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Research Article

# Conditions and Antimicrobial Resistant Profiles of Campylobacter Species from Cow Milk Samples in Oromia Region, Ethiopia

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## Abstract

Campylobacter is one of the major causes of gastroenteritis and is commonly transmitted through the consumption of raw milk or improperly pasteurized milk. A cross-sectional study was conducted from January 2019 to March 2020 in four study sites in the Oromia region of Ethiopia to isolate, identify, and estimate the prevalence of Campylobacter species in milk samples and to determine their antibiotic susceptibility pattern. A total of 384 cow milk samples were randomly chosen from 192 samples of raw milk from farmers and collectors and 192 samples of pasteurized milk from processors and retailers. Standard bacteriological techniques and PCR were used to isolate and identify Campylobacter spp. Of the total 384 milk samples, 35 (9.1%) were found to be positive for Campylobacter spp. The prevalence of Campylobacter spp. was highest in collector raw milk (13.5%), farmer raw milk (12.5%), and pasteurized milk (5.2%).The antibiotic susceptibility test was performed using the disc diffusion method. The most prevalent Campylobacter spp. isolated from milk samples was Campylobacter jejune (C. jejuni) (100%). The overall prevalence of Campylobacter in dairy value chains, including producer, collector, processor, and retailer, was 12.5%, 13.5%, 5.2%, and 5.2%, respectively. Cold storage, material type for making collection rooms, calibrating the pasteurizer machine, restricting milk handlers that are sick, means of transportation, and maintaining temperature during transportation had a statistically significant association. 100% and 8.6% of the Campylobacter isolates were sensitive to ciprofloxacin and chloramphenicol, respectively. However, all of the isolates were resistant to ampicillin, clindamycin, oxytetracycline, and trimethoprim. Moreover, 80% of the C. jejuni were resistant to tetracycline and streptomycin. 26% of the species developed ciprofloxacin degradation. The result of this study revealed the prevalence and risk factors of Campylobacter species in raw and pasteurized milk samples. Hence, there is a chance of acquiring infection via the consumption of raw or undercooked milk. Thus, the implementation of hygienic practices from the producer to the retailer's market, proper handling to avoid cross-contamination and proper pasteurization are very important in preventing Campylobacter infection.

## Keywords

Foodborne, Campylobacter, Thermophile, Fluoroquinolone

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# 1. Introduction

*Campylobacter* species are gram-negative, microaerophilic bacteria that are commonly found in the intestines of animals and birds [28]. They are recognized as one of the most common causes of bacterial gastroenteritis in humans worldwide and can also cause systemic infections in humans, including Guillain-Barre syndrome (GBS) and reactive arthritis (RA) [27]. The disease burden of *Campylobacter* infections is significant, with an estimated 96 million cases annually worldwide [28]. Dairy products are the major reservoirs for many foodborne pathogens, such as *Campylobacter* species, non-Typhi serotypes of *Salmonella enterica*, Shiga toxin-producing strains of *Escherichia coli*, and *Listeria monocytogenes* [16]. Globally, 500 million cases of gastroenteritis with acute diarrhea have been reported per year [23].

In sub-Saharan Africa, 3.8 million deaths of children fewer than 5 years old are reported annually; of those, 25% are caused by diarrheal diseases, of which *Campylobacter* is one of the most frequently isolated [6]. Infections with these organisms occur more frequently than do infections due to *Salmonella* species, *Shigella species*, and *Escherichia coli* O157: H7 [11]. *Campylobacter* spp. are colonizing the intestinal by the of a wide variety of wild and domestic animals, including humans. Humans infected by the ingestion of infected and raw animal products especially meat, milk, and milk products, contaminated drinking water, direct contact with animals, fecal runoff of domestic animals and especially chickens, and contaminating surface water act as the main source of organisms [2]. In Ethiopia, there is limited information on the antimicrobial resistant patterns of *Campylobacter* species in cow milk. Cow milk is an important source of nutrition and income for many households in Ethiopia and is often consumed raw or minimally processed [10]. The consumption of raw or contaminated milk can be a source of human *Campylobacter* infections and may also contribute to the spread of antibiotic-resistant strains of *Campylobacter* [7].

Several studies have been conducted in different parts of Ethiopia to investigate the antimicrobial susceptibility pattern of *Campylobacter* in animal and human populations. A study conducted in Addis Ababa reported a prevalence of 12.9% in cows [41] while another study conducted in Jimma reported a prevalence of 37.9% [17]. One study conducted in the Oromia region found a prevalence of 11.7% [42]. A study conducted in the Oromia region, Bishoftu, reported a prevalence of 23.7% in humans with gastroenteritis [36], while another study conducted in the same region found a prevalence of 26.2%. Other studies conducted in different regions of Ethiopia have reported prevalence rates ranging from 1.9% to 69.6% [5]. However, there is limited information on the prevalence of *Campylobacter* in cow's milk in Ethiopia. In Ethiopia, there are several factors that may contribute to the transmission of *Campylobacter* species in cow milk. These include poor hygiene practices during milking and storage, a lack of access to clean water, and limited awareness of the risks associated with consuming raw or contaminated milk [38]. Furthermore, uncontrolled use of in livestock production for animal growth, which is contribute to the emergence of antibiotic-resistant *Campylobacter* strains.

Moreover, *Campylobacter* with resistance to antimicrobial agents has also been implicated worldwide [33]. The use of antimicrobial agents in dairy cows has resulted in the emergence and dissemination of antimicrobial-resistant bacteria, including antimicrobial resistant *Campylobacter*, which has a potentially serious impact on food safety in both animal and human health. A few studies were done in different parts of Ethiopia to find out how common enteric Campylobacteriosis is and how well antibiotics work against it in people and foods that come from animals [42, 16]. Therefore, this study was carried out to know the risk factors, and their antimicrobial susceptibility pattern of clinically important *Campylobacter* species from milk in the Oromia region of Ethiopia. Furthermore in the Oromia region, Ethiopia, the prevalence of *Campylobacter* species in cow milk is not well documented, and the risk factors associated with its contamination are not clear. Due to the high consumption of raw or minimally processed cow milk in Ethiopia, there is a potential risk of *Campylobacter* infection. Therefore, there is a need to determine the isolation, and risk factors associated with *Campylobacter* species in cow milk to establish effective control measures and reduce the risk of human infections.

# 2. Material and Methods

## 2.1. Descriptions of the Study Area

The study areas are Debrezeit, Assella, Fiche, and Holeta towns, which are located in Oromia region, Ethiopia.

## 2.2. Study Design

The experimental research design was used to isolate and identify Campylobacter bacteria from cow milk samples for the determine their antibiotics resistant profile, prevalent and associated risk factors in dairy value chain from March 2019 to May 2020.

## 2.3. Collection and Transport of Samples

A total of 384 milk samples, comprising raw (n = 192) and pasteurized (n = 192) milk, were collected from the farmer (n = 96), collector (n = 96), processor (n = 96), and retailer (n = 96) of these four study areas. All samples were aseptically collected and placed in sterile universal Falcon tubes to prevent cross-contamination and immediately transported to the microbiology laboratories, Ethiopian conformity assessment, and enterprises using an icebox with ice packs.

## 2.4. Risk Factor Survey Data Collection

Surveys were conducted to identify potential risk factors associated with *Campylobacter* contamination in milk. These surveys included questionnaires or on-site evaluations to identify potential sources of contamination, such as poor hygiene practices, inadequate milk storage, insufficient cleaning of milking equipment, socio-demographic characteristics, and others in dairy value chains. Local languages were used to ensure the reliability of the information; the respondents were interviewed in their local language. All the questionnaires were checked for completeness and consistency every day.

## 2.5. Isolation and Characterization of Campylobacter Species

Isolation and detection of *Campylobacter* spp. were done following [24] methods. In detail, 10 ml of milk sample was aseptically transferred into 90 ml of Preston broth using the next preparation: Nutrient broth No.2 (Oxoid, CM0067) with 5% laked horse blood (Hardy Diagnostics, 10052-808), and Preston *Campylobacter* Supplement (Oxoid, SR0204E) in a sterile stomacher bag and homogenized for 30 sec, then incubated at 41.5 C for 24 hours in the microaerophilic environment using a gas generating system Campy Gen sachet (Oxoid, CN0035): 5% oxygen, 10% carbon dioxide, and 85% nitrogen.The enriched sample was then streaked onto Charcol Cefoperazole Deoxycholate Agar (CCDA, Oxoid, CM 739) containing *Campylobacter* selective supplement containing cefoperazole and amphotericin B (CCDA selective supplement SR0155E) with 5% laked horse blood and kept in a gas jar containing *Campylobacter* gas packing systems to maintain the microaerophilic condition for 4 hours at 41.5℃. The presumptive *Campylobacter* colonies were identified based on growth appearance on mCCDA medium at 41.5 ℃ after 48 h.

The genomic DNA of the presumptive colonies of *Campylobacter* was extracted using the boiling method, according to [25]. Then, 2.5 l of each extracted genomic DNA sample was run in an agarose gel electrophoresis and visualized under UV light. Then, a genome-based polymerase chain reaction (PCR) was done as described by [15].using the following genus- and species-specific primers. Each PCR reaction mixture was performed in a 25-l total volume containing 2.5 l of template DNA, 12 l of GoTaq Green Master Mix (Promega), 0.125 l of forward and reverse primers (100 M) targeting the *C. jejune hipO* gene, 0.25 l of forward and reverse primers (100 M) targeting the *C. coli glyA* gene, 0.05 l of each forward and reverse primer (100 M) targeting the *Campylobacter*-specific 23S rRNA sequence, and 9.65 l of nuclease-free water. Amplification was carried out with thermal cycling conditions of initial denaturation at 95 ℃ for 6 min, followed by 30 cycles of denaturation at 95 ℃ for 0.5 min, annealing at 59 ℃ for 6 min, extension at 72 ℃ for 0.5 min, and a final extension at 72 ℃ for 7 min. Finally, the PCR products were separated by running on a 1.5% (w/v) agarose gel containing 5 ul of gel red (5 mg/ml stock concentration, Biotium). Electrophoresis was conducted in a horizontal equipment system for 40 min at 120 V using 1X TAE buffer (40 mM Tris, 1 mM EDTA, and 20 mM glacial acetic acid, pH 8.0). The amplicons were visualized under UV-light gel documentation, and their molecular weights were estimated by comparing them with a 100-bp DNA molecular weight marker (Solis BioDyne, Tartu, Estonia). Each PCR run included a positive control (DNA extracted from *Campylobacter jejune* ATCC 29428) and a negative control nuclease-free water (Table 1).

***Table 1.*** *List of Primers for confirmation of Campylobacter genus, species C. jejuni, and C. coli.*

| Primer | Size (bp) | Sequence (5'–3') | Target gene | location (bp) |
|---|---|---|---|---|
| CJF | 323 | ACTTCTTTATTGCTTGCTGC | C.jejunihipO | 1662–1681 |
| CJR | | GCCACAACAAGTAAAGAAGC | | 1984-1965 |
| 23SF | 650 | TATACCGGTAAGGAGTGCTGGAG | 23SrRNA | 380-738 |
| 23SR | | ATCAATTAACCTTCGAGCACCG | | 4456-4435 |
| CCF | 126 | GTAAAACCAAAGCTTATCGTG | C. coli glyA | 337–357 |
| CCR | | TCCAGCAATGTGTGCAATG | | 462–444 |

## 2.6. Antimicrobial Susceptibility Testing

The *Campylobacter* spp. isolates were screened for in vitro antimicrobial susceptibility using the standard agar disc diffusion method as recommended by Clinical and Laboratory Standards Institutions (CLSI) on Mueller-Hinton Agar (Millipore, 70192) without being supplemented with 5% lactate of horse blood. The following nine different antibiotic discs, with their concentrations given in parentheses, were used in

the antibiogram testing: Ampicillin(AMP)(10µg), Chloramphenicol (C)(30µg), Erythromycin (E)(15µg), Gentamycin (CN)(10µg), Ciprofloxacin(CPFX)(5ug), Streptomycin (S)(10µg), Tetracycline (TE)(30µg), and Sulfamethoxazole-trimethoprim(SXT)(25µg) (Oxoid Company, Hampshire, England). After 48 h of microaerophilic incubation at 37 ℃, the clear zones of inhibition of bacterial growth around the antibiotic discs, including the disc diameter for individual antimicrobial agents, were measured and then translated into sensitive (S), intermediate (I), and resistant (R) categories according to the interpretation table of the CLSI [32].

### 2.7. Statistical Analysis

The data was analyzed using SPSS version 23 (IBM, USA) statistical software. Logistic regression and the chi-square ($\chi 2$) test were applied to assess the prevalence of *Campylobacter* spp. and the risk factor associations. For all tests, p-values less than 0.05 were considered statistically significant.

## 3. Results

### 3.1. Prevalence of Campylobacter in Cow Milk Sample

The results showed that among 384 milk samples collected along the dairy value chain, 35 (9.1%) were positive for *Campylobacter* spp. The highest prevalence of 13.5% were found in the collector value chain, which is found to be 2.6 times more likely to have *Campylobacter* contamination as compared to other value chains. Relatively, the lowest 5.2% prevalence of *Campylobacter* spp. were observed in the retailer's value chain (Table 2). Along dairy value chains, there is no significant difference ($p > 0.05$) in *Campylobacter* spp. prevalence between producers, retailers, and processors in the value chain (Table 2). Producers were following collectors with the highest prevalence of 12.5% and were 2.6 times more likely to have *Campylobacter* contamination as compared to processors and retailers.

***Table 2.*** *The apparent prevalence of Campylobacter in raw cow milk across dairy value chains.*

| Conditions | Risk factors | Animals examined | Positives | Apparent prevalence | 95% CI | OR | $X^2$ | p-Value |
|---|---|---|---|---|---|---|---|---|
| Value chain | Producer | 96 | 12 | 12.5 $^{AB}$ | 3.3-37.3 | 2.9 | 7.33 | 0.055 |
| | Collector | 96 | 13 | 13.5 $^{B}$ | 8.0-21.9 | 2.6 | | |
| | Processor | 96 | 5 | 5.2 $^{A}$ | 1.0-22.4 | 1 | | |
| | Retailer | 96 | 5 | 5.2 $^{A}$ | 1.0-22.4 | 1 | | |
| | Total | 384 | 35 | 9.1 | 6.6-12.4 | | | |

*Columns that share the same letters do not have a statistically significant difference.
** CI- Confidence Interval; OR- Odds Ratio; X2- Chi-square.

### 3.2. Prevalence of Campylobacter Species in the Different Cow Milk Samples

All Campylobacter spp. isolated and identified from raw and pasteurized milk samples were C. jejune. The prevalence of C. jejune in raw and pasteurized milk samples was found to be 71.4 and 28.57%, respectively (Table 3).

***Table 3.*** *The prevalence of Campylobacter species among different cow milk samples.*

| Sample Type Prevalence | *Campylobacter* spp. | |
|---|---|---|
| | *C. jejuni* | *C. coli* |
| Raw milk (n= 25) | 25 (71.43%) | 0 (0%) |
| Pasteurized milk (n= 10) | 10 (28.57%) | 0 (0%) |
| Total (n = 35) | 35 (100%) | 0 (0%) |

*n - number of positive; %- percent per hundred

All *Campylobacter* spp. isolated and identified from raw and pasteurized milk samples were *C. jejune*. The prevalence of *C. jejune* in raw and pasteurized milk samples was found to be 71.4 and 28.57%, respectively (Table 3).

### 3.3. Risk Factors for Campylobacter Contamination at the Milk Producer Value Chain

Among the risk factors are hygienic practices at the farm level, good milking practices, barn construction material, barn condition, udder cleaning, cleanness of udder drying cloth, cow health associated with mastitis, milk filtering, hygiene of material for filtering, and milk handling material, which showed no statistically significant association with *Campylobacter* prevalence. where milk storage conditions had a statistically significant association (Table 4). Of the 96 farmers who do not use refrigerators for milk storage, 25 (26%) were found to be positive for *Campylobacter* species. Farmers who used refrigerators were always less likely to be positive for *Campylobacter* prevalence than those who did not use them (OD = 3.4 (3.8–71.6), P = 0.05).

***Table 4.*** *Risk factors associated with milk contamination by Campylobacter spp. at producers' value chain.*

| Conditions | Risk factors | Animals examined | Positives | Apparent prevalence | 95% CI | OR | $X^2$ | p-Value |
|---|---|---|---|---|---|---|---|---|
| Good milking practice | Yes | 52 | 7 | 13.5 | 4.2-22.7 | 1.2 | 0.10 | 0.7562 |
| | No | 44 | 5 | 11.4 | 2.0-20.7 | 1 | | |
| Barn construction material | Concrete floor | 52 | 7 | 13.5 | 4.2-22.7 | 1.2 | 0.10 | 0.7562 |
| | Cement floor barn | 44 | 5 | 11.4 | 2.0-20.7 | 1 | | |
| Hygienic barn | Poor | 21 | 4 | 19.0 | 2.3-35.8 | 1.97 | 0.97 | 0.3256 |
| | Good | 75 | 8 | 10.7 | 3.7-17.7 | 1 | | |
| Udder wash with warm water | Yes | 94 | 12 | 12.8 | 6.0-19.5 | | - | - |
| | No | 2 | 0 | 0.0 | - | - | | |
| Appearance of cleanness of drying clothes | No cloth | 47 | 5 | 10.6 | 1.8-19.5 | 1 | 1.02 | 0.7966 |
| | Somewhat dirty | 25 | 4 | 16.0 | 1.6-30.4 | 1.13 | | |
| | Very dirty | 13 | 1 | 7.7 | 6.8-22.2 | - | | |
| | Visibly clean | 11 | 2 | 18.2 | 4.6-41.0 | - | | |
| Cow had mastitis | Yes | 27 | 3 | 11.1 | 0.7-23.0 | - | 0.07 | 0.7948 |
| | No | 69 | 9 | 13.0 | 5.1-21.0 | - | | |
| Milk filtered | No | 88 | 12 | 13.6 | 6,5-20.8 | - | - | - |
| | Yes | 8 | 0 | - | - | - | | |
| Material for filtration | No use anything | 8 | 0 | - | | - | 0.00 | 0.9985 |
| | Cloth | 44 | 6 | 13.6 | 3.5-23.8 | 1 | | |
| | Plastic | 37 | 5 | 13.5 | 2.5-24.5 | 0.99 | | |
| | Wire | 3 | 1 | 14.3 | 11.6- 40.2 | 1.06 | | |
| Milk handling material | Aluminum | 6 | 1 | 16.7 | 13.2-46.5 | 1.5 | 0.35 | 0.8381 |
| | Plastic | 5 | 1 | 20.0 | 15.1-55.1 | 1.9 | | |
| | Mazzi | 85 | 10 | 11.8 | 4.9-18.6 | 1 | | |
| Refrigerator used | No | 25 | 6 | 24.0 | 3.8-71.6 | 3.4 | 3.66 | 0.0428 |
| | Yes | 71 | 6 | 8.5 | 3.8-17.6 | 1 | | |

| Conditions | Risk factors | Animals examined | Positives | Apparent prevalence | 95% CI | OR | $X^2$ | p-Value |
|---|---|---|---|---|---|---|---|---|
| Total | | 96 | 12 | 12.5 | 7.2-20.7 | | | |

* CI- Confidence Interval; OR- Odds Ratio; $X^2$- Chi-square

## 3.4. Risk Factors for Campylobacter in Raw Milk at the Milk Collection Value Chain

Five (5.20%) of the 96 milk collectors who used soil floor material tested positive for *Campylobacter* species, compared to 91 (94.79%) who used cement floor material. This indicates that the milk collector who used a soil floor was 12.2 times (OD = 12.2 (10.4–95.1), P = 0.0115) more susceptible to *Campylobacter* contamination, but other factors like, the maintained temperature during transportation, the type of milk filter, cooling for preservation, the source of water for washing, and the milk handling equipment were not a statistically significant risk for the contamination of raw milk with *Campylobacter* (Table 5).

***Table 5.*** *Risk factors for Campylobacter spp. contamination in the milk collector value chain.*

| Conditions | Risk factors | Animals examined | Positives | Apparent prevalence | 95% CI | OR | $X^2$ | p-Value |
|---|---|---|---|---|---|---|---|---|
| Maintained tem during transportation | No | 12 | 3 | 25.0 | 3.8-73.6 | 2.5 | 1.32 | 0.2508 |
| | Yes | 84 | 10 | 11.9 | 6.5-20.7 | 1 | | |
| | Total | 96 | 13 | 13.5 | 8.0-21.9 | | | |
| Milk filtered up on receipt | No | 36 | 7 | 19.4 | 3.1-64.6 | 2.2 | 1.66 | 0.1974 |
| | Yes | 60 | 6 | 10.0 | 4.6-20.5 | 1 | | |
| | Total | 96 | 13 | 13.5 | 8.0-21.9 | | | |
| Filter type | Plastic filter | 60 | 6 | 10.0 | 4.6-20.5 | 1 | 1.66 | 0.1974 |
| | Piece of cloth | 36 | 7 | 19.4 | 3.1-64.6 | 2.2 | | |
| | Total | 96 | 13 | 13.5 | 8.0-21.9 | | | |
| Cooling for preservation | Yes | 68 | 9 | 13.2 | 1.5-61.0 | 1 | 0.02 | 0.8917 |
| | No | 28 | 4 | 14.3 | 5.5-32.4 | 1.1 | | |
| | Total | 96 | 13 | 13.5 | 8.0-21.9 | | | |
| Material of collection room | Cement floor | 91 | 10 | 11.0 | 6.0-19.2 | 1 | 6.38 | 0.0115 |
| | Soil floor | 5 | 3 | 35.0 | 10.4-95.1 | 12.2 | | |
| | Total | 96 | 13 | 13.5 | 8.0-21.9 | | | |
| Source of water for washing | Tap water | 96 | 13 | 13.5 | 8.0-21.9 | - | 0 | - |
| | Ground water | 0 | 0 | - | - | - | | |
| | Total | 96 | 13 | 13.5 | 8.0-21.9 | | | |
| Milk handling equipment | Plastic container | 72 | 10 | 13.9 | 7.6-23.9 | 1 | | |
| | Muzzican | 0 | 0 | 0 | 0-0 | - | 0.87 | 0.3514 |
| | Aluminum can | 24 | 3 | 25.0 | 3.8-73.8 | 2.1 | | |
| | Total | 96 | 13 | 13.5 | 8.0-21.9 | | | |

* CI- Confidence Interval; OR- Odds Ratio; $X^2$- Chi-squar

### 3.5. Risk Factors for Campylobacter spp. Contamination at the Milk Processing Value Chain

The culture-positive rate of *Campylobacter* speces among study subjects who could not calibrate the pasteurizer machine was 24.0%. Milk processors who did not calibrate the pasteurizer had a 20.8% greater likelihood of testing positive for *Campylobacter* infection than those who calibrated the milk pasteurization system. The processors who restricted milk handlers who are sick from working with milk were more protected from *Campylobacter* infection compared to those who did not restrict milk handlers by 20.80% (Table 6).

*Table 6. Risk factors associated with contamination of processors' pasteurized milk by Campylobacter spp.*

| Conditions | Risk factors | Animals examined | Positives | Apparent prevalence | 95% CI | OR | $X^2$ | p-Value |
|---|---|---|---|---|---|---|---|---|
| Source of water for equipment washing | Tap water | 29 | 0 | 0 | - | - | 0.00 | . |
| | Groundwater | 67 | 5 | 7.5 | 3.2-16.7 | - | | |
| Restricting milk handlers that are sick work with milk | Yes | 24 | 5 | 20.8 | 8.9-41.3 | - | 0.00 | . |
| | No | 72 | 0 | 0 | - | - | | |
| Pasteurizer was calibrated annually | No | 24 | 5 | 20.8 | 8.9-41.3 | - | 0.00 | . |
| | Yes | 72 | 0 | 0 | - | - | | |
| Efficacy of pasteurization was verified | No | 12 | 0 | 0 | - | | 0.00 | . |
| | Yes | 84 | 5 | 6.0 | 2.5-13.5 | - | | |
| Maintained cold chain during transportation | Yes | 33 | 0 | 0 | - | - | 0.00 | . |
| | No | 63 | 5 | 7.9 | 3.3-17.7 | - | | |
| Microbiological test for pasteurization efficiency test | Yes | 12 | 0 | 0 | - | | 0.00 | . |
| | No | 84 | 5 | 6.0 | 2.5-13.5 | - | | |
| Total | | 96 | 5 | 5.2 | 2.2-11.9 | | | |

* CI- Confidence Interval; OR- Odds Ratio; $X^2$- Chi-square

### 3.6. Risk Factors for Campylobacter spp. Contamination at the Milk Retail Value Chain

A higher culture-positive rate of *Campylobacter* species had been observed in retailers who used the four-wheel drive as a means of transportation during milk delivery to the retailer market compared to using cold trucks. Milk retailers who used four-wheel drive for transportation were found to be 8.3 times more affected than those who used cold trucks (OD= 8.3 (5.8-42.7), P =0.02, (Table 7). Pasteurized milk not maintained at cold storage during transportation had 17 (17.7%), an 8.3-fold higher probability of contamination than those maintained at cold storage during transportation: 79 (82.3%) (OD = 8.3 (5.8-42.7), P = 0.02), as shown in Table 7.

*Table 7. Risk Factors Associated with contamination of retailer's pasteurized milk by C.jejune.*

| Conditions | Risk factors | Animals examined | Positives | Apparent prevalence | 95% CI | OR | $X^2$ | p-Value |
|---|---|---|---|---|---|---|---|---|
| Anyone from the shop attended | No | 92 | 5 | 5.4 | 2.3-12.4 | - | 0.00 | . |

| Conditions | Risk factors | Animals examined | Positives | Apparent prevalence | 95% CI | OR | $X^2$ | p-Value |
|---|---|---|---|---|---|---|---|---|
| training related to the safety and quality of milk | Yes | 4 | 0 | 0 | - | - | | |
| Total | | 96 | 5 | 5.2 | 2.2-11.9 | | | |
| Means of transportation for delivering milk to retail shop | Four wheels | 17 | 3 | 17.6 | 5.8-42.7 | 8.3 | 4.79 | 0.0287 |
| | Cold truck | 79 | 2 | 2.5 | 0.1-37.1 | 1 | | |
| Total | | 96 | 5 | 5.2 | 2.2-11.9 | | | |
| Pasteurized milk is maintained cold during transportation | Yes | 79 | 2 | 2.5 | 0.1-37.1 | 1 | 4.79 | 0.0287 |
| | No | 17 | 3 | 17.6 | 5.8-42.7 | 8.3 | | |
| Total | | 96 | 5 | 5.2 | 2.2-11.9 | | | |
| A separate refrigerator is used for milk and dairy foods | Yes | 78 | 3 | 3.8 | 0.2-53.0 | 1 | 1.29 | 0.2552 |
| | No | 18 | 2 | 11.1 | 2.8-35.2 | 3.1 | | |
| Total | | 96 | 5 | 5.2 | 2.2-11.9 | | | |

* CI- Confidence Interval; OR- Odds Ratio; $X^2$- Chi-square

### 3.7. Antimicrobial Susceptibility Pattern of Campylobacter Species

Among *Campylobacter* spp. isolated from the different dairy products, 91% were susceptible to Ciprofloxacin and 20% were susceptible to Chloramphenicol. However, all the isolates (100% each) had shown resistance to Ampicillin, Oxytetracycline (100%), Clindamycin (100%), and Trimethoprim (100%). Among the 35 isolates of *C. jejuni*, 82.8% were resistant to streptomycin and tetracycline (Table 8). Moreover, 25.7% of *C. jejuni* isolates developed a capability to degrade Ciprofloxacin antibiotics, as shown in Table 8.

***Table 8.*** *In vitro antimicrobial sensitivity pattern of Campylobacter species.*

| Antibiotics | R No. (%) | I No. (%) | S No. (%) |
|---|---|---|---|
| AM | 35(100) | 0 | 0 |
| TE | 29(82.8) | 0 | 0 |
| S | 29(82.8) | 0 | 0 |
| C | 0 | 0 | 7(20) |
| J | ND | ND | ND |
| CIP | 0 | 3(8.6) | 32(91) |
| NA | ND | ND | ND |
| CLN | 35(100) | 0 | 0 |
| OT | 35(100) | 0 | 0 |
| W | 35(100) | 0 | 0 |
| KF | ND | ND | ND |
| TOTAL= 35 | | | |

Where: ND: not done, S: sensitive, R: resistant, I: intermediate, W: trimethoprim, CIP: ciprofloxacin, C: chloramphenicol, CLN: clindamycin, AMP: ampicillin, TE: tetracycline, OT: ox tetracycline, S: streptomycin, KF: cephalothin, J: gentamicin, NA.

## 4. Discussion

The pooled prevalence of *Campylobacter* spp. among the dairy value chain in these four study areas was 35 (9.1%) (Table 2). Raw cow milk with a higher prevalence of 13.5% in the collector value chain and a lower prevalence of 5.2% in the processor and retailer value chains was found among the value chain actors. The prevalence of Campylobacter spp. in producers was 12.5 %. This was relatively lower than the prevalence of 20.6% reported by [8] in raw milk collected from different dairy farms in Ethiopia, and [42] also reported (61.2%) the prevalence of Campylobacter in raw milk samples collected from milk collection centers and (41.8%) in retail markets. Another study conducted by [14] also found that the prevalence of Campylobacter was 55.6% in milk collection centers and 16.7% in raw milk retail markets. [30], also found a 63.2% prevalence of Campylobacter in raw milk from collection centers and a 42.1% prevalence in retail markets. A study by [42] investigated the prevalence and antimicrobial resistance of Campylobacter spp. in raw milk

shops in and around Addis Ababa, Ethiopia, and found that 4.4% of the raw milk samples were positive for Campylobacter spp.

The study of [37] also found zero prevalence of Campylobacter spp. in raw milk and milk products in the Hawassa area of Ethiopia. These variability's in the prevalence of Camphylobacter *spp.* might be due to the methods of analysis and variability in the location of sample collection areas and sampling seasons of the dry and wet seasons, which affect the possibility of milk contamination with Campylobacter spp. The prevalence of Campylobacter spp. in this study was relatively medium as compared to the previous studies; this might be associated with the hygiene awareness of the value chain actors in these study areas. Since the area was a major milk shade area, most non-governmental and governmental organizations were providing different short training and support on milk hygiene and milk safety improvement awareness creation for each value chain actor.

The milk samples collected from producers had the highest prevalence (12.5%). The milk samples collected from farmers were found to be 0.3 times more likely to have *Campylobacter* compared to milk collected from milk collectors and 2.9 times more likely to have *Campylobacter* compared to milk collected from processors and retailers. The difference in the prevalence of *Campylobacter* between sources of milk samples was found to be statistically significant (P 0.05) (OR = 2.9, CI = 3.30–37.30). This might be due to an extra chance of acquiring contamination from cow udders and teats, the cow barn, and the source of water for washing.

Among the 35 samples positive for Campylobacter spp., 25 (71.43%) were found in raw milk across the value chain. And 10 samples (28.57%) were found in pasteurized milk. It is a well-known fact that raw milk appeared to be a significant source of microbial contaminants, including Campylobacter spp., as compared with pasteurized milk [34]. Pasteurization has the potential to kill most pathogens, but its efficacy depends on key factors. In this study, the prevalence of *Campylobacter* spp. in pasteurized milk might be due to poor pasteurization efficiencies or post-contamination. Wide variation (0–96%) in the prevalence of Campylobacter in milk samples had been reported in different countries. These variations in Campylobacter spp. prevalence might be due to differences in cow barn conditions, types of water for washing udders, and the quality of the milking process. In this study, the prevalence of Campylobacter spp. in pasteurized milk was 25.2%. This was comparable to the finding reported from a previous study done by [16], (55.8%) in Pakistan. However, it was higher than the findings reported by [35], which were 0% in England. According to [42] report, the prevalence of Campylobacter spp. in raw milk and pasteurized milk in Ethiopia was 54.3% and 9.1%, respectively. It is important to note that the prevalence of Campylobacter in milk can vary depending on several factors, such as farming practices, hygiene practices during milk production and processing, and storage and transportation conditions [1, 42]. Therefore, it is important to follow good hygiene practices and proper food safety protocols to minimize the risk of contamination and transmission of Campylobacter and other harmful microorganisms in milk.

In the current study, the microbiological and PCR characterization of *Campylobacter* isolates revealed that C. jejuni predominated over other species. The prevalence of C. jejuni in raw and pasteurized milk was found to be 100%. Campylobacter jejuni has been reported to be the most frequent species recovered from foods of animal origin, especially milk samples [22]. These findings were in agreement with the findings of [40], who reported 100% C. jejuni in dairy products. A study conducted by [39] in Ireland found that Campylobacter jejuni was more frequently isolated from raw milk samples than Campylobacter coli (69.6% vs. 30.4%). Similarly, a study by [26] in Greece reported a higher prevalence of Campylobacter jejuni in raw milk samples compared to Campylobacter coli (86.1% vs. 13.9%). Another study conducted by [13] in Spain found that Campylobacter jejuni was the most prevalent species isolated from both raw milk (70.8%) and cheese (73.3%). This implies that C. jejuni is the dominant contaminant species in dairy products among the species of Campylobacter.

In this study, several risk factors were assessed at each dairy value chain and correlated with the prevalence of Campylobacter spp. At the producer or farmer level, among several potential risk factors, the lack of a refrigerator in their home, no cooling after milking, and lack of cool transportation to the milk collection center had a significant contribution to Campylobacter contamination. This is in line with the study conducted by [4], who identified several risk factors associated with Campylobacter contamination in the milk value chain, including poor milking hygiene practices, inadequate milk storage facilities, a lack of proper waste disposal practices, and cold transportation facilities that are significantly related to Campylobacter spp. To reduce the risk of *Campylobacter* contamination, it is important to implement effective hygiene and sanitation practices. This includes improving animal health management, implementing good milking practices, providing adequate infrastructure and facilities, and promoting awareness about the importance of good hygiene practices at all stages of the value chain, specifically at the initial stages on the farm.

In this study, milk collection material also showed significant risk factors for Campylobacter infection in milk collection centers. This can be explained by the rare use of cement floors for making collection rooms, which is new information compared to previous studies. The milk separation room with a soil floor had a significantly increased risk of Campylobacter contamination. This might be due to dust contamination from the soil on the floor.

In this study, calibrating the pasteurizer machine and restricting milk handlers that are sick from working with milk were the most common factors (20.8%) and had a statistically significant association with the prevalence of Campylobacter

species among milk processors. A related study done by [9] also found a 3.6% prevalence of Campylobacter in pasteurized milk in Ethiopia. An earlier study in the United Kingdom by [18] also found a high prevalence of Campylobacter in pasteurized milk. This might be due to an improper pasteurization process, post-contamination during filling or packaging, or a lack of quality control at the processing plant. So, when processing plants, there have to be a quality check and monitoring mechanisms before distributing their products to the user.

On the other hand, high contamination rates were seen in milk retailers who have four-wheel drives for delivering milk to the retailer's shop or restaurant, which indicates the direct association between Campylobacter species infection and maintained temperature during transportation, as already pointed out by the presence of this pathogen in the retailer's milk. Related results were reported by [3], who found a 25.4% prevalence of Campylobacter in milk retailers. [16] also reported a high prevalence of Campylobacter spp. in milk retailers in Oman.

Antibiotic resistance is a global health concern, and its development in dairy products is an increasing challenge. In Ethiopia and Africa, as well as globally, the misuse of antibiotics in livestock production and agriculture has led to the emergence and spread of antibiotic-resistant bacteria, posing a threat to human and animal health. Antibiotic resistance in Campylobacter is emerging globally and has already been described by several authors and recognized by the WHO as a problem of public health importance [19]. Campylobacter spp. resistance to antibiotics can be transferred from different sources to humans.

Antibiotic resistance in Campylobacter has become a growing concern globally, including in Ethiopia. The development of antibiotic-resistant Campylobacter strains is a serious public health threat as it reduces the effectiveness of antibiotics in treating infections caused by these bacteria. Antibiotic susceptibility patterns have been determined in previous studies conducted in Ethiopia, which showed 80%-100% of isolates from food animals were sensitive to antimicrobial agents [31]. In the current study, 35 C. jejuni isolates were investigated for their antimicrobial susceptibility pattern, and all (100%) Campylobacter isolates were resistant to ampicillin, and 82.8% were resistant to two or more antibiotics. Related reports in Ethiopia by [36] also showed that 61.1% of the *Campylobacter* isolates from raw milk and 67.2% of the isolates from cheese were resistant to at least one antibiotic, and the most common antibiotics to which the isolates were resistant were tetracycline, ciprofloxacin, and nalidixic acid. Globally, the antibiotic-resistant Campylobacter strain has also become a significant concern. A study conducted in 24 European countries by [29] found that Campylobacter was the most commonly reported cause of foodborne infections in humans and that there was a high prevalence of antibiotic-resistant Campylobacter strains in humans, poultry, and other food-producing animals. The most common antibiotics to which Campylobacter was resistant were ciprofloxacin and tetracycline.

In general, several studies have reported that resistance to beta-lactam antibiotics is high in food animals. The resistance rate of Campylobacter isolates (82.8%) to tetracycline in the present study was comparable with the findings of [12], (79.9%), but higher than that of [21], (6%). The resistance level to streptomycin in the current study was 82.8%, which was higher than reports from Thailand [20]. Drug-resistant isolates have always remained susceptible to ciprofloxacin and chloramphenicol. In the present study, the developed capability to degrade ciprofloxacin antibiotics was 25.7%, which was new and comparable to the previous finding. Hence, the current antimicrobial resistance finding might be because antibiotics can be bought for human or animal use without a prescription, and similarly, in countries like Ethiopia without standard regulation and treatment guidelines, antibiotics are often overprescribed by health workers and veterinarians and overused by the public.

## 5. Conclusion and Recommendations

*Campylobacter* species are a major cause of foodborne illness globally, including in Ethiopia. The prevalence of *Campylobacter* in dairy products along the milk value chain in Ethiopia is a matter of concern, with varying rates reported in different studies. Additionally, antibiotic resistance among *Campylobacter* species is an increasing problem, as it limits treatment options and increases the risk of treatment failure. The present study revealed the prevalence of *Campylobacter* in raw and pasteurized milk samples across the dairy value chains of producers, milk collection centers, processors, and retailers. Based on this finding, it is recommended that measures be taken to improve the hygiene and safety of dairy products along the milk value chain in Ethiopia. This can be achieved through the implementation of good agricultural practices (GAPs) and good manufacturing practices (GMPs) to minimize the contamination of dairy products. Additionally, increasing awareness of food safety among farmers, processors, and consumers is crucial to preventing the spread of *Campylobacter* and other foodborne pathogens.

The use of antibiotics in animal husbandry should also be controlled, as it contributes to the emergence and spread of antibiotic-resistant *Campylobacter* strains. The development and implementation of a national surveillance system for antibiotic resistance among *Campylobacter* species in dairy products are also recommended to monitor the situation and inform appropriate intervention strategies. Moreover, further studies should be needed to identify the most likely antibiotics to develop resistance and strains of *Campylobacter* with a high potential for resistance gene development. In conclusion, the prevalence of *Campylobacter* species and their antibiotic resistance profile in dairy products along the milk value chain in Ethiopia is a public health concern. Improving food safety through the implementation of GAPs and GMPs, increasing

awareness of food safety, and controlling the use of antibiotics in animal husbandry are crucial steps in reducing the burden of *Campylobacter* infections and antibiotic resistance in Ethiopia.

## Abbreviations

| | |
|---|---|
| VBNC | Viable but Non Culturable |
| WHO | World health Organization |
| PCR | Polymerase Chain Reaction |
| rRNA | Ribosomal Ribose Nucleic Acid |
| C.jejuni | Campylobacter Jejuni |
| GAPs | Good Agricultural Practice |
| GMPs | Good Manufacturing Practice |

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## Author Contributions

Adane Eshetu Haile is the sole author. The author read and approved the final manuscript.

## Conflicts of Interest

The author declares no conflicts of interest.

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