

Occurrence, genomic diversity, antimicrobial resistance and virulence profile of *Campylobacter* spp. from children, an abattoir and butcher shops in central Ethiopia

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Abstract

Background

Campylobacter spp. are significant contributors to global diarrheal disease and associated with severe infection in children in low- and middle-income countries. In Ethiopia, diarrheal diseases are often driven by poor sanitation, unsafe food and water, and close human–animal contact. Underreporting is common due to limited sampling, diagnostic capacity, surveillance, knowledge gaps of antimicrobial resistance (AMR) and pathogen genomics. This study assessed the occurrence, genomic diversity, AMR and virulence profiles of *Campylobacter* spp. from diarrheic children and abattoir sources.

Methods

A cross-sectional investigation was conducted from November 2023 to September 2024 in Addis Ababa, Ethiopia. A total of 660 samples were collected from slaughterhouses (n = 192), butcher shops (n = 84) and children at health center (n = 384) and analyzed according to ISO 10272-1:2017. *Campylobacter* spp. were confirmed using MALDI-TOF MS, tested for AMR by disc diffusion and further characterized by whole- genome sequencing.

Results

Campylobacter spp. were detected in 9.9% of pediatric stools and 6.3% of abattoir samples, with highest occurrence in cattle feces (14.6%) and carcasses (8.3%). *Campylobacter jejuni* predominated, while *C. coli* and *C. fetus* were detected only in children. Whole-genome analysis showed substantial genetic diversity, identifying eight sequence types (STs) belonging to seven clonal complexes (CCs). The most common were ST-460 and ST-1365, and CC-443 and CC-460. The occurrence of *Campylobacter* spp. in feces, carcasses and humans sharing similar STs showing small genetic difference indicates contamination during slaughter and suggests potential transmission to humans or exposure to same sources. Furthermore, two human *C. jejuni* isolates belonged to CC-21, a clonal complex considered a generalist lineage capable of colonizing and persisting across wide range of hosts. High AMR was observed for tetracycline (91.5%), ciprofloxacin (70.2%) and erythromycin (55.3%) with 74.5% of the isolates being multidrug resistant. Genomic analysis identified multiple AMR determinates and conserved virulent genes in *C. jejuni*, indicating strong resistance and pathogenic potential.

Conclusion

These findings show that genetically diverse and AMR *Campylobacter* circulates among human and abattoir sources in Ethiopia, with evidence of carcass contamination and potential transmission. This

highlights the importance of strengthened hygiene practices, careful antimicrobial use and integrated One Health genomic surveillance.

Introduction

Campylobacter species are among the most frequently reported bacterial causes of diarrheal disease worldwide (1, 2) and represent a substantial contributor to childhood diarrhea in low- and middle-income countries (LMICs) (3). Despite their global importance much of the current understanding of *Campylobacter* is derived from studies in high-income countries, where infections are strongly associated with handling or consuming chicken meat (4). In contrast, in many low-income settings, *Campylobacter* infections are endemic and largely affect children under 2 years of age (5) as well as immunocompromised individuals (6). Transmission occurs mainly through consumption of contaminated food products, including undercooked meat and unpasteurized milk. Environmental and socioeconomic factors such as inadequate sanitation, unsafe drinking water and close human–animal contact further facilitate the spread of the pathogen (6–8). The incidence and mortality associated with campylobacteriosis are highest in low-income settings (9). However, the true burden of the disease is often underestimated because of weak surveillance systems, limited diagnostic capacity and the common occurrence of asymptomatic infections (10). Consequently, important gaps remain in understanding the epidemiology and transmission in LMICs such as Ethiopia.

In Ethiopia, *Campylobacter* is recognized as an important cause of pediatric gastroenteritis, with reported prevalence ranging from 5% to 20% across regions (11–14). In Addis Ababa, detection rates of 13–18% have been documented from human and environmental samples (15, 16). In addition, abattoir-based studies have identified *Campylobacter* contamination in beef carcasses, with prevalence estimates ranging from 5.6% to 24.8% across carcasses and contact surfaces (17–19). These studies showed that *Campylobacter* is a pathogen of public health relevant in Ethiopia within the One Health domain.

Genomic diversity is fundamental to understanding *Campylobacter* transmission, virulence and AMR evolution (20). Comparative genomic analyses demonstrate extensive strain variation driven by horizontal gene transfer, recombination, and mobile genetic elements, contributing to emerging multidrug resistance (MDR) lineages (21). Large-scale genomic datasets have also revealed a global increase in AMR in *C. jejuni* and *C. coli* over the past 23 years, particularly to tetracyclines, fluoroquinolones, and aminoglycosides (22). Key drivers of this trend include antibiotic misuse, poor sanitation, and governance-related factors such as weak regulation, limited health infrastructure, poor coordination between human and animal health (23).

In low-income countries such as Ethiopia, genomic studies of *Campylobacter* remain limited though emerging data are beginning to provide important insights into *Campylobacter* transmission. Studies of rural households have identified substantial *C. jejuni* sequence-type diversity, with poultry serving as a major reservoir for infant exposure (24). Phylogenetic clustering between children and environmental sources has also been reported (13). Despite these advances, genomic evidence from urban settings,

abattoirs, meat pathways and campylobacteriosis among children remains scarce. Moreover, the absence of national surveillance, limited genomic analysis capacity and reliance on selective culture methods hinder accurate estimation of disease burden, leaving *Campylobacter* underrecognized as a significant diarrheal pathogen. To address these gaps, this study aimed to investigate the occurrence, AMR and genomic characteristics of *Campylobacter* spp. in low-income urban settings of central Ethiopia. Specifically, we sought to (i) determine the prevalence of *Campylobacter* in diarrheic children at health center and meat-derived samples, (ii) characterize their AMR profiles, (iii) examine sequence-type diversity, clonal complexes and phylogenetic relationships and (iv) assess potential epidemiological links between human and animal reservoirs.

Material and Methods

Study area, population and design

This cross-sectional study was carried out from November 2023 to September 2024 in Addis Ababa, Ethiopia. Addis Ababa is the capital city of the country and located in the central highlands. Samples were collected from two sources. Clinical samples were obtained from children with diarrhea who visited Nefas Silk Sub-City Woreda 11 Health Center for treatment. Beef-related samples were collected from the Addis Ababa Abattoir Enterprise and nearby butcher shops in the woreda 11 Health Center with the shop owners' permission. Children under one year of age who were presented with diarrhea during the visit to the health center were included in the study after consent was obtained from their caregivers. The sample size was calculated using Thrusfield (25) formula by assuming an expected prevalence of 50%, a 95% confidence level and an absolute precision of 0.05, which resulted in 384 clinical samples. For beef-derived samples, the sample size was determined based on a previously reported *Campylobacter* prevalence of 9.3% in meat from Addis Ababa and Debre Zeit (26) using the same formula. In total, 276 samples were collected, including 192 from the abattoir and 84 from butcher shops. At the abattoir, 48 cattle fecal samples were taken before slaughter and 48 corresponding carcass swabs collected after slaughter. In addition, 32 hands, 32 aprons and 32 knives swabs were sampled from the specific butchers who slaughtered the sampled cattle. At the butcher shop, 84 samples were collected, comprising 21 carcass swabs, 21 cutting board swabs, 21 knife swabs and 21 hand swabs.

Sample collection and transport

Fresh stool samples were obtained by laboratory personnel from children presented with diarrhea at the Health Center as part of routine diagnostic procedures. Immediately after collection, samples were placed in sterile 50 mL screw-capped containers containing Cary-Blair transport medium (Oxoid, UK). The samples were kept at 4°C at the health center for no longer than 24 hours and then shipped in a cold box with ice packs to the Health Biotechnology Laboratory, institute of Biotechnology, Addis Ababa University, taking about 30 minutes. Cattle fecal samples were collected aseptically directly from the

rectum using sterile disposable gloves. About 5–10 g of feces was placed into sterile 50 mL screw-capped containers containing Cary-Blair transport medium and transported immediately to the laboratory for analysis. Carcass swab samples were collected according to the ISO 17604 standard (27) by rubbing the carcass surface horizontally and vertically several times at the 12 recommended anatomical sites using sterile sponge swabs moistened with sterile saline. Swab samples were also collected from the hands of workers, aprons, knives and cutting boards using sterile sponge swabs moistened with sterile saline. Each swab was placed in a clean plastic bag with 20 mL of Cary-Blair transport medium. Samples were collected daily from abattoir and butcher shops. From collection to arrival at the laboratory, the process took up to six hours, during which the samples were kept cool in an icebox with ice packs. All samples were processed immediately upon arrival.

Isolation and Identification of *Campylobacter* spp.

The analyses were performed according to ISO 10272-1:2017 (28). Human stool and cattle fecal samples were surface streaked onto modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA; Oxoid, UK) supplemented with SR0155E and incubated at 42°C for 48 h. Swab samples were enriched in Preston broth (Oxoid, UK) supplemented with SR0155E (Oxoid, UK) and incubated at 42°C for 48 h, then surface streaked onto mCCDA and incubated at 42°C for 48 h. All incubations were carried out under microaerobic conditions generated using CampyGen (Oxoid, UK).

Presumptive *Campylobacter* colonies were sub-cultured onto blood agar (Oxoid, UK) containing 5% defibrinated horse blood (Thermo Scientific) and incubated at 42°C for 24–48 h under microaerobic conditions. Suspected *Campylobacter* colonies were identified using a matrix-assisted laser desorption/ionization time of flight mass spectrometry system (MALDI-TOF MS) (Zybio EXS3000, China) according to the manufacturer's instruction. Confirmed isolates were preserved at – 20°C in brain heart infusion broth (HiMedia, India) containing 20% glycerol.

Antimicrobial susceptibility testing

Confirmed *Campylobacter* isolates were analyzed for AMR using disc diffusion method, following the European Committee on Antimicrobial Susceptibility Testing guidelines (29). Antibiotics were selected based on availability and clinical/veterinary relevance in Ethiopia. Inoculated plates were incubated at 42°C for 24 + 24 h under microaerobic conditions. Inhibition zones were measured after 24 and 48 h of incubation and interpreted according to EUCAST breakpoints where available. For antibiotics without EUCAST breakpoints, i.e. streptomycin, norfloxacin and nalidixic acid isolates were considered resistant only when the diameter of the inhibition zone measured 0 mm. Multidrug resistance was defined as resistance to ≥ 3 antibiotic classes.

Whole-genome sequencing and genomic analysis

Genomic DNA was extracted from the confirmed *Campylobacter* isolates using the EZ-10 Spin Column Genomic DNA Minipreps Kit (BioBasic, Markham, ON, Canada). DNA quality and concentration were assessed using NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) and Qubit fluorometer (Invitrogen, USA). Paired-end sequencing libraries (151 bp) were prepared using the Illumina Nextera XT DNA Library Preparation Kit and sequenced on an Illumina NovaSeq X platform at SciLifeLab, Sweden.

Raw reads were quality-checked using FastQC (v0.11.9) (30) and trimmed with fastp (v0.23.4) (31). High-quality reads were *de novo* assembled using SPAdes (v3.15.5) (32) and assembly quality was assessed using QUAST (v5.2.0) (33), and contigs shorter than 500 bp were removed. Species identification was performed using KmerFinder v3.2 and SpeciesFinder v2.0 (Center for Genomic Epidemiology (CGE), Technical University of Denmark (DTU), for species and subspecies classification (34, 35) accepting results > 80% query coverage and a PASS confidence score. Out of the 50 *Campylobacter* isolates subjected to whole-genome sequencing only 20 isolates (13 human, 4 cattle feces, and 3 carcass swabs) yielded high-quality assemblies suitable for downstream analysis. The remaining isolates were excluded due to low sequencing coverage, potential contamination, or incomplete assemblies.

Genome annotation was conducted using Prokka v1.14.6 (36) and pan-genome analysis was performed with Roary v3.13.0 (37). Genes were categorized as core ($\geq 99\%$ of genomes), soft-core (95–99%), shell (15–95%), and cloud ($\leq 15\%$) based on their frequency among isolates. Core-genome SNPs analysis was identified by aligning the 18 *C. jejuni* genomes to the reference strain *C. jejuni* subsp. *jejuni* NCTC 11168 (GenBank: GCF_000009085.1) using Parsnp v2.1.5 (38) and a maximum-likelihood phylogeny was inferred. Phylogenetic trees were visualized in iTOL (v6.5.1) (39), with associated metadata. *In silico* MLST was performed using MLST v2.23.0 (Seemann; Bioconda distribution), against the PubMLST database (40), using the standard seven-locus *Campylobacter* MLST scheme, and clonal complexes were assigned accordingly (databases accessed November 2025). Antimicrobial resistance determinants were identified using ABRicate v1.0.1 (41) with the CARD database (2024-07) and AMRFinderPlus v3.12.8 (database 2024-07-22.1) (42). Virulence associated genes were detected using ABRicate v1.0.1 with the VFDB database (November 2025), applying $\geq 75\%$ identity and $\geq 80\%$ coverage threshold. Results were processed in Python to generate a presence–absence matrix and visualized as a heatmap in iTOL (39).

Results

Occurrence of *Campylobacter*

A total of 660 samples were analyzed from children from diarrhea, abattoir and butcher shops in Addis Ababa. Among the 384 stool samples obtained from diarrheic children ≤ 1 year of age, *Campylobacter* was detected in 38 samples (9.9%). *Campylobacter jejuni* was the predominant species (92.1%), followed by *C. coli* (5.3%) and *C. fetus* (2.6%) (Table 1). *Campylobacter fetus* subsp. *fetus* was detected in a child who had recently visited a rural household with mixed livestock for one week and presented to the health center with diarrhea.

Of the 192 abattoir samples, *Campylobacter* were detected from 12 (6.3%), all identified as *C. jejuni*. Detection was highest in cattle fecal samples (14.6%) and carcass swabs (8.3%), with a single isolate recovered from apron swabs (3.1%). No *Campylobacter* was detected in hand or knife swabs from slaughterhouse workers, nor in any of the 84 butcher shops samples (Table 1).

Table 1

Occurrence and distribution of *Campylobacter* species across different sample types in Addis Ababa, Ethiopia.

Sample Type		No. of samples analyzed	Identified <i>Campylobacter</i> species (%)			
			<i>C. jejuni</i> (%)	<i>C. coli</i> (%)	<i>C. fetus</i> (%)	Total (%)
Diarrheic children (≤ 1 year)		384	35 (9.1)	2 (0.5)	1 (0.3)	38 (9.9)
Abattoir	Cattle fecal samples	48	7 (14.6)	0 (0)	0 (0)	7 (14.6)
	Carcass swabs	48	4 (8.3)	0 (0)	0 (0)	4 (8.3)
	Apron swabs	32	1 (3.1)	0 (0)	0 (0)	1 (3.1)
	Hand swabs	32	0 (0)	0 (0)	0 (0)	0 (0)
	Knife Swab	32	0 (0)	0 (0)	0 (0)	0 (0)
Butcher shops	Carcass swabs	21	0 (0)	0 (0)	0 (0)	0 (0)
	Cutting board swabs	21	0 (0)	0 (0)	0 (0)	0 (0)
	Knife swabs	21	0 (0)	0 (0)	0 (0)	0 (0)
	Hand swabs	21	0 (0)	0 (0)	0 (0)	0 (0)
Total		660	47 (7.1)	2 (0.3)	1 (0.2)	50 (7.5)

Phenotypic Antimicrobial Susceptibility Testing

All *C. jejuni* isolates demonstrated resistance to at least one antimicrobial agent. The highest resistance was observed to tetracycline (91.5%), followed by ciprofloxacin (70.2%) and erythromycin (55.3%), with lower resistance observed to gentamicin (36.2%), nalidixic acid (17.0%), norfloxacin (10.6%) and streptomycin (4.3%). Multidrug resistance was identified in 74.5% of isolates (Table 2). The *C. coli* isolate exhibited resistance to tetracycline and ciprofloxacin, whereas *C. fetus* was resistant only to tetracycline.

Table 2. Antimicrobial resistance pattern of *Campylobacter jejuni* from human and abattoir sources in Addis Ababa, Ethiopia.

Resistance pattern	Human (n = 35)	Cattle (n = 7)	Carcass (n = 4)	Apron (n = 1)	Total (n = 47)
Tet	3	1	0	0	4
Cip + Ery	2	0	0	0	2
Cip + Tet	3	1	0	0	4
*Cip + Ery + Tet	8	2	1	1	12
*Ery + Tet + Str	0	1	1	0	2
*Cip + Tet + Nor	1	0	0	0	1
*Cip + Ery + Tet + Gen	7	1	2	0	10
*Cip + Tet + Nal	4	0	0	0	4
*Ery + Tet + Gen	3	0	0	0	3
*Ery + Tet + Nor + Nal + Gen	4	0	0	0	4

Abbreviations: Cip, ciprofloxacin; Ery, erythromycin; Tet, tetracycline; Nor, norfloxacin; Str, streptomycin; Nal, nalidixic acid; Gen, gentamicin.

Rows marked with * indicate MDR.

Genome characteristics of *Campylobacter*

The draft genome assemblies of the *Campylobacter* isolates displayed the following characteristics: among the 18 *C. jejuni* isolates, the median genome size was 1.69 Mb with a GC content of 31%. The single *C. coli* isolate had a genome size of 1.80 Mb with a GC content of 31%, and the *C. fetus* isolate had a genome size of 1.91 Mb with a GC content of 34%.

Pan-Genome Composition

The *C. jejuni* pan-genome comprised 3,359 genes, including 1,201 core genes (35.8%) shared across all isolates, with no soft-core genes detected. The remaining 2,158 genes (64.2%) formed the accessory genome, consisting of 1,021 shell genes (30.4%) and 1,137 cloud genes (33.8%). Pan-genome analysis was not performed for *C. coli* and *C. fetus* because of the limited number of isolates, which rendered the analysis unreliable.

Multilocus Sequence Typing (MLST) and clonal complex

MLST analysis of 20 *Campylobacter* (18 *C. jejuni*, one *C. coli* and one *C. fetus*) genomes revealed eight distinct STs among *C. jejuni* isolates distributed across eight different clonal complexes while four isolates lacking defined STs (Figure 1). The most represented clonal complex were CC-443 and CC-460

(n = 3 each), followed by CC-353, CC-206 and CC-21 (n = 2 each), and single representatives of ST-22 and ST-464. Sequence type ST-460 was the most prevalent (n = 3), detected in human, cattle and carcass samples originating from the same animal. Other *C. jejuni* STs included ST-1365 (n = 3, clinical), ST-5 (n = 2, clinical), ST-227 (n = 2, cattle and corresponding carcass) and ST-22, ST-251, ST-883, and ST-464 (n = 1 each, clinical). The *C. coli* isolate was ST-9011 belonging to CC-828 and the *C. fetus* isolate was assigned to ST-11, which did not cluster within a defined complex.

Core-Genome SNP Variation and Phylogeny

Core-genome SNP analysis of the 18 *C. jejuni* isolates showed pairwise differences ranging from 17 to 20,763 SNPs, indicating the presence of both related strains and highly diverse lineages. The closest lineage was isolates of ST-227 and ST-460 detected from cattle feces and corresponding carcass sample differed by 35 SNPs and 17 SNPs, respectively. Similarly, ST-460 was also detected in a clinical isolate with a pairwise difference of 52 SNPs. Clinical isolates of ST-5 and ST-1365 differed by 17 and 34 SNPs respectively. In contrast, some isolates, including ST-22 and an unassigned clinical strain, showed high divergence (>18,000 SNPs), reflecting substantial genomic diversity (S1 File). The core-genome SNP-based maximum-likelihood phylogeny (Figure 1) revealed multiple small clades irrespective of source, with short branches among related isolates and long branches separating highly divergent lineages.

Genomic determinants of AMR

Among *C. jejuni* isolates, AMR-associated determinants, including efflux pump components, resistance genes and point mutations, were identified in all 18 genomes, with each genome carrying four to six determinants (Figure 1, Table 3). All *C. jejuni* isolates harbored the complete *cmeABC* efflux operon and its regulator *cmeR*. Members of the OXA-61 family of β -lactamases, including *blaOXA-193* and *blaOXA-576*, were detected in 78% of isolates, while *blaOXA-61*(G→T) variant occurred in 11%. Tetracycline resistance-encoding genes were identified in 10 isolates, with *tet*(O) most prevalent (n = 7) across human, cattle feces, carcass samples. Mosaic *tet*(O/M/O) genes were identified in two isolates, while one isolate carried an untyped *tet* gene. Macrolide resistance-associated *rpIV* A103V mutations were found in 10 isolates, and fluoroquinolone resistance-associated *gyrA* T86I mutations were present in six isolates across human and animal sources. Aminoglycoside resistance was rare, limited to a single *aadE*-positive isolate from cattle feces. Among non-*jejuni* species, *C. coli* carried an OXA-family β -lactamase gene (*blaOXA-193*) and a partial *cmeBC* efflux operon, while *C. fetus* lacked detectable AMR determinants.

Table 3. Antimicrobial resistance determinates in 18 *C. jejuni* isolates from humans and abattoir samples in Ethiopia.

Target Antibiotic	Genes(s)/Mutation	No. of isolates	Percentage
Erythromycin, tetracycline, ciprofloxacin, nalidixic acid, norfloxacin, chloramphenicol	<i>cmeA</i> , <i>cmeB</i> , <i>cmeC</i> , <i>cmeR</i>	18	100%
Tetracycline	<i>Tet</i> , <i>tetO</i> , <i>tet(O/M/O)</i>	10	56%
Amoxicillin, ampicillin, cefalexin	<i>blaOXA-193</i>	9	50%
	<i>blaOXA-576</i>	3	17%
Amoxicillin, ampicillin, cefalexin	<i>blaOXA-61_G-57T</i>	2	11%
Gentamicin, streptomycin	<i>aadE</i> (<i>aad(6)</i> family)	1	7%
Nalidixic acid, ciprofloxacin, norfloxacin	<i>gyrA</i> (T86I)	6	33%
Erythromycin	<i>rplV</i> (A103V)	10	56%

Virulence Gene profile of *Campylobacter* isolates

In silico screening of *C. jejuni* against the VFDB identified between 84 and 105 virulent isolated genes (VFs) per genome (mean = 91), whereas *C. coli* and *C. fetus* contained 61 and 0 VFs, respectively (Figure 2, S1 File). All *C. jejuni* isolates carried a complete set of flagellar and motility genes, including basal-body and hook gene (*flgA–flgS*), filament-associated proteins (*flaC*, *fliD*, *fliS*) and regulatory elements (*rpoN*). Energy metabolism and stress-response genes, including *cysC* (94.4%), *htrB* (94.4%), *eptC* (77%), and *rpoN* (100%), were highly prevalent and >90% of isolates carried the full pseudaminic acid biosynthesis cluster (*pseA–pseI*, *ptmA/B*). Core LOS/LPS biosynthesis genes *gmhA* (100%), *gmhA2* (77.7%), *gmhB* (100%), *hddA* (77.7%), *hddC* (66.6%), *hldD* (100%), and *hldE* (66.6%) were widely conserved. Flagellar Type III secretion system (fT3SS) structural components were also highly conserved, with *flhA* and *flhB* in 100% of isolates, *fliP/Q/R* in 88–100%, with invasion effectors *ciaB* and *ciaC* present in all *C. jejuni* and *C. coli* isolates. Chemotaxis genes (*CheA*, *CheY*, *CheW*, *CheV*) were universally present *C. jejuni*. Capsule biosynthesis and export genes (*kpsC–kpsT*) were detected in all *C. jejuni* genomes and core LOS loci (*Cj1416c*, *Cj1417c*, *Cj1419c*, *Cj1420c*), along with key LOS genes such as *waaC* (100%), *waaV* (100%), and *hddC* (72%), were highly conserved. Adhesion and invasion genes *cadF* and *jlpA* were detected in all *C. jejuni* isolates, while *pebA* and *porA*, and were present in 100% and 55.5%, respectively. The cytolethal distending toxin operon (*cdtABC*) was detected in all *C. jejuni* isolates but absent in *C. coli*.

Discussion

This study combines epidemiological and genomic approaches to better understand *Campylobacter* in children attending health centers and in abattoir sources in Addis Ababa, central Ethiopia. The prevalence of *Campylobacter* among diarrheic children in this study was comparable to earlier studies in Ethiopia (11,12) and sub-Saharan Africa (43). However, it was higher than the prevalences reported by

Belina et al. (13) and Abay et al. (44), but lower than those reported by Mulu et al. (14) in Ethiopia and Karikari et al. (45) in Ghana. These variations may be related to differences in study methods and categories, sociodemographic characteristics, seasonality and exposure to livestock or contaminated environments. They may also reflect differences in the study population, as this study included only infant presenting to a health center for treatment, which may represent more severe cases. In contrast, most of the cited studies included broader age groups attending health facilities and at home, which may partly explain the observed differences in prevalence.

The prevalence of isolated *Campylobacter* at the abattoir (6.3%) was mainly associated with cattle feces (14.6%) and carcass swabs (8.3%), consistent with reports from other Ethiopian municipal abattoirs (17, 18). This finding suggests that carcasses were contaminated with cattle feces during slaughter, with potential for transmission to humans, as consumption of raw meat is common in the area. The small genetic difference (17-35 SNPs) observed in corresponding cattle feces isolates further support possible contamination during processing. In contrast, the absence of *Campylobacter* in butcher shop samples may reflect its limited survival under aerobic conditions during open-air carcass hanging practices in the area.

Campylobacter jejuni was the most frequently identified species, in line with earlier Ethiopian reports (11, 14, 18) and with global patterns of human campylobacteriosis (7). The detection of *C. fetus* subsp. *fetus* in an infant is unusual, as this species is rarely reported in young children and is typically associated with zoonotic transmission from sheep and cattle (46). In this case, the child had spent a week visiting a rural household with mixed livestock (including sheep, cattle, donkeys, and chickens) and developed diarrhea while on the way home. The child was brought to the health center immediately after returning. During the visit, the child played with the animals and crawled on barn floors contaminated with manure. The household had recently experienced abortions and deaths among small ruminants and a sheep was even slaughtered on the barn floor while the child was present. These conditions likely facilitated exposure to *C. fetus*, highlighting the role of direct animal contact, unhygienic practices and environmental contamination in zoonotic transmission.

The pan-genome analysis of *C. jejuni* revealed a relatively small conserved core genome and a large, variable accessory genome consistent with previous findings in Ethiopia (47). This reflects substantial genetic heterogeneity. The predominance of accessory genes reflects an open pan-genome shaped by horizontal gene transfer and strain-specific adaptations (20, 49). Such genomic plasticity supports ecological flexibility, host adaptation, AMR and virulence variation, emphasizing the importance of ongoing genomic surveillance. Three *C. jejuni* sequence types (ST-1365, ST-883, and ST-251) identified in this study had been previously reported in Ethiopia (13, 24). In contrast, *C. jejuni* ST-460, ST-227, ST-5, ST-464 and ST-22, as well as *C. coli* ST-9011 and *C. fetus* ST-11, are reported here for the first time in the country. These findings highlight the considerable genetic diversity of *Campylobacter* strains circulating in Ethiopia.

Core-genome SNP analysis revealed substantial genetic diversity among *C. jejuni* isolates, with pairwise differences ranging from 17 to 20,763 SNPs aligns with established knowledge that *C. jejuni* exhibits high genetic diversity (50). Several STs identified in our study have also been reported internationally. ST-5, ST-251 and ST-22 are commonly associated with human infections and poultry reservoirs are widely documented in the PubMLST database (51) and individual studies (50, 52, 53). These findings indicate that many of the STs observed in our study are not geographically restricted and can circulate across diverse regions and host species, underscoring their global dissemination and zoonotic potential. Overall, phylogeny shows multiple lineages circulating across human and abattoir sources, with no clear host-specific clustering, reflecting a mix of recent transmission and a diverse background population.

Certain *Campylobacter jejuni* CCs, such as CC-21, CC-45, CC-48, and CC-206, are considered “generalist” lineages because their ability to colonize a wide range of hosts, such as poultry, cattle, wild birds, and humans, rather than being host-specific (49). In this study, CC-21 was detected in cattle, carcasses, and humans, highlighting its transmission potential and ability of generalists to spread across multiple hosts.

The pathogenesis of *Campylobacter* is complex and not yet fully understood (54), largely due to its high genetic variability and frequent recombination (55). In this study, *C. jejuni* isolates carried a largely conserved set of virulent genes associated with motility, chemotaxis, adhesion, invasion and toxin production, in line with previous reports (55-58). The presence of flagellar components and the fliC machinery highlights their role in colonization and host-cell invasion, while the widespread detection of adhesion genes and invasion effectors (*ciaB*, *ciaC*) supports a strong capacity for intestinal infection (57, 59). The *cdtABC* operon, responsible for cytolethal distending toxin production, was detected in all *C. jejuni* isolates, confirming its conserved role in host-cell damage (54). In addition, key LOS/LPS and capsule biosynthesis genes were consistently present, indicating maintained mechanisms for immune evasion, serum resistance and host interaction (57, 58). Overall, the circulating *C. jejuni* strains showed most extensive and conserved virulent profiles, suggesting strong pathogenic potential.

The high AMR levels, particularly to tetracycline, fluoroquinolones and macrolides, are consistent with recent findings (8, 16, 18). Whole genome sequencing revealed that all the genotyped *C. jejuni* isolates harbored the *cmeABC-cmeR* efflux system. This efflux pump in *Campylobacter* spp. is not specifically targeted against a single antibiotic but confers multidrug resistance by actively pumping out multiple types of antibiotics out of the bacterial cell (60, 61). Its presence in isolates from human, carcass and cattle feces highlights its role in antimicrobial resistance dissemination along the human–animal–food interface. Genotype–phenotype concordance was incomplete for several antimicrobials, as reported previously (61, 62). Tetracycline resistance genes were widespread, predominantly as *tet(O)*, followed by mosaic *tet(O/M/O)* and one untyped *tet* gene, across cattle, carcasses and human isolates. These findings align with previous studies identifying *tet(O)* as the main tetracycline resistance determinant mediated by ribosomal protection proteins (63, 64), while the mosaic variant reflects ongoing recombination and genetic diversification in *Campylobacter* (65). Notable, although 56% of the sequenced *C. jejuni* isolates carried the *tet(O)* and *tet(O/M/O)* genes, 91.5% displayed phenotypic resistance to tetracycline, suggesting additional mechanisms beyond these known genes may contribute

to tetracycline resistance. Most isolates carried OXA-61 family β -lactamases, particularly blaOXA-193 and blaOXA-576, consistent with their role in intrinsic β -lactams resistance (61, 66). The blaOXA-61_G-57T promotor mutation identified in two isolates, which is known to enhance gene expression and contribute to high level β -lactam resistance *C. jejuni* (62, 67). Mutations associated with clinically important resistance were also common. The gyrA_T86I mutation conferring resistance fluoroquinolone as ciprofloxacin and nalidixic acid, was detected in one-third of the *C. jejuni* isolates, while the rplV_A103V mutation associated with reduced macrolide (64, 68,69) occurred in more than half of the isolates. Given the importance of macrolides as first-line therapy, these findings are clinically relevant and likely reflect antimicrobial selection pressure in food-animal production. Aminoglycoside resistance was rare in this study, consistent with previous studies (62), but highlights the potential for emergence and spread through horizontal gene transfer. Overall, the resistance patterns observed among *C. jejuni* isolates from children clinical cases and food sources are concerning, reflecting both local antimicrobial use practices and broader global evolutionary trends. This emerging threat makes empirical treatment challenging and highlights the need for continuous surveillance and responsible use of antimicrobials.

Conclusion

This study highlights the public health significance of *Campylobacter* in Ethiopia, showing its circulation among humans and abattoir sources, including carcass contamination during slaughter and potential transmission to human. The detection of *C. fetus* subsp. *fetus* in an infant underscores the zoonotic risks associated with close human–livestock interactions. High genetic diversity, widespread AMR and largely conserved virulence profiles, particularly in *C. jejuni*, indicates the adaptability and pathogenic potential of these strains. These findings emphasize the need for improved hygiene practices, prudent antimicrobial use and implementation of One Health strategies to reduce transmission and protect vulnerable populations.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki (70) and received Ethical approval from Institutional Review Board of the Institute of Biotechnology, Addis Ababa University (Approval No. IOB/LB/2016/2024). Caregivers were informed about the study objectives in their local language and voluntary verbal consent was obtained and recorded. Abattoir and butcher shop-based samples were collected with the permission from the abattoir authorities and butcher shop owners. No individual or sample identities are disclosed.

Consent for publication

N/A.

Competing interests

The authors declare no competing interests.

Authors' contributions

FZ led the study, including conceptualization, data curation, analysis, laboratory work, methodology development and visualization. FZ also drafted the manuscript and contributed to its review and editing.

TST, HA, SB and IH contributed to the study design and methodology, supervised the project, secured funding, provided resources and contributed to reviewing and editing the manuscript.

Availability of data and materials

All data supporting the findings of this study are included within the manuscript and its supplementary materials. The whole genome sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1449354.

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Figures

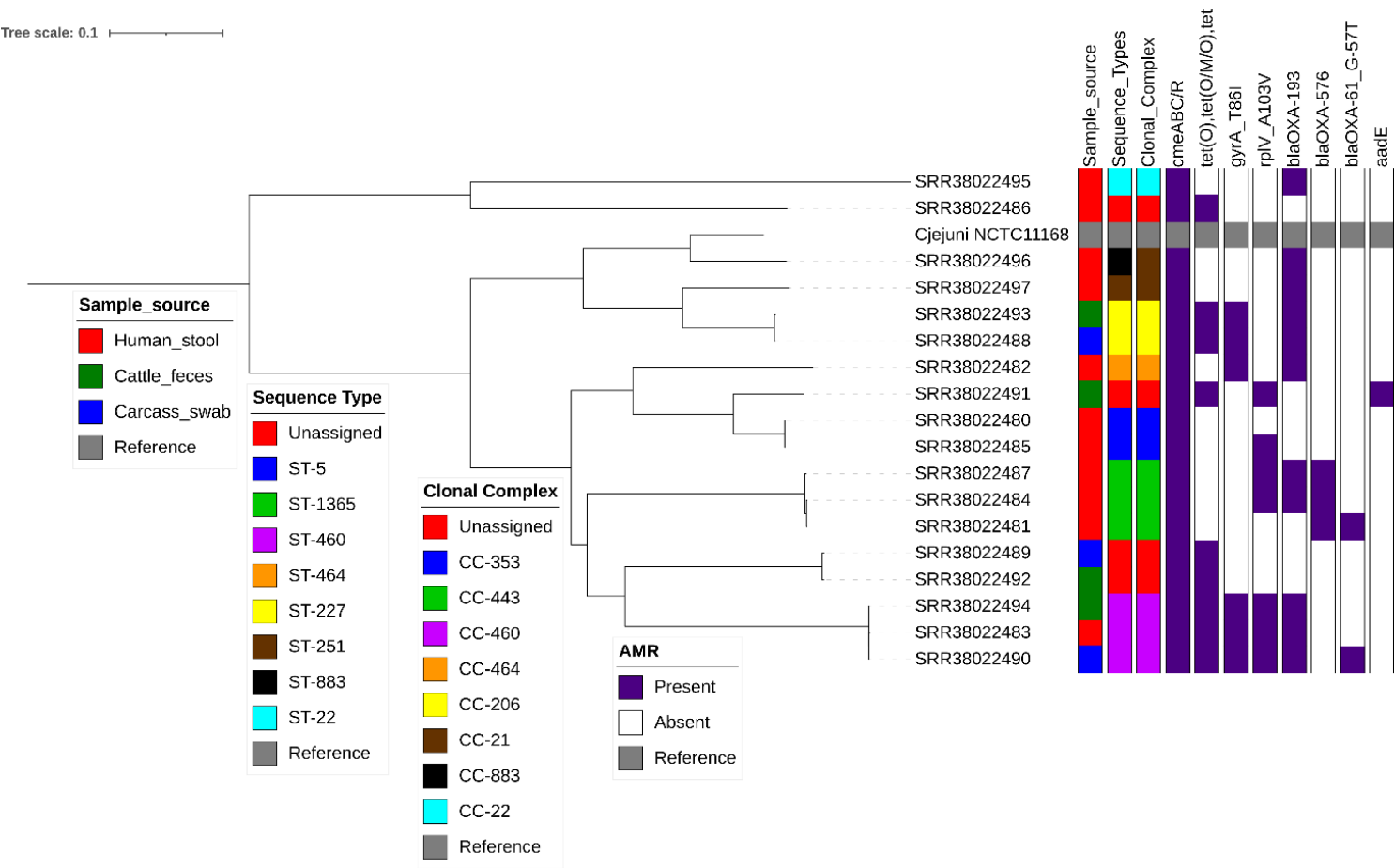


Figure 1

Maximum-likelihood core-genome SNP phylogeny of 18 *C. jejuni* from human and abattoir sources in Ethiopia. The reference strain NCTC11168 was included for comparison. Annotation strips indicate sample source, sequence type (STs), clonal complex (CCs), and presence of key AMR genes and associated mutations

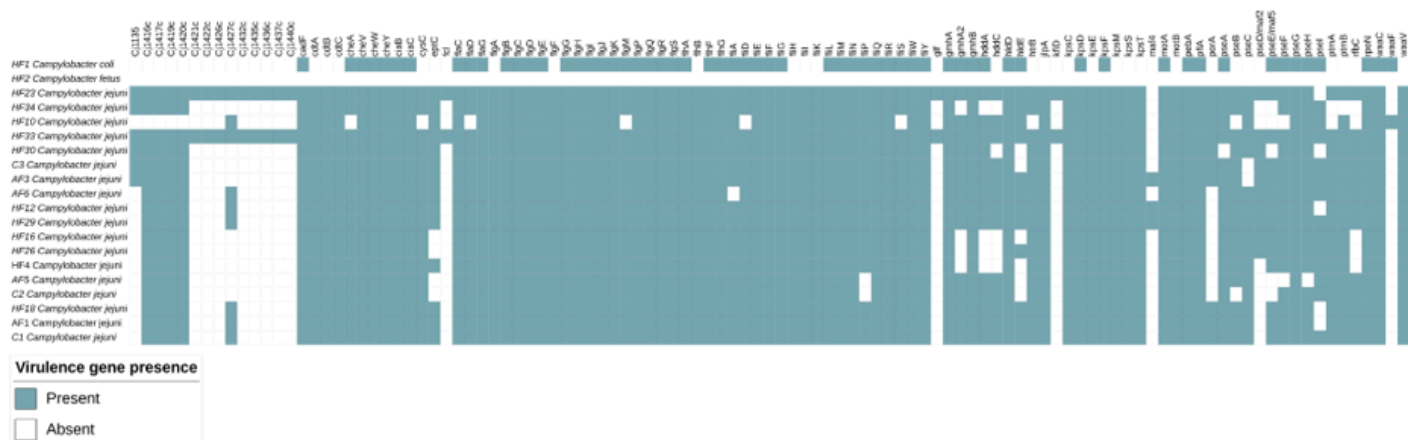


Figure 2

Heatmap of virulence-associated genes in *Campylobacter* isolates from human and abattoir sources in Ethiopia. Presence (blue) and absence (white) of 107 virulence-associated genes were determined using ABRicate v1.0.1 against the VFDB database (updated November 2025). Columns represent individual isolates, grouped by source, showing patterns of conserved and variable virulent genes across human and animal-derived strains.

Supplementary Files

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