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DEPARTMENT OF VETERINARY MICROBIOLOGY, PARASITOLOGY AND
POULTRY HEALTH

MASTER OF SCIENCE IN ONE HEALTH

**ISOLATION AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF
CAMPYLOBACTER FROM DIARRHEIC HUMAN PATIENTS, POULTRY, AND
ENVIRONMENTAL SOURCES ALONG ASSESSMENT OF POULTRY
BIOSECURITY IN BISHOFTU, CENTRAL ETHIOPIA.**

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Study on Isolation and Antimicrobial Susceptibility Profile of Campylobacter from Diarrheic Human Patients, Poultry, And Environmental Sources along Assessment of Poultry Biosecurity in Bishoftu, Central Ethiopia.

The Thesis Submitted to Addis Ababa University College of Veterinary Medicine and Agriculture, in Partial Fulfillment of the Requirement for the Degree of Master of Science in One Health.

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CAMPYLOBACTER FROM DIARRHEIC HUMAN PATIENTS, POULTRY, AND
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STATEMENT OF THE AUTHOR

First, I declare that this thesis is my own work and is not submitted to any institution elsewhere for the grant of any academic degree, diploma or certificate and all sources of materials used for this Thesis have been properly acknowledged.

This Thesis has been submitted in partial fulfillment of the requirements for the M.Sc. degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University Library to be made available to borrowers under the rules of the library.

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LIST OF ABBREVIATIONS

AMR	Antimicrobial Resistance
CB	Cary-Blair
CDT	Cyto lethal Distending Toxin
CFU	Colony Forming Unit
CLSI	Clinical Laboratory Standard Institute
DALYs	Disability Adjusted Live Years
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
mCCDA	Modified Cefoperazone Charcoal Deoxy cholate Agar
MDR	Multi Drug Resistance
PCR	Polymerase Chain Reaction
PEP	Personal Protective Equipment
PH	Potential of Hydrogen
rRNA	ribosomal Ribonucleic Acid
WHO	World Health Organization
WOAH	World Organization for Animal Health
VBNC	Viable But Non-Culturable

ABSTRACT

Campylobacteriosis is one of the main causes of foodborne bacterial gastroenteritis worldwide, with poultry acting as the principal reservoir. Even though, it is actively circulating across the human, poultry, and environmental interface, with poor biosecurity practices the coordinated surveillance is lacked. Accordingly, the cross-sectional study was conducted in Bishoftu city, Ethiopia, from October 2025 to May 2026 to isolate and identify *Campylobacter* from diarrheic human patients, poultry, and environmental water sources, determine the antimicrobial resistance profiles of the isolates, and assess community biosecurity practices. In total, 380 samples were obtained, including 140 diarrheal samples, 180 chicken cloacal swabs, and 60 environmental water samples. Isolation was done on mCCDA under microaerophilic conditions at 42°C, and the isolates were then confirmed using biochemical tests. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method with 5% of defibrinated horse blood against twelve antimicrobial agents for twenty one isolates. Its overall prevalence is 10.78%, with source-specific prevalence's of 5% in human patients, 15.6% in chicken cloacal swabs, and 10.0% in environmental water samples. Important risk factors for human infection included direct contact with animals ($\chi^2 = 46.93$), age ($\chi^2 = 18.42$), irregular handwashing after latrine use, and eating undercooked meat, all having significant p value. At the farm level, the absence of rodent control ($\chi^2 = 16.62$) and the lack of regular cleaning and disinfection ($\chi^2 = 4.77$) with significant p-value were the strongest predictors of poultry colonization. Among 21 isolates tested for susceptibility, highest resistance were cephalothin, erythromycin, tetracycline, Cotrimoxazole, Amoxicillin-clavulanic acid and Ciprofloxacin with resistance profile of 100%, 71.4%, 71.4%, 66.7%, 61.9% and 42.9% respectively, whereas the highest susceptible rate of isolates were nalidixic acid (100%) and meropenem (76.2%) . MDR was identified in 85.7% (18/21) of isolates, with most showing resistance to four to five antimicrobial classes across three sampling source. These findings highlight the urgent need for integrated One Health interventions that include stronger farm biosecurity, prudent antimicrobial stewardship in animal production, and Implementing targeted community health education in the study area.

Keywords: *Antimicrobial resistance, Biosecurity, Campylobacter, Diarrheic patients, Multidrug resistance, One Health, Poultry.*

1. INTRODUCTION

1.1. Background

Food safety is a significant barrier to the socio-economic development of countries across the globe and has become a major concern due to the emergence of foodborne pathogens. Approximately 600 million people are infected with foodborne pathogens by consuming contaminated food, with approximately 420,000 deaths worldwide (Madilo *et al.*, 2023) which account for about 30% of deaths in children less than five years of age. Not only that foodborne illness has public health and economic significance but also curtails social life particularly in middle and low-income countries. Campylobacteriosis is one of the common causes of foodborne bacterial human gastroenteritis with an estimated 1 in 4 diarrheal diseases worldwide (WHO, 2020).

Campylobacter species are responsible for more than 95 million foodborne illnesses and over 21,000 deaths globally (Thomas *et al.*, 2020). They are a gram-negative, flagellated motile, non-spore forming, spirally curved bacteria, which colonizes the mucosal surfaces of the intestinal tracts, oral cavities and urogenital tracts of most warm blooded animals (Pal, 2017) and *Campylobacter jejuni* and *Campylobacter coli* are the most common cause of human bacterial gastroenteritis (Badjo *et al.*, 2024).

Different risk factors such as international travels, consumption of undercooked chicken, environmental exposure and direct contact with farm animals contribute to human Campylobacteriosis (Kaakoush *et al.*, 2015; Pal, 2017). *Campylobacter* is often found in water and soil and the results of studies revealed the potential of transmission of *Campylobacter* species between animals, humans and water sources (Deblais *et al.*, 2023). Several sources of *Campylobacter* in poultry farms such as insects, rodents, wild birds, and the environment around poultry farms has been reported (Hald *et al.*, 2016; Taha-Abdelaziz *et al.*, 2023).

Despite being widely colonized, *Campylobacter* is largely commensal in poultry and exists in high numbers without causing any harm on host (Ijaz *et al.*, 2018; Wagenaar *et al.*, 2023) and causes an acute self-limiting disease in human characterized by diarrhea, fever and abdominal cramps (Igwaran and Okoh, 2019).

Antibiotics have been widely used for treatment and prevention of diseases in humans and animals also as growth promotion in food animals (Maharjan *et al.*, 2020; Mdegela *et al.*, 2021). Antibiotics administered to food animals leave the residues in animal bodies that reach human bodies through the consumption of animal products. This creates an opportunity for selection of antibiotic resistance bacteria such as *Campylobacter* species. The incidence of drug resistant *Campylobacter* outbreaks has increased by 44% from 2013 to 2019 with drug resistant *Campylobacter* infection doubling in the last two decades (Poudel *et al.*, 2021).

Previous studies conducted regarding *Campylobacter* in Ethiopia have reported prevalence in the range of 6.04%-68.8% in humans (Kebede *et al.*, 2017; Deblais *et al.*, 2023), 7.1%-99.5% in food animals (Chala *et al.*, 2021; Deblais *et al.*, 2023), 4.1%-21.6% in food of animal origin (Dadi and Asrat, 2009; Debelo *et al.*, 2022) and 10.5%- 43.2% in water (Chala *et al.*, 2021; Deblais *et al.*, 2023). In addition, the studies conducted on the antimicrobial susceptibility profile of *Campylobacter* species in humans and animals have identified the occurrence of multidrug resistant *Campylobacter* species (Chala *et al.*, 2021; Behailu *et al.*, 2022). Although several studies in Ethiopia have documented the prevalence and antimicrobial resistance patterns of *Campylobacter* in humans, food animals, foods of animal origin, and environmental samples, these investigations were conducted separately within individual reservoirs. Consequently, there is limited evidence on the epidemiological linkage and potential transmission dynamics of *Campylobacter* among chickens, humans, and the farm environment. Furthermore, the role of poultry as a source of environmental contamination and human exposure has not been comprehensively investigated using a One Health approach. Therefore, the source attribution, circulation patterns, and transmission interfaces of antimicrobial-resistant *Campylobacter* strains among chickens, humans, and environmental compartments remain poorly understood in Ethiopia.

1.2. Statement of the problem

As indicated in the previous studies, the incidence and prevalence of *Campylobacter* infection had increased over the last 10 years, and was expected to challenge global health in the future (Behailu *et al.*, 2022). *Campylobacter* diarrheal disease among children is a huge problem in Ethiopia (Abay *et al.*, 2024). The previous studies conducted in Ethiopia focused on humans, food animals and foods of animal origin. The role of different environments such as water source, and interaction among humans, animals and environment as potential risk factors and sources of transmission of *Campylobacter* to humans and animals has not been explored.

There is also limited work conducted regarding the risk factors associated with human and poultry infection with *Campylobacter* species in Ethiopia. Previous studies revealed that the biosecurity measure implementation practice at chicken farms was poor and several chicken farms were located in nearby residential areas (Ismael *et al.*, 2021) and this poses a biosecurity risk as well as public health problems. Poor biosecurity practices contribute to the easy access of *Campylobacter* to the farm.

Previous studies conducted revealed that the level of poor food safety practices was remarkably high in Ethiopia (Tamiru *et al.*, 2022). Consumption of contaminated food due to poor food safety practices are the main sources and contribute to high prevalence of human *Campylobacter* infection. On the other hand, Poultry producers utilize many antimicrobials by mixing with feed and water resulting in high prevalence of antimicrobial resistance in Poultry (Mdegela *et al.*, 2021). Since poultry are the main reservoir for *Campylobacter* species the information on antimicrobial resistance from Poultry and humans in Bishoftu town and around is significant. Moreover, updated information is needed on the sources of *Campylobacter* infection, contribution of the environment like water, disease prevalence and antibiotic resistance profile in the study area.

1.3. Research question

The study was guided by the following four core research questions:

- ✓ What is the prevalence of *Campylobacter* from human, poultry, and environmental sources in the selected study area?
- ✓ Are there associations between poor biosecurity practice with the prevalence of the *Campylobacter* in the poultry farms and diarrheic human patients?
- ✓ Are there antimicrobial resistant *Campylobacter* in the samples from chicken, human and water sources?

1.4. Significance of the study

This study holds substantial scientific and public health significance as it is the one health oriented investigation in the study area, to simultaneously examine *Campylobacter* across human diarrheic patients, poultry and environmental water sources within a unified framework. Unlike previous Ethiopian studies that examined individual host populations in isolation, this research establishes cross domain transmission linkages, identifies modifiable risk factors at both the human and farm levels, and characterizes the antimicrobials resistance profile of isolates from all three sources simultaneously. The finding that 85% of the isolates exhibited multi drug resistance, these findings provide the first localized evidence base needed to design integrated, multi sectoral interventions targeting poultry biosecurity, community health educations, rational antimicrobials use in the animal production especially poultry productions, and revision of empirical treatment protocols for diarrheal disease in the study area and similar peri urban Ethiopian setting.

1.5. Objectives of the Study

General objective

Isolation and Antimicrobial susceptibility profile of Campylobacter from diarrheic human patients, poultry, and environmental sources along assessments of community biosecurity in Bishoftu, central Ethiopia, using one health framework.

Specific objectives

- To isolate and identify Campylobacter from diarrheic human patients, chicken and environmental samples.
- To assess the poultry Biosecurity across poultry farms owners
- To assess antimicrobial resistance profile of isolated Campylobacter.

2. LITERATURE REVIEW

2.1. Description of *Campylobacter species*

The name *Campylobacter* is derived from the Greek ‘campylos’ meaning ‘curved’ and ‘baktron’ meaning ‘rod’. *Campylobacter* species are small (0.2-0.9µm wide and 0.2-5µm long) gram-negative microorganisms, non-spore-forming a spirally curved shape (**Figure 1**), which colonizes the mucosal surfaces of the intestinal tracts, oral cavities, or urogenital tracts of most warm-blooded animals. Thirty-two species and 13 subspecies of the bacteria were identified. The majority of the *Campylobacter* species except *C.gracilis*, *C. hominis*, *C. ureolyticus*, and *C. blaseri* which are non-motile are mobile of spiral movement caused by a polar flagellum present on one or both ends of the cell except *C. showae* that has multiple flagella (Gilbert *et al.*, 2018, Kaakoush *et al.*, 2015, Facciola *et al.*, 2017).

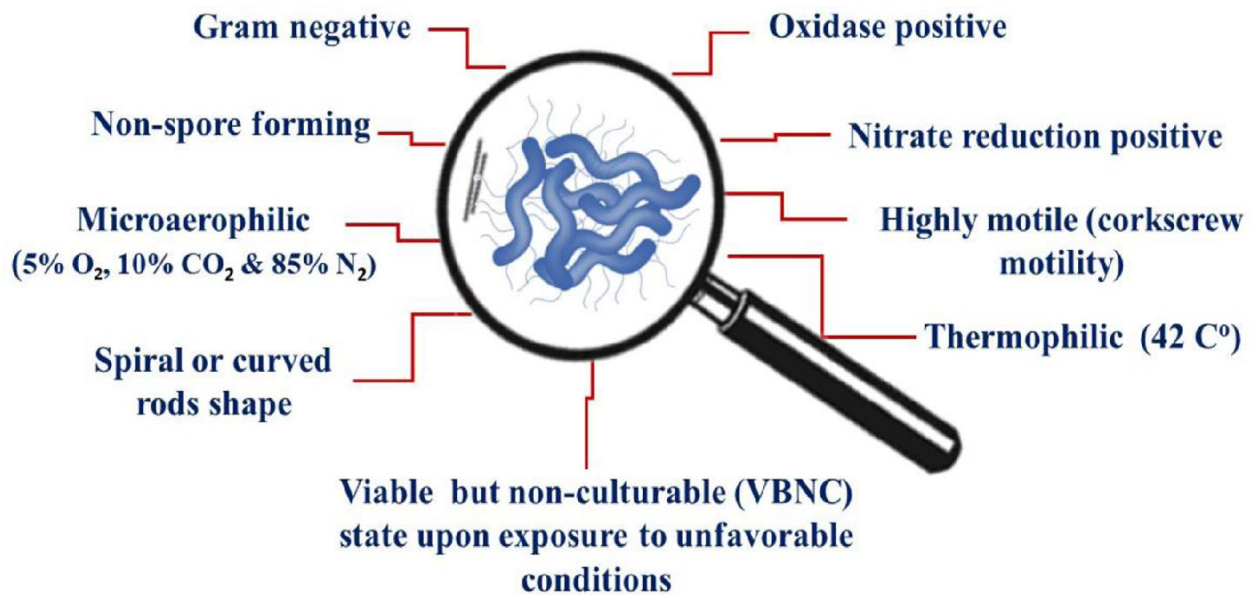


Figure 1: The morphological, biochemical and physiological characteristics of *Campylobacter* species.

Source: El-Saadony *et al.*, 2023

2.2. Growth and survival characteristics of *Campylobacter species*

Campylobacter species can grow at pH between 6.5 and 7.5 and temperature ranging from 37°C to 42°C. They are unable to grow above 55°C and below 30°C with optimum growth temperature of 40-42°C which makes them thermophilic (Chlebicz and Śliżewska, 2018). The high optimum growth temperature (42°C) distinguishes the thermophilic *C. jejuni* from most other *Campylobacter* species which is due to the absence of cold shock protein genes that play a role in low-temperature adaptation (Keto-Timonen *et al.*, 2016; Hakeem and Lu, 2021). The bacterium grows well at an optimal water activity of 0.997 and does not grow with water activity less than 0.987. The majority of *Campylobacter* species are microaerophilic as they have the ability to survive and grow in an environment with low oxygen tension containing nearly 10% of CO₂, 5% of O₂ and 85% of N₂ (Facciola *et al.*, 2017).

2.3. Risk factors associated with *Campylobacteriosis*

A number of risk factors contribute to exposure of humans to *Campylobacteriosis*. International travel is the most important risk factor followed by consumption of undercooked chicken, environmental exposure and direct contact with farm animals. Travel related infections are associated with consumption of contaminated meat and water (Kaakoush *et al.*, 2015). Contaminated animal products, especially poultry meat and unhygienic handling of food items, pose a human risk factor for sporadic *Campylobacter* infections. Unhygienic slaughtering procedures and poor handling of meat and its products may provide an avenue for human contamination in abattoirs. The enteric nature of *Campylobacter species* constitutes the risk of cross-contamination from the fecal matter of different animals during flaying and evisceration (Too *et al.*, 2023).

Animal contact is a major risk factor for acquiring human *Campylobacteriosis*, and this is due to occupational exposure to animals in a slaughterhouse, pet shop, farm, or zoo. Wild birds are considered notable reservoirs of *Campylobacter* and often contaminate the environment through fecal droppings. Children are at risk of ingesting *Campylobacter* in the open playgrounds because of exposure to contaminated fecal matter (Osborne *et al.*, 2016; Fravalo *et al.*, 2021).

Water is an effective vehicle for transmission of *Campylobacter species* to humans and animals and contaminated water has been responsible for a number of outbreaks in different countries. Water bodies such as lakes and rivers have been associated with Campylobacteriosis because of contamination with animal feces, draining of sewage effluent, discharge from slaughterhouses, and slurry that is used in agricultural farms. Campylobacter can remain infective in water for over 120 days (Kaakoush *et al.*, 2015; Too *et al.*, 2023). Szczepanska *et al.*, 2017 reported contaminated surface water as a potential reservoir of Campylobacter, with 16.8% of total and 17.5% of river water samples testing positive for Campylobacter in Poland. Similarly, study conducted in Addis Ababa revealed 22.5% and 8.3% prevalence of *Campylobacter species* in surface water and municipal tap water respectively (Chala *et al.*, 2021)

Campylobacter infections are predominantly common in children less than five and elderly people above seventy-five years and immunocompromised peoples are at the higher risk. Children are found to be more susceptible to acquiring Campylobacteriosis through animal contact during play (Levesque *et al.*, 2013). Chronic gastrointestinal disease conditions, gastric ulcers, celiac disease, liver disease, asthma, or diabetes have been predisposing factors for Campylobacteriosis (Fravalo *et al.*, 2021).

2.4. Sources of infection and transmission

Faecal-oral route is the principal mode of transmission for *Campylobacter species* and humans get the infection by consumption of unpasteurized milk, untreated water, fresh chicken, and uncooked meat of cattle, pig and sheep, which are contaminated with the organisms. Close contact with diseased animals and their discharges can also transmit the infection. Clinically healthy animals may also be a source of infection. Swimming in natural resources of water may pose a risk to Campylobacter infection. Humans may act as vectors by transferring the organism into poultry production areas with contaminated clothing and foot wear. Poultry is implicated in 50%-70% of human Campylobacter infections. Ingestion of contaminated poultry is the primary mode of infection in developed parts of the world while ingestion of contaminated water is commonly responsible for Campylobacter infections

in developing parts of the world (Kaakoush *et al.*, 2015, Pal, 2017). The usual route of Campylobacter transmission in animals is horizontal transmission through ingestion of Campylobacter with contaminated water, feed, soil and fomites (Little *et al.*, 2010). Flies play a crucial role in the transmission of Campylobacter infection from contaminated sources (Kreling *et al.*, 2020).

Various possible sources of Campylobacter in poultry farms such as insects, rodents, wild birds, other livestock farms, the environment around and within poultry farms like soil, puddle and water sources and residual Campylobacter population from previous flock has been reported (Hald *et al.*, 2016; Taha-Abdelaziz *et al.*, 2023).

2.5. Campylobacter Diagnosis and Species Identification Methods

2.5.1. Specimen collection

Appropriate specimen types for Campylobacter testing are liquid or semi-soft stool and rectal swabs. Campylobacter may also be recovered from specimens such as blood and tissue, mainly liver for *C.lari*. Specimens should be collected during the acute phase of the diarrheal illness before antibiotic treatment is initiated. Urine-free stool and swabs should be collected in a sterile, airtight container containing modified Cary-Blair (CB) transport medium (Too *et al.*, 2023). In poultry, Campylobacter can be isolated from fresh feces or cloacal swabs and for detection of Campylobacter by culture freshly voided faces should be collected (WOAH, 2024).

2.5.2. Isolation and identification Methods,

The conventional method for isolating the common enteric Campylobacter species from faecal samples is primary plating on selective media followed by incubation at 42°C in a micro aerobic atmosphere. Though feces often contain large numbers of viable Campylobacter making their detection easily possible by direct plating on selective media, food products and environmental samples tend to have fewer numbers of stressed

Campylobacter cells, thus, an enrichment step in liquid medium before plating on solid agar plates is indicated. Enrichment broths like Bolton broth, Campylobacter enrichment broth and Preston broth are available to be used before plating (Natsos *et al.*, 2019).

Microscopic observation of direct smear or wet preparation, in the case of liquid faeces, may reveal the presence of curved rods characteristic of Campylobacters. Dark field microscopy may also reveal the morphology and the darting motility of Campylobacter species. Moreover, the direct Gram-stain with carbol-fuchsin counterstain method, though underutilized, may provide a presumptive result within 30 minutes of receipt of a fecal sample in the laboratory with relatively high sensitivity and at low cost (WOAH, 2024). Culturing steps of Campylobacter species follow these steps, after 48 hours' incubation enriched from field collected sample, a loopful of culture from each sample was inoculated onto modified Cefoperazone Charcoal Deoxycholate Agar, the mostly recommended and selective media for isolation of Campylobacter species. The inoculated plates were incubated under a microaerophilic environment provided by gas generating sachets containing 5% O₂, 10% CO₂ and 85% N₂ and incubated at 37° C and 42° C for 48 hours.

After incubation for 48 hours, all presumptive Campylobacter colonies were selected from each petri dish and checked for Gram staining (morphological characteristics), catalase, oxidase, hippurate test, nitrate test and motility as previously described by (WOAH, 2024). Then, suspected colonies were sub-cultured onto Columbia blood agar with 5% (v/v) sheep blood or Brain heart infusion agar 5% (v/v) sheep blood and incubated as described above. Colonies with slightly pink, round, convex, smooth and shiny, with a regular edge were preserved (WOAH, 2024). Further investigation of Campylobacter *species* level by molecular confirmation conducted from preserved suspected colony in -80oc in Brain heart infusion Broth with 20% glycerols.

2.6. Public Health Concern of Campylobacteriosis

Campylobacteriosis has long been recognized among the most common responsible causes of enteritis and gastroenteritis in humans both in adults and pediatric patients. Pathogenic Campylobacter species known to be implicated in human infections are *C. jejuni*, *C. concisus*, *C. hyointestinalis*, *C. insulaenigrae*, *C. sputorum*, *C. helveticus*, *C. rectus*, *C. lari*, *C. fetus*, *C. mucosalis*, *C. coli*, *C. upsaliensis* and *C. ureolyticus* (Heredia and García, 2018). These pathogenic Campylobacter species are grouped into major human enteric pathogens (*C. jejuni*, *C. jejuni subsp. jejuni*, *C. jejuni subsp. doley*, *C. coli* and *C. fetus*) and minor enteric pathogens (*C. concisus*, *C. upsaliensis*, *C. lari* and *C. hyointestinalis*) (Igwaran and Okoh, 2019).

Campylobacter species, particularly Campylobacter *jejuni* and Campylobacter *coli* are the most common of human bacterial gastroenteritis in the developed world and are responsible for over 95% of human Campylobacteriosis (Badjo *et al.*, 2024). The major infection caused by Campylobacter is acute diarrhea, which can be triggered by an ingested dose of a few as 500-800 CFU from the bacterial cells. However, the ingestion of 100 bacterial cells or less could also correlate with human infection (Al-Khresieh *et al.*, 2022).

Campylobacter species are significant zoonotic causes of bacterial food-borne infection and farm animals are the major reservoir of Campylobacter species (De Vries *et al.*, 2018). Campylobacter foodborne infection is a problem and an economic burden to the human population which causes about 8.4% of the global diarrhea cases. Campylobacter foodborne infection is a global concern because of the emerging Campylobacter species involved in both human infections and Campylobacter foodborne outbreaks. It is estimated that Campylobacter are responsible for more than 95 million foodborne illnesses and over 21,000 deaths globally (Thomas *et al.*, 2020). According to the reports of WHO, Africa have the highest burden of foodborne diseases per capita, with a median of 2455 foodborne Disability Adjusted Life years (DALYs) per 100,000 inhabitants of which 5.7% attributed to Campylobacter *species* (Havelaar *et al.*, 2015; WHO, 2015).

In developed countries, cases of Campylobacteriosis have exceeded those caused by other enteric bacteria in recent years. The Campylobacter species are isolated from patients with infections of the alimentary tract with a frequency of about 3-4 times higher than in *Salmonella* or *Escherichia coli* (EFSA, 2015). In developing countries where Campylobacter is endemic, infection is usually limited to children, with infection ratios decreasing with age, suggesting that early exposure may lead to the development of protective immunity (Kaakoush *et al.*, 2015).

2.7. Campylobacteriosis in Poultry

Campylobacter can multiply in the gut epithelium of almost all warm-blooded host species. Poultry is the predominant natural host for Campylobacter species possibly due to their elevated body temperature (Barker *et al.*, 2020) and the pathogen colonizes the poultry gut in high numbers without causing any disease or loss in production with greater risk in commercial chicken (Ijaz *et al.*, 2018; Wagenaar *et al.*, 2023). Campylobacter species is a commensal in broiler chickens with multiplication up to 10^{10} colony-forming units (CFU) per gram of droppings. *C. jejuni* colonizes the chicken cecum with relatively high numbers and more recently has been demonstrated to be pathogenic to chicken causing prolonged inflammation, damage of the gut, and diarrhea depending on the genetics of the host and the strain of infection (Ijaz *et al.*, 2018). Campylobacter *jejuni* is the most commonly isolated species from the confirmed cases of poultry Campylobacteriosis followed mainly by *C. coli* and the other species including Campylobacter *lari*, *fetus*, and *upsaliensis* that have been reported to cause infection in both humans and poultry (Facciola *et al.*, 2017; Indykiewicz *et al.*, 2021).

In poultry flocks, natural colonization of chicks occurs within 2 – 3 weeks of hatching via horizontal contamination from the environment and other birds and birds typically remain colonized for life. Since domestic and wild birds are the microorganism's primary reservoir, they mostly carry up to 10^9 CFU Campylobacter per gram of cecal contents. The microorganism can then spread from the intestines of poultry to meat during processing (Johnson *et al.*, 2017). Ingestion of 35CFU of Campylobacter was sufficient to induce multiplication in chicken tissues (Urdaneta *et al.*, 2023).

The organism is highly infectious and in each colonized flock up to 100% of birds can be Campylobacter-positive and significant proportions of broilers produced worldwide are colonized with these organisms (Vandeplas *et al.*, 2010; Wagenaar *et al.*, 2023). Campylobacter colonizes the gut of broiler chicken and can be transferred to the carcass during slaughter processing and spread further to retail chicken meat. Cross-contamination arising from poor hygienic handling of raw chicken is considered the leading risk factor impacting human exposure to Campylobacter (Asuming-Bediako *et al.*, 2019)

2.8. The Role of the Environment as Reservoir of Campylobacter

Campylobacter is often found in the environment, including water sources and soil, where it usually indicates recent fecal contamination from animals, sewage, or agricultural run-off. The results of previous studies revealed the potential transmission of Campylobacter species between animals, humans and water sources (Chala *et al.*, 2021, Deblais *et al.*, 2023). The capability of Campylobacter to live outside the host and adapt to different environmental circumstances demonstrate show Campylobacter can survive via the food production chain. Campylobacter infection is often endemic in areas with untreated drinking-water, poor sanitation and close contact with animals and environmental sources (Firleyanti *et al.*, 2016, Habib *et al.*, 2023). Campylobacter *jejuni* can survive for up to 6 days in chicken droppings after excretion, making them a potential source of transmission to the environment, particularly when manure is used as a fertilizer (Coorey *et al.*, 2018).

The presence of Campylobacter in many animal species not only contributes to the prevalence of food-to-human transmission, but also environment-to-human transmission due to the abundance of agricultural contaminants in the environment (Johnson *et al.*, 2017). The bacteria have the ability to survive in the agricultural environments and can occur in soil, dust, air and in abiotic surfaces and transmission through organic fertilizer is considered (Kreling *et al.*, 2020).

2.9. Antimicrobial Susceptibility of *Campylobacter*

Antibiotics have been widely used for treatment and prevention of diseases in humans and animals also as growth promotion in food animals (Maharjan *et al.*, 2020; Mdegela *et al.*, 2021). Antibiotics administered to food animals leave the residues in animal bodies that reach human bodies through the consumption of animal products. This creates an opportunity for selection of antibiotic resistance bacteria such as *Campylobacter*. Antibiotic resistant bacteria could result in severe infections that result in disability and death mostly in young children, cancer patients and those who are older than 60 years (Murray *et al.*, 2022). About 700,000 deaths have been reported to occur each year due to antibiotic resistance bacterial infection worldwide (Aljeldah, 2022) with prevalence of antibiotic resistance in foodborne bacteria reported to range from 21%-100% (Hassani *et al.*, 2022).

Worldwide rapid increase in the proportion of *Campylobacter* strains resistant to antimicrobial agents has evolved (Mughal, 2018). As antibiotic resistance becomes increasingly prevalent in *Campylobacter*, the need for novel antimicrobial strategies to reduce *Campylobacter* in poultry and poultry products becomes more critical. This is primarily driven by the need to reduce the economic and human health burden incurred by antibiotic-resistant *Campylobacteriosis* (Johnson *et al.*, 2017).

Investigation of antibiotic susceptibility of *Campylobacter* species showed an increase in the rates of resistance to Ciprofloxacin (35.7%) and tetracycline (21.4%) (Abay *et al.*, 2024). Worku *et al.*, 2024 conducted study of antibiotic susceptibility on isolates from diarrheic children and reported the resistance levels of *C. jejuni* to ciprofloxacin, erythromycin, and tetracycline to be 22.70%, 4.50%, and 27.30% respectively. The study of antimicrobial resistance profile in food animals in Gambella region of Ethiopia indicated the rate of resistance of *C. jejuni* to ampicillin, chloramphenicol, clindamycin, gentamicin, ciprofloxacin, norfloxacin, tetracycline, cephalotin, nalidixic acid and erythromycin were 46.6%, 61.5%, 54.6%, 50%, 80.5%, 64.9%, 82.2%, 100%, 3.8 and 60.3%, respectively. Similarly, the rate of resistance of *C. coli* to ciprofloxacin and cephalotin were 29.6% and 100% respectively (Abamecha *et al.*, 2015). A study conducted on an antimicrobial

resistance profile in Jimma town on cattle investigated showed that 84.2% of the isolates showed multi-drug resistance (against three-to-six drug classes) (Debelo *et al.*, 2022). Multidrug resistant *Campylobacter* species have also been reported from the United States (Poudel *et al.*, 2021), Ghana (Karikari *et al.*, 2017) and China (Yang *et al.*, 2023).

2.10. The Status of *Campylobacter* in Ethiopia

In Ethiopia, there are only few reports from different parts of the country on the prevalence of *Campylobacter* species isolated from humans (Asrat *et al.*, 1997; Mitike *et al.*, 2009; Lengerh *et al.*, 2013; Beyene and Haile amlak, 2014; Mulatu *et al.*, 2014; Kebede *et al.*, 2017; Behailu *et al.*, 2022), animals (Kassa *et al.*, 2006; Woldemariam *et al.*, 2010; Chanyalew *et al.*, 2013; Abamecha *et al.*, 2015) and animal source foods (Dadi and Asrat, 2009; Ababa, 2015; Eshetu *et al.*, 2023). The previous author concentrated on a single component of the triad. Only a few studies used one health approach by assessing the prevalence of *Campylobacter* species in humans, animals, animal source foods and the environment such as water sources and equipment used for food processing (Chala *et al.*, 2021; Debelo *et al.*, 2022). That means, although those studies provided evidence on the occurrence and importance of *Campylobacter* species in Ethiopia, linkage between the human, animals and the environment is not explored. As a result, it is difficult to pinpoint if *Campylobacter* species are transmitted between animals, the environment and humans.

Those previous studies from different parts of the country revealed that the prevalence of *Campylobacter* species ranges from 6.04% -68.8% in human ((Kebede *et al.*, 2017; Deblais *et al.*, 2023). The study on the human *Campylobacter* prevalence conducted in 1992 on diarrheic patients at Tikur Anbessa Hospital reported the prevalence of 10.8% from a total 630 examined patients (Asrat *et al.*, 1997). Similarly, the study conducted at Dembia district, Hawasa, Jimma and Gondor areas on diarrheic children reported the prevalence of *Campylobacter* at 10.5%, 6.8%, 11.6% and 15.4% respectively (**Table 1**). Different reports indicated that *C. jejuni* and *C. coli* were predominately isolated *Campylobacter* species from human stool samples in Ethiopia (Asrat *et al.*, 1997; Chala *et al.*, 2021; Debelo *et al.*, 2022).

Table 1: Prevalence's of Campylobacter species in human patients from different parts of Ethiopia.

Study area	Sample origin	Number of positive (%)	<i>C.jejuni</i>	<i>C.coli</i>	unidentified	References
Akaki Kality	Human	10(10.1%)	5(50%)	1(10%)	4(40%)	(Chala <i>et al.</i> , 2021)
Jimma	Human	50(11.6%)	-	-	50(100%)	(Beyene and Haileamlak, 2014)
Dembia district	Human	16(10.5%)	-	-	16(100%)	(Mitike <i>et al.</i> , 2009)
Tikur Anbessa H	Human	68(10.8%)	56(82.4%)	12(17.6 %)	-	(Asrat <i>et al.</i> , 1997)
Hawasa	Human	16(6.8%)	-	-	16(100%)	(Behailu <i>et al.</i> , 2022)
Hawasa	Human	20(12.7%)	-	-	20(100%)	(Mulatu <i>et al.</i> , 2014)
Hawasa	Human	13(6.04%)	-	-	13(100%)	(Kebede <i>et al.</i> , 2017)
Gondor	Human	44(15.4%)	40(90.9%)		4(9.1%)	(Lengerh <i>et al.</i> , 2013)
Bahir Dar	Human	17(8%)	16(94.1%)	1(5.9%)	-	(Ewnetu and Mihret, 2010)
Mekele	Human	14(6.5%)	12(85.7%)		2(14.3%)	(Abay <i>et al.</i> , 2024)
Haramaya	Human	933(68.8%)	-	-	933(100%)	(Deblais <i>et al.</i> , 2023)
Bahir Dar	Human	30(7%)	22(73.3%)	8(26.7 %)	-	(Worku <i>et al.</i> , 2024)

The study conducted in the Nuer zone of Gambella in 2015 on 368 various food animals (cattle, sheep, chicken and goats) to isolate and identify Campylobacter species revealed 208 (56.5%) prevalence in fecal samples. The highest prevalence was recorded in chickens (86.6%), followed by cattle (48%), sheep (39%) and goats (33.3%) (Abamecha *et al.*, 2015). Similarly, the study conducted in urban and rural farm animal settings in Jimma on various

species of food animals isolated *Campylobacter* species from 192 (39.6%) out of 485 fecal samples with the highest prevalence recorded in chickens (68.1%) followed by pigs (50%), sheep (38%) and cattle (12.7%) as reported by Kassa et al (2006) (**Table 2**).

Similar study conducted in Addis Ababa to determine prevalence of thermotolerant *Campylobacter* strains in retail raw meat products, revealed the prevalence *Campylobacter* species to be 50 (9.3%) out of 540 meat samples with the highest prevalence (21.7%) recorded in chicken meat, followed by sheep meat (10.5%), pork meat (8.5%), goat meat (7.6%) and beef (6.2%) (Dadi and Asrat, 2009).

In Addis Ababa, the study conducted using One Health approach revealed the highest prevalence of *Campylobacter* species in surface water samples (22.5%), followed by cattle feces (18.5%), poultry cloacal swabs (13.0%) and Humans (10.1%); with the lowest prevalence in municipal tap water (8.3%) and goat feces (7.1%) (Chala *et al.*, 2021). In general, data from different parts of the country indicated that *Campylobacter* species are prevalent in humans, different animal species, and animal products as well as their living environments. In Ethiopia, studies conducted on human, food animals, animal source food and environment (water and soil) indicated the pooled prevalence of *Campylobacter* to be higher in food animals (48%) than in the environment (37.2%) and humans (27%)(**Table 2**)

Table 2: Prevalence's of Campylobacter species in food animals and their environment in Ethiopia.

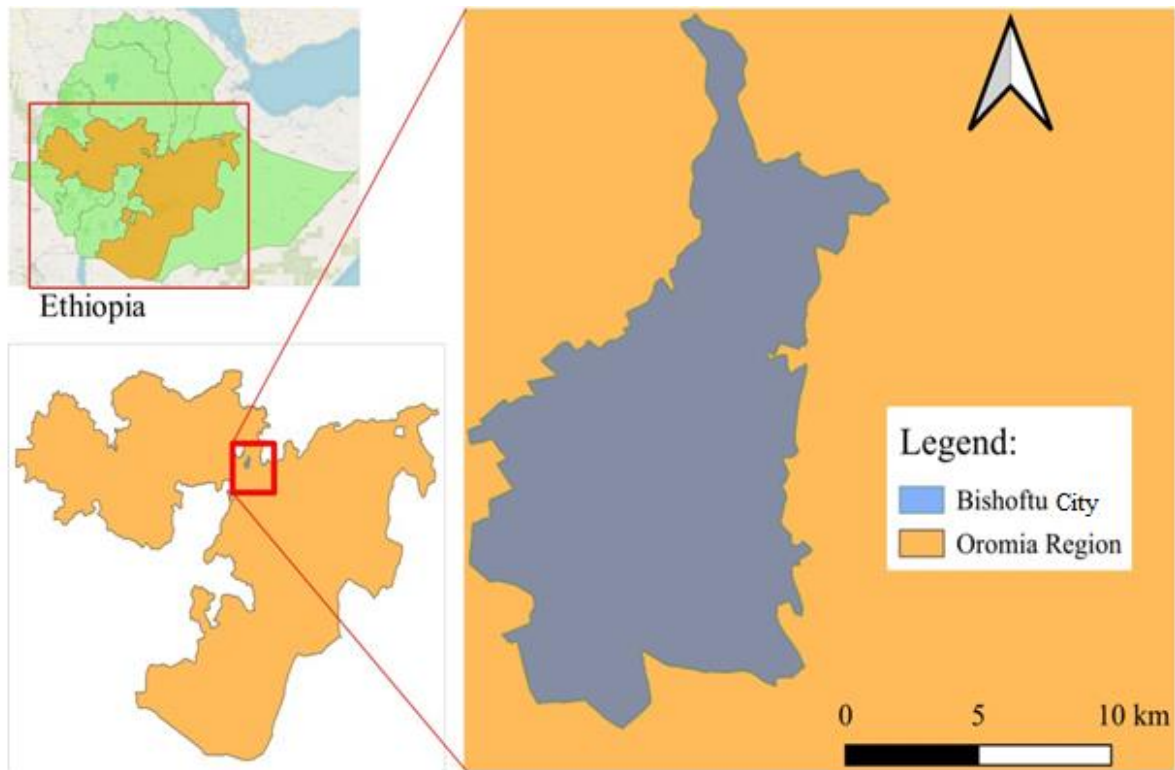
Study area	Sample origin	Number of positive (%)	Campylobacter species isolated					References
			<i>C.jejuni</i>	<i>C.coli</i>	<i>C.lari</i>	<i>C.fetus</i>	Other	
Akaki Kality	Cattle	25(18.5%)	6(24%)	-	-	10(40%)	9(36%)	(Chala <i>et al.</i> , 2021)
	Poultry	9(13%)	2(22.2%)	2(22.2%)		1(11.1%)	4(44.4%)	
	Water	18(10.5%)	3(16.7%)	3(16.7%)		2(11.1%)	10(55.5%)	
Gambella	Cattle	85(48%)	64(75.3%)	15(17.6%)	6(7.1%)	-	-	(Abamecha <i>et al.</i> , 2015)
	Chicken	84(86.6%)	73(86.9%)	10(11.9%)	1(1.2%)	-	-	
	Chicken meat	13(21.6%)	11(84%)	1(8%)	1(8%)	-	-	
Jimma	Cattle	26(12.7%)	14(53.8%)	10(38.5%)	2(7.7%)	-	-	(Kassa <i>et al.</i> , 2006)
	Chicken	130(68.06%)	105(80.8%)	21(16.2%)	4(3%)			
Bahir Dar	Chicken	160(72.7%)	148(92.5%)	12(7.5%)	-	-	-	(Ewnetu and Mihret, 2010)

3. MATERIALS AND METHODS

3.1. Description of study area

The study was conducted in Bishoftu city, which is located in the central highlands of Ethiopia at latitude of 8°45'N and a longitude of 38°59'E, with an average altitude of 1850 meters above sea level (**Figure 2**). The town has an annual rainfall of 866 mm of which 84% is in the long rainy season (June to September). The dry season extends from October to February. The mean annual maximum and minimum temperature is 26°C and 14°C respectively, with mean relative humidity of 61.3% (Getu, 2022).

The area is characterized by bimodal rainfall with an average of 1100 mm with the highest percentage of rainfall is received during the long rainy season covering June to September and the remaining in the short rainy season extending from March to May. It has embraced favorable environment, different bird species and historical heritages that makes it the tourist attraction sites in Ethiopia and both national and international tourist visit every year (Tesfaye, 2019). On the other hand, it is one of the urban centers where market oriented small, medium and large scale intensive broiler and layers poultry farms are flourishing rapidly and it is a place where there is open chicken markets are active throughout the year and serve as central market for adjacent regions (Tekelemariam *et al.*, 2022). The poultry production in the area is dominated by small scale farms with poor application of biosecurity practice (Waktole *et al.*, 2023). The flourishing of poultry farms with poor biosecurity, increasing of travel to town due to tourist attraction nature of the town and being chicken market place throughout the year contribute for the prevalence *Campylobacter* species both in chicken and humans.



Source: The map was created using ArcMap 10.7.1

Figure 2: Map of the study area

3.2. Study design

The cross-sectional study was conducted from October 2025 to May 2026 to estimate the prevalence of *Campylobacter* from poultry farms, diarrheic patients who were attending Bishoftu city Health facilities and environmental water sources samples.

3.3. Study population

The study populations were human patients with signs and symptoms of gastrointestinal diseases, especially those with acute diarrhea attending government hospitals and other health centers during the study period. The other study population were chicken from randomly selected intensive poultry farms who rearing small scale, medium and large-scale farm in the city

consistent with classifications used in previous studies (Gure *et al.*, 2024) and other environmental samples from various water sources.

3.4. Sample size determination

The sample size was calculated for two independent populations using previous 10% and 13% prevalence of *Campylobacter species* for human and poultry respectively (Chala *et al.*, 2021) at 5% of desired absolute precision and 95% confidence interval. Using the formula recommended by Thrustfield (2005).

$$n = \frac{1.96^2 P_{exp} (1-P_{exp})}{d^2}$$

Where, n = required sample size, P_{exp} = expected prevalence, d = desired absolute precision
Accordingly, 140 and 180 samples were collected from human and poultry respectively. The sample size for water samples was conveniently determined based on the available water sources that are in use for poultry farm and household drinking and other daily activities in the study area. Furthermore, around 60 water samples were collected from various water sources. Specifically, 20 water samples from pipe water and tankers from where drinking water distributed to the whole town and to the surrounding community, 22 water samples from surface water which is freely used for livestock and other wildlife and 18 water samples were collected from water sources that are in use in the poultry farm(**Figure 3**).

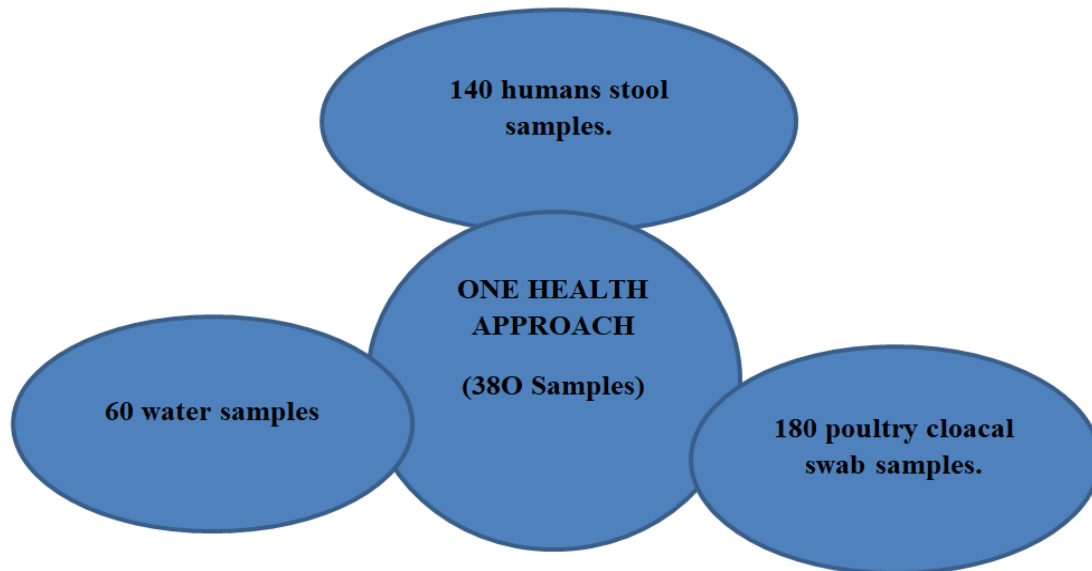


Figure 3: Diagrammatic description of source of sample collection and sample size

3.5. Inclusion and Exclusion Criteria

For human participants, any individual of any age and gender who visited clinics or hospitals in Bishoftu city and was actively experiencing diarrhea during the study period without being under any form of antibiotics treatment two weeks prior to sampling and after obtaining their consent were selected. With regard to chickens, any chicken irrespective of its breed and age from randomly selected poultry farms which include those of different sizes including large, medium, and small scales as registered with the Urban Agriculture Office in Bishoftu city and which was not on any form of antibiotics treatment two weeks prior to sampling was selected.

For environmental water sources, inclusion criteria included those sources whose water fell into one of the three operationally defined water sources: piped or tanker delivered drinking water, surface water sources that were available to livestock and wildlife, or water sources used in poultry farming. In the case of human participants, inclusion was limited to those individuals who had not been administered any antimicrobial therapy within two weeks preceding enrollment; similarly, any poultry under antimicrobial treatment or within the relevant withdrawal period were not included. Also excluded were sources of water those were either inaccessible or structurally un-suitable for sampling.

3.6. Sampling techniques and sample collection.

3.6.1. Chicken sample

Poultry farms were identified by consulting Bishoftu city administration bureau of urban agriculture. Based up on the recorded data information from Bishoftu city administration bureau of urban agriculture, eighteen (18) poultry farms were selected by simple random sampling using lottery system after providing each farm specific number, based on the scale of flock as described by Gure *et al.*, (2024). Mean that, four large scale poultry farms, from 6 large scale poultry farms recorded, ten medium scale poultry farms from 60 medium scale poultry farms recorded and four small scale poultry farms from 20 small scale poultry farms recorded in bishoftu city were selected by simple random sampling using lottery system after providing each farm specific number. From selected large scale poultry farms, medium scale poultry farms and small scale poultry farms, 15 chickens, 10 chickens and 5 chickens were randomly selected for cloacal swab sample collection from each farms respectively. Totally 180 chickens were randomly selected as per sample size calculated for the poultry domains. Individual chicken was selected at random and fecal samples were collected directly from cloaca using swabs.

All necessary precautions were taken to avoid contamination from outside of the cloaca using sterile cotton tipped swab. All sampled chicken were marked to avoid repeated sampling and each sample was labeled with unique code. Sample collection was done in sterilized containers and cold chain was maintained during transportation. Chicken on antimicrobial treatment or treated during the previous two weeks at the time of contact was excluded. Immediately after collection the fecal samples were placed in universal bottle containing Cary-Blair transport medium that prevent swab content from drying and toxic effects of oxygen (WOAH, 2024).

3.6.2. Human patients

Similarly, government hospitals and health centers was identified by consulting Bishoftu city administration bureau of health. In Bishoftu city there are 6 hospitals, 30 clinics and 11 health centers (Bishoftu city administration bureau of health, 2026). From this, 2 hospitals and 5 clinics

were randomly selected for sampling. From each selected health care facilities 20 Stool specimens were collected from diarrheic patients at health care facilities by medical personnel, particularly by medical laboratory workers. Approximately 3-5 grams of fresh stool specimen was collected from humans into leak proof and clean stool screw capped bottles using sterile applicator stick immediately after direct defecation. Human patients on antimicrobial treatment or treated during the previous two weeks at the time of contact were excluded. Immediately after collection the fecal samples were placed in universal bottle containing Cary-Blair transport medium that prevent swab content from drying and toxic effects of oxygen (WOAH, 2024).

3.6.3. Environmental samples

Water samples were taken from various water sources used in poultry farms, for cleaning utensils and drinking water during sampling chicken farm and human patients in order to assess the prevalence of *Campylobacter* species in water. The containers were labeled with the sources of water and individual identifiers. 60 water samples were collected from environmental water sources. Specifically, 20 water sample from pipe water and septic tank system from where drinking water distributed to the whole town and to surrounding community, 22 water sample from surface water which is freely used for livestock and others wild life and 18 water sample were collected from water sources that are used in the poultry farms.

Finally, the collected sample from each three domains were placed in an ice box containing ice pack with an approximate temperature of 2-8° C and was transported to the Microbiology laboratory of Addis Ababa University College of Veterinary Medicine and Agriculture. **Annex 9** indicated sample collection from field and transportation to the Microbiology laboratory of Addis Ababa University College of Veterinary Medicine and Agriculture.

3.7. Isolation and Phenotypic Identification of *Campylobacter* Species

A selective enrichment was initially performed on all samples to resuscitate the bacteria exposed to the stress and enable the recovery of the isolates when present at low concentration in the tested samples as described by Corry and Atabay (2011). Initially, the human stools and chicken

cloacal swab samples were homogenized and water samples were filtered using filter membrane. Water samples were filtered through a 0.45µm sterile nitrocellulose filter. Membrane filtered water and homogenized human stools and cloacal swab of poultry were inoculated in 30ml of Bolton enrichment broth containing Campylobacter growth supplement with 5% (v/v) defibrinated horse blood. Afterward, the homogenized fecal samples and filtered water were inoculated into 30 mL Bolton enrichment broth and incubated for 48 hours at 37° C and 42 ° C under microaerophilic condition 5% O₂, 10% CO₂ and 85% N₂ using gas generating sachet as described by Szczepanska *et al* (2017).

After 48 hours' incubation, a loopful of culture from each sample was inoculated onto modified Cefoperazone Charcoal Deoxycholate Agar (mCCDA), the mostly recommended and selective media for isolation of Campylobacter. The inoculated plates were incubated under a microaerophilic environment provided by gas generating sachets containing 5% O₂, 10% CO₂ and 85% N₂ and incubated at 37° C and 42° C for 48 hours. typical Campylobacter colonies on mCCDA appears moist, grey, flat, and spreading (often following the streak lines), Grey or grayish-brown in color and Sometimes showing a distinct metallic sheen or smooth and shiny appearance as it indicated in **Annex 11**. To eliminate growth of other gram negative bacteria which can resist composition of mCCDA and account for cephalosporin-resistant Gram-negative breakthrough organisms on mCCDA, a rigorous multi-temperature and selective physiological screening checkpoint system was implemented. Suspect colonies exhibiting characteristic moist, greyish, flat, or spreading (direct growth on inoculated line) morphology on mCCDA were simultaneously cross-inoculated onto MacConkey agar plates and incubated at 37°C in ambient air, while separate mCCDA subcultures were maintained under microaerophilic conditions at variable temperatures of 25°C, 37°C, and 42°C for 48 hours.

Definitive presumptive Campylobacter genus allocation was exclusively assigned to isolates that strictly demonstrated zero growth on MacConkey agar (confirming successful elimination of enteric like Pseudomonas or atypical E. coli), no growth at 25°C and 37°C in aerobic conditions, but robust growth restricted to 42°C under microaerophilic conditions. Isolates satisfying these metabolic criteria were subsequently sub-cultured onto non-selective Brain Heart Infusion agar to obtain pure, highly viable biomass for definitive phenotypic batteries. Then, suspected

colonies were sub-cultured onto Columbia blood agar with 5% (v/v) sheep blood or Brain Heart Infusion agar with 5% (v/v) defibrinated horse blood and incubated.

Phenotypic confirmation from pure Brain Heart Infusion agar colonies was verified via gram staining by revealing distinct gram negative bacteria, faintly staining, curved, spiral, or characteristic "gull-wing" rods under microscope (**Annex 10**). a positive catalase test was revealed by immediate bubble formation, a positive oxidase test, and a wet-mount motility assay demonstrating rapid, characteristic "darting" or corkscrew-like motility as previously described by (WOAH, 2024) and Resistance and susceptible pattern to Nalidixic acid and to Cephalotin also used as one of biochemical test for identification (**Annex 5**). Only isolates displaying the complete, unaltered matrix of these morphological and biochemical markers were conventionally confirmed and recorded as *Campylobacter* positive. Positive colonies were inoculated in Brain Heart Infusion Broth and incubated for 48 hours at the same condition for preservation with 20% glycerol addition and further investigation. Thus, such isolate was preserved in Brain Heart Infusion Broth under -80°C for long term preservation with 20% glycerol or -20°C for certain short term preservation for further test if needed and certain period subsequently advanced to standardize Antimicrobial Susceptibility Testing.

3.8. Antimicrobials Susceptibility patterns of the *Campylobacter*.

Out of 41 conventionally isolated *Campylobacter* species, 21 isolates were subjected to antimicrobial susceptibility test using the standard Kirby-Bauer disk diffusion assay on Muller-Hinton agar with addition of 5% (v/v) defibrinated horse blood, according to Clinical Laboratory Standards Institute guidelines (CLSI, 2024). Around 2-3 colonies were picked from culture and suspended in 5 mL of 0.85% normal saline and the turbidity of broth culture was adjusted to 0.5 McFarland standards. Then, sterile cotton swab was dipped into the suspension and streaked on the entire surface of Muller-Hinton agar. The inoculated plates were dried at room temperature for 3-5 minutes and discs are placed on the inoculated Muller-Hinton agar using disc dispenser. After 24 hours of incubation, the diameter of clear zone of inhibition was measured in mm using ruler and interpreted and determined as susceptible, intermediate, and resistant, the all procedure and protocol for Antimicrobials Susceptibility patterns of the

Campylobacter indicated in detail in **Annex 6**. The antibiotics discs used in this study are given in **Table 3** in the table below. Reference strains of Campylobacter jejuni and coli were obtained from the Ethiopian Biodiversity Institute and used as positive controls.

Table 3: Breakpoints of disk diffusion methods used to determine antimicrobial susceptibility of Campylobacter isolates.

Class of antibiotics	Antibiotics	Code	Conc.(microg ram)	Zone Diameter(nearest whole mm)		
				S	I	R
Tetracyclines	Tetracycline	TET	30	≥26	23-25	≤22
Aminoglycosides	Gentamicin	CN	10	≥15	13-14	≤12
Macrolides	Erythromycin	ERY	15	≥16	13-15	≤12
Sulfonamides	Co-trimoxazole	COT	15	≥16	11-15	≤10
Quinolones	Nalidixic Acid	NA	30	≥19	14-18	≤13
Fluoroquinolones	Ciprofloxacin	CIP	5	≥24	21-23	≤20
Amphenicols	Chloramphenicol	CHL	30	≥19	14-18	≤13
B-lactam (Penicillin's, Cephalosporin's, Carbapenem)	Amoxicillin-clavulanic acid****	AMC	30	≥20	16-19	≤15
	Cephalothin	CEP	30	-	-	-
	Meropenem*	IPM	10	≥23	20-22	≤19
	Ceftriaxone**	CTX	30	≥21	14-20	≤13
	Cefixime*	CFM	5	≥19	16-18	≤15

Source: (CLSI, 2024). Four antibiotics*, **, ***, **** breakpoints adopted from Enterobacteriaceae, CLSIM100 breakpoints.

MARI: Multiple Antibiotic Resistance Index: calculated using the following formula:

MARI = Number of antibiotics to which the isolate is resistant

Total number of antibiotics tested

If MARI scores greater than **0.20** indicates a high probability of environmental exposure with the use of antibiotics.

3.9. Questionnaire data collection

After securing the consent, questionnaires were used to collect information from human case on the family size, drinking water sources, latrine usage, hand washing practice, contact with chicken and contact history with diarrheal patients. Similarly, on poultry farm data including biosecurity, water sources, personal protecting equipment usage and contact with other animals were recorded using questionnaire and observing the farm. Questionnaire data on potential risk factors associated with human infection with *Campylobacter* were collected. Structured questionnaires were utilized and translated into local languages (Afaan Oromo & Amharic language) human questionnaires' form, poultry farm questionnaires' form and written consent format are indicated in Annex 7, 8, 12 respectively.

3.10. Data management and analysis

All data were recorded on data collection sheet prepared for this purpose and exported to excel spread sheet and analyzed using STATA version 17 and R-studio. Descriptive analysis was made to compute frequency and proportion. Odd ratio was calculated to see the association between dependent and independent variables. The Pearson chi-square (X^2) test was used to assess the associations between explanatory variables, and observed differences were considered statistically significant when $P < 0.05$.

3.11. Ethical Considerations

The study was conducted in full compliance with institutional, national, and international ethical standards governing research involving human participants and animals. Ethical clearance for the animal component of the study was sought from the Institutional Animal Research Ethics Committee of the College of Veterinary Medicine and Agriculture (CVMA), Addis Ababa University. Approved Animal Ethical clearance certificate with **Ref no:** NM/ERC/08/115/18/2026 (Annex 13).

Similarly, ethical approval for procedures involving human participants was obtained from the Aklilu Lemma Institute of Pathobiology (ALIPB) Institutional Research Ethics Review Committee, Addis Ababa University. Approved Human Ethical clearance certificate with **Ref no:** ALIHR-IRERC-004/2018/2026 (Annex 14).

All research procedures were adhering to the ethical principles outlined in the Declaration of Helsinki for human research and in the WOAHA Terrestrial Animal Health Code for animal welfare. Participation of human subjects, including residents and wastewater treatment plant workers, were strictly voluntary. The objectives, benefits, and potential risks of the study were clearly explained to all participants, and written informed consent was obtained prior to sample collection. Participants have the right to withdraw from the study at any time without penalty. Personal identifiers were removed from all data to ensure confidentiality and privacy.

Animal sampling was carried out with minimum distress to the animals, following humane handling and restraint procedures. Only non-invasive sample types (such as fecal swabs) were collected, and all animal handling was performed by trained personnel under veterinary supervision.

All biological samples and data were used solely for the purposes of this research. The study team was ensured safe handling, transport, and disposal of biological materials in accordance with biosafety regulations of Addis Ababa University and Ethiopian national standards.

4. RESULTS

4.1. Overall Prevalence of Campylobacter from Various Sources.

The prevalence of Campylobacter in all the samples considered in this study was 10.78%. From among the different sample types assessed, the highest prevalence was observed in poultry cloacal swabs, which were 15.5% (28/180; 95% CI: 10.2-20.9%), followed by environmental water samples at 10% (6/60; 95% CI: 2.1 and followed by human stool samples at 5% (7/140; 95% CI: 1.34–8.65) as it indicated in table 4.

Table 4: The prevalence's of Campylobacter across all tested samples.

Source of samples	Observation	Positive	Prevalence (%)	95% CI
Human stools	140	7	5	1.34-8.65
Poultry cloacal swabs	180	28	15.5	10.2-20.9
Water sample	60	6	10	2.18-17.82
Total	380	41	10.78	7.65-13.92

4.2. Association of Campylobacter in human samples and variables using chi square.

The Prevalence of Campylobacter amongst the 140 diarrheic humans was 5% (7/140; 95% CI: 1.34-8.65). Age was established to be a highly significant risk factor for acquiring the organism ($\chi^2 = 18.42$, $P = 0.000$). The seven positive cases of Campylobacter infection were observed among children only (17.5%). Statistically significant association was also noted among those under marital status ($\chi^2 = 15.06$, $P = 0.000$) as it indicated in **table 5**. In addition, all positive cases were recorded from unmarried subjects, with a prevalence of 15.2%. Occupations were found to have a significant association ($\chi^2 = 9.27$, $P = 0.026$). Family dependents had a prevalence of 11.3% with all positive cases. Gender, family size, residential place, educational level, and drinking water source did not show statistically significant associations with Campylobacter positivity (all $P > 0.05$).

Table 5: Socio-demographic Characteristics of respondent and Risk Factors for Human Campylobacteriosis (N=140)

Variables	Categories	Observation	No. of +ve (%)	χ^2	P-Value
Gender	Female	69(49.3)	4(5.8)	0.1820	0.670
	Male	71(50.7)	3(4.2)		
Age	Children	40(28.6)	7(17.5)	18.4211	0.000
	Adult	52(37.1)	0(0)		
	Old	48(34.3)	0(0)		
Marital status	Unmarried	46(32.9)	7(15.2)	15.0572	0.000
	Married	94(67.1)	0(0)		
family size	<5	64(45.7)	1(1.6)	2.9328	0.087
	>5	76(54.3)	6(7.9)		
Residential place	Rural	75(53.6)	5(6.7)	0.9447	0.331
	Urban	65(46.4)	2(3.08)		
Level of education	No formal Education	76 (54.3)	7(9.2)	6.2050	0.184
	Primary Education	5(3.6)	0(0)		
	Secondary Education	8(5.7)	0(0)		
	Higher level Diploma + and above	35 (25.0) 16(11.4)	0(0) 0(0)		
Occupation	Daily labor	0(0)	0(0)	9.2699	0.026
	Farmer	13(9.3)	0(0)		
	Student	48(34.3)	0(0)		
	Employed	17(12.1)	0(0)		
	Unemployed	0(0)	0(0)		
	Family dependent	62(44.3)	7(11.3)		
sources of water for drinking	Tab water	65(46.4)	2(3.1)	2.4161	0.299
	spring water	62(44.3)	5(8.1)		
	well water	13(9.3)	0(0)		

4.3. Assessment of study participant's practices towards diarrheic disease prevention.

Having contact with domestic animals and *Campylobacter* positivity was found to be significant ($\chi^2 = 4.55$, $P = 0.000$). As the Table 6 below indicates, all seven positive cases belonged to those respondents who had exposure to animals (5.6%). Exposure to poultry was also one of the strongest risk factors in this table ($\chi^2 = 46.93$, $P = 0.000$). Out of the 19 people exposed to poultry, 7 (36.8%) were positive for *Campylobacter*, as against zero positives who had contact with pets, cattle, and shoats.

Irregular handwashing after latrine was another factor that was found to have an association with the infection ($\chi^2 = 16.62$, $P = 0.000$). All positive cases had irregular handwashing behavior; they washed their hands "sometimes" (16.3%), and there were no positives among those who always washed their hands. Consumption of undercooked meat was also statistically significant in relation to the infection ($\chi^2 = 5.18$, $P = 0.023$). All seven positive cases had consumed undercooked meat (19%), whereas none of the other respondents had.

Hand washing practice before and after cooking and food preparations were both significant risk factors for *Campylobacter* infections ($\chi^2 = 5.18$, $P = 0.023$), with people who never washed their hands having much more positive results (33.3%) than those who always washed their hands (4.4%). Factors such as visiting poultry farms, eating raw fruits and vegetables, type of cleaning agents used, use of protective devices, and types of wastes disposal were not significant ($P > 0.05$)(Table 6).

Table 6: Assessment of study participant's practices toward human diarrheic disease prevention.

Variables	Categories	Observation	+ve (%)	χ^2	P-Value
Contact with any domestic animals	No	15 (10.7)	0 (0)	4.5464	0.000
	Yes	125 (89.3)	7 (5.6)		
To which domestic animals have contact?	poultry	19 (14)	7(36.8)	46.9252	0.000
	pet	60 (42.9)	0(0)		
	cattle	23 (16.4)	0(0)		
	shoat	23 (16.4)	0(0)		
	none	15 (10.7)	0(0)		
Do you visit poultry farm in the last two weeks?	No	97 (69.3)	7(7.2)	3.2664	0.071
	Yes	43 (30.7)	0(0)		
Do you contact with diarrheic person with in the last two weeks?	No	85 (60.7)	7(8.2)	4.7678	0.029
	Yes	55 (39.3)	0(0)		
Hand washing before food preparation	No	3 (2.1)	1(33.3)	5.1812	0.023
	Yes	137(97.9)	6(4.4)		
Hand washing before and after touching cooked food	No	3 (2.1)	1(33.3)	5.1812	0.023
	Yes	137 (97.9)	6(4.4)		
Having toilet at your home	No	0 (0)		-	-
	Yes	140(100)			
How often you wash your hand	Always	97(69.3)	0(0)	16.6218	0.000

after using latrine	sometimes	43(30.7)	7(16.3)		
Do you consume chicken meat or other animals products	No	2(1.4)	0(0)	0.1068	0.744
	Yes	138 (98.6)	7(5.1)		
Eating undercooked meat	No	103(73.6)	0(0)	5.1812	0.023
	Yes	37(26.4)	7(19)		
Do you consume of raw fruits and vegetables	No	41(29.3)	0(0)	3.0516	0.081
	Yes	99(70.7)	7(7.1)		
Which cleaning agents use for cleaning utensils before and after food preparation?	Soap	40(28.6)	0(0)	2.3523	0.097
	Detergent	27(19.3)	0(0)		
	water only	73(52.1)	7(9.6)		
Use of PPE during contact with poultry	No	43(30.7)	7(16.3)	0.7583	0.289
	Yes	97(69.3)	0(0)		
Wash hands with soap after handle poultry and cleaning barn	No	80(57.1)	7(8.75)	0.1068	0.470
	Yes	60(42.9)	0(0)		
How do you dispose the poultry wastes?	using glove	14(10.0)	0(0)	3.4903	0.175
	using bare hand	84(60.0)	7(8.3)		
	using shovel	42(30.0)			

4.4. Prevalence of Campylobacter in poultry samples and Poultry farm owner or workers practice assessment towards diarrheic disease prevention

The prevalence of Campylobacter in poultry farm categories was found to be 15.5% (28/180). As it indicated in the **table 7** there was no statistical significance between flock size, Age of Chicken, and Distance from Main Roads on campylobacter positivity: p-values were greater than 0.05. One of the statistically significant factors shown by the table is that farms without rodent control had $\chi^2 = 16.62$, and P-value = 0.000 (p-value less than 0.05). The prevalence of campylobacter was 28.75% on farms without rodent control, while only 5% on farms with active rodent control programs.

The lack of disinfection and cleaning program was another significant factor with a p-value of 0.029 ($\chi^2 = 4.77$). The prevalence of campylobacter in this category was 24.4%, while for those with active disinfection and cleaning programs it was 6.7%. All other farm management factors including distance from nearby farms, exchanging equipment and workers, water source, litter management, movement of workers between barns, presence of wild birds, and frequency of PPE usage were not statistically significant (p-values greater than 0.05)(**Table 7**).

Table 7: Farm characteristics and practice assessment of Poultry farm owner or workers towards diarrheic disease prevention (N=180)

	Categories	observation	Positive	χ^2	P-Value
Types of Poultry farms	layer	50 (27.8)	8(16)	0.7190	0.698
	broiler	80 (44.4)	14(17.5)		
	Dual purpose	50 (27.8)	6(12)		
Flock size	large	60(33.3) 40(22.2)	14(23.33) 14(35%)	0.4145	0.520
	medium	100(55.6)	8(8)		
	Small	20(11.1) 40(22.2)	6(30) 6(15)		
Age in weeks	<6 weeks	40(22.2)	8(20)	0.8141	0.846
	6-8 weeks	40(22.2)	6(15)		

	8-18 weeks	80(44.4)	11(13.75)		
	>=18 weeks	20(11.1)	3(15)		
Is there any other poultry farm nearest to this poultry farm?	No	40(22.2)	6(15)	0.345	0.764
	Yes	140(77.8)	22(15.7)		
Does your farm have fence and gate?	No	0(0)	0(0)	-	-
	Yes	180(100)	28(15.5)		
Do you use foot and tire bath at the gate?	No	0(0)	0(0)	-	-
	Yes	180(100)	28(15.5)		
Have you exchange equipment and worker with other farms?	No	130(72.2)	22(16.9)	0.6663	0.414
	Yes	50(27.8)	6(12)		
Do you allow any visitor entry to your farm without restriction?	No	180(100)	28(15.5)	-	-
	Yes	0(0)	0(0)		
Do you have rodent control method you use in your farm?	No	80(44.4)	23(28.75)	16.6218	0.000
	Yes	100(55.6)	5(5)		
Can wild bird access to the farm?	No	100(55.6)	9(9)	0.4145	0.520
	Yes	80(44.4)	19(23.75)		
Do you have regular cleaning and disinfection program?	No	90(50.0)	22(24.4)	4.7678	0.029
	Yes	90(50.0)	6(6.7)		
How often you clean feeding and watering trough?	Every day	40(22.2)	4(10)	0.7190	0.698
	Every 2 days	40(22.2)	8(20)		
	Once a week	100(55.6)	16(16)		
How you manage a litter?	Uses for compost	90(50.0)	16(17.8)	0.6767	0.411
	Stored and sold	90(50.0)	12(13.3)		
How often workers use personal protective equipment?	Always	50 (27.8)	6(12)	0.6663	0.414
	Sometimes	130(72.2)	22(17)		

4.5. Prevalence of Campylobacter in environmental samples

Campylobacter was isolated from six out of the 60 samples obtained from different water sources supplying humans and poultry in Bishoftu city, giving an isolation rate of 10% (**Table 4**). No statistically significant differences were seen in the association between Campylobacter isolation and the source of the water ($P = 0.237$), the location of the source of the water ($P = 0.411$), or the sampled water source site ($P = 0.860$). As shown in **Table 8** below, importantly, all samples from municipal tap water sources ($n = 20$) were found negative for Campylobacter, while surface water (lakes and streams; 13.6%) and bird farm water (16.7%) gave positive results.

Regarding the sampling water source site, samples collected from Birbirs Foka showed the highest percentage of isolation (13.6%), followed by Gandaa Biftu fi Calalaqa (12.5%). These sites represent open surface waters. The lowest percentage of isolation rate was seen at Lake Hora (5.9%), while Xadacha and Ganda Gorba reservoirs were all negative for campylobacter

Table 8: Assessment of Campylobacter among environmental sample, specifically water source used for both human and poultry as source of drinking in Bishoftu city, Ethiopia (N=60)

Study Variable	Categories	Observation	No. of +ve (%)	χ^2	P-Value
Water source	Municipal Tap water	20 (33.3)	0(0)	2.8788	0.237
	Surface water	22 (36.7)	3(13.6)		
	Water used by poultry farms	18(30)	3(16.7)		
Location of Water source	Storage tank/Reservoir	4 (6.7)	0(0)	2.6470	0.411
	Pipeline network	16 (26.7)	0(0)		
	Lake and stream	22 (36.7)	3(13.6)		
	From each poultry farm	18(30)	3(16.7)		
Sampled site	Xadacha reservoir site	3 (5)	0(0)	1.3102	0.860
	Ganda Gorba Reservoir site	2 (3.3)	0(0)		
	Babogaya(Birbirs foka)	22(36.7)	3(13.6)		
	Gandaa Biftu fi calalaqa	16(26.7)	2(12.5)		
	Lake Hora Area	17(28.3)	1(5.9)		

4.6. Antimicrobial Susceptibility Pattern of Campylobacter

4.6.1. Overall Antibiotic Susceptibility Profile of Campylobacter Isolates (n = 21)

The antimicrobial susceptibility testing of 21 *Campylobacter* isolates recovered from human, poultry, and environmental samples against 12 specific drugs revealed a notably high level of resistance across multiple antibiotic classes. As indicated in **Table 9**, among all antibiotics tested, Cephalothin recorded the highest resistance rate, with all 21 isolates (100%) being resistant in this finding study. Erythromycin and Tetracycline each exhibited high resistance rates, recorded at 71.4%. Co-trimoxazole and Amoxicillin-clavulanic acid also showed high resistant following Erythromycin and Tetracycline by showing that closely with a resistance rate of 66.7% and 61.9% respectively while Ciprofloxacin and Gentamycin relatively showed moderate resistance about 42.9% and 33.3% respectively. Ceftriaxone, Chloramphenicol and Cefixime showed comparatively lower but still considerable resistance, each at resistance rate of 28.6%. Meropenem, a carbapenem antibiotic, showed the second-best efficacy with no resistance recorded but five isolate showed intermediate record in spite of its high susceptibility rate of 76.2% recorded. Strikingly, Nalidixic Acid was the only antibiotic to which all isolates (100%) were fully susceptible.

Table 9: Overall Antibiotic Susceptibility Profile of *Campylobacter* Isolates (n = 21)

AMR class	Antimicrobial drugs	R (%)	I (%)	S (%)
Tetracyclines	Tetracycline	15(71.4)	3(23.8)	3(14.3)
Aminoglycosides	Gentamycin	7(33.3)	6 (28.6)	8(38.1)
Macrolides	Erythromycin	15(71.4)	2(9.5)	4(19.0)
Sulfonamides	Co-trimoxazole	14(66.7)	5(23.8)	2(9.5)
Quinolones	Nalidixic Acid	0(0)	0(0)	21(100)
Fluoroquinolones	Ciprofloxacin	9(42.9)	5(23.8)	7(33.3)
Amphenicols	Chloramphenicol	6(28.6)	7(33.3)	8(38.1)
B-lactam(Beta-lactam)	Amoxicillin-clavulanic acid	13(61.9)	3(14.3)	5(28.8)
	Cephalothin	21(100)	0(0)	0(0)
	Meropenem	0(0)	5(23.8)	16(76.2)
	Ceftriaxone	6(28.6)	8(38.1)	7(33.3)
	Cefixime	6(28.6)	9(42.9)	6(28.6)

4.6.2. Resistance Pattern of *Campylobacter* by Sample Source

When the resistance patterns were disaggregated by sample source, human diarrheic patients (n=4), poultry (n=14), and water sample (n=3), notable source-specific variation was observed, though resistance was found across all three sources. As indicated in the **Table 10**, Cephalothin maintained a universal 100% resistance rate across all sample sources (human, poultry, and water). The resistance of Co - trimoxazole and erythromycin distributed across entire sampling source by the following rate for erythromycin the high, record 75% in human isolates, 71.4% in poultry isolates, and 66.7% in water isolates, whereas for Co – trimoxazole resistance reached 100% in water isolates, 64.3% in poultry, and 50% in human isolates.

Tetracycline resistance was greatest in human isolates (75%), followed by water (66.6%) and poultry (57.1%). Amoxicillin-clavulanic acid resistance was highest in human isolates (75%), followed by water (66.7%) and poultry (57.1%). Ciprofloxacin resistance was 38.1% in poultry, 4.8% in water and no resistance isolates for Ciprofloxacin in human isolates. Notably, meropenem resistance was no detected across three domains of samples. It was remained susceptible for majority isolates though some isolates showed intermediate records. Chloramphenicol resistance was remarkably high in water isolates (100%) but considerably lower in poultry (14.3%) and human (25%) isolates. Nalidixic Acid and meropenem remained effective recording 0% resistance in isolates from all three sources. However unlike Nalidixic Acid, meropenem showed intermediate records among some isolates.

Table 10: Resistance Pattern of *Campylobacter* by Sample Source.

AMR class	Antimicrobial drugs	Human(n=4) No (%)	Poultry(n=1 4) No (%)	Water(n=3) No (%)	Total(n=21) No (%)
Tetracyclines	Tetracycline	3(75)	8(57.1)	2 (66.6)	13(61.9)
Aminoglycosides	Gentamycin	2(50)	4(28.6)	1(33.3)	7(33.3)
Macrolides	Erythromycin	3(75)	10(71.4)	2(66.7)	15(71.4)
Sulfonamides	Co-trimoxazole	2(50)	9(64.3)	3(100)	14(66.7)
Quinolones	Nalidixic Acid	0(0)	0(0)	0(0)	0(0)
Fluoroquinolones	Ciprofloxacin	0(0)	8(38.1)	1(4.8)	9(42.9)
Amphenicols	Chloramphenicol	1(25)	2(14.3)	3(100)	6(28.6)
B-lactam(Beta-lactam)	Amoxicillin-clavulanic acid	3(75)	8(57.1)	2(66.7)	13(61.9)
	Cephalothin	4(100)	14(100)	3(100)	21(100)
	Meropenem	0(0)	0(0)	0(0)	0(0)

Ceftriaxone	2(9.5)	4(19)	0(0)	6(28.6)
Cefixime	2(9.5)	3(14.3)	1(4.8)	6(57.1)

Note: AMC: Amoxicillin-Clavulanic acid; CEP: Cephalothin; CHL: Chloramphenicol; CIP: Ciprofloxacin; CRO: Ceftriaxone; ERY: Erythromycin; FOX: Cefoxitin; GEN: Gentamicin; MEM: Meropenem; SXT: Sulfamethoxazole-Trimethoprim; TET: Tetracycline.

4.6.3. Distribution of Isolates by Number of Specific Drugs Resisted

Analysis of the drug resistance spectrum at the individual drug level revealed a striking diversity of resistance combinations among the 21 *Campylobacter* isolates. As indicated in table 11, the largest proportion of isolates, 38.1% exhibited resistance to six specific antibiotics simultaneously, representing the most frequently encountered resistance depth in this study. Four and Five-drug resistance was the second most common pattern, observed in four isolates in each case 19% and followed by seven drugs resistance in 3 isolates 14.3% Two and three drug resistance was recorded in only one isolates 9.5%.

In this study, MARI values of isolated *Campylobacter* (n=21) were highly disturbing when taking into consideration against 12 antibiotics used (**Table 11**). MARI values attained were ranging from 0.17 to 0.58 giving a mean average value of MARI **0.444**.

Table 11: Distribution of Isolates by Number of Specific Drugs Resisted (n=21)

No. specific antibiotics resistant	Isolate no (%)	Sampling source	MARI (a/12)	Resisted antibiotics	Freq. of isolate
Two	1(4.8)	P	0.17	SXT & CEP	1
Three	1(4.8)	P	0.25	AMC, CEP & CFM	1
Four	4(19)	H, P, W	0.33	SXT, AMC, CEP & CFM	1
				TET, CIP, SXT & CEP	1
				ERY, SXT, CHL & CEP	2

Five	4(19)	P	0.42	TET GEN AMC CEP & CRO GEN, ERY, CIP, CEP & CFM TET, ERY, SXT, AMC & CEP TET, ERY, AMC, CEP & CFM	1 1 1
Six	8(38.1)	H, P, W	0.50	TET, GEN, ERY, SXT, AMC & CEP TET, GEN, ERY, CHL CEP & CRO TET, ERY, AMC, CEP, CFM & CRO TET, GEN, ERY, SXT CIP, & CEP TET, ERY, SXT, CIP, AMC, & CEP TET, ERY, SXT, CIP, CEP & CRO, ERY, CIP, AMC, CEP, CFM, & CRO TET, ERY, SXT, CHL, AMC, & CEP	1 1 1 1 1 1 1
Seven	3(14.3)	P, W	0.58	TET, ERY, SXT, CIP, CHL, AMC, & CEP GEN, ERY, SXT, CIP, AMC, CEP & CRO TET, GEN, SXT CIP CHL, AMC & CEP	1 1 1
Total	21(100)	-	Mean MARI: 0.444	Overall MARI Range 0.17 to 0.58	

Note: AMC: Amoxicillin-Clavulanic acid; CEP: Cephalothin; CHL: Chloramphenicol; CIP: Ciprofloxacin; CRO: Ceftriaxone; CFM: Cefixime; ERY: Erythromycin; GEN: Gentamicin; MEM: Meropenem; NAL: Nalidixic Acid; SXT: Sulfamethoxazole-Trimethoprim; TET: Tetracycline. Sample source:* H: Human; P: Poultry; W: Water.

4.6.4. *Distribution of Isolates by MDR pattern of Campylobacter isolates*

As indicated in the **table 12 below**, a total of 18 out of 21 isolates (85.7%) were classified as multidrug resistance (MDR). The majority of MDR isolates demonstrated simultaneous resistance to five antibiotic classes was observed in 6(28.6%) isolate, representing the highest degree of multi-class resistance recorded. Resistance to four antibiotic classes was observed in 5(23.8%) isolates predominantly from poultry and water sources. three antibiotic classes resistance was recorded in 4(19%) isolates, whereas six- antibiotic class resistance in isolates 3(14.3%), the latter involving both water and poultry isolates.

Table 12: Multi drug resistance patterns of Campylobacter isolate (N=21)

Resistance category	Isolate no (%)	Resistance Pattern	Freq. of isolate	Source of sample
Against three AMC	4(19)	TET, MAC & β L	2	H, P
		TET, AMI & β L	1	
		MAC, FQL, & β L	1	
Against Four AMC	5(23.8)	TET, AMI, FQL& β L	1	P, W
		AMI, MAC, FQL & β L	1	
		TET, MAC, SUL & β L	1	
		MAC, SUL, AMI & β L	2	
Against Five AMC	6(28.6)	TET, AMI, MAC, SUL & β L	1	H, P, W
		TET, AMI, MAC, AMP & β L	1	
		TET, MAC, SUL, FQL & β L	2	
		AMI, MAC, SUL, FQL, & β L	1	
		TET, MAC, SUL, AMP & β L	1	
Against Six AMC	3(14.3)	TET, AMI, MAC, SUL, FQL& β L	1	P,W
		TET, MAC, SUL, FQL, AMP & β L	1	
		TET, AMI, SUL, FQL, AMP & β L	1	
Total MDR	18(85.7)			

Note: TET: Tetracyclines; AMI: Aminoglycosides; MAC: Macrolides; SUL: Sulfonamides; FQL: Fluoroquinolones; QN: Quinolones AMP: Amphenicols; β L: β -lactam; including (CEP: Cephalosporins; CAR: Carbapenems). Sample source:* H: Human; P: Poultry; W: Water.: AMC: antimicrobial class.

5. DISCUSSION

5.1. Overall Prevalence of *Campylobacter* from Various Sources

The overall prevalence of *Campylobacter* among all samples tested in the current study was 10.78% (41/380). This value is lower than the combined prevalence observed by Chala *et al.* (2021) that reported from a human (10.1%), poultry cloacal swab samples (13%), cattle (18.5%), and water (10.5%) in a One Health approach in peri-urban Addis Ababa.

Additionally, the present finding is considerably lower than the highest prevalence recorded by Deblais *et al.* (2023) in Haramaya, Eastern Ethiopia, where *Campylobacter* was detected in 68.8% of humans, 93.2% of poultry, 99.5% of cattle, and 43.2% of water samples. When compared further with other studies, the 10.78% overall prevalence in present finding is higher than the 9.3% prevalence of *Campylobacter* in retail raw meat products in Addis Ababa reported by Dadi and Asrat (2009).

5.2. Prevalence and Risk Factors Associated with Human *Campylobacter* Infection

The source-specific present finding prevalence of diarrheic human patients was 5% (7/140). This present findings falls within the lower range of prevalence values reported in previous Ethiopian studies on human *Campylobacteriosis*. Human prevalence has been shown by several other studies carried out in different regions of Ethiopia, where prevalence rates of 10.8% were seen in Tikur Anbessa Hospital, Addis Ababa (Asrat *et al.*, 1997); 10.5% in Dembia District, Northwest Ethiopia (Mitike *et al.*, 2009); 11.6% in Jimma (Beyene and Haileamlak, 2014); 15.4% in Gondar (Lengerh *et al.*, 2013). More recent prevalence rates include 7% for under- five years diarrheic children from Bahir Dar by Worku *et al.* (2024), and 6.8% and 12.7% from two independent studies performed in Hawassa by Behailu *et al.* (2022). Although the current prevalence rate of 5% appears much lower than these earlier reports, it is almost the same with those seen at Hawassa 6.04% (Kebede *et al.*, 2017) and Mekelle 6.5% (Abay *et al.*, 2024) respectively.

In the present finding age was found as the most important risk factor in human infections caused by *Campylobacter* species ($\chi^2 = 18.42$, $P = 0.000$), where only under five years children age group of 17.5% showed positive for campylobacter. This finding has strong support from the global epidemiology of Campylobacteriosis. In comparison to the global review carried out by Kaakoush *et al.* (2015), in which the authors stated that Campylobacteriosis is a disease in developing countries that mostly occurs in children and decreases with age as people get exposed to the disease and develop an immune response against it. Likewise, Lengerh *et al.* (2013) in Gondar, Ethiopia, and Behailu *et al.* (2022) in Hawassa reported that the risk of *Campylobacter* was highly dependent on the age of patients.

The present studies reveal that contact with poultry was the significant human risk factor to acquire campylobacteriosis. This assessed with 36.8% of people with poultry exposure being positive for campylobacter while none were found to be positive for *Campylobacter* in those without poultry exposure ($\chi^2 = 46.93$, $P = 0.000$). These results have a strong agreement with the previously existing findings in regard to worldwide reports of Campylobacteriosis transmission (Kaakoush *et al.*, 2015; Wagenaar *et al.*, 2023). Additionally, this is further discussed as poultry contact poses risk to humans by the work conducted by Chala *et al.* (2021) in peri-urban Addis Ababa based on the One Health approach.

Additionally, the present finding was telling the irregular handwashing after latrine usage had a strong statistical associations with *Campylobacter* positivity ($\chi^2 = 16.62$, $P = 0.000$), whereby all the subjects found to be positive for *Campylobacter* were those that washed their hands only "sometimes". This finding similar with the existing documents with the observation by Tamiru *et al.* (2022) that the prevalence of poor food safety practices, such as insufficient hand washing, was high in Ethiopia and considered the main contributors to the transmission of enteric pathogens. Undercooked meat ingestion emerged as another statistically associated risk factor for with *Campylobacter* positivity ($P = 0.023$), considering the thermal lability of *Campylobacter*, meaning that it dies when cooked at the right temperature but survives in poorly cooked poultry and meat (Kaakoush *et al.*, 2015; Too *et al.*, 2023). Also, Osbjer *et al.* (2016) in Cambodia found

poor handling and consumption of poultry as some of the important risk factors for *Campylobacter* infections.

5.3. Prevalence and Farm-Level Risk Factors Associated with Poultry *Campylobacter* Colonization

The present finding's source-specific prevalence of chickens was 15.5% (28/180) and this is relatively lower when compared to the majority of previous studies in Ethiopia. For instance, Kassa *et al.* (2006) noted significantly higher prevalence of 68.06% in chickens from Jimma, while Abamecha *et al.* (2015) reported much higher prevalence of 86.6% in chickens from the Gambella region. Similarly, Ewnetu and Mihret (2010) in Bahir Dar recorded significantly higher prevalence of 72.7% in chicken cloacal samples and similarly high prevalence of 93.2% in poultry was noted in the study conducted by Deblais *et al.* (2023) in Haramaya. In contrast, lower prevalence than present finding, 13% in poultry cloacal swabs collected in peri-urban Addis Ababa was noted in the study by Chala *et al.* (2021), where a similar One Health approach was used as the present finding with significantly nearest prevalence of 15.5% with present study. This also highlights the importance of Bishoftu city as the central chicken market for surrounding areas and the significant effect of even moderate prevalence at farm level on public health through contamination at slaughter and market levels (Tekelemariam *et al.*, 2022).

Among all biosecurity practices evaluated, the lack of rodent control was found to be the most statistically significant risk factor associated with *Campylobacter* positivity in poultry ($\chi^2 = 16.62$, $P = 0.000$). This revealed by 28.75% prevalence in poultry farms with no rodent control compared to 5% in farms with a rodent control program, which is more than five times higher. It is very clear that the evidence from Hald *et al.* (2016) and Taha-Abdelaziz *et al.* (2023) supports this result, since rodents have been confirmed to act as mechanical vectors of *Campylobacter* in poultry farms.

The lack of regular cleaning and disinfection was the second most statistically significant biosecurity risk factor associated with *Campylobacter* positivity in poultry farms ($\chi^2 = 4.77$, $P = 0.029$). There was a 24.4% prevalence of *Campylobacter* in farms with no regular cleaning and

disinfection practices compared to 6.7% in farms with such practices, which means almost four times higher prevalence. There is substantial evidence from Taha-Abdelaziz *et al.* (2023).

Furthermore the present study highlight that the lack of proper disinfection processes on the majority of the poultry farms of study area observed in this study is in line with the general lack of biosecurity, as seen from the findings of Waktole *et al.* (2023). Similarly the previous authors analyzed significant biosecurity concerns in poultry farming in Central Ethiopia quantitatively and identified non-compliance with biosecurity principles among other issues.

It is worth noting that flock size, age of the chickens, type of farms, and proximity to major roads were not significantly linked to the positivity of *Campylobacter* on the poultry farms in this study ($P > 0.05$). This suggestion supported by previous finding from the findings of Ijaz *et al.* (2018), the campylobacter positivity level of adherence to biosecurity guidelines and not the size or age of production that influences risk of exposure to campylobacteriosis.

5.4. Prevalence of *Campylobacter* in Environmental Water Samples

Environmental water sample source-specific prevalence of *Campylobacter* was 10% (6/60). Detection of *Campylobacter* in surface water in this study was lower and related with the prevalence of 22.5% and 8.3% for surface water and tap water obtained from peri-urban Addis Ababa, as described by Chala *et al.* (2021). However, while surface water prevalence (13.6%) was lower in the current study compared to that observed by Chala *et al.* (2021), the trend in both studies was consistent, that surface water had more burden compared to treat piped water.

Unlike Chala *et al.* (2021), however, the prevalence (43.2%) detected in Haramaya town in the current study was much higher than the 10% prevalence reported in this study. This may be because Haramaya town is characterized by less development in terms of water treatment facilities compared to Bishoftu city, which has a relatively well-developed municipal water system. Moreover, the isolation of *Campylobacter* in water from poultry farm (16.7%) is of great public health importance. According to Kaakoush *et al.* (2015), as supportive thought water

bodies such as rivers and lakes develop association with campylobacteriosis when contaminated with animal faeces, sewage outflow, slaughter house effluents, and agricultural slurries.

5.5. Antimicrobial Resistance Profile of Campylobacter Isolates

Regarding resistance of campylobacter isolates against twelve antibiotic, Cephalosporins showed 100% resistance rate as reported in the present study was supported by the idea of the biological property of cephalosporins resistance in Campylobacter is due to the presence of outer membrane protein and β -lactamase enzyme (Abamecha *et al.*, 2015; Facciola *et al.*, 2017). In fact, with similar report of present findings, Abamecha *et al.* (2015) in Gambella, Ethiopia, reported that Campylobacter jejuni and Campylobacter coli isolated from food animal showed 100% resistance to cephalothin.

The resistance to ciprofloxacin at 42.9% is greater compared to various results previously published in Ethiopia. For instance, in their findings in Mekelle, Abay *et al.* (2024) reported ciprofloxacin resistance of 35.7% in Campylobacter isolates collected from under-five years diarrhea affected children while in their investigations carried out in Bahir Dar, whereas the lower report recorded by Worku *et al.* (2024) noted ciprofloxacin resistance of 22.70% in Campylobacter jejuni strains isolated from under-five diarrhea affected children. Nevertheless, this level of resistance obtained herein is smaller than very high resistance obtained by Abamecha *et al.* (2015) in Gambella, who had obtained ciprofloxacin resistance of 80.5% in Campylobacter jejuni isolates from food animals.

The resistance to erythromycin, 71.4%, recorded in this investigation is also greater than the previous study done by Abamecha *et al.* (2015), which had found a 60.3% erythromycin resistance level in C. jejuni in Gambella region. The high erythromycin resistance in the entire three sample sources (human, poultry, and water) recorded in this research clearly demonstrates the dissemination of erythromycin resistance within the human-animal-environment interface in the study area.

Similarly with erythromycin resistance tetracycline resistance was detected in 71.4% of isolates overall in the present study, with the highest rate in human isolates (75%), followed by water (66.6%) and poultry (57.1%). The previous finding by Worku *et al.* (2024) in Bahir Dar reported 27.30% tetracycline resistance in *C. jejuni* from diarrheic children, which was lower than present studies reported in human case and Abamecha *et al.* (2015) in Gambella reported 82.2% tetracycline resistance in *C. jejuni* from food animals which was higher than present finding. The 71.4% tetracycline resistance in the present study evidence supported by the reports of tetracycline is one of the most widely used antibiotics globally in both human medicine and veterinary practice, and has also been used extensively as a growth promoter in food animal production in Ethiopia, creating sustained selection pressure (Maharjan *et al.*, 2020; Mdegela *et al.*, 2021).

In sharp contrast to the widespread resistance report across most antibiotic classes, nalidixic acid demonstrated complete efficacy against all 21 isolates tested in this study (100% susceptibility). When we compare this finding with previously studies by Abamecha *et al.* (2015) reported 3.8% nalidixic acid resistance in *C. jejuni* from food animals in Gambella, which, while low resistance level. Furthermore the carbapenems sub class drugs, such as meropenem showed no resistance against all 21 isolates. Unlike nalidixic acid existence of intermediate isolate among poultry and water samples irrespective of human isolates told us, it necessitates imposing biosecurity measures that separate poultry waste from the common water source, reduce agricultural selection pressures on resistance genes, and protect carbapenems as a last resort for humans before this transition stage can leap across the zoonotic bridge (Bukari *et al.*, 2025).

With respect to individual drug resistance base as showed in (table 11), the range of MARI values was between 0.17 and 0.58. The overall mean value for individual MARI was 0.444. More importantly, 95.2% (n=20) of the isolates showed higher than 0.20 MARI, which is considered as the high-risk threshold value globally with respect to individual antibiotics As previous stated by Krumperman (1983). Simultaneously, emergence risk due to drug resistance and selective pressure is clearly evident through the emergence of high values of MARI in relation to the individual antibiotics.

Out of twenty one, 18 isolates (85.7%) reported as MDR in present finding, with the majority exhibiting simultaneous resistance to four to five antibiotic classes. In comparison, the present finding of MDR rate is considerably higher than that of 84.2% multi-drug resistance reported by Debelo *et al.* (2022) in cattle isolates from Jimma town in which resistance was against three to six drug classes. Chala *et al.* (2021) from peri-urban Addis Ababa higher finding of the present study was reported MDR *Campylobacter* species 95.5% which is higher than present finding.

5.6. One Health Perspective: Linking Human, Poultry, and Environmental Findings

The overall results reveal the existence of a contamination chain in Bishoftu city. *Campylobacter* is found in chickens (15.5%), contaminated to the environment through chicken excreta and farm runoff water, found in surface water and farm water sources (13.6% and 16.7% respectively), and passed on to humans mainly through contact with chickens and consuming undercooked meat (5% in general, 17.5% in children, with contact with chicken as the major risk factor). The contamination pattern is similar to what Chala *et al.* (2021) found in their one-health approach study on the existence of *Campylobacter* strains shared among humans, livestock, and environmental sources around peri-urban Addis Ababa. In contrast to this study, the current study proves this contamination chain in Bishoftu city with respect to its poultry market and close proximity between farms and communities (Ismael *et al.*, 2021; Waktole *et al.*, 2023).

The alignment of AMR resistance profiles among the different sample types represents yet another important consideration from the point of view of One Health. The previous researchers have mainly been preoccupied with studying individual elements of the transmission cycle suggested by Debelo *et al.* (2022) and Chala *et al.* (2021). Meanwhile, the findings of the current study are necessary to inform efforts at developing coordinated antimicrobial stewardship strategies within the poultry production, water management, and training and health education, which is in line with the recommendations made by Johnson *et al.* (2017).

5. CONCLUSION AND RECOMMENDATIONS

The current findings demonstrate that *Campylobacter* is present and circulating across all three study compartments. The statistical analysis revealed that factors such as age, direct contact with poultry, irregular handwashing after latrine use, and consumption of undercooked meat were drive the transmission of *Campylobacter* in human setting. On the farm side, the absence of rodent control measures and the lack of regular cleaning and disinfection programs were the two strongest determinants of poultry colonization, while other variables did not significantly associate with infection. These reveal that, *Campylobacter* infection is more associated with quality of biosecurity and hygiene practices, than by the scale of production. Of the 21 isolates tested against 12 antibiotics drugs, the highest resistance was cephaloxin (100%) (71.4%) were recorded for erythromycin and tetracycline simultaneously. followed by Co-trimoxazole, Amoxicillin-clavulanic acid and Ciprofloxacin with resistance profile of 66.7%, 61.9% and 42.9% simultaneously. Controversially NA and meropenem showed no resistance across three samples sources. Largest proportion of specific drug resistance pattern lay to six specific antibiotics. Again 85.7% of the tested isolates were recorded for MDR pattern, with the majority simultaneously resistant to four to five antibiotic classes. The convergence of similar resistance profiles across human, poultry, and water isolates strongly suggests that antimicrobial resistance is disseminated across the human–animal–environment interface in study area. The co-occurrence of MDR strains across all three sources highlighted the urgency of addressing this circuit through integrated, multi-sectoral strategies rather than isolated single-sector interventions. The study thus demonstrates that a One Health framework is not only conceptually appropriate for understanding *Campylobacter* epidemiology in this setting, but is practically indispensable for designing effective control responses.

The following recommendations are proposed based on the present findings of the study:

- Routine biosecurity protocol and hygiene practices should be implemented on all poultry farms.
- Targeted community health education should prioritize mothers and caregivers of young children
- Routine surveillance of campylobacter and antimicrobial resistance in human clinical specimens should be formally established in study area and surrounding areas to enable evidence based managements of campylobacteriosis at the local level.
- Future investigations should expand environmental sampling beyond surface, drinking water and farm water sources to inclusive popular campylobacter niches like soil, poultry litter, and equipment's swabs to build a comprehensive models of the ecosystem reservoirs.
- PCR characterization and molecular genotyping of campylobacter species isolates recovered from sampling source should be definitively characterize campylobacter pathogenic species level and the transmission linkages gene suggested by shared antimicrobial resistance profiles reported in present study.

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7. ANNEXES

Annex 1: Microscopic identification of morphology and motility

One drop of sterile saline is placed on the slide. Colonies from the mCCDA plates are mixed with saline and place cover slip above the colonies and then examine the slide under microscope. The striking character of Campylobacter is their helical or curved shape. They have flagella, usually single, at one or both poles and are highly motile, spinning around their long axes and frequently reversing directions.

Annex 2: Gram staining

One drop of saline placed on a slide and colonies from the mCCDA plates are mixed with saline and smeared over the surface of the slide. Then, the smears allowed drying thoroughly and the smears are fixed by passing the slide, smear up, quickly through Bunsen flame three times. After cooling the smears can be stained Crystal violet (60seconds), Gram's Iodine (60seconds), Ethanol (60seconds) and Carbon Fuchsine (60%). Between each staining reagent the smear is washed under a gently running tap. Gram negative bacteria (like Campylobacter) are decolorized and stained red by the counter-stain (Carbol fuchsine). Campylobacter are curved or gull shaped forms. Old cultures may contain coccoid bacteria.

Annex 3: Test for Catalase

Put a colony at small spot on the slide (do not make suspension; just dry) and put one drop of 3% H₂O₂ on the spot with bacterial material. Finally examine for evolution of gas, which indicate catalase activity.

Annex 4: Test for Oxidase

Transfer a colony to a filter paper and soak the filter in oxidase solution. The appearance of a blue color within 10 seconds indicates the positive result.

Annex 5: Biochemical test of *Campylobacter* species

Characteristics	<i>C. jejuni</i>	<i>C. coli</i>	<i>C. lari</i>	<i>C. fetus</i>	<i>C. hyointestinalis</i>	<i>C. upsalensis</i>
Growth at 25°C	-	-	-	+	D	-
Growth at 35-37°C	+	+	+	+	+	+
Growth at 42 °C	+	+	+	D	+	+
Nitrate reduction	+	+	+	-	+	+
H ₂ S, TSI	-	+	-	+	+	-
Catalase	+	+	+	+	+	-
Oxidase	+	+	+	+	+	+
Motility	+	+	+	+	+	+
Hippurate hydrolysis	+	-	-	-	-	-
Resistance to Nalidixic acid	S	S	R	S	R	S
Resistance to Cephalotin	R	R	R	R	S	S

D = Some +, some – S = Susceptible R= Resistant

Source: (Corry and Atabay, 2011)

Annex 6: Procedure for Antimicrobials Susceptibility Testing of Campylobacter

A). Preparing the Inoculum

- ✚ Pure culture of Campylobacter isolated using selective media (mCCDA or Blood Agar) after an incubation period of 24 to 48 hours under microaerophilic conditions.
- ✚ Sterile loop/swab inoculates the isolated colony to a tube of sterile physiological saline (85%NACL) or Mueller-Hinton Broth.
- ✚ The bacterial suspension should then be thoroughly mixed and its turbidity made similar to that of 0.5 McFarland standards. This should be done as soon as possible because the cells may die when left long in the broth.

B). Inoculating the Test Medium

- ✚ Sterilize a cotton swab in the autoclave and dip it into the prepared bacterial suspension.
- ✚ Carefully wipe it against the inner side of the test tube above the line of fluid level to remove excess solution.
- ✚ Coat the agar surface with the swab in such a way that a confluent growth can be achieved by spreading it in three directions in the test plate (Mueller-Hinton Agar + 5% Defibrinated Horse Blood).
- ✚ The agar surface should then be allowed to dry for about 3 to 5 minutes (not more than 15 minutes).

C). Application of Antimicrobial Disks

- ✚ Place the selected antimicrobial disks (e.g., Erythromycin, Ciprofloxacin, and Tetracycline) on the inoculated agar plates with the help of sterile forceps or an automated disk dispenser.
- ✚ The separation between these disks should be at least 24 mm to avoid any overlapping inhibition zones.
- ✚ Carefully push down the disks with the help of a sterile needle or forceps.

D). Incubation

- ✚ The inoculated plates need to be inverted and placed in a gas jar/incubator that creates a microaerophilic environment.
- ✚ Let these plates grow for 24-48 hours under the temperature of 42°C (Some strains will require 48 hours incubation for a clear zone).

E). Zone of Inhibition Measurement

- ✚ After the specified incubation time, look for a uniform growth in the plates.
- ✚ With the help of a calibrated ruler or calipers, measure the diameters of complete inhibition zones (along with the diameter of the disk).
- ✚ This measurement should be done from the back side of the plates while keeping them under a non-reflecting black background.

F). Interpretation of Results

- ✚ Zone diameters must be recorded for each antimicrobial used.
- ✚ The zone diameters should be interpreted as either Susceptible (S), Intermediate (I), or Resistant (R) based on comparisons between observed zone diameters to interpretive criteria for *Campylobacter jejuni/coli* according to the most recent CLSI or EUCAST guidelines.

G). Quality Control

- ✚ Special attention must be paid to the quality of results obtained from these tests. As such, appropriate quality control strains should be included for testing, such as *Campylobacter jejuni* ATCC 33560, in parallel with isolates from the clinical or environment setting.

Annex 7: Questionnaire Format for Human Participants

1. Name /code of the patients -----Age----- Sex-----
2. Residential place A) ☐ Urban B) ☐ Rural
3. What is your level of education?
A) ☐ Illiterate B) ☐ Primary level C) ☐ Secondary level D) ☐ College and above

4. What is your family size?
A) ☐ Less than five B) ☐ Greater than five
5. What is your occupation?
A) ☐ Day labor B) ☐ Farmer C) ☐ Merchant D) ☐ Employed
6. Which water sources you use for drinking?
A) ☐ Tap water B) ☐ well C) ☐ Spring
7. Do you have contact with any domestic animals? A) ☐ Yes B) ☐ No
8. If yes, which animal species? A) ☐ chicken B) ☐ pet C) ☐ cattle D) ☐ sheep E) ☐ goat
9. Do you work or visit poultry farm in the last two weeks? A) ☐ Yes B) ☐ No
10. Do you have any contact with diarrheic person with in last two weeks A) ☐ yes B) ☐ No
11. Have you washing your hands before food preparation? A) ☐ Not at all B) ☐ Yes, sometimes
C) ☐ Yes, always
12. Do you wash your hands before touching cooked food A) ☐ Yes B) ☐ No
13. Do you have toilet at your home for sanitation? A) ☐ No, open defecation B) ☐ Yes, use toilet
14. How often you wash your hands after using latrine A) ☐ Not at all B) ☐ Yes, sometimes C) ☐ Yes, always
15. Do you consume chicken meat or other animal products A) ☐ Yes B) ☐ No
16. Do you consume of raw fruit and vegetables A) ☐ Yes B) ☐ No
17. Which cleaning agents you use for cleaning utensils before and after food preparation?
A) ☐ Use water only B) ☐ Water and soap

Annex 8: Questionnaire Format for poultry farm Participants

1. Farm name -----
2. What is your level of education?
A) ☐ Illiterate B) ☐ Primary level C) ☐ Secondary level D) ☐ College and above
3. Flock size

A) ☐ Small scale (500-1000chickens) B) ☐ Medium scale (1000-10000 chickens) C) ☐ Large scale (>10,000 chickens)

4. Age of chicken in weeks

A) ☐ <6 weeks B) ☐ 6-8 weeks C) ☐ 8-18 weeks D) ☐ >=18 weeks

5. What is the distance of your farm from main roads?

A) ☐ 0-20m B) ☐ 51-100m C) ☐ 101-300m D) ☐ >300

6. Are there other poultry farm on nearest distance? A) ☐ <500m B) ☐ > 500

7. Does your farm have fence and gate? A) ☐ Yes B) ☐ No

8. Do you use foot and tire bath at the gate? A) ☐ Yes B) ☐ No

9. Have you exchange equipment and worker with other farms? A) ☐ Yes B) ☐ No

10. Do you allow of visitor entry to your farm? A) ☐ Yes B) ☐ No

11. Do you have rodent control method you use in your farm? A) ☐ Yes B) ☐ No

12. Can wild bird access to the farm? A) ☐ Yes B) ☐ No

13. From which sources you use water for chicken and cleaning equipment's?

A) ☐ Pipe water B) ☐ well C) ☐ spring

14. Do you have regular cleaning and disinfection program? A) ☐ Yes B) ☐ No

15. How often you clean feeding and watering trough?

A) ☐ every day B) ☐ every other day C) ☐ Once a week D) ☐ Sometimes

16. How you manage a litter? A) ☐ Uses for compost B) ☐ Stored and sold C) ☐ reuse litter

17. Can your farm allow the movement of workers between barns? A) ☐ Yes B) ☐ No

18. How often workers use personal protective equipment?

A) ☐ not at all B) ☐ Yes, sometimes C) ☐ Yes, always

Annex 9: Picture taken up on during sample collection and laboratory work.



Image 1: sample collection from field and poultry.



Image 2: picture taken during laboratory work.

Annex 10: Some of Pictures of Laboratory findings

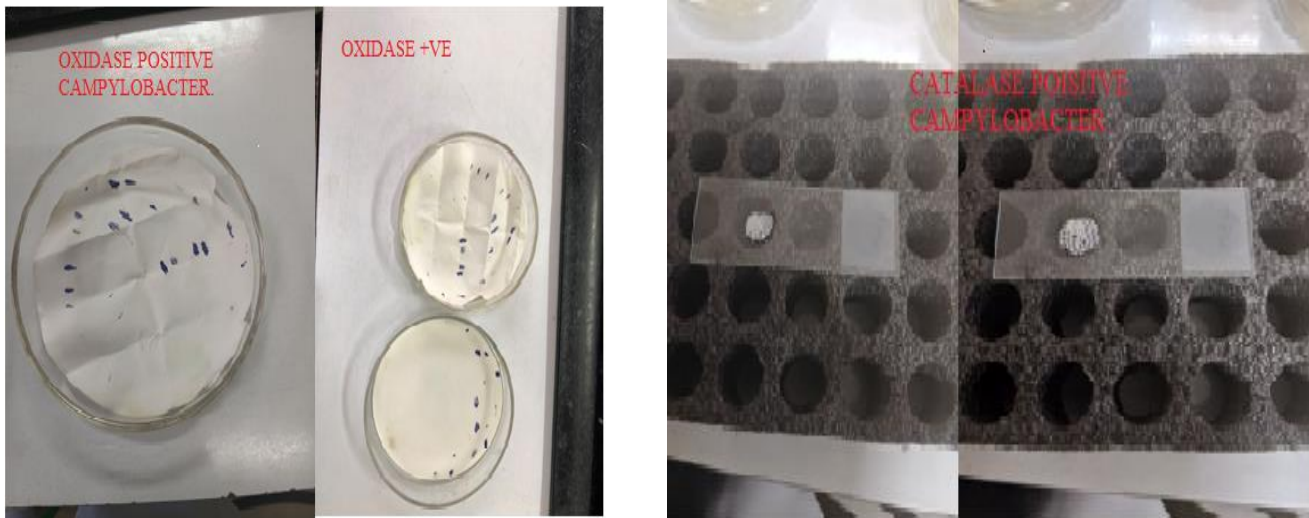


Image 1: (right): Catalase positive results of Campylobacter. Image 2 (left): Oxidase positive results of Campylobacter.



Image 3: gram staining of Campylobacter (by revealing distinct gram negative bacteria, faintly staining, and curved, spiral, or characteristic "gull-wing" rods under microscope)



Image 4: Media and supplements used for the culture of Campylobacter.



Image 5: Antibiotics disk used for AST Testing and Recording Measurements of diameter based on CLIS, 2024

Annex 11: Colony appearance *Campylobacter* from MCCDA

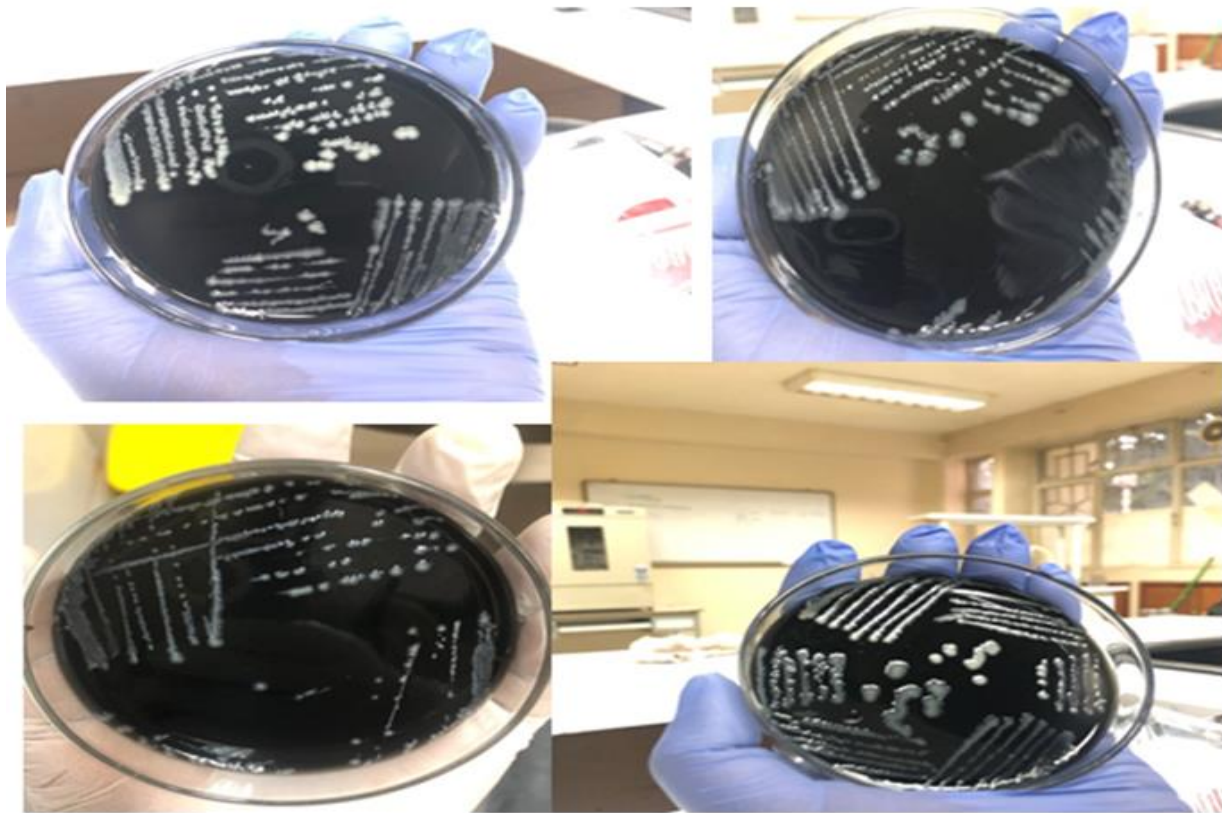


Image 1: moist, grey, flat, and spreading, Grey in color and sometimes showing a distinct metallic sheen or smooth and shiny appearance.

Annex 12: Written Consent Form

English Version

S/No.: _____

Card No.: _____

Full Name: _____

I, the undersigned, confirm that the details of the study titled:

“Isolation and Antimicrobial Susceptibility Profile of Campylobacter from Diarrheic Human Patients, Poultry, and Environmental Sources along Assessment of Community Biosecurity in Bishoftu, Central Ethiopia.”

Being conducted in Bishoftu city at _____ Health Center, have been clearly explained to me. I understand the study’s purpose, potential benefits, possible risks, and the confidentiality measures being taken to protect my personal information. I have had the opportunity to ask questions, and I understand that I may withdraw from the study at any time without penalty or loss of benefits. I am also aware that my data would be anonymized using numerical codes rather than my name. I understand that I would not receive any direct personal benefit except the clinical services related to the stool examination. Additionally, I agree that part of my stool sample may be sent to the laboratory for further diagnostic testing and may be reused in future research under the same project scope. With full understanding and without any coercion, I voluntarily agree to participate in this study.

Participant’s Signature: _____

Name of the Person Obtaining Consent: _____

Signature of the Person Obtaining Consent: _____

Date: _____

AFAAN OROMOOTIIN

Unka Walii-galtee Barreeffamaa
Gosa Afaan Oromoo

Lakk. Tartiibaa: _____

Lakk. Kaardii: _____

Maqaa Guutuu: _____

Ani, namni maqaan koo asii gadiitti mallataye, ibsi waa’ee qorannoo mata-duree:

“Isolation and Antimicrobial Susceptibility Profile of Campylobacter from Diarrheic Human Patients, Poultry, and Environmental Sources along Assessment of Community Biosecurity In Bishoftu, Central Ethiopia.”

(Adda baasuu fi Haala Madaqama Farra-baafee dhibee 'Campylobacter' kan namoota garaa kaasaan qabaman, indaanqoo, fi naannoo irraa argaman xiinxaluu, waliin qorannoo eegumsa fayyaa hawaasaa Magaalaa Bishoftu, Giddu-galeessa Itoophiyaatti gaggeeffamu).

Magaalaa Bishoftu keessatti Buufata Fayyaa _____ keessatti gaggeeffamaa jiru kun, sirriitti kan naa ibsame ta’uu koo nan mirkaneessa. Kaayyoo qorannichaa, faayidaa argamuu danda’u, miidhaawwan dhufuu danda’anii fi tarkaanfiiwwan icciti odeeffannoo dhuunfaa koo eeguuf fudhataman hubadheera. Gaaffii gaafachuuf carraa kan nan argadhe yoo ta’u, yeroo kamiyyuu dhiibbaa fi faayidaa dhabuun malee qorannicha keessaa ba’uu akka danda’u nan hubadha. Akkasumas, odeeffannoon koo maqaa kootiin osoo hin taane lakkoota addaan baasaniin iccitiin isaa akka eegamu nan beeka. Tajaajila yaalaa qorannoo faandii (sagaraa) waliin walqabatu malee, faayidaa dhuunfaa kallattii yoo xiqqaate akka hin arganne nan hubadha. Dabalataan, kofoon (faandiin) koo qorannoo dabalataatiif gara laaboraatoorii akka ergamu fi pirojektiidhuma kana keessatti qorannoo gara fuulduraatiif deebi’ee hojiirra ooluu akka danda’u nan walii-gala. Hubannoo guutuudhaan fi dhiibbaa tokko malee, fedha kootiin qorannoo kana keessatti hirmaachuuf nan walii-gala.

Mallattoo Hirmaataa: _____

Maqaa Nama Walii-galtee Fudhate qopheesse: _____

Mallattoo Nama Walii-galtee Fudhate qopheesse: _____

Guyyaa: _____

Annex 13: Animal ethical clearance certificate

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Research Ethics Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/08/115/18/2026

Name of Applicant: **Getachew Shelfa Beriso (DVM, MSc Student)**

Address: Department of Microbiology, Parasitology and Poultry Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Isolation, risk factor assessment and antimicrobial susceptibility profile of campylobacter species from diarrheic human patients, poultry and environmental samples, Bishoftu, Central ethiopia*

Date of application: **December, 2025**

Nature of the project: **Field investigation**
Target animal species: **Domestic chicken**
Number of animals involved: **180**
Study area: **Central Ethiopia**

Minutes No. and date of review: **VM/ERC/08/18/026, 27/03/2026**

The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of Student Getachew Shelfa Beriso.

Procedures on human subjects require separate ethical clearance from appropriate body

Professor Getachew Terefe (DVM, PhD)
Chairman



(Signature)
Signature

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Please quote Our Ref. No. When replying

ፋክስ
Fax 251-11-4339933

ስልክ
Tel. +251 114338450

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P.O.x. Box)34

ቢሽቶቱ አ.ተ.ዋ.ዲ
Bishoftu, Ethiopia

Annex 14: Human ethical clearance certificate

		አዲስ አበባ ዩኒቨርሲቲ አክሊሌ ለማ የጤና ምርምር ኢንስቲትዩት	Addis Ababa University Aklilu Lemma Institute of Health Research
Ethical Clearance Certificate			
Ref. No.: ALIHR-IRERC-004/2018/2026			
Date: June 9, 2026			
Title: [Identification, Molecular Detection and Antimicrobial Resistance Profile of <i>Campylobacter</i> Species from Diarrheic Patients, Poultry and Water in and Around Bishoftu Town, Central Ethiopia]			
Principal Investigator: Getachew Shelfa,			
Recommendation by the ALIHR-IRERC			
Dear: Getachew,			
The ALIHR-IRERC has reviewed your above mentioned research proposal and noted its merit. The IRERC would like to remind you, as PI, to submit progress reports of the work every 6 months and the final report upon completion of the study. Furthermore, you are expected to notify the ALIHR-IRERC ahead of time for any amendment or modification in the protocol or premature suspension or termination of the study.			
STATUS: <u>Approved</u>			
National Ethics Review Board Clearance: <u>not required</u>			
IRERC Chairperson: <u>Nigatu Kebede, Prof.</u>		IRERC Secretary: <u>Abaye Animut, PhD</u>	
Signature: 		Signature: 	
Approved by			
Name: <u>Prof. Wakgari Deressa, Director, ALIHR</u>			
Signature: 			
Date: _____			
CC:			
• IRERC Office			
• CPR Office			
• ALIHR-IRERC			
			
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SEEK WISDOM, ELEVATE YOUR INTELLECT, AND SERVE HUMANITY			

ISOLATION AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF CAMPYLOBACTER FROM DIARRHEIC HUMAN PATIENTS, POULTRY, AND ENVIRONMENTAL SOURCES ALONG ASSESSMENT OF COMMUNITY BIOSECURITY IN BISHOFTU (Getachew

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