዎታል ብለን አናስብም ሆኖም ግን በጥናቱ ላይ ሆነዉ ጉዳት ከደረሰብዎ ለሚመለከታቸዉ የጥናቱ አባላት በመንገር ተገቢዉ ህክምና እነዲሰጥዎት ይደረጋል፡፡

**14. በዚህ ጥናት ተሳተፊ ከሆነኩ መብቶቼ ምን ምን ናቼዉ?**

በጥናቱ ለመሳተፍ ከዎሰኑ በኃላ ሃሳብዎን መቀየር ይችላሉ፤በዚህ ጥናት ለመሳተፍ በመወሰነዎ ማንኛዉም መብትዎ አይነካም፤በምርምሩ ላይ ሃሳብዎን ሊያስቀይሩ የሚችሉ አዳዲስ መረጃዎች ይደርስዎታል፤

በ ኢትዮጵያ ብሄራዊ የምርምር ስራዎች ግምገማ ኮሚቴ ዉሰጥ ለምርምሩ አባላት ተጠሪ የሆነዉ የተgሙ የግምገማ ቦርድ ፣የኢትዮጵያ ማህበረሰብ ጤና ኢንስቲትዩት ፣ጎንደር ዩኒቨርስቲ ፣ሃረማያ ዩኒቨርሲቲ፣የካቲት 12 ሆስፒታል፣ አሃዮ ስቴት ዩኒቨረሲቲ እና ዓለም ዓቀፍ የእንስሳት ምርምር ተgም ይህን ምርምር በተgሞቹ መመሪያ ፣ለሚመለከታቸዉ ግዛት እና ፌደራል ህጎች እንዲሁም የ ምርመር ተሳታፊዎች መብት እና ደህንነት ለመጠበቅ በዩኒቨርሲቲዎች የወጡ ደንቦች መሠረት ገምግመዉ ተቀባይነቱን አረጋግጠዋል፡፡

**15.ጥናቱን የሚመለከቱ ጥያቄዎችን መልስ መስጠት የሚችል ማነው?**

በጥናቱ ላይ ለሚነሱ ጥያቄዎች፣ስጋቶች፣አቤቱታዎች ወይም በጥናቱ ምክንያት ማንኛዉም አይነት ቅሬታዎች ካላችሁ ፤

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በጥናቱ በመሳተፍዎ ስለሚያገኙት መብት ፣ስለሌሎች ጥናቶችም ለመወያየት እነዲሁም የጥናቱ አካል ባለሆኑ ሰዎች ለሚነሱ ቅሬታዎች ፤

- የኢ.ህ.ጤ.ኢ ሳይንሳዊ እና የስነ-ምግባር ግምገማ ኮሚቴ (Tel: +251118685503/15)

## Appendix VIII. Parental Permission for Child's Participation in Research (Afan Oromo version)

**Mata Duree Qorannoo:** Qorannoo baakteriyaa qoricha damdamatan, kan karaa nyaataa daddarban dhukkubsattoota dhibee garaa-kaasaa wal qabatee irratti magaalotaa Ethiopia sadii keessatti geggeeffamu

**Qorattoota:** Amete Mihret, Dr. Woldaregay Erku, Dr. Alem Abrha, Dr. Eyasu Tigabu

**Deeggertoota yookiin Ispoonsara qorannichaa:** Instiitiyuutii Fayyaa Hawaasummaa Itoophiyaa (EPHI), Bill fi Melinda Gates Foundation, Waajjira Hojii Misooma Idil-Addunyaa UK, Yuunivarsiitii Finfinnee

Akkam jirtu?

Maqaan koo _____ jedhama. Ani projektii fayyaa hawaasummaa keessatti qorattoota waliin Yunivarsiitii Finfinnee irraa, akkasumas Ohio State University, fi Instiitiyuutii Fayyaa Hawaasummaa Itoophiyaa keessatti kan argamu; dabalataanis Dhaabbata Qorannoo beeyiladaa Addunyaa (ILRI) kan eejensii mootummaa ta'e fi Ministira Fayyaa jala jiru waliin hojjachaan jira. Qorannichi baay'ina dhukkuboota bakteeriyaa nyaataan daddarban kan sababa dhukkuba garaachaa (dhibee garaa-kaasaa) ta'an Itoophiyaa keessatti hangam akka ta'e tilmaamuuf hojjetama. Kanaafuu daa'imni keessan qorannoo kana keessatti akka hirmaataniif kan filataman akka carraa ta'eeti yookaan akka tasaati.

Yoo daa'imni keessan qorannoo kana irratti hirmaate, saamuda sagaraa daa'ima keessanii gosoota baakteeriyaa nyaataan daddarban adda addaa 4 afuriif ni qoratama. Dabalataanis, akka armaan gadiitti isin gaafatama:

- Gaaffii waa'ee mucaa keessanii, Fakkeenyaaf, umrii fi haala barnoota daa'ima keessanii.
- Ji'a darbe keessa namni daa'ima keessan waliin jiraatu nyaata irraa kan ka'e dhukkubsatee jiraachuu fi dhiisuu isaa. Akkasumas, mallattoolee akkamii akka argisiise, yeroo hammamiif dhukkubsatee, dhaabbata fayyaa yoo deeme, kunuunsa kamiyyuu yoo argate fi kunuunsaaf waan kaffaltan yoo jiraate.

- Gaaffii waa'ee qabiinsa manaa fakkeenyaaf bishaan eessa irraa argattu?, horii manaa kamuu yoo qabaattan?
- Waa'ee nageenya nyaataa fi dhukkuboota nyaata irraa nama qabanii beektuu?

**Kun unka hayyama maatii hirmaannaa qorannoo daa'imaatiif;** Waa'een qorannoo kanaa odeeffannoo barbaachisaa ta'e of keessaa qaba. Mee yeroo keessan fudhadhaatii odeeffannoo kenname sirriitti ilaalaa. Mucaan keessan akka hirmaatu hayyamuu fi dhiisuu keessan murteessuu keessan dura ykn yeroo kamiyyuu yeroo qorannichaa gaaffii gaafachuuf bilisa ta'aa.

**Hirmaannaan daa'ima keessanii guutummaatti fedhii keessaniin kan raawwatamudha**. Isin ykn mucaan keessan qorannoo kana irratti hirmaachuu fi dhiisuu filachuu dandeessu. Isin ykn mucaan keessan amma yoo itti walii galtan illee booda yaada keessan jijjiiruu dandeessu. Mucaan keessan qorannoo kana irratti akka hirmaatu hayyamtanis hayyamuu baattanis, kunuunsi inni dhaabbata fayyaa kana keessatti argatu itti fufa. Yoo daa'imni keessan qorannoo kana irratti akka hirmaatu hayyamuu dhiisuu filattan, daa'imni keessan kunuunsi dhaabbata fayyaa kana keessatti yeroo baay'ee kennamu ni kennamaaf. Kun hin jijjiiramu.

**Mucaan keessan qorannoo kanarratti hirmaachuun faayidaa qabaachuus ta'e fayyadamuu dhiisuu danda'a**. Mucaan keessan qorannoo kana irratti yoo hirmaate faayidaan kallattiin jiraachuu dhiisuu danda'a. Faayidaan battalaa hawaasaaf ta'us jiraachuu dhiisuu danda'a, garuu daataan maddisiifamu dhaloota egeree fayyadamoo taassisuu danda'a.

**Isiniif fi mucaan keessan odeeffannoo haaraa yeroo qorannichaa uumamu kamiyyuu kan murtoo itti fufuu fi dhiisuu keessan irratti dhiibbaa uumuu danda'u isiniif in kennamaaf**. Yoo daa'imni keessan akka hirmaatu hayyamtan, unka kana akka mallatteessitan isin gaafatama. Koppii unkaa kanaas isiniif in kennama.

**Odeeffannoo Ijoo waa'ee Qorannoo Kanaa Irratti**

1. **Qorannichi maaliif geggeeffamaa jira?**

Qaamoleen fayyaa hawaasummaa fayyummaa nyaataa eegsisuuf hojjachaa jira. Haa ta'u malee, namoota Itoophiyaa keessaa hangamtu nyaata faalama qabuun akka dhukkubsatu hin beekamne. Kanaafuu Qorannichi odeeffannoo barbaachisoo ta'an

walitti guuruun waggaa keessatti namoota Itoophiyaa keessa dhibee baakteeriyaa karaa nyaata faalameen dhufan fi tilmaamuuf kan qophaa'edha

2. **Namoonni meeqa qorannoo kana keessatti ni hirmaatu?**

Walii galatti namoonni 2,304 ta'an qorannoo kana keessatti hirmaatu.

3. Mucaan koo qo'annaa kana irratti yoo hirmaate maaltu ta'a?

Yoo daa'imni keessan qorannoo kana irratti hirmaate, saamuda sagaraa isaanii gosa baakteeriyaa adda addaa 4 afuriif ni qoratama. Akkasumas, waa'ee daa'ima keessanii fi aadefannaa nyaataa isaa/ishee gaaffii muraasa gaafachuus ni barbaanna. Akkasumas seenaa yaalaa yeroo garaacha ykn garaa-kaasaa daa'imaa ammaa ilaalchisee (mallattoo akkamii akka qabdu, yeroo hammamiif akka dhukkubsatte, kunuunsa fayyaa akkamii akka argatte fi baasii yaalaaf baastan) ilaaluu barbaanna. Gaaffii fi deebii gabaabaan kun daqiiqaa 20-30 fudhachuu danda'a.

4. **Mucaan koo qorannaa keessa jiraachuu dhiisuu yookiin adda kutuu ni danda'aa?**

Isinis ykn mucaan keessan yeroo barbaaddanitti qorannoo dhaabuu dandeessu. Mucaan keessan akka qooda fudhatu hayyamtanis hayyamuu baattanis wanti jijjiiramu hin jiru. Murtoon keessan hariiroo gara fuula duraa hojjettoota qorannichaa, kilinika fayyaa, ykn dhaabbilee qorannoo kana keessatti hirmaatan kamiyyuu waliin qabdu irratti dhiibbaa hin geessisu.

5. **Mucaan koo qorannoo kana keessatti hirmaachuun balaa, miidhaa cinaa qorannoon wal qabate ykn miira namatti hin tolle akkamiin eeguu danda'a?**

Mucaan keessan saamuda sagaraa kennuu isaatiin rakkina xixiqqoo fi miira namatti hin tolle mudachuu danda'a. Gareen qorannoo keenyaa miira namatti hin tolle kana xiqqeessuuf of eeggannoo barbaachisaa ta'e ni taasisa.

6. **Mucaan koo qorannaarratti argamuun faayidaa akkamii irraa eeguu danda'a?**

Mucaa keessaniif faayidaan kallattiin jiraachuu dhiisuu danda'a, garuu daataa maddisiifamu dhaloota dhufuuf faayidaa qabaachuu danda'a. Yoo baakteeriyaa sagaraa

daa'ima keessanii keessatti aragame fayyaa isaa irratti dhiibbaa uumuu danda'u arganne, odeeffannoo sana hakiima isaaf qoodnee daa'ima keessan kutaa wal'aansaa barbaachisaa ta'e waliin ni walqabsiifna.

7. **Mucaan koo qorannaa irratti yoo hin hirmaanne filannoowwan biroo akkamii qaba?**

Mucaan keessan hirmaachisuu dhiisuu filachuu dandeessu. Mucaan keessan qorannoo kana irratti akka hin hirmaanne yoo filattan, kunuunsi dhaabbata fayyaa kana keessatti yeroo baay'ee kennamu ni kennamaaf. Kun hin jijjiiramu

8. **Odeeffannoon mucaa kootii qo'annoo wajjin walqabatu iccitiin ni qabamaa?**

Odeeffannoon pirojektii qorannoo kana irraa walitti qabnu iccitii ta'ee ni eegama. Adda baastonni dhuunfaa hin fayyadamu. Sanadni odeeffannoo elektirooniksii kun bakka qorattoota qofaaf qaqqabummaan kennamutti jecha icciitii fi qulfii fi furtuunis ni eegama. Odeeffannoon qorannoo daa'ima keessanii iccitii akka ta'uuf carraaqqiin ni taasifama. Haa ta'u malee, yeroon itti odeeffannoon kun gadhiifamuu qabu jiraachuu danda'u. Fakkeenyaaf, galmeewwan gareewwan armaan gadiitiin ilaalamuu danda'u:

- Miseensota garee qorattootaa
- Bulchiinsa Nyaataa fi Qoricha Itiyoophiyaa (EFDA) dabalatee manneen hojii to'atan .
- Boordii Gamaaggama Dhaabbilee ykn Waajjira Hojii Qorannoo Itti Gaafatamummaa Koree Gamaaggama Naamusa Qorannoo Biyyaalessaa Itoophiyaa (NRERC); Dhaabbata Fayyaa Hawaasaa Itiyoophiyaa (EPHI), Yuunivarsiitii Gondar, Yuunivarsiitii Haramaya, Hospitaala Yekaatit 12, Yuunivarsiitii Ohaayo, Inistiitiyuutii Qorannoo Beeyladaa Idil-addunyaa
- Ispoonsara yoo jiraate ykn ejensiin qorannicha deeggaru.

Unka hayyamaa mallatteessuudhaan, qorattoonni odeeffannoo daa'imni keessan kennuu fi galmee yaalaa isaa qorannoo ammaa kanaaf akka argatan fi akka fayyadaman hayyamuuf walii galtee keessan mirkaneessu qabdu.

9. **Odeeffannoon daa'ima kootii kan adda baafame qorannoo gara fuula duraatiif qoodamu yookiin immoo fayyaduu danda'aa?**

Odeeffannoon qo'annoo adda baafame hayyama dabalataa malee qorattoota birootiin fayyadamuu ykn qoodamuu ni danda'a. Odeeffannoon kun iccitii ta'ee eegamee waggoota dheeraaf kan kuufamee fi itti fayyadamu ta'a.

**Saamuda sagaraa daa'ima keessanii qorannoo gara fuula duraatti fayyadamuuf erga qoratamee booda qabachuu dandeenyaa?**

_____ Saamuda sagaraa daa'ima kootii qabattee gosa qorannoo qorannoo gara fuula duraa KAMIYYUU itti fayyadamuu dandeessa.

_____ Qorannoo gara fuula duraatiif saamuda sagaraa daa'ima kootii kaa'uu HIN dandeessu. Saamuda mucaa koo erga qoratamee booda akka gatamun fedha.

**Qorannoowwan walqabatan itti aanan irratti hirmaachuuf fedhii akka qabdan gaafachuuf ammas isin qunnamuu dandeenyaa?**

 _____ Gara fuulduraatti fedhii koo qorannoowwan kanaan walqabatan biroo irratti akkan hirmaadhuuf na qunnamuu dandeessu.

 _____ Gara fuulduraatti fedhii koo qorannoowwan kanaan walqabatan biroo irratti hirmaachuuf na gaafachuuf na qunnamuu HIN DANDEESSAN.

**Qorannoo kanaaf galmee yaalaa daa'ima keessanii argachuu dandeenyaa?**

 _____ Galmee yaalaa mucaa koo argachuu dandeessu

 _____ Galmee yaalaa mucaa koo argachuu hin dandeessan.

**10. Qorannoo kana irratti hirmaachuun koo baasii maali na baassisa?**

Mucaan keessan qorannoo kana irratti hirmaachuuf baasiin baasu tokkollee hin jiru.

**11. Ani ykn mucaan koo qorannaa kana irratti hirmaachuu keenyaaf kaffaltiin ni kaffalama?**

Isiniif fi mucaan keessan qorannoo kana irratti hirmaachuu keessaniif kaffaltiin isiniif hin kaffalamu.

**12. Mucaan koo qorannaa kana irratti hirmaachuu isaatiin yoo miidhame maaltu ta'a?**

Yeroo qorannichaa mucaan keessan miidhamuun isaa hin oolu. Yoo daa'imni keessan qorannichaan miidhame, maaloo hatattamaan hojjettoota qo'annichaa beeksisaa, kutaa kunuunsa barbaachisaa ta'e waliin ni wal quunnamsiisna.

**13. Mucaan koo qorannoo kana irratti yoo hirmaate mirgi isaa maali?**

Mucaan keessan yeroo booda yaada isaa jijjiiree qorannoo dhaabuu danda'a yoo amma walii galeellee. Unka kana mallatteessuudhaan mirga seeraa dhuunfaa qabaachuu dandeessu kamiyyuu hin kennitu. Isiniif fi mucaan keessan odeeffannoo haaraa, yoo jiraate, kan yeroo qorannichaa uumamu kan murtoo qorannicha itti fufuu fi dhiisuu keessan irratti dhiibbaa uumuu danda'u ni kennamaaf.

Boordii Gamaaggama Dhaabbilee Koree Gamaaggama Naamusa Qorannoo Biyyaalessaa Itoophiyaa (NRERC) keessatti qorannoo dhimmoota namootaa irratti itti gaafatamummaa qabu; Inistiitiyuutiin Fayyaa Hawaasaa Itiyoophiyaa, Yuunivarsiitii Gondar, Yuunivarsiitii Haramaya, Hospitaala Yekaatit 12, Yuunivarsiitiin Naannoo Ohaayo fi Dhaabbanni Qorannoo Beeyladaa Idil-addunyaa pirojektii qorannoo kana gamaaggamuun fudhatama kan qabu ta'uu isaa, akkaataa qajeelfama isaaniin, dambii naannoo fi federaalaa hojiirra jiruu fi imaammata Yuunivarsiitii mirgaa fi nageenya hirmaattota qorannichaa eeguuf qophaa'een fudhatama akka qabu argataniiru.

**14. Gaaffilee qorannoo ilaalchisee ani dhiheessuuf eenyutu deebii kennuu danda'a?**

Gaaffii, yaadaa, ykn komii waa'ee qorannichaa yoo qabaattan, ykn sababa hirmaannaa qorannichaatiin daa'imni keessan miira tasgabbii dhabuu akka qabu yoo isinitti dhaga'ame, Amete Mihret, amete2004@gmail.com, fi 0913773888 qunnamuu dandeessu.

Gaaffii waa'ee mirga daa'ima keessanii akka hirmaataa qorannoo kanaatti yookiin yaaddoowwan qorannoon walqabatan biroo yookiin komii nama garee qorannoo keessaa tokko hin taane waliin mari'achuuf, kanneen armaan gadii qunnamuu dandeessu.

- Koree gamaaggama saayinsii fi naamusaa EPHI (SERO) (Dr. Geetachoo Addis, Teessoo bilbilaa: 0912100756)

**Unka hayyamaa mallatteessuu**

Unka kana dubbiseera (ykn namni tokko naaf dubbiseera). Mucaan koo qorannoo irratti akka hirmaatu hayyama akkan kennu na gaafatamuu isaa nan beeka. Gaaffii gaafachuuf carraa argadheera, deebiis akka na gammachiisutti argadheera. Mucaan koo qorannoo kana irratti akka hirmaatu akkan hayyamuuf fedhii kootiin waliin gala.

Unka kana mallatteessuun mirga seera qabeessa kamiyyuu kan na dhorku hin jiru. Koppii unka kanaa naaf kennama.

**Maqaa hirmaataa maxxanfame**

| **Maqaa warraa/guddistuu maxxanfame** | **Mallattoo/Ashaaraa qubaa warraa/guddistuu** |
|---|---|
| | **AM/PM** |
| **Hariiroo hirmaataa wajjin qabu** | **Guyyaa fi sa'aati** |

**Qorataa/Hojjetoota Qorannoo**

Mallattoowwan armaan olii gaafachuun koo dura qorannoo hirmaataa ykn bakka bu’aa isaaf ibseen jira. Barreeffama kana keessatti bakki duwwaa hin jiru. Koppiin unka kanaa hirmaataaf ykn bakka bu’aa isaaf kennameera.

| **Maqaa nama hayyama aragatee maxxanfame** | **Mallattoo nama hayyama argatee** |
|---|---|
| | **AM/PM** |
| | **Guyyaa fi sa'aatii** |

## Appendix IX. Assent to Participate in Research (Age 12-17 years) (English version)

**Study Title:** Molecular features, Epidemiology and Antimicrobial Resistance profile of Selected Foodborne Bacterial Pathogens Among Diarrheal Patients in Three Cities in Ethiopia

**Researchers:** Amete Mihret Teshale, Dr. Woldaregay Erku, Dr. Alem Abrha, Dr. Eyasu Tigabu

**Sponsor**: Bill and Melinda Gates Foundation, UK Department for International Development,

Addis Ababa University

Hello,

My name is_____. I am working on a public health project with

researchers at Addis Ababa University, The Ohio State University and Ethiopian Public Health Institute along with the International Livestock Research Institute, a government agency under the Ministry of Health. This study is working to estimate the burden of diarrheal disease caused by selected bacterial pathogens in under fifteen children in Ethiopia. You have been selected to take part in this study is by chance.

If you take part in this study, your stool sample will be tested for 4 different types of bacteria. Additionally, you will be asked:

 Questions about yourself. For example, your age and education you have completed.

 Whether anyone living with you has gotten sick from food in the past month. Also, what symptoms he/she had, how long he/she was sick, if he/she went to a health facility, if he/she received any care and if he/she paid anything for care.

 Questions about the house. For example, where the house gets water from and if you have any animals.

 What you know about food safety and getting sick from food.

**This is a consent form for research participation.** It contains important information about this study. Please take your time and consider the information provided carefully.

Feel free to ask questions before deciding whether you would like to take part in our study or not or at any point during the study.

**Your participation is entirely voluntary.** You can choose whether or not to take part in this study. You can change your mind later even if you agree now. Whether or not you choose to take part in this study, the care you receive at this health facility will continue. If you choose not to take part in this study, you will be offered care commonly offered at this health facility. This will not change.

**You may or may not benefit from taking part in this study.** There may not be any direct benefit for you if you take part in this study. There may not be any immediate benefit to the society, but the data generated may benefit future generations.

**You will be provided with any new information that develops during the study that may affect your decision whether or not to continue.** If you decide to take part, you will be asked to sign this form. You will receive a copy of the form.

**Key Information About This Study**

**1.What is this study about?**

Public health groups are working to make food safer. However, it is unclear how many Ethiopians get sick from eating contaminated food. This study will help determine how many people in Ethiopia get sick from foodborne bacterial pathogens each year.

**2.What will I need to do if I am in this study?**

If you take part in this study, your stool sample will be tested for 4 different types of bacteria. Also, we will also like to ask you a few questions about yourself and your eating habits. We would also like to review your medical history regarding your current diarrhea episode (what symptoms you have, how long you've been sick, what healthcare you've received and the costs of care). This short interview may take 20-30 minutes of your time.

**3. Can I stop being in the study?**

You can stop being in the study at any time. Nothing will change whether you choose to take part or not. Your decision will not affect your future relationship with study staff, the health clinic, or any of the organizations involved in the study.

**4. What bad things might happen to me if I am in the study?**

It is possible you will experience discomfort while giving a stool sample. We will take

the necessary precautions to minimize this discomfort.

**5.What good things might happen to me if I am in the study?**

You will not benefit directly from being in this study. If the lab result contains the targeted bacterial pathogens, the result will be provided for the healthcare provider to support the healthcare service.

**6.Will I be given anything for being in this study?**

You will not be given anything to be in this study.

**Can we contact you again to ask for your interest to participate in subsequent related studies?**

- _____ You may contact me in the future to ask for my interest to be involved in other related studies.
- _____ You may NOT contact me in the future to ask for my interest to be involved in other related studies.

**Can we keep your stool sample after it has been tested to use in future studies?**

- _____ You may keep my stool sample and use it for ANY type of future research study.
- _____ You may NOT keep my stool sample for a future study. My sample will be destroyed after it is tested.

**Can we access your medical record for this study?**

- _____ You can access my medical record
- _____ You can't access my medical record

**7.Who can answer my questions about the study?**

For questions, concerns, or complaints about the study, or if you feel you have any discomfort as a result of the study, you may contact Amete Mihret, by amete2004@gmail.com, 0913773888.

For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who part of the research team is not, you may contact the following

- EPHI's Scientific and Ethics review Committee (SERO) (Dr. Getachew Addis, Tel address: 0912100756)

**Signing the assent form**

I have read (or someone has read to me) this form. I have had a chance to ask questions before making up my mind. I want to be in this research study.

**AM/PM**

| **Signature, fingerprint, or printed name of subject** | **Date and time** |
|---|---|

**Investigator/Research Staff**

I have explained the research to the participant before requesting the signature above. There are no blanks in this document. A copy of this form has been given to the participant or his/her representative.

| **Printed name of person obtaining assent** | **Signature of person obtaining assent** |
|---|---|

**AM/PM**

**Date and time**

# Appendix X. Assent to Participate in Research (Amharic version)

**በጥናቱ ውስጥ ለመሳተፍ ስምምነት**

**የጥናቱ ርዕስ:** የተቅማጥ በሽታ የሚያመጡ ከምግብ ጋር ተዛማጅነት ያላቸው የተመረጡ ባክቴሪያዎችን ስርጭትና ለፀረ-ባክቴሪያ መድሃኒቶች መላምድ ሁኔታ ለማውቅ የተቅማጥ በሽታ ካለባቸው ታማሚዎች በተመረጡ ሶስት ከተሞች በኢትዮጵያ ውስጥ የሚደረግ ጥናት::

**ተመራማሪዎች ፡ አመተ ምህረት፣** ዶ/ር ወልዳረጋይ እርቁ፣ ዶ/ር አለም አብርሃ፣ ዶ/ር ኢያሱ ጥጋቡ

**ድጋፍ ሰጪ፡ የኢትዮጵያ ህብረተሰብ ጤና ኢንስቲትዩት ፣** ቢል እና መሊንዳ ጌትስ ፋዉነዳሽን ፣ የእነግሊዝ አለም አቀፍ ልማት መምሪያ።

ስሜ_____ይባላል። የምሰራው በህበረተሰብ ጤና ምርምር ከአዲስ አበባ ዩኒቨርስቲ፣ ከአሃዮ ስቴትስ ዩኒቨርስቲ፣ ኢንተርናሽናል ላይቭስቶክ ምርምር ተቋም ጋር በመተባበር ነው። ይህ ምርምርም የተቅማጥ በሽታ የሚያመጡ ከምግብ ጋር ተዛማጅነት ያላቸው የተመረጡ ባክቴሪያዎችን ስርጭትና ለፀረ-ባክቴሪያ መድሃኒቶች መላመድ ሁኔታ ለማውቅ የተቅማጥ በሽታ ካለባቸው ታማሚዎች በተመረጡ ሶስት ከተሞች በኢትዮጵያ ውስጥ የሚደረግ ጥናት ነው። ለዚህ ጥናት የተመረጥከው/ሽው በኣጋጣሚ ነው።

**በምርምሩ ለመሳትፍ የስምምነት ፎርም፡**

ይህ ሰነድ ስለምርምሩ ጠቃሚ መረጃዎቹን ያካትታል፣ እባክዎት ጊዜዎትን ወስደዉ በጥንቃቄ ያንብቡት:: በምርምራችን ተሳታፊ ለመሆን ከመዎሰንዎ በፊት ጥያቄ ለመጠየቅ ነጻ ይሁኑ::

**የእርስዎ ተሳትፎ ሙሉ በሙሉ በፈቃደኝነትዎ ላይ ተመሰረተ ነዉ**

በዚህ ምርምር መሳተፍ ወይም አለመሳተፍ የእርስዎ ምርጫ ነው:: አብረውን ለመስራት ከወሰኑ በላም ሀሳብዎን መቀየር ይችላሉ:: በዚህ ምርምር ለመሳተፍ ቢስማሙ ወይም ባይስማሙም በዚህ ጤና ተቋም የሚያገኙት አገልግሎት ላይ ምንም አይነት ተፅእኖ አይፈጥርም::የእርስዎ ዉሳኔ ከተመራማሪዎች ጋር የሚኖርዎትን የወደፊት ግንኙነት ላይ ተፅእኖ አይኖረዉም::

**በዚህ ምርምር ተጠቃሚ ሊሆኑም ላይሆኑም ይችላሉ**

ከዚህ ምርምር በቀጥታ ተጠቃሚ ላይሆኑ ይችላሉ ፣ ማህበረሰቡም ቢሆን በፍጥነት ተጠቃሚ ላይሆን ይችላሉ ነገር ግን ከዚህ ምርምር የሚገኘዉ መረጃ ለወደፊቱ ትዉልድ ይጠቅማል ብለን እናስባለን::

**በምርምሩ ላይ ሃሳብዎን ሊያስቀይሩ የሚችሉ አዳዲስ መረጃዎች ይደርስዎታል፤**

በዚህ ተስማምተዉ የምርምሩ አካል ለመሆን ከወሰኑ እዚህ ፎርም ላይ እንዲፈርሙ ይጠየቃሉ::የፎርሙ ቅጂም ይደርስዎታል::

**1. ጥናቱ ለምን አስፈለገ?**

የህብረተሰብ ጤና ቡድኖች የምግብን ጤናማነት እየሰሩ የሚገኙ ቢሆንም በኢትዮጵያ በምግብ መበከል ምክንያት የሚታመሙ ሰዎች ቁጥር በግልፅ አይታዎቅም።ይህ ጥናት መረጃዎችን ከማሰባሰብ በዘለለ ት ምን ያህል ኢትዮጵያዉያን በምግብ ብክለት ምክንያት እንደሚታመሙ ለማዎቅ ይረዳናል።

**2. ምን ያህል ሰዎች በዚህ ጥናት ላይ ይሳተፋሉ?**

በአጠቃላይ 2304 ሰዎች በዚህ ጥናት ይሳተፋሉ።

**3. በዚህ ጥናት ተሳታፊ ከሆንኩ ምን ይፈጠራል?**

በዚህ ጥናት ተሳታፊ ከሆኑ ለላቦራቶሪ ምርመራ የሰጡት የሰገራ ናሙና ለሶስት የተለያዩ ተህዋስያን ምርመራ ይደረግለታል። በተጨማሪም ስለ ጤና ተቋሙ እነዚህን እና መሰል ጥያቄዎች ይጠየቃሉ፤

- የ ጤና ባለሙያዎች ቁጥር
- የ ላቦራቶሪ እና የ ህክምና ክትትል ልምድ
- ለ ተቅማጥ ነክ በሽታዎች ተቅማጥ ነክ በሽታ ታማሚዎችን ለማከም ዎጭዉ ምን ያክል ነዉ
- የተቅማጥ ነክ በሽታዎች እና የምግብ ደህንነት ላይ ያለዎት ጠቅላላ እዉቀት

**4. በጥናቱ ለመሳተፍ ምን ያህል ጊዜ ይዎሰዳል**?

ይህ መጠይቅ ከ 10 ደቂቃ ያልበለጠ ይዎስዳል.

**5. ጥናቱን ማቋረጥ እችላለሁ?**

በማንኛዉም ሰዓት ጥናቱን ማቆም ይችላሉ ፤ሃሳብዎን ስለቀየሩ ምንም የሚፈተር ነገር የለም ፤ከተመራማሪዎች ጋር የሚኖርዎት ግንኙነት ላይ ተፅእኖ አይኖረዉም።

**6. የዚህ ጥናት ተሳታፊ በመሆኔ ምን አይነት አደጋዎችን ፤ የጎንዮሽ ጉዳቶችን፤ምቾት የሚነሱ ነገሮችን ልጠብቅ?**

የ ጥናቱ ተሳታፊዎች መልስ ከጥናቱ ዉጭ ያሉ ሰዎች ሊያገኙት ይችላሉ ።የዚህን ተፅእኖ ለመቀነስ ሲባል የጥናቱ መረጃ እንደተሰበሰበ ማንነተዎ ከጥናቱ ላይ ይዎጣል።

**7. ምን አይነት ጥቅሞችን ከዚህ ጥናት ላገኝ እችላለሁ?**

በቀጥታ ከጥናቱ ተጠቃሚ ላይሆኑ ይችላሉ ፤ ነገርግን የሚሰበሰበዉ መረጃ ለመጪዉ ትዉልድ ጠቃሚ ይሆናል።

**8.በዚህ ጥናት ተሳታፊ ባለሆን ሌሎች ምን ምንርጫዎች ይኖሩኛል?**

በዚህ ጥናት ላለመሳተፍ መምረጥ ይችላሉ ፤ በዚህም ምንም አይነት ቅጣትም ይሁን ጥቅምዎ አይነካም።

**9. በዚህ ጥናት ላይ ያሉ ግላዊ መረጃዎች ምንያህል የተጠበቀ ነዉ?**

ከዚህ ጥናት የሚሰበሰቡት መረጃዎች በጥንቃቄ ይያዛሉ፤ ግላዊ መለያዎችን አይጠቀምም፤የዲጅታል መረጃዎች በይለፍ ቃል ይቆለፋል ፤ መረጃዎች የሚቀመጡበት ቦታ የሚቆለፍ ሲሆን ቁልፉም ለተመራማሪዎች ብቻ

ይሰጣል፡፡ የጥናቱ ተሳታፊዎችን መረጃ ለመጠበቅ በተቻለን አቅም እንጥራለን፡፡ የተሳታፊዉ መረጃ በሚከተሉት አካላት ሊታዩ ይችላሉ

- የምርምር ቡድኑ አባላት
- በተቆጣጣሪ ኤጀንሲዎች ፤ እንደ ኢትዮጵያ ምግብ እና መድሀኒት አስተዳደር እና ልሎችም
- የተgሙ ግምገማ ቦርድ ፣በ ኢትዮጵያ ብሄራዊ የምርምር ግምገማ ኮሚቴ፣የኢትዮጵያ ማህበረሰብ ጤና ኢንስቲትዩት፣ጎንደር ዩኒቨርሲቲ ፣ሃረማያ ዩኒቨርሲቲ፣የካቲት 12 ሆስፒታል፣ አሃዮ ስቴት ዩኒቨርሲቲ፣አለም አቀፍ የእንስሳት ምርምር ኢንስቲትዩት
- ጥናቱን የሚደግፉ አካላት

**10. በዚህ ጥናት የተሰበሰበዉ መረጃ በሌሎች ጥናቶች ጥቅም ላይ ይዉላል?**

በዚህ ጥናት የተሰበሰበዉ ማንነት የማይገልፅ መረጃ ለሌሎች ምርመሮች ጥቅም ላይ ካለ ተጨማሪ ስምምነት ሊዉል ይችላል ፤ይህም መረጃ ለአመታት በጥንቃቄ በማሰቀመጥ ጥቅም ላይ ይዉላል፡፡

**የሰገራ ናሙናዎትን ለዚህ ምርመራ ከተጠቀምንበት በኋላ ለወደፊት ተጨማሪ ጥናት እንዲቀመጥ ፈቃደኛ ነዎት?**

_____የሰገራ ናሙናዬን ማስቀመጥና ወደፊት ለሌላ ለማንኛውንም አይነት ጥናት መጠቀም ትችላላችሁ

_____የሰገራ ናሙናዬን ማስቀመጥና ለሌላ ጥናት መጠቀም አትችሉም፡፡ ናሙናው መወገድ አለበት

**ወደፊት በተመሳሳይ ጥናቶች ላይ በመካፈል ፍላጎትዎን ለመጠየቅ እንደገና እንነጋገርዎ እንችላለን?**

_____ ወደፊት በተመሳሳይ ጥናቶች ላይ ለመካፈል ፍላጎት እንደሌኝ ለመጠየቅ ማነጋገር ይችላሉ።

_____ ወደፊት በተመሳሳይ ጥናቶች ላይ ለመካፈል ፍላጎት እንደሌኝ ለመጠየቅ መነጋገር አትችሉም።

**ለዚህ ጥናት የጤና መዝገብዎን ማግኘት እንችላለን?**

_____ የጤና መዝገቤን ማግኘት ትችላላችሁ።

_____ የጤና መዝገቤን ማግኘት አትችሉም።

**11. በዚህ ጥናት ለመሳተፍ ምን ያህል ወጪ ያስፈልጋል?**

በዘህ ጥናት ለመሳተፍ ምንም አይነት ወጪ አያስፈለግም

**12. በዚህ ጥናት ለሚሳተፍ ክፍያ ይኖረዋል?**

በዚህ ጥናት ለሚሳተፉ ምንም አይነት ክፍያ አይኖረዉም

**13.በዚህ ጥናት ተሳታፊ ስለሆነኩ ጉዳት ቢደርስብኝስ?**

በዚህ ጥናት ላይ ሆነዉ ጉዳት ይደረስብዎታል ብለን አናስብም ሆኖም ግን በጥናቱ ላይ ሆነዉ ጉዳት ከደረሰብዎ ለሚመለከታቸዉ የጥናቱ አባላት በመንገር ተገቢዉ ህክምና እነዲሰጥዎት ይደረጋል፡፡

**14. በዚህ ጥናት ተሳተፊ ከሆነኩ መብቶቼ ምን ምን ናቼዉ?**

በጥናቱ ለመሳተፍ ከዎሰኑ በኃላ ሃሳብዎን መቀየር ይችላሉ፤በዚህ ጥናት ለመሳተፍ በመወሰነዎ ማንኛዉም መብትዎ አይነካም፤በምርምሩ ላይ ሃሳብዎን ሊያስቀይሩ የሚችሉ አዳዲስ መረጃዎች ይደርስዎታል፤

በ ኢትዮጵያ ብሄራዊ የምርምር ስራዎች ግምገማ ኮሚቴ ዉሰጥ ለምርምሩ አባላት ተጠሪ የሆነዉ የተgሙ የግምገማ ቦርድ ፣የኢትዮጵያ ማህበረሰብ ጤና ኢንስቲትዩት ፣ጎንደር ዩኒቨርስቲ ፣ሃረማያ ዩኒቨርሲቲ፣የካቲት 12 ሆስፒታል፣ አሃዮ ስቴት ዩኒቨረሲቲ እና ዓለም ዓቀፍ የእንስሳት ምርምር ተgም ይህን ምርምር በተgሞቹ መመሪያ ፣ለሚመለከታቸዉ ግዛት እና ፌደራል ህጎች እንዲሁም የ ምርመር ተሳታፊዎች መብት እና ደህንነት ለመጠበቅ በዩኒቨርሲቲዎች የወጡ ደንቦች መሠረት ገምግመዉ ተቀባይነቱን አረጋግጠዋል፡፡

**15.ጥናቱን የሚመለከቱ ጥያቄዎችን መልስ መስጠት የሚችል ማነው?**

በጥናቱ ላይ ለሚነሱ ጥያቄዎች፣ስጋቶች፣አቤቱታዎች ወይም በጥናቱ ምክንያት ማንኛዉም አይነት ቅሬታዎች ካላችሁ ፤

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- የኢ.ህ.ጤ.ኢ ሳይንሳዊ እና የስነ-ምግባር ግምገማ ኮሚቴ (Tel: +251118685503/15)

## Appendix XI. Assent to Participate in Research (Afan Oromo version)

**Mata Duree Qorannoo:** Qorannoo baakteriyaa qoricha damdamatan, kan karaa nyaataa daddarban dhukkubsattoota dhibee garaa-kaasaa wal qabatee irratti magaalotaa Ethiopia sadii keessatti geggeeffamu

**Qorattoota:** Amete Mihret, Dr. Woldaregay Erku, Dr. Alem Abrha, Dr. Eyasu Tigabu

**Deeggertoota yookiin Ispoonsara qorannichaa:** Instiitiyuutii Fayyaa Hawaasummaa Itoophiyaa (EPHI), Bill fi Melinda Gates Foundation, Waajjira Hojii Misooma Idil-Addunyaa UK, Yuunivarsiitii Finfinnee

Akkam jirtu?

Maqaan koo _____ jedhama. Ani projektii fayyaa hawaasummaa keessatti qorattoota waliin Yunivarsiitii Finfinnee irraa, akkasumas Ohio State University, fi Instiitiyuutii Fayyaa Hawaasummaa Itoophiyaa keessatti kan argamu; dabalataanis Dhaabbata Qorannoo beeyiladaa Addunyaa (ILRI) kan eejensii mootummaa ta'e fi Ministira Fayyaa jala jiru waliin hojjachaan jira. Qorannichi baay'ina dhukkuboota bakteeriyaa nyaataan daddarban kan sababa dhukkuba garaachaa (dhibee garaa-kaasaa) ta'an Itoophiyaa keessatti hangam akka ta'e tilmaamuuf hojjetama. Kanaafuu Isin qorannoo kana keessatti akka hirmaattaniif kan filatamtan akka carraa ta'eeti yookaan akka tasaati.

Qorannoo kana keessatti kan hirmaattan yoo ta'e, sampliin (saamuda) sagaraa keessanii gosa baakteeriyaa adda addaa afur 4 irratti ni qoratama. Dabalataanis, gaaffilee tokko tokko isin in gaafanna. Isaanis?

- Gaaffii waa'ee mataa keessaniin wal qabatu ta'e; fakkeenyaa umrii fi sadarkaa barnootaa isin xumuurtan
- Ji'a darbe keessatti namni isin waliin jiraatuu keessaa namni dhibee garaachaa yookiin dhibee garaa-kaasaa nyaataan daddarban kanaan dhibame jiraachuu fi jiraachuu dhabuu isaa. Namni dhukkubsate yoo jiraate, mallattoo akkamii agarsiisee?, yeroo hangamiif dhukkubsatanii?, namni dhukkubsate kun yaalaaf gara buufata fayyaa deemaniiru? Yoo deeman ta'e yaala argataniiru? Yaala argataniiru yoo ta'e yaala tolaa moo kaffaltiin?

- Gaaffii waa'ee qabiinsa manaa fakkeenyaaf bishaan eessa irraa argattu?, horii manaa kamuu yoo qabaattan?
- Waa'ee nageenya nyaataa fi dhukkuboota nyaata irraa nama qabanii beektuu?
- **Kun unka waliigaltee hirmaannaa qorannoo ti;** Unki kun odeefannoo barbaachisaa qorannoo kanaaf tajaajilan of keessaa qaba. Kanaaf yeroo keessan fudhaatii odeefannoo kenname sirriitti ilaala. Qorannoo keenya irratti hirmaachuuf fedha qabachuu fi dhiisuu keessanis ta'e yeroo qorannoo irra jirtanittis ta'e yeroo kamitti iyyuu murteessuu keessanin dura gaaffii kamuu gaafachuuf bililisa ta'aa gaafadhaa!.
- **Hirmaannaan keessan guutummaatti fedhii ofii keessaniin kan ta'uudha.** Hirmaachuuf fedhii qabaattanis ta'e hin qabaanne filachuu dandeessu. Amma yoo waliigaltee keessan kennitanis, boodarra yaada keessan jijjiiruu ni dandeessu. Hirmaachuu dhiiftaniyyuu tajaajilli isin buufata fayyaa kana irraa ni argattu. Kun kan hin jijjiiramneedha.
- **Qorannoo kana irratti hirmaachuu keessaniin fayyadamaa ta'uus dhiisuus dandeessu**. Qorannoo kana irratti hirmachuu keessaniif faayidaan kallattiin isiniif dhufuu dhiisuu mala. Faayidaan battalaa hawasaaf ta'us jiraachuu dhiisuu danda'a garuu daataan maddisiifamu dhaloota egereef bu'aa guddaa buusa.
- **Odeeffannoo haaraan yeroo qorannochaa uumamu kamiyyuu kan murtoo itti fufuu yookiin dhiisuu keessan irratti dhiibbaa uumuu danda'u yoo jiraate isiniif kennama** Yoo hirmaachuuf murteessitan, unka kana akka mallatteessitan isin gaafanna. Mallattoo keessan booda, copy unka kanaa ni argattu.

**Odeeffannoo Ijoo waa'ee Qorannoo Kanaa Irratti**

1. **Qorannichi maaliif geggeeffamaa jira?**

Qaamoleen fayyaa hawaasummaa fayyummaa nyaataa eegsisuuf hojjachaa jira. Haa ta'u malee, namoota Itoophiyaa keessaa hangamtu nyaata faalama qabuun akka dhukkubsatu hin beekamne. Kanaafuu Qorannichi odeeffannoo barbaachisoo ta'an walitti guuruun waggaa keessatti namoota Itoophiyaa keessa dhibee baakteeriyaa karaa nyaata faalameen dhufan fi tilmaamuuf kan qophaa'edha

2. **Namoonni meeqa qorannoo kana keessatti ni hirmaatu?**

Walii galatti namoonni 2,304 ta’an qorannoo kana keessatti hirmaatu.

3. **Qorannoo kana keessatti yoon hirmaadhe maaltu ta’a?**

Sampliin yookiin samuudi keessan bakteeriyoota nyaataan daddarban afur irratti qoratama. Akkasumas seenaa yaalaa keessan kan garaacha yookiin garaa-kaasaa kan ammaa ilaalchisee (mallattoo akkamii akka qabdan, yeroo hammamii akka dhukkubsattan, kunuunsa fayyaa akkamii akka argattan fi baasii kunuunsaa yookiin yaalaa baastan) ilaaluu barbaanna. Gaaffii fi deebii gabaabaan kun yeroo keessan daqiiqaa 20-30 fudhachuu danda'a.

4. **Yoon qorannoo kana keessa jiraadhe wanti hamaan akkamii na mudachuu danda’a?**

Yeroo saamuda sagaraa kennitu miira tasgabbii dhabuun si mudachuu danda’a. Miidhaa kana xiqqeessuuf of eeggannoo barbaachisu ni goona.

5. **Yoon qorannaa irratti argamu wanti gaariin akkamii na mudachuu danda’a?**

Qorannaa kana keessatti argamuun kallattiin faayidaa hin argattu. Bu’aan mana yaalaa paatojeenoota baakteeriyaa xiyyeeffannoo argatan yoo qabaate, bu’aa argame sanaan bu’uura godhachuun tajaajila eegumsa fayyaa ni kennama.

6. **Qorannoo kana keessa waanan tureef wanti naaf kennamu jiraa?**

Qorannoo kana keessatti akka argamtuuf waa siif hin kennamu.

**Qorannoowwan kanaan walqabatan itti aanan irratti hirmaachuuf fedhii akka qabdan gaafachuuf ammas isin qunnamuu dandeenyaa?**

_____ Gara fuulduraatti fedhii koo qorannoowwan kanaan walqabatan biroo irratti akkan hirmaadhuuf na qunnamuu dandeessu.

_____ Fuulduratti fedhii koo qorannoowwan kanaan walqabatan biroo irratti hirmaachuuf na gaafachuuf na qunnamuu HIN DANDEESSAN.

**Saamuda sagaraa keessan qorannoo gara fuula duraatti fayyadamuuf erga qoratamee booda ol kaa’uun qabachuu dandeenyaa?**

_____Saamuda sagaraa koo qabattee gosa qorannoo qorannoo gara fuula duraa KAMIYYUUf itti fayyadamuu dandeessa.

_____ Qo'annoo gara fuula duraatiif saamuda sagaraa koo kaa'uu HIN dandeessan. Saamuda koo erga qoratamee booda haa gatamu.

**Qorannoo kanaaf galmee yaalaa keessan argachuu dandeenyaa?**

_____ Galmee yaalaa koo argachuu dandeessu

_____ Galmee yaalaa koo argachuu hin dandeessan

**7. Gaaffiiwwan qorannoo ani dhiheessu ilaalchisee eenyutu deebii kennuu danda'a?**

Gaaffii, waan yaaddoo isinitti ta'u, ykn komii waa'ee qorannichaa yoo qabaattan, ykn sababa qorannichaatiin miira namatti hin tolle yoo isinitti dhaga'ame, **Amete Mihret**, **amate2004@gmail.com** ykn **+251-913773888** qunnamuu dandeessu.

Gaaffii waa'ee mirga keessan akka hirmaataa qorannoo kanaatti yookiin nama qaama garee qorannoo hin taane waliin yaaddoowwan ykn komii qorannoon walqabatan biroo irratti mari'achuuf, qunnamuu dandeessu.

- Koree gamaaggama saayinsii fi naamusaa EPHI (SERO) (Bilbila: +251 118685503/15)

**Unka hayyamaa mallatteessuu**

Unka kana dubbiseera (ykn namni tokko naaf dubbiseera). Murtoo koo osoon hin murteessiin dura gaaffii gaafachuuf carraa argadheera. Qorannoon kana keessatti argamuun barbaada.

**AM/PM**

| | |
|---|---|
| **Mallattoo, ashaaraa qubaa, ykn maqaa hirmaataa** | **Guyyaa fi sa'aatii** |

**Qorataa/Hojjetoota Qorannoo**

Mallattoowwan armaan olii gaafachuun koo dura qorannoo hirmaataa ykn bakka bu’aa isaaf ibseen jira. Barreeffama kana keessatti bakki duwwaa hin jiru. Koppiin unka kanaa hirmaataaf ykn bakka bu’aa isaaf kennameera.

| | |
|---|---|
| **Maqaa nama hayyama aragatee maxxanfame** | **Mallattoo nama hayyama argatee** |
| | **AM/PM** |
| | **Guyyaa fi sa'aatii** |

## Appendix XII. Standard Operating Procedures

**Laboratory Analysis of the Non-typhoidal *Salmonella* (NTS), Shiga-toxin producing *Escherichia coli* (STEC), and *Campylobacter* spp., (CAMPY).**

### 1. Isolation and Identification

Isolation and identification of STEC, *Salmonella*, *Shigella* was conducted using the conventional culture techniques, followed by a set of biochemical, and the detection of *Campylobacter* conducted as follows (Figure 1).

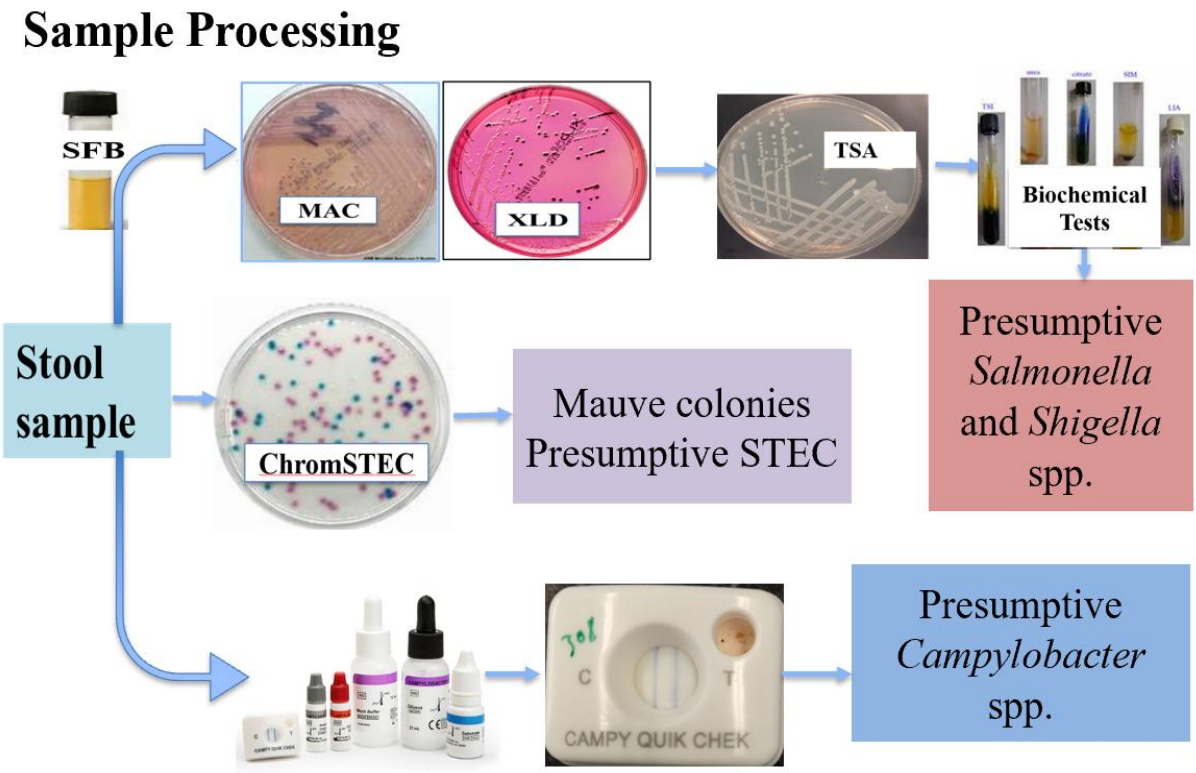


**Figure 1.** Schematics of stool sample processing for the isolation, identification and detection of the targeted foodborne bacterial pathogens.

### 1.1. Identification of *Salmonella species*

Stool samples were inoculated using the Selenite-F-broth enrichment broth and incubated aerobically at 35±2°C overnight. Samples of the enriched broth were sub-cultured on the MacConkey (MAC, Difco™, USA) and Xylose-Lysine-Deoxycholate (XLD, Difco™, USA) agar plates and incubated aerobically at 35±2°C overnight (Figure 1). The plates were examined for the

presence of hydrogen-sulfide producing non-fermenting colonies (reddish colony with and without black center) on XLD, and moist colorless colonies on MAC agar. Identification of SALM were conducted using biochemical tests i.e., Triple Sugar Iron Agar (TSIA, Difco™, USA), Lysine Iron Agar (LIA, Difco™, USA), Simmon Citrate Agar (SCA, Difco™, USA), Urease Agar (UA, Difco™, USA), and Sulfide Indole Motility Agar (SIMA, Difco™, USA). The genus Salmonella were confirmed using Magnetic Induction Cycler qPCR (MIC qPCR) by targeting the *invA* gene.

### 1.2. Identification of Shiga-toxin Producing *Escherichia coli*

Stool samples were plated on to chromogenic media (ChromSTEC agar, France) and incubated aerobically at 35±2°C overnight (Figure 1). The presumptive STEC positive results were detected, if any mauve colonies (as described by media manufacturers) were present on the ChromSTEC agar (Figure 2). Confirmation of STEC was conducted using MIC qPCR machine by targeting (s*tx1*/*stx2*) and *eae* genes according to the ISO/TS 13136 guideline (ISO/TS 13136.,2012) and manufacturer's instruction.

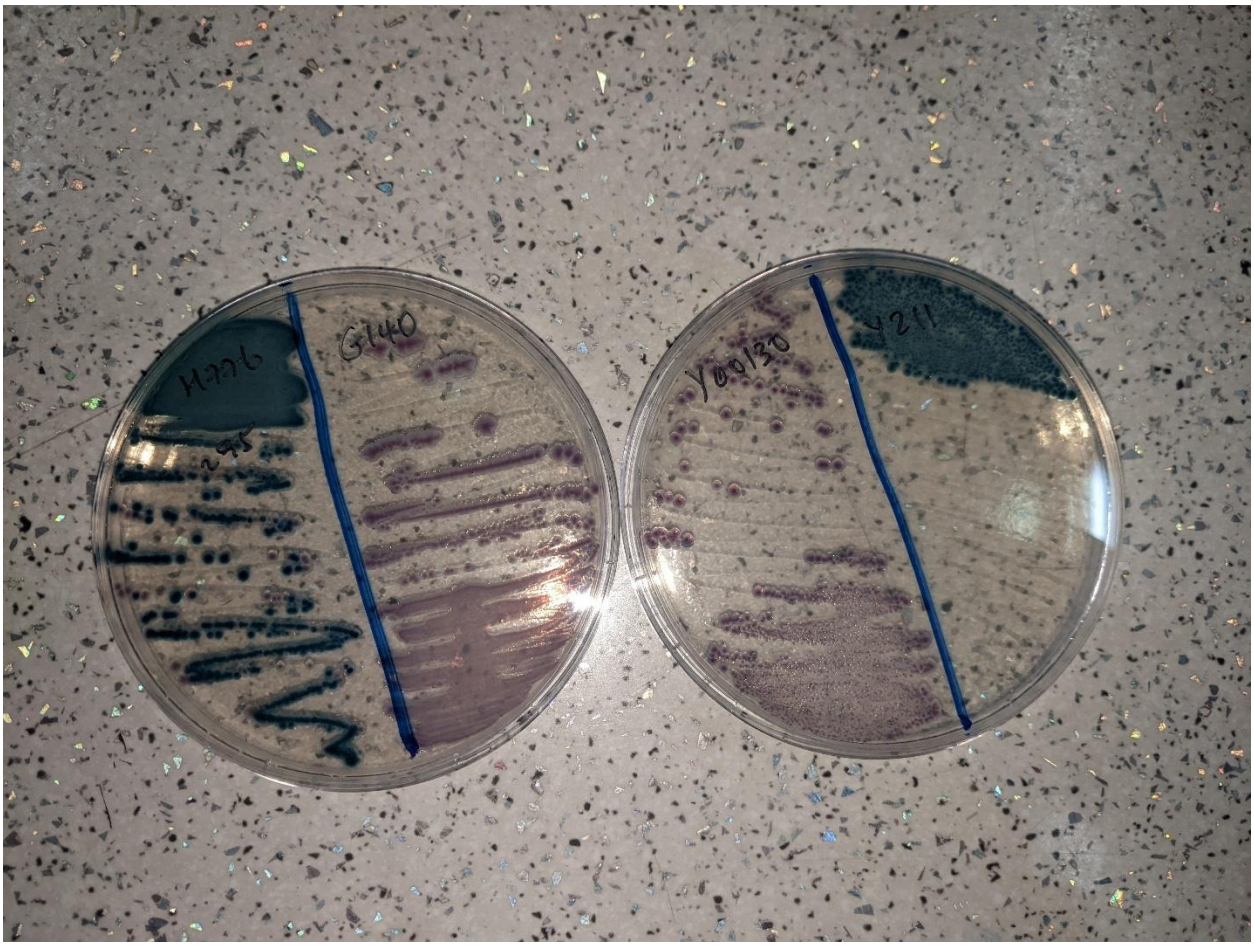

**Figure 2.** The colony characteristics of STEC (mauve colonies), and other non-STEC pathogens (blue colonies) on the ChromSTEC agar.

### 1.3. Detection of Campylobacter species

Detection of CAMPY were conducted using an immunoassay technique (Campy Quick Check, USA) based on the manufacturer's instruction (TECHLAB.,2020) (Figure 1). The kit was examined after 10minute for the formation of purple lines in the control and test area of the kit and reported as presumptive CAMPY positive according to the manufacturer's instruction. The immunoassay method was used for the detection of the *C. coli*, *C. jejuni*, *C. lari*, and *C. upsaliensis* species. The test is intended for use with preserved fecal specimens in transport media and unpreserved fecal specimens. The CAMPYLOBACTER QUIK CHEK™ test uses antibodies that recognize a *Campylobacter*-specific antigen in human fecal samples. The device contains a Reaction Window with two vertical lines of immobilized antibodies. The test line ("T") contains antibodies against a *Campylobacter*-specific antigen. The control line ("C") contains anti-IgG antibodies. The Conjugate consists of antibodies to a *Campylobacter*-specific antigen coupled to horseradish peroxidase. Briefly, diluent (750µL) was added to the test tube; one drop of conjugate was added to each tube containing the diluent; and the stool specimen (25µL) was added into the tube of diluent-conjugate mixture. The diluted specimens were vortexed for 5 to 20 seconds and incubated for 30 minutes at room temperature. The diluent-conjugate-stool mixture (500µL) was then transferred into the sample well of the membrane device and incubated for 15 minutes at room temperature. During the incubation, the *Campylobacter*-specific antigens in the sample bind to the antibody-peroxidase conjugate. The antigen-antibody complexes migrate through a filter pad to a membrane where they are captured by the immobilized anti-*Campylobacter* antibodies in the line. Wash buffer (300µL), and 2 drops of substrate were added before examining the test result. The appearance of two blue lines (one for the control and one for the test) within 10 minutes was indicative of *Campylobacter* positive results. CAMPY positive stool samples were stored at –80$^{o}$C and used for the molecular confirmation by using the MIC qPCR machine and targeting the *Campylobacter* species specific 16S rRNA genes.

### 2. Antibiotic Susceptibility Testing

Phenotypic antibiotic susceptibility testing (AST) was conducted for isolates of STEC, *Salmonella* spp., and *Shigella* spp. using the Phoenix M50 machine (Becton Dickinson, USA) according to

the manufacturer's instruction (Becton Dickinson, n.d) (Figure 3). Briefly, the BD Phoenix AST method is a broth-based micro-dilution test that uses a redox indicator to detect an organism's growth in the presence of an antibiotic agent. Changes to the indicator and bacterial turbidity are continuously measured to determine bacterial growth. In this study, five pure colonies of each isolate were suspended in BD Phoenix AST Broth, and the suspension density was measured using a densitometer (BD PhoenixSpec™ Nephelometer) (Garcia, 2008) that was designated with a 0.5 McFarland standard. The standardized inoculum was then used to inoculate BD Phoenix panels, gram-negative AST 501 (NAST/501) and emerge NMIC/474 panels, which were then placed in the machine at 35$^{o}$C, and tested every 20 minutes for up to 16 hours at 35°C. The results were displayed on the minimum inhibitory concentration (MIC) form and interpreted as Susceptible or Resistant based on the BD Phoenix, Software version, V7.21A. Each isolate was tested for ampicillin (0.5-32µg/mL), ciprofloxacin (0.25-4µg/mL), trimethoprim-sulfamethoxazole (0.5/9.5-16/304µg/mL), amikacin (0.5-64µg/mL), gentamicin (0.25-16µg/mL), cefazolin (0.5-32µg/mL), cefuroxime (1-64µg/mL), ceftriaxone (0.5-64µg/mL), ceftazidime (0.5-64µg/mL), cefepime (0.5-64µg/mL), ampicillin-sulbactam (0.5/0.25-32/16µg/mL), ceftazidime-avibactam (0.25-32µg/mL), ceftolozone-tazobactam (0.25-32µg/mL), piperacillin-tazobactam (0.5/4-128/4µg/mL), meropenem (0.125-32µg/mL), azithromycin (0.5-64µg/mL), fosfomycin (16-256µg/mL), and colistin (0.5-4µg/mL).

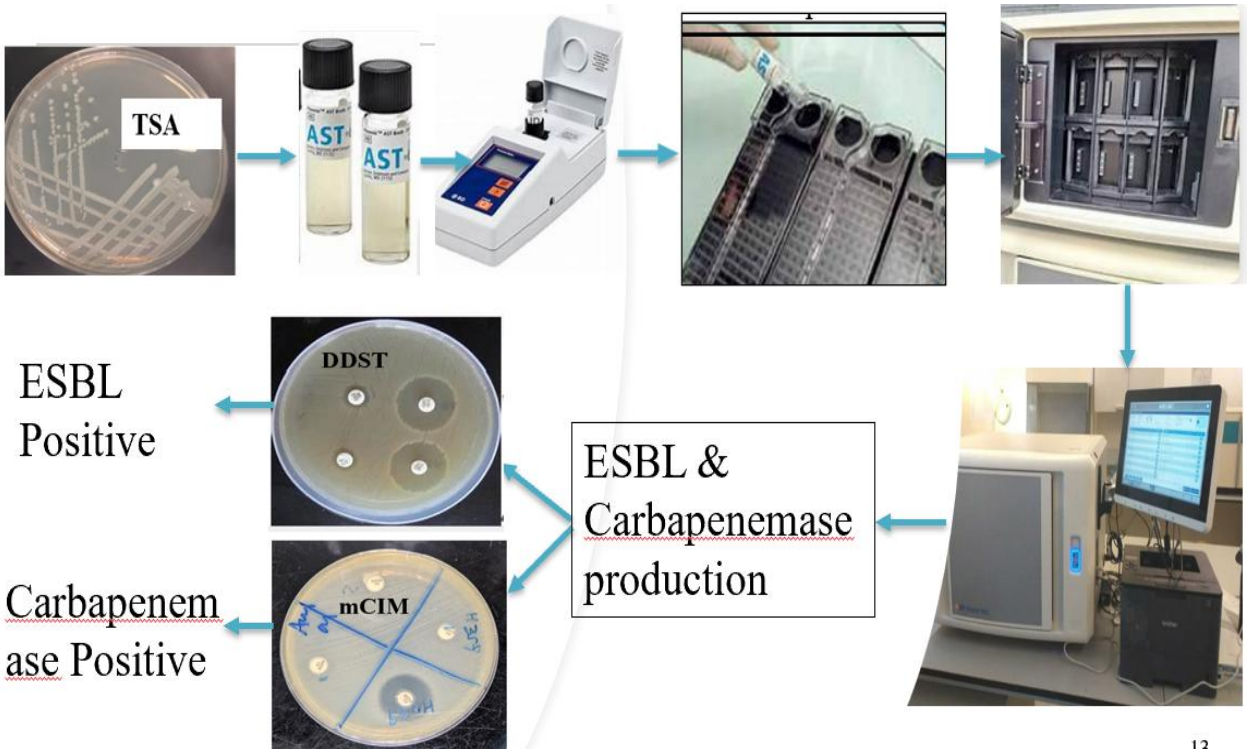


**Figure 3.** Antibiotic susceptibility testing.

## 3. Molecular Confirmation

### 3.1. DNA Extraction: Extraction from Pure Bacterial Colony

Extraction of DNA for the NTS and STEC confirmation were conducted from the pure colony using the boiling methods. Frozen presumed NTS and STEC isolates were sub-cultured on to the Trypticase-soya agar (TSA), incubated aerobically at 35±2°C overnight. Then, approximately 5 pure colonies from the TSA were suspended into 250µl of sterile nuclease free water in the Eppendorf tube to have suspension. The colony suspension was centrifuged at 14000 rpm for 10 minutes. The supernatants were discarded, and 250µl of nuclease free water were added to the cell pellets, followed by boiling in preheated heat block at 100°C for 10 minutes. Then, the suspension was cooled down for 15 minutes, and centrifuged at 14000 rpm for 10 minutes, and the supernatants were transferred to new sterile Eppendorf tube, dilute 1:5, and used as DNA template for our PCR testing. The amount and purity of the extracted DNA were measured with a NanoDrop apparatus (Thermo Fisher Scientific, Waltham. Massachusetts, United States).

### 3.2. DNA Extraction: Extraction from The Stool Samples

For the CAMPY confirmation, the DNA extraction was done from the frozen stool samples using the Fast Stool DNA Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol (QIAamp® Fast DNA Stool Mini Handbook, Feb,2020). Frozen stool samples, weighing approximately 180–220 mg was placed in a 2 ml microcentrifuge tube and placed on ice. Then, 1 ml InhibitEX Buffer was added into each stool sample, and vortexed continuously for 1 min or until the stool sample was thoroughly homogenized, followed by heating the suspension for 5 min at 70-95°C, and vortexed for 15 s. The suspension was centrifuged at 12000 rpm for 1 min to pellet stool particles, and 200 µl supernatant were transferred to the tube containing 15 µl proteinase K, and 200 µl Buffer AL were added and vortexed for 15 s. The mixture was incubated at 70°C for 10 minutes, followed by addition of 200 µl of ethanol (96–100%) to the lysate, and mixed by vortexing, transfer all the lysates prepared so far (600µl lysate) into the QIAamp spin column, close the cap and centrifuge at full speed for 1 minute, repeated this step 2x to remove debris from the DNA suspension. Then, 500 µl of the wash buffer AW1 and AW2 were added stepwise, and pipet 200 µl Buffer ATE directly onto the QIAamp membrane, incubated for 1 minute at room temperature, then centrifuged at full speed for 1 minute to elute DNA, and this suspension was diluted 1:5, and used as DNA template for the PCR analysis. The amount and purity of the extracted DNA were measured with a NanoDrop apparatus (Thermo Fisher Scientific, Waltham. Massachusetts, United States).

### 3.3. PCR testing (MIC qPCR Analysis)

The PCR confirmation of the NTS, STEC, and the CAMPY were conducted using the MIC qPCR machine (MIC qPCR Guideline). Besides, the ISO 6579:2002, and ISO/TS 13136.,2012 guidelines were also used for NTS and STEC confirmation respectively.

**Table 1**. Primers and Probes used for PCR confirmation of Non-typhoidal *Salmonella*, Shiga-toxin Producing *Escherichia coli*, and *Shigella* spp., and *Campylobacter spp*.

| **Bacteria** | **Target gene** | **Primer and Probe Sequence** | **Amplicon size (bp)** | **References** |
|---|---|---|---|---|
| Genus *Salmonella* | *invA* | Forward (Sal1598): AACGTGTTTCCGTGCGTAAT | 95 | Heymans et al., 2018 |
| | | Reverse (Sal1859): TCCATCAAATTAGCGGAGGC | | |
| | | Probe: [6FAM] TGGAAGCGCTCGCATTGTGG[BHQ1] | | |
| *S*. Typhimurium | *STM4200* | Forward: CACCTGATATAGAGTCCAA | 101 | |
| | | Reverse: CACCTGATATAGAGTCAA | | |
| | | Probe: [Cyanine] AAGGTATTCTTGACTGAACAATGCC[BHQ2] | | |
| *S*. Enteritidis | *SEN1392* | Forward: GGATATGAGGTGCGTTTA | 77 | |
| | | Reverse: CAGTGCCGGAATTATCTC | | |
| | | Probe: [TxRd]CACCATGACCCGCAGACG[BHQ2] | | |
| *Shigella* spp. | *ipaH* | Forward: TGAAGGAAATGCGTTTCTATG | 181 | Lin et al 2010 |
| | | Reverse: AGGGAGAACCAGTCCGTAAA | | |

| | | | | |
|---|---|---|---|---|
| | | Probe: HEX-CACGGCCGAAGCTATGGTC AGAAGCCGTG | | |
| | *lacY* | Forward: ACCAGACCCAGCACCAGATAAG | 101 | Pavlovic et al., 2011 |
| | | Reverse: CTGCTTCTTTAAGCAACTGGCGA | | |
| | | Probe: FAM-CATACATATTGCCCGCCAGTACAGAC-BBQ | | |
| STEC | *stx1* | Forward: TTTGTYACTGTSACAGCWGAAGCYTTACG | 131 | Perelle et al., 2004 |
| | | Reverse: CCCCAGTTCARWGTRAGRTCMACRTC | | |
| | | Probe: [6FAM] CTGGATGATCTCAGTGGGCGTTCTTATGTAA[BHQ1] | | |
| | *stx2* | Forward: TTTGTYACTGTSACAGCWGAAGCYTTACG | 128 | |
| | | Reverse: CCCCAGTTCARWGTRAGRTCMACRTC | | |
| | | Probe: [HEX]TCGTCAGGCACTGTCTGAAACTGCTCC[BHQ1] | | |
| | *eae* | Forward: CATTGATCAGGATTTTTCTGGTGATA | 137 | |
| | | Reverse: CTCATGCGGAAATAGCCGTTA | | |
| | | Probe: [Cyanine5] TAGTCTCGCCAGTATTCGCCACCAATACC[BHQ2] | | |
| *Campylobacter* genus | *16S rRNA* | Forward: GATGACACTTTTCGGAGCGTAA | 197 | Platts-Mills et al, 2014 |
| | | Reverse: GCTTGCACCCTCCGTATTACC | | |

| | | | | |
|---|---|---|---|---|
| | | Probe: [6FAM] CGTGCCAGCAGCC[BHQ1] | | |
| *C. jejuni* | *hipO* | Forward: TGCACCAGTGACTATGAATAACCGA | 124 | Vondrakova et al., 2014 |
| | | Reverse: TCCAAAATCCTAACTTGCCATT | | |
| | | Probe: [HEX]TTGCAACCTCACTAGCAAAATCCACAGCT[BHQ1] | | |
| *C. coli* | *gly A* | Forward: CATATTGTAAAACCAAAGCTTATCGTG | 133 | Vondrakova et al., 2014 |
| | | Reverse: AGTCCAGCAATGTGTGCAATG | | |
| | | Probe:FAM-TAAGCTCCAACTTCATCCGCAATCTCTCTAAATTT-Eclipse | | |
| IAC | | Forward: AGTTGCAGTGTAACCGTCATGT | 145 | FDA, 2021 |
| | | Reverse: TCGACGAGACTCTGCTGTTAAG | | |
| | | Probe: AGTTGCAGTGTAACCGTCATGTACCAGTAATCTGCGTCGCACGTGTGCACCTAGTCTAATCACTTATGACTCAGATAACTTAACAGCAGAGTCTCGTCGA | | |

**Note:** "W" and "Y" in our sequence are ambiguous nucleotides. "W" stands for "A" or "T", and "Y" stands for "G" or "C". In the sequence of (stx) In the sequence Y is (C, T), S is (C, G), W is (A, T), R is (A, G), M is (A, C).

### 3.3.1. Non-typhoidal Salmonella Confirmation

NTS was confirmed using monoplex PCR test by targeting the *Salmonella*-specific *invA* gene, and the specific primers pairs and probe sequences (Table1). A total volume of 20µl was used through a combination of the primer pair (300nm,1µl each) for the *invA* gene and internal amplification control (IAC), dual fluorescent labelled probes (150nm, 1µl), IAC template (0.075 pg/m/0.002 pg, 1µl), and master mix (1X, 10µl), nuclease free water (2µl), and finally the DNA template (1µl each) was added. The assay includes a one-cycle initial denaturation stage of 95ºC for 2 minutes, followed by the denaturation of 95ºC for 10 seconds, and annealing/extension steps at the 60ºC for 60 seconds for a total of 50 cycles. The IAC was included in the PCR assays to detect the presence of PCR inhibitors and reduce false-negative results, positive control was conducted using the *S. enteritidis* (ATCC® 14028) serotype and nuclease-free water was used as a negative control.

### 3.3.2. Shiga toxin-producing *Escherichia coli* Confirmation

The multiplex qPCR was used for the detection of *stx1*, *stx2*, and *eae* genes of STEC for confirmation using the assay protocol, sequences of primer, and probes (Table 1). A total volume of 20µl was used through a combination of the primer pair (300nm,1µl each), dual fluorescent labelled probes (150nm, 1µl), and master mix (1X, 10µl), and finally the DNA template(1µl) were added. The assay includes a one-cycle of initial denaturation stage of 95ºC for 2 minutes, followed by the denaturation stage of 95ºC for 10 seconds, and annealing/extension stage of 60ºC for 10 seconds for a total of 50 cycles. The positive and negative control testing were conducted using the *E. coli* O157:H7 (ATCC 35150) and nuclease-free water respectively.

### 3.3.3. *Shigella* species confirmation

The PCR run conditions for *Shigella* spp. were set according to a previously used protocol (Lin et al., 2010) with modifications to optimize detection of *Shigella* spp. Since enteroinvasive *Escherichia coli* (EIEC) also found positive for the *ipaH* gene, a Touchdown PCR approach was implemented to enhance both specificity and sensitivity (Korbie & Mattick, 2008). The 20µl reaction volume comprised primer pairs (1µl), probe (1µl), master mix (12.5µl), nuclease-free water (0.5µl), and template (1µl). The cycling conditions for the PCR began with an initial denaturation step at 95°C in 3 minutes. This was followed by 5 cycles of amplification, each consisting of denaturation at 95°C for 10 seconds, annealing at 58°C for 20 seconds, and

extension at 72°C for 15 seconds, then, 40 cycles of denaturation at 94°C for 10 seconds, annealing at 55°C for 20 seconds, and extension at 72°C for 15 seconds. The Touchdown strategy, with a higher annealing temperature in the first few cycles, reduced non-specific amplification and preferentially amplified the target *Shigella* sequences. For the quality control, *S. dysenteriae* ATCC 13313 was used as positive control, and nuclease-free water as the negative control. The isolates were confirmed as *Shigella* spp. by detecting the *ipaH* gene, followed by the absence of *lacY* gene.

### 3.3.4. Campylobacter Species Confirmation

The confirmation of CAMPY was conducted using the multiplex MIC qPCR using an assay protocol using the sequence of primers and probes (Table 1). The assay condition of one-cycle pre-amplification stage (initial denaturation) at 95$^{o}$C for 10 minutes, followed by the denaturation of 95$^{o}$C for 15 seconds and annealing/extension 55$^{o}$C for 60 seconds with a total of 50 cycles. a total of 20µl reaction volume was used with the composition of primer pairs (1µl each), probe (1µl), master mix (1X, 12.5µl), and water (0.5µl). *C. jejuni* (ATCC 29411), and *C. coli* (ATCC 43478) were used as a positive control, and nuclease-free water was used as a negative control.

### 3.3.5. Molecular Confirmation of antibiotic resistance genes

#### 3.3.5.1. Extended spectrum Beta-lactamase and Carbapenemase production

Phenotypic confirmation of ESBL was performed using the double disk diffusion method (CLSI, 2024) at the Ethiopian Public Health Institute and Armauer Hansen Research Institute. A combined disk assay was conducted using ceftazidime (30µg) vs ceftazidime-clavulanic acid (30/10µg), and cefotaxime (30µg) vs cefotaxime-clavulanic acid (30/10µg) disks. A bacterial colony of 18-24hrs old from a well-isolated growth on TSA was selected, and a bacterial suspension was prepared in sterile saline to match the turbidity of a 0.5 McFarland standard. Then, the suspension was inoculated onto the Mueller-Hinton agar plate, ensuring that the entire surface is evenly covered. Then, ceftazidime (30µg) and ceftazidime-clavulanic acid (30/10µg), and cefotaxime (30µg) and cefotaxime-clavulanic acid (30/10µg) was placed side by side in the distance of 24mm from the centre of the disks. The plates were incubated at 35 ± 2°C 16-18hrs aerobically. Following incubation, the zones of inhibition around each antibiotic disk were measured and compared. The production of ESBL was determined by

observing the expansion of ≥ 5 mm of the diameters of combined disks (ceftazidime-clavulanic acid and cefotaxime-clavulanic acid) compared to the individual disks (ceftazidime and cefotaxime) alone. *K. pneumoniae* (ATCC 700603) and *E. coli* (ATCC 25922) were used for positive and negative testing, respectively.

Phenotypic confirmation of carbapenemase production was conducted on isolates that were resistant to the meropenem or imipenem antibiotics using modified carbapenemase inactivation methods as described by the previously designed protocol (Seman et al., 2022). Briefly, a loopful (1µL) of the test isolates, taken from an overnight trypticase soya agar (TSA, Oxoid, UK) culture, was suspended in 2 mL of trypticase soya broth (TSB, Oxoid, UK). A 10 µg meropenem disk was then added to the broth, ensuring it was fully immersed, and the mixture was incubated at 37°C aerobically for approximately 4 hours. After incubation, the meropenem disks were carefully removed using a 10 µL inoculation loop and transferred onto Mueller-Hinton agar plates (MHA, Oxoid, UK), which had been freshly inoculated with a 0.5 McFarland standard suspension of the carbapenem-susceptible *E. coli* ATCC®25922. Following overnight incubation, results were interpreted following CLSI guidelines (CLSI, 2024).

#### *3.3.5.2. Molecular characterization of antimicrobial resistant genes (ESBL and carbapenemase)*

#### *3.3.5.1. DNA extraction*

DNA extraction for the confirmation of the ESBL genes was carried out using the QIAgen kit (Qiagen, n.d.). Briefly, the frozen bacterial culture was sub-cultured onto TSA (Tryptic Soy Agar) plates and incubated at 35°C ± 2°C. A single colony was then inoculated into 5 mL TSB (Tryptic Soy Broth, Oxoid, UK) and incubated overnight at 35°C ± 2°C. Following incubation, 3 mL of the broth culture was centrifuged, and the supernatant was discarded. The resulting sediment was used as the source for DNA extraction, which was performed according to the guidelines provided in the QIAgen protocol (Qiagen, n.d.).

The DNA extraction for the confirmation of the carbapenemase genes were conducted using the thermal/NaOH methods (CDC, 2024). Briefly, the frozen bacterial culture was sub-cultured onto TSA plates and incubated at 35°C ± 2°C. To prepare the samples, 25 µl of molecular-grade water was added to each microcentrifuge tube, and a loopful (1 µl) of colonies was

suspended in the molecular-grade water. The tubes were vortexed and 25 µl of 0.1 N NaOH was added, vortexed again, and heated at 95-99°C for 10 minutes. Then, cooled on a pre-cooled block, 18 µl of 0.5 M Tris-HCl (pH 8.0) and 400 µl of nuclease-free cold water was added and gently mixed by inversion. The tubes were centrifuged at 13,200 rpm for 3 minutes, and then 400 µl of the lysate was transferred to a labelled storage tube, after which the lysates were stored at -20°C to -30°C until use.

#### *3.3.5.2. PCR protocol*

Confirmation of ESBL production was performed using conventional PCR, a previously used protocol (Seman et al., 2022) using the primers sequences on Table 2. First a master mix was prepared with a total reaction volume of 25 µL, which included the following components: 12.5 µL of Taq Plus 2X Master Mix, 1.5 mM of $MgCl_2$, 5.5 µL of nuclease-free water, 1 µL of forward multiplex primers (bla-CTX-M, bla-TEM, bla-SHV), 1 µL of reverse multiplex primers (bla-CTX-M, bla-TEM, bla-SHV), and 5 µL of DNA template. To prepare the primer multiplex, the initial concentration of the primers (10 µM) was diluted to 2 µM. This was achieved by adding 5 µL each of forward bla-SHV, bla-CTX-M, and bla-TEM primers to a total volume of 85 µL of nuclease-free water. The same dilution was performed for the reverse primers. The thermal cycling conditions were optimized as follows: an initial denaturation step at 95°C for 3 minutes, followed by 40 cycles consisting of denaturation at 95°C for 15 seconds, annealing at 53°C for 1 minute, and extension at 72°C for 2 minutes. A final extension step was conducted at 72°C for 7 minutes. This process was carried out using a thermal cycler (BIO-RAD, California, USA). Known ATCC strains, *K. pneumoniae* (ATCC 700603), were used as positive controls for the ESBL procedure, while *E. coli* (ATCC 25922) served as the negative control. For gel electrophoresis, 1.5 grams of agarose gel was dissolved in 100 mL of 1X TAE (Tris-acetate-EDTA) buffer, making a 1.5% agarose gel. The solution was boiled until clear, cooled slightly, and then dispensed into the gel casting tray. After the PCR amplification, the products were visualized using gel electrophoresis. The PCR samples were loaded after mixing 10µL of the PCR product with 2 µL of loading dye. The gel electrophoresis was run for 55 minutes. Following electrophoresis, the gel was visualized using a gel imaging system (Geldoc, Cleaver Gel Pro), and a molecular weight ladder (Promega) was used to determine the sizes of the amplified products (Figure 4).

**Table 11.** The primer sequences for detection of enzymes the ESBL confirmation

| **Target gene** | **Sequence** | **Amplicon Size(bp)** | **References** |
|---|---|---|---|
| ***bla-TEM*** | Forward: TTTCGTGTCGCCCTTATTCC<br>Reverse: ATCGTTGTCAGAAGTAAGTTGG | 403 | Seman et al., 2022 |
| ***bla-SHV*** | Forward: CGCCTGTGTATTATCTCCCT<br>Reverse: CGAGTAGTCCACCAGATCCT | 293 | Seman et al., 2022 |
| ***bla-CTX-M*** | Forward: AGACTGGGTGGCATTGAT | 676 | Murugan et al., 2018 |

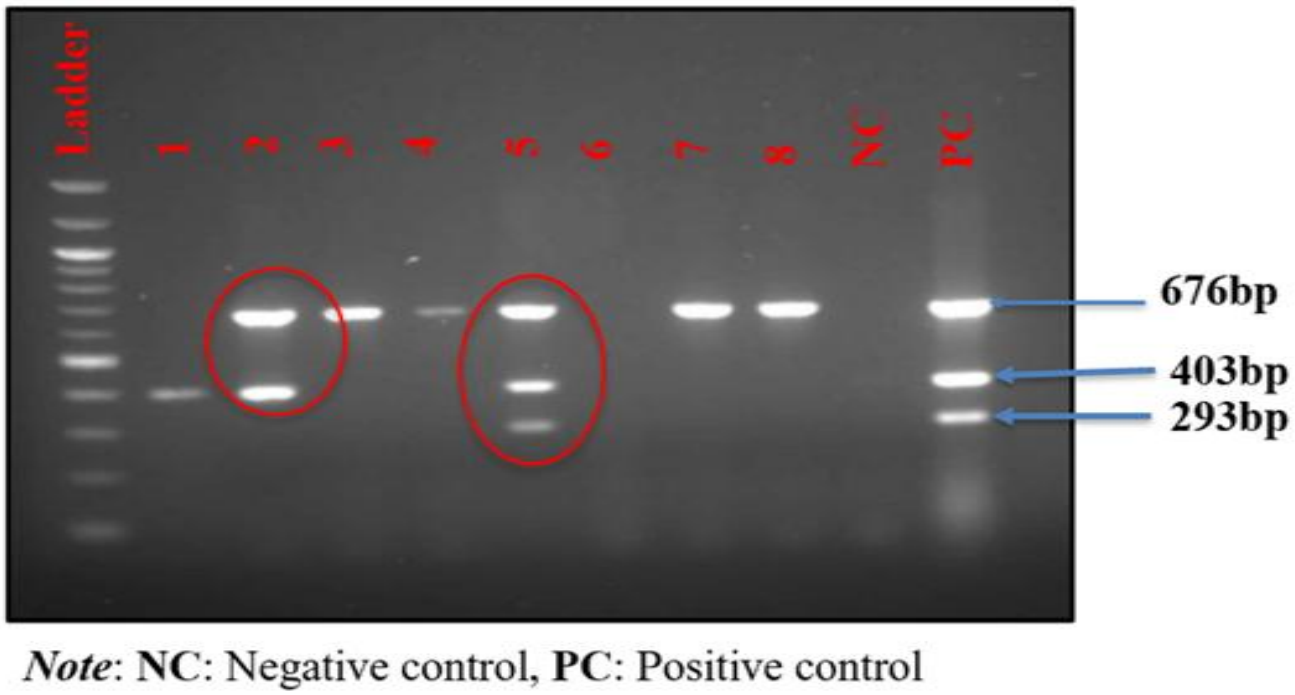


**Figure 4.** Gel electrophoresis of ESBL enzyme encoding genes among the STEC isolates. NC: Negative control, PC: Positive control.

The molecular characterization of carbapenemase genes was conducted by targeting four common carbapenemase genes: bla-OXA-48-like, bla-VIM, bla-KPC, and bla-NDM, following the recommendations of the CDC and other previous studies (CDC, 2024; Lutgring et al., 2018; Sabour et al., 2024). The characterization was performed using a TaqMan probe-based Real-Time PCR assay on the Applied Biosystems Fast 7500 Real-Time PCR System (Applied Biosystems, Inc., Foster City, CA). Two groups of multiplex PCRs were designed during assay development: 1) the bla-OXA-48-like and bla-VIM gene-targeting group, and 2) the bla-KPC and bla-NDM-targeting assay. In both assays, the 16S rRNA gene, a universal bacterial standard, was used as an endogenous control to validate the lysate and amplification in each reaction. The assay simultaneously detected bla-OXA-48-like (including multiple variants) and bla-VIM genes in a single reaction using lysates from Gram-negative bacteria. The sequences of primers and probes were indicated in Table 3.

For the PCR reaction, a 20 µL volume of reaction solution was prepared in which 2 µL of template DNA was added into 18 µL of master mix containing 10 µL of KiCqStart Probe qPCR Ready Mix, 5 µL of the "Master P (0.5 µL of primers for each target gene (20 µM) and 0.5 µL of each probe (10 µM))", and 3 µL of sterile reagent-grade I water. The thermal cycling conditions included an enzyme activation step at 95°C for 3 minutes, followed by 35 cycles at 95°C for 3 seconds for denaturation, then an annealing and extension step at 60°C for 30 seconds. The control setup for the bla-VIM and bla-OXA-48-like assay included *P. aeruginosa* AR Bank #0054 as a positive control for the bla-VIM gene and *K. pneumoniae* AR Bank #0039 as a positive control for the bla-OXA-48-like gene. Negative controls included bla-OXA-48-like-negative and bla-VIM-negative *K. pneumoniae* ATCC BAA-1706, along with a No Template Control (NTC), which consisted of sterile reagent-grade water to confirm the absence of contamination. The same procedure was followed for the detection of bla-KPC and bla-NDM genes. The results were interpreted based on Ct values between 10 and 30, and the 16S rRNA gene should be positive all the time to make the reaction valid (Figure 5).

**Table 12.**The primer and probe sequences for the carbapenemase confirmation.

| **Target genes** | **Sequence** | **Amplicon size (bp)** | **Reference** |
|---|---|---|---|
| bla-OXA-48-like | Forward: ACG GGC GAA CCA AGC AT | 538 | Lutgring et al., 2018 |
| | Reverse: GCG ATC AAG CTA TTG GGA ATT T | | |
| | Probe: FAM-TTA CCC GCA TCT ACC-BHQ1 | | |
| bla-VIM | Forward: GAA AAA CAC AGC GGC ACT TCT | 390 | Sabour et al., 2024 |
| | Reverse: CACGCGTTACRGGAAGTCCAA | | |
| | Probe: HEX-CGGAGATTGARAAGCA-BHQ1 | | |
| *16S* rRNA | Forward: TGGAGCATGTGGTTTAATTCGA | 232 | Lutgring et al., 2018 |
| | Reverse: TGCGGGACTTAACCCAACA | | |

| | | | |
|---|---|---|---|
| | Probe: CY5b-CACGAGCTGACGACARCCATGCA-BHQ3 | | |
| bla-KPC | Forward: GGCCGCCGTGCAATAC<br>Reverse: GCCGCCCAACTCCTTCA<br>Probe: FAM-TG ATA ACG CCG CCG CCA ATT TGT-BHQ1 | 399 | Rasheed et al., 2013<br>CDC, 2024 |
| bla-NDM | Forward: GAC CGC CCA GAT CCT CAA<br>Reverse: CGC GAC CGG CAG GTT<br>Probe: HEX-TG GAT CAA GCA GGA GAT-BHQ1 | 621 | Rasheed et al., 2013<br>CDC, 2024 |
| *16S* rRNA | Forward: TGG AGC ATG TGG TTT AAT TCG A<br>Reverse: TGC GGG ACT TAA CCC AAC A<br>Probe: CY5a -CA CGA GCT GAC GAC R C CAT GCA-BHQ3 | 232 | Rasheed et al., 2013<br>CDC, 2024 |

***Note**: R = Mixture of A and G at this position, not necessarily equimolar; CY5 b = An alternative fluorophore that can be used at this position is Quasar 670; CY5 a = An alternative fluorophore that can be used at this position is Quasar 670.*

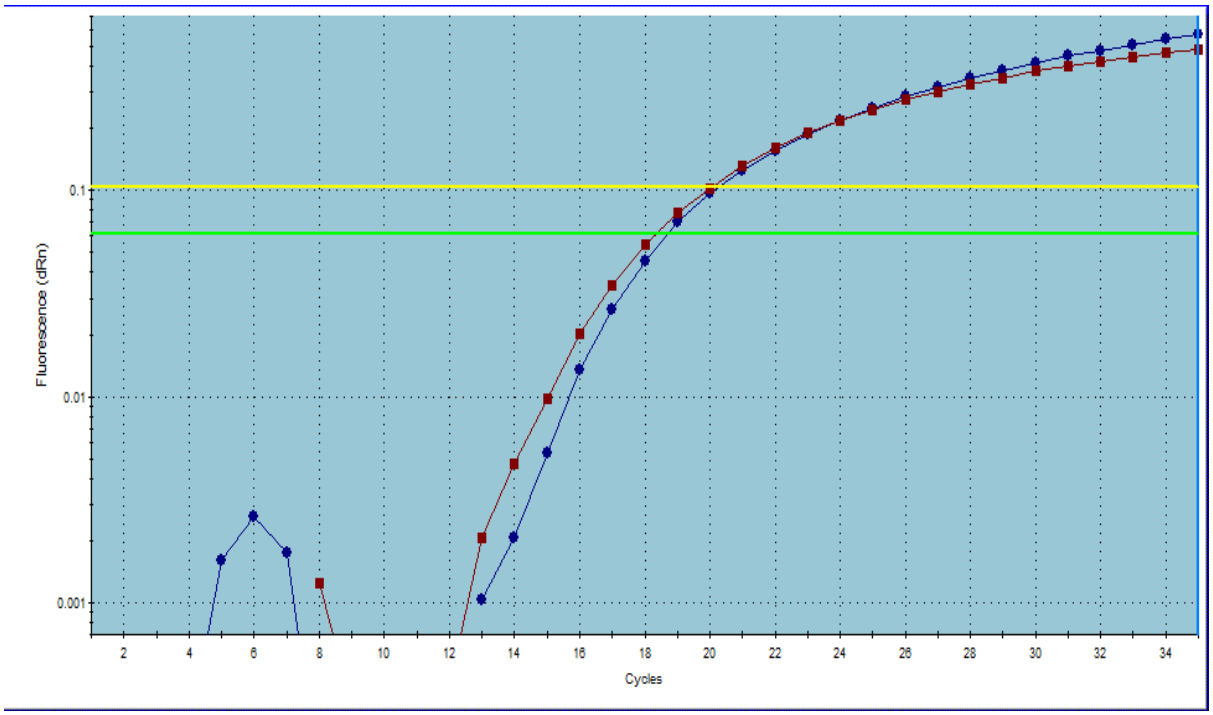


**Figure 5**. The amplification plot of the detection of *KPC-NDM* among the STEC isolates using the Real-time fluorescence technique.

## 4. Quality assurance

Positive and negative quality control tests are critical for monitoring the overall implementation of the laboratory diagnosis. These help for the development of accurate and reliable data for the study. This can be achieved using positive and negative quality checks. The known quality control organism required for each of the tests in the microbiology laboratory should be conducted accordingly. The following ATCC strains were used for the study of media and reagents according to the manufacturer's instruction.

*E. coli* ATCC 25922, *Proteus mirabilis* NCTC 10975, *S. enteritidis* ATCC® 14028, *Shigella flexneri* 12022, *E. coli* O157 ATCC 35150, *C. coli* ATCC 43478, *C. jejuni* ATCC 29411.

**Media Preparation, Handling & Disposal**

**Overview**

Growth medium is a solid or liquid substance designed to support the growth of microorganisms or cells. There are different types of media for growing different types of microorganisms or cells. Each medium requires a different set of preparation steps and reagents, and these steps must be conducted properly to be effective. Thus, it is very important to follow certain guidelines during media, and particularly selective supplement preparation to ensure media are properly made. This includes sterility during preparation of media, storage of reagents, and appropriate storage of finished media. Remember: A good medium is KEY in the success of your experiment.

**Tips on handling media**

- Powder to prepare media must be stored appropriately. Read the label of the medium and pay close attention to the storage conditions of each medium (i. e. check if it needs to be stored at 10-30°C, refrigeration temperature, freezer, dry environment etc.).
- Clean lab coats and gloves need to be worn during preparation of media, since contamination of media is a major concern that can produce false results.
- Use plastic weigh boats or paper for weighing media powder.

- Use measuring cylinder to accurately measure the amount of water used to prepare media.
- Use distilled or deionized (DI) water to prepare media. Do not use tap water.
- For mediums which do not require autoclaving (i.e., those that require just boiling), use sterile (autoclaved) distilled water.
- Always prepare larger volumes of media than what is needed (approx. 100 ml extra) to account for the loss of media volume during autoclaving and pouring or aliquoting.
- Autoclave gloves need to be worn when using the autoclave or when handling hot glassware to protect yourself from injury due to contact with hot objects.
- Allow at least a day before the experiment to make your media, unless the medium needs to be prepared on the day of experiments (e.g., MKTTn). Preparation of media takes time, do not rush, and pay attention to details to ensure all the components of the media are properly weighed, diluted, and processed.

The standard autoclave condition (unless otherwise stated) is 121°C at 15 psi for 15 minutes. If large volumes of media per container are autoclaved, the time of autoclaving may need to be increased. 121°C at 15 psi for 20 minutes is also acceptable, however longer autoclaving will result in media browning and changed nutritional composition, which may affect the outcomes of your experiments. To ensure that your media is sterile, use a control medium plate (i.e., incubate a non-inoculated plate or a tube of medium along with your samples to control for sterility).

**General Procedures for Preparing Media**

**Agar Petri Dishes**

**1.** Calculating Media Amounts:

a. Calculate the number of media needed:

i. A standard plate (100 mm x 15 mm) requires $\pm$15 mL of agar.

ii. If 100 plates are needed, then $\pm$1500 mL of agar is needed.

iii. Always make a larger volume of agar than what is needed (approx. 100 ml extra) to account for loss of media during autoclaving and pouring or aliquoting.

b. Follow the medium label instructions for preparation:

i. Instructions are typically written to make 1 L of medium or in some cases for 0.5 L medium. If a larger volume is needed, use a ratio equation to calculate the mass required for the desired number of media.

For example, when you need to prepare 400 mL of a medium that requires 30 g of base medium powder for 1 L of medium, calculate the required mass of base medium powder as follows:

30 g / 1000 mL = X g / 400 mL

X = (30 g * 400 mL) / 1000 mL

X = 12 g of base medium powder and 400 mL of DI water

**2.** Media Making Tips:

a) Always prepare media in a bottle with at least 1/3 empty space (e.g., prepare 700 mL medium in a 1000 mL bottle). This is particularly important when preparing agar media that foam and can overflow during autoclaving. By following these guidelines, you will avoid media loss and damage to your autoclave.

b) Label bottles with the type of media made, your first and last name initials and date prepared. Mark bottles using autoclave tape to verify that the autoclaving was conducted at sufficiently high temperature. For example, a bottle of Tryptic Soy Broth (TSVB) made by Jessie Vipham (JV) on May 15, 2019, should be labelled as follows: TSB 15/05/2019 JV

**3.** Preparing Media:

a) Pour a fraction of the water volume necessary into the bottle before putting in the media powder. This will prevent caking of powders on the bottom of the bottle. After powder is mixed with water, finish pouring the rest of the water into the bottle.

b) Make sure that media powder is fully dispersed in the water. This can be done by manually shaking the bottle, or by inserting a stir bar and mixing the media on a

stirring plate. Note: media with high quantity of powder will need to be mixed with a stirring plate; some media may also need to be boiled before autoclaving to prevent caking of the agar on the bottom of the bottle after autoclaving.

c) Boil or autoclave media using the instruction on the media label, to achieve sterility. Do not close the lid on the bottle too tight before autoclaving, to prevent pressure build-up during heating, which can break the bottle. Best practice is to completely close the lid and then open it for half a turn or one full turn. If media is boiled, watch your media constantly and do not let it boil over.

d) While the media is sterilizing, arrange petri dishes on the lab bench top. Depending on the quality of air in your lab, you may need to pour your media in the biosafety cabinet to avoid contamination from air. If the air quality is poor, pouring the media in proximity of the Bunsen burner will help, but will not completely control the contaminants. The best way to test the quality of air in your lab is to leave a nutrient-rich non-selective agar (e.g., BHI, TSA, NA) on the top of the bench for a few hours, with the lid open, and then incubate it at 35 °C for 24 – 48 h and examine the number of colonies.

e) Once the sterilization is completed, allow the media to cool to $\pm$50°C before plating. A good rule of thumb is to plate the media when the media is cool enough to be touched by gloved hands. Avoid shaking the medium prior to pouring into Petri dishes to prevent bubbles on top of the agar, which will make the agar suboptimal for streaking.

***Note:*** **do not overcool prepared agar as it WILL solidify! Check its temperature by touching the bottle regularly. If you need to keep your agar in the water bath, make sure that every bottle is dried, sprayed with 70% ethanol and wiped before pouring the medium. That will reduce the chance of microbial contamination from water bath.**

a) Pour the desired volume in each petri dish aseptically (approximately 15 mL per standard petri dish). Swirl the plate slightly to ensure the dispersion of agar. If the bubbles are created, you may remove them by flaming the surface of the agar plate, however, that may not be necessary.

b) Allow plates to cool on the countertop. Do not move the plates before they are completely solid, as this will disturb the polymerization of agar. When cooled,

invert plates so the lid is on the bottom. It is recommended to dry the plates for approximately 15 – 20 min in a biosafety cabinet before storing them in the fridge to reduce the moisture and prevent condensation during the cold storage.

c) Store plates upside-down (lid side on the bottom) in plastic bags in the refrigerator (-4 °C). Seal and label the bag.

**Broth**

1. Calculate the number of media needed:
    a. Check the protocol to see what volume you need.
    b. Always prepare an extra volume of agar than what is needed (approx. 100 ml extra) to account for loss of media during autoclaving, pouring or aliquoting.
2. Follow the package instructions for preparation:
    a. Instructions are typically written for 1 L or 0.5 L of media. If other volume is needed, use the ratio equation to calculate the grams needed (refer to Agar Petri Dish procedure for sample calculation).
3. Follow steps 3-7 from the Agar Petri Dish procedure.
4. While medium is sterilizing, arrange tubes in racks on the countertop or in the biosafety cabinet, as needed.
5. Once sterilization is completed, allow the media to cool.
6. Aseptically aliquot the desired volume of medium in each tube using a pipette or a dispenser. Place caps onto the tubes.
7. Store broths in the refrigerator to prolong the shelf life.

**Storage**

- Prepared media (agar and broths) that are not used immediately must be stored in the refrigerator. Antibiotic stock must be stored in the freezer.
- For quality purposes, do not use agars and broths that are stored more than three weeks in the refrigerator.

**Identifying contamination on prepared media**

It is best practice to inspect prepared media for contamination before using. Media can be contaminated due to several reasons such as a breach in sterility during plating/dispensing, improper storage of media etc. Following are signs that indicate your media are contaminated:

- Fungal growth on agar: fuzzy, filamentous or hairlike growths and visible.
- Bacterial growth on agar: presence of bacterial colonies on an un-inoculated agar.
- Color change of media (both agar and broth).
- Turbid broth: cloudy broth, substance floating in the broth.

****Contaminated media must not be used to conduct experiments and must be properly discarded (i.e., needs to be autoclaved before discarding). If you believe that a batch of plates may have been contaminated, place them in a 37°C incubator or a warm location overnight, then inspect for signs of contamination as described above. ****

**Disposal of inoculated or contaminated media**

Inoculated or contaminated media must be sterilized before they are disposed of or cleaned. These are the steps used to sterilize and dispose inoculated media in plastic petri dish and tubes:

1. Secure Petri dish or plastic tubes in a plastic autoclave bag. It is a best practice to "double bag" (i.e. put the autoclaved bag filled with media inside another empty autoclave bag) to avoid leakage or place the bag in a secondary container (a plastic container that will contain the liquid if your bag breaks).
2. Seal the bag using autoclave tape.
3. Sterilize at 121°C at 15 psi for 30 minutes.
4. Let cool and dispose the autoclave bag in a trash bin. Never pour liquid agar into the sink, as it will clog the drain.

**Disposal of inoculated media in glassware**:

1. Secure glass tubes in metal racks.
2. Mark glassware or racks with autoclave tape.
3. Ensure that all the lids are slightly open to allow for pressure release.

4. Sterilize at 121°C at 15 psi for 30 minutes.
5. Drain contents into the sink under running tap water. Wash glassware using detergent and rinse using distilled water. Never pour liquid agar into the sink, as it will clog the drain.
6. Let dry.

***Salmonella* spp. Media Preparation Guide**

**Selenite F broth**

Principle: Selenite F Broth is the medium used for the selective enrichment of *Salmonella* spp from both clinical and food samples. It is a buffered Lactose Peptone Broth to which Sodium Biselenite is added as the selective agent. Casein enzymic hydrolysate provides nitrogenous substances, carbon compounds and vitamin sources. Lactose is a carbohydrate source which maintains the pH of medium. Selenite is reduced by bacterial growth and alkali is produced. An increase in pH lessens the toxicity of the selenite and results in overgrowth of other bacteria. The acid produced by bacteria due to lactose fermentation serves to maintain a neutral pH. Sodium phosphate maintains a stable pH and lessens the toxicity of selenite. Sodium selenite inhibits many species of gram-positive and gram-negative bacteria including enterococci and coliforms. Do not incubate the broth longer than 24 hours as inhibitory effect of selenite decreases after 6-12 hours of incubation. It is used as a selective enrichment for the cultivation of *Salmonella* spp, an enrichment medium for the isolation of some species of *Shigella, and t*he broth is also recommended for the transport of strains of *Vibrio cholerae,* because these organisms can survive 2 to 5 days in Sodium Selenite Broth.

Preparation of Selenite F Broth

1. Add 4.0 gm sodium selenite powder to distilled/deionized water (500ml).
2. Add the remaining 19.0 gm powder to above solution and distilled/ deionized water and bring the total volume to 1.0 liter.
3. Gently heat and bring to boiling.
4. Dispense into sterile test tubes (10ml each)
5. Sterilize in boiling water bath or at 0 psi pressure at 100°C for 10 minutes.

6. **DO NOT AUTOCLAVE.**
7. Cool to room temperature prior to use.

**Xylose Lysine Deoxycholate (XLD)**

Type: Agar, Supplements: None

Suspend 56.68 grams of base media powder in 1000 ml sterile distilled water. Heat with frequent agitation until the medium boil. BE CAREFUL. Once the medium begins to boil, the bubbles will move up the flask very quickly, and the medium will boil over if not removed from heat. The bubbles should reach the upper part of the flask before quickly removing the medium from heat. If the medium appears grainy and translucent, the flask should be heated again. If the medium appears transparent and does not contain grainy agar crystals, the medium can begin cooling. DO NOT AUTOCLAVE OR OVERHEAT. Transfer immediately to a water bath at 50°C, if available (if not, cool down on a bench top). After cooling, pour into sterile Petri plates.

**Note:** It is recommended not to prepare large volumes that will require prolonged heating at once. Plan the preparation of this agar well ahead of time. There are many things that can go wrong with this agar and the instructions need to be followed exactly to achieve the desired quality of the medium. For example, if not heated as instructed, the solids in the agar will not completely dissolve, the agar will not solidify as expected and may fall out of the petri dishes when incubated.

**MacConkey Agar**

Suspend 49.53 grams of dehydrated medium in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well before pouring sterile Petri plates.

**STEC Media Preparation Guide**

**CHROMagarTM STEC**

Step1. Preparation of the base CHROMagarTM STEC base

- Disperse slowly 30.8 g of powder base in 1 L of purified water.
- Stir until agar is thickened.
- Heat and bring to boil (100 °C) while swirling or stirring regularly.

- DO NOT HEAT TO MORE THAN 100 °C.
- DO NOT AUTOCLAVE AT 121 °C.
- Warning 1: If using an autoclave, do so without pressure.
- Advice 1: For the 100 °C heating step, mixture may also be brought to a boil in a microwave oven: after initial boiling, remove from oven, str gently, then return to oven for short, repeated bursts of heating until complete fusion of the agar grains has taken place (large bubbles replacing foam).

• Cool in a water bath to 45-50 °C. Swirl or str gently to homogenize.

Step2. Preparation of the Supplement (S) and Mix of the prepared mix.

- Aseptically rehydrate ONE vial with 10 mL of sterile water. Use 1 vial rehydrated supplement per 1 liter of media. 5 liters, needs 5 vial supplements.
- Swirl well until complete dissolution.
- Add this rehydrated solution to the CHROMagarTM STEC base cooled at 45-50 °C.
- Swirl gently to homogenize.

Step 3. Pouring plate

- Pour into sterile Petri dishes.
- Let it solidify and dry

Storage:

- Store in the dark before use.
- Prepared media plates can be kept for one day at room temperature.

Advice 2: Plates can be stored for up to one month under refrigeration (2/8 °C) if properly prepared and protected from light and dehydration.

Advice 3: If not fully used, rehydrated CHROMagarTM STEC supplement can be stored for up to 2 months at 2/8 °C.

**Bacterial Isolates Storage Media Preparation**

**Trypticase Soy Broth plus 20% glycerol**

Trypticase soy broth (BD)---------- 30.0g

ETF (endotoxin-free) $H_2O$---------- 800.0 ml

Glycerol (Baker) ---------- 200.0 ml

1. Add water to dehydrated trypicase soy broth base; heat to dissolve the medium completely.
2. Add glycerol and dispense in 1.0ml aliquots in 12mm x 75 mm sterile, sealed tubes. Sterilize by autoclaving for 15min at 121$^{o}$c.
3. Store at 4$^{o}$C or -20$^{o}$C (long term) or at room temp until used. Storage conditions: Refrigerated, 4-10$^{o}$C (long-term) or room temp. Shelf life: 1 year

## Appendix XIII. List of Published Papers

**Publication 1**. Prevalence of Shiga toxin-producing *Escherichia coli, Salmonella,* and *Campylobacter* species among diarrheal patients from three major hospitals in Ethiopia. PLOS One. Prevalence of Shiga toxin-producing Escherichia coli, Salmonella, and Campylobacter species among diarrheal patients from three major hospitals in Ethiopia | PLOS Global Public Health.

**Publication 2**. Molecular Detection and Antibiotic Susceptibility Pattern of Shiga-toxin Producing *Escherichia coli* among Children with Diarrhea in Addis Ababa, Gondar, and Harar, Ethiopia. https://jommid.pasteur.ac.ir/article-1-729-en.html.

**Publication 3**. Epidemiological Insights into *Shigella* Infections in Ethiopia: A Study of Prevalence, Antimicrobial Resistance, and Associated Factors.