Campylobacter Gastroenteritis Among Under-Five Children in Southwest Ethiopia

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Background: Under-five children are at an increased risk for foodborne illnesses because of the ingenuousness of their immune system. Although Campylobacter species are one of the bacterial etiologies of gastroenteritis, Campylobacter gastroenteritis among under-five children is not well considered in Ethiopia. Therefore, this study aimed at exploring the prevalence, associated risk factors, and antibiotic susceptibility patterns of Campylobacter species among under-five children with diarrhea.

Methods: The institution-based cross-sectional study was conducted among under-five children with diarrhea at Jimma Medical Center, southwestern Ethiopia from January 5 to April 21, 2020. Stool samples were collected and inoculated into Campylobacter agar medium. Isolation and identification were done using standard bacteriological techniques. Antibiotic susceptibility testing was conducted on Mueller-Hinton agar supplemented with 10% sheep blood using disk diffusion techniques. Bivariate and multivariate logistic regressions were used to assess the associated risk factors.

Results: A total of 214 under-five children were enrolled. The prevalence of Campylobacter infection was 8.9%. Absence of caretakers’ handwashing before preparation of food [AOR = 3.7, 95% CI: (1.2–10.8)], direct contact with domestic animals [AOR = 3.6, 95% CI: (1.0–12.7)], and consumption of raw dairy products [AOR = 4.5, 95% CI: (1.4–13.9)] are the factors associated with Campylobacter infection. Some Campylobacter species were found to be resistant to most available antibiotics.

Conclusion: The magnitude of Campylobacter gastroenteritis indicates the need for routine isolation and identification of Campylobacter species from all under-five children clinically diagnosed with diarrhea. Species that are resistant to the drug of choice for Campylobacteriosis are also emerging. Health education on the importance of pasteurization of milk and caretakers’ handwashing can mitigate the transmission. Mechanism of handling of domestic animals should be considered to reduce transmission of zoonotic diseases like Campylobacteriosis.

Keywords: Campylobacter species, prevalence, antibiotic susceptibility pattern, Ethiopia

Introduction

Globally, under-five mortality has reduced from 51 deaths in 2010 to 39 deaths per 1000 live births in 2018. However, a significant number of under-five children are losing their lives. Sub-Saharan Africa was the region with the highest under-five mortality in the world with an average mortality rate of 78 deaths per 1000 live births in 2018.¹

In 2016, 9% of all the global under-five mortality was attributed to diarrhea, of which 90% of deaths occurred in Low- and Middle-Income Countries (LMICs). Estimated 290,724 deaths in sub-Saharan Africa were attributed to diarrhea.² Campylobacter spp. are one of the most common bacterial pathogens associated with diarrhea among under-five children along with Enterohemorrhagic Escherichia coli (EHEC), Enterotoxigenic Escherichia coli (ETEC), Salmonella spp., Shigella spp., Vibrio spp., and Yersinia spp. in different parts of the world.³ Most species are part of the commensal flora of a wide range of animals including food-producing animals like chickens, pigs, and ruminants (eg. sheep, goats, and cattle).⁴ Most Infection with Campylobacter spp. may also result in extra-gastrointestinal manifestations like Guillain-Barre Syndrome (GBS), Miller Fisher Syndrome, bacteremia/septicemia, meningitis, and reactive arthritis.⁶ Due to their ability to colonize most domestic and wild animals, consumption of undercooked meat/poultry,
unpasteurized dairy products, contaminated drinking water, and direct contact with farm animals are found to be risk factors for infection with *Campylobacter* spp.7

Worldwide, *Campylobacter* spp. are responsible for up to 400–500 million infection cases each year.8 In 2010, *Campylobacter* spp. were one of the most common bacterial agents of diarrhea and were responsible for about 96 million illnesses, 21,374 deaths, and 2.1 million Disability Adjusted Life Years (DALYs) globally. The African regions bore more than half (50.6% of the total DALYs per 100,000 population) of the global *Campylobacter* disease burden. The global morbidity rate of 2010 caused by *Campylobacter* infections was relatively higher as compared with *Shigella* spp. (1.2 million DALYs), *Vibrio cholera* (1.7 million DALYs), and Enterotoxigenic *Escherichia coli* (2.0 million DALYs).9

In Europe, *Campylobacter*iosis remains the most reported gastrointestinal disease since 2005 and affected over 220,000 people in 2019.10 The global burden of *Campylobacter* spp. is not confined only to its ability of infecting humans, rather the emergence of drug-resistant *Campylobacter* spp. also became a global concern.11 Increasing trends of resistance by *Campylobacter* spp. against the fluoroquinolone class of antibiotics have been observed in European countries.12

The magnitude of *Campylobacter* infection among under-five children in developing countries is under the shadow; as there are no surveillance systems and routine diagnostic mechanisms in line seen in many developed countries.13 The possible reasons are the absence of a well-equipped laboratory required for isolation and identification of *Campylobacter* spp., the absence of a national surveillance program involving *Campylobacter* gastroenteritis, and the bacteriological characteristics of the organism (fastidiousness).

As a result, the above challenges make the rates and burden of *Campylobacter* infection remain hidden and/or underestimated in Ethiopia. Therefore, this study aimed to explore the isolation rate, antibiotic resistance pattern, and associated risk factors of *Campylobacter* spp. among under-five children in southwestern Ethiopia.

**Materials and Methods**

**Study Setting and Period**
The study was conducted at Jimma Medical Center (JMC) from January 5 to April 27, 2020. JMC is one of the teaching and referral hospitals in the southwestern part of Ethiopia and serves a catchment population of 15–20 million. The hospital has 600 beds and about 1620 staff, providing services for more than 218,000 outpatients, 16,000 inpatients, 14,200 emergencies, and 5900 deliveries annually. The pediatric ward at the center has a bed capacity of 110, admits about 2030 children per year, and serves 4680 children per year as an outpatient. The pediatrics clinic has 78 health professionals, of which 12 are pediatricians, 21 are residents and 45 are pediatric nurses.

**Study Design and Study Population**
A hospital-based cross-sectional study was conducted among all under-five children presented with acute diarrhea at JMC pediatric clinic during the study period.

**Sample Size and Sampling Technique**
A single population proportion formula was used to calculate the minimum sample size.14 Considering a 95% confidence interval, 16.7% previous prevalence of *Campylobacter* spp. among under-five children in Ethiopia,15 and a 5% margin of error, the sample size was 214. A consecutive sampling technique was used to achieve the required sample size.

**Eligibility Criteria**

**Inclusion Criteria**
All under-five children who presented with acute diarrhea at JMC during the study period were included.

**Exclusion Criteria**
All under-five children presented with acute diarrhea who had been treated with antibiotics in the last 7 days prior to data collection.
Data Collection Procedures
Socio-demographic data of both caretakers and under-five children were collected through face-to-face interviews using a structured questionnaire. Height, weight, and age of the children were recorded to assess the nutritional status, and presented as indices of height-for-age (HFA), weight-for-age (WFA), and weight-for-height (WFH). Each index was recorded as the Z-score value of HFA, WFA, and WFH calculated from WHO-Anthro software version 3.2.2 (WHO, Geneva, Switzerland). Accordingly, the Z-score values of < -2 standard deviation for HFA, WFA, and WFH were considered as stunting, underweight, and wasting, respectively. Z-score values between -2 standard deviations and 2 standard deviations were considered normal, and Z-score values of > 2 standard deviations for WFH were considered overweight.16

Sample Collection and Processing
Freshly passed stool specimens were collected using a swab and placed in a 6-mL Cary-Blair transport medium (HKM, Hong Kong, China) prepared in a tube. The samples were then transported to Jimma University Medical Microbiology Laboratory within 2 hours of collection. Stool samples of study participants were directly inoculated into Campylobacter agar base media (HiMedia, Mumbai, India) supplemented with Campylobacter Supplement-I/Blazer-Wang (HiMedia, Mumbai, India) (having 1.250IU Polymyxin B, 5 mg of Vancomycin, 2.500 mg of Trimethoprim, 1 mg of Amphotericin B, and 7.500 mg of Cephalothin) and 10% defibrinated sheep blood. An inoculated medium was incubated at 42°C for 48 hours using a 3.5-L anaerobic jar in a micro-aerobic atmosphere of approximately 5–10% O₂, 5–10% CO₂, and 85% N₂ produced using gas generating sachets, CampyGen (Oxoid Ltd, Hampshire, England).17,18

Isolation and Identification of Campylobacter spp
Grayish, flat, and moistened colonies, with a tendency to spread, and having a metallic sheen on Campylobacter agar media were considered. A saline wet mount was performed and microscopically examined for the characteristic darting/corkscrew motility. Gram staining was also performed, and Gram-negative rod, curved, or spiral bacteria having sea-gull wing appearance were considered. Catalase and oxidase-positive organisms were sub-cultured on blood agar and incubated at 42°C for 48 hours. Colonies having characteristics of non-hemolytic, shiny, and colorless to grayish with irregular or round-edged nature were confirmed as Campylobacter spp.17 The isolated strains were differentiated to the species level based on phenotypic classification methods, which depend on the hydrogen sulfide production in triple sugar iron agar and susceptibility to nalidixic acid (30 µg) and cephalothin (30 µg).19

Antimicrobial Susceptibility Testing
The antimicrobial susceptibility patterns of the isolated strains were determined using Kirby-Bauer disk diffusion technique. Mueller-Hinton agar (HiMedia, Mumbai, India) supplemented with 5% defibrinated sheep blood was prepared on a 90 mm petri dish. The isolated Campylobacter spp. were mixed into sterile normal saline and bacterial suspension having turbidity equivalent to 0.5 McFarland standards was prepared. Using a sterile cotton swab, a bacterial suspension was streaked all over the surface of the prepared Mueller-Hinton agar (HiMedia, Mumbai, India) supplemented with 5% defibrinated sheep blood. Antimicrobial disks of ampicillin (AMP) (10µg), amoxicillin with clavulanic acid (AUG) (30µg), gentamicin (GEN) (10µg), ciprofloxacin (CIP) (5µg), ceftiraxone (CTX) (30µg), erythromycin (E) (15µg), chloramphenicol (C) (30µg), meropenem (MEM) (10µg), trimethoprim-sulfamethoxazole (SXT) (25µg) and azithromycin (AZM) (15) (all Liofilchem, Abruzzo, Italy) was applied over the inoculated plates and incubated micro-aerobically at 42°C for 24 hours. Susceptibility patterns toward nalidixic acid (NA) (30µg) and cephalothin (CEF) (30 µg) were additionally used for species-level identification. The panel of antibiotics was chosen following Clinical and Laboratory Standards Institute (CLSI) guidelines from the previous studies and recent reports of resistance to Campylobacter spp.20,21 Analysis of the diameter of zones of inhibition for erythromycin (E), ampicillin (AMP), ciprofloxacin (CIP), and nalidixic acid (NA) was performed according to the CLSI guideline for Campylobacter spp. from the previous studies.21,22 The rest were done according to CLSI guideline breakpoints for Enterobacteriaceae and were interpreted as susceptible (S), intermediate (I), and resistant (R).23
Data Processing and Statistical Analysis

Data were entered into Epi-data version 3.1 and exported to IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, NY, USA) for statistical analysis. Descriptive statistics were computed to determine the prevalence of Campylobacter infections and the frequencies of other variables. The bivariate logistic regression was conducted and all independent variables with a p-value ≤0.25 in a bivariate logistic regression analysis were taken as candidate variables for possible associated factors. Multivariable logistic regression analyses were computed to identify factors associated with Campylobacter infection. The strength of the association was indicated by the odds ratio (OR) at a 95% confidence interval (CI). A p-value of ≤0.05 was considered indicative of a statistically significant association.

Quality Control

A dummy medium of Campylobacter agar was incubated at 42°C for 48 hours for sterility checking. The sterile Campylobacter agar media and Campylobacter supplement were checked using control strains of Campylobacter jejuni (ATCC 33560) and Escherichia coli (ATCC 25922) for growth and partial or complete inhibition characteristics, respectively. All antibiotic disks and Mueller-Hinton agar medium were checked for correct functionality by control strain of Escherichia coli (ATCC 25922).

Results

Socio-Demographic Characteristics of the Study Participants

A total of 214 under-five children with diarrhea were enrolled. More than half, 109 (50.9) were males and about 54% were living in rural areas. The age of study participants ranged from 1 to 56 months, with a mean age of 15.78 months (±11.8 SD). Most of the children, 79 (36.9%) were between 12 and 24 months of age. Majority of the children’s caretakers, 75 (35%) had no level of education and about 76.6% had latrine at the household level (Table 1).

Table 1 Socio-Demographic Characteristics of the Study Participants at Jimma Medical Center, Southwest Ethiopia (N = 214)

| Variables                                | Categories       | Freq. (%) |
|------------------------------------------|------------------|-----------|
| Age of the child (in months)             | <6               | 38 (17.8) |
|                                          | 6–11             | 57 (26.6) |
|                                          | 12–23            | 79 (36.9) |
|                                          | >24              | 40 (18.7) |
| Sex of the child                         | Male             | 109 (50.9)|
|                                          | Female           | 105 (49.1)|
| Place of residence                       | Urban            | 114 (53.3)|
|                                          | Rural            | 100 (46.7)|
| Maternal/Female caretakers’ occupational status | Housewives     | 145 (67.8)|
|                                          | Government employees | 39 (18.2)|
|                                          | Merchants        | 21 (9.8)  |
|                                          | Others*          | 9 (4.2)   |
| Maternal/Female caretakers’ level of education | No level of education | 75 (35.0)|
|                                          | 1–8              | 41 (19.2) |
|                                          | 9–12             | 26 (12.1) |
|                                          | Diploma          | 47 (22.0) |
|                                          | First degree and above | 25 (11.7)|
| Paternal/male caretakers’ occupational status       | Farmers         | 79 (38.6) |
|                                          | Government employee | 78 (38.0)|
|                                          | Merchants        | 38 (18.5) |
|                                          | Others*          | 10 (4.9)  |

(Continued)
Behavioral and Clinical Characteristics of the Study Participants
Most of the caretakers, 172 (80.4%) had a habit of handwashing after using the toilet. The highest number of caretakers, 147 (68.7%) had a habit of handwashing before preparing food for their children. More than half, 143 (66.8%) used treated water for drinking. Only 9 (4.2%) of the children had a chance of eating raw/undercooked meat and about one-fourth of the children, 52 (24.3%) had a habit of eating raw dairy products. Three fourth of the under-five children had vomiting (Table 2).

Table 1 (Continued).

| Variables                               | Categories   | Freq. (%) |
|-----------------------------------------|--------------|-----------|
| Presence of a latrine in the household  | Yes          | 164 (76.6)|
|                                         | No           | 50 (23.4) |
| Source of drinking water of the household | Private tap  | 108 (50.5)|
|                                         | Public tap   | 90 (42.0) |
|                                         | Spring       | 16 (7.5)  |

Notes: *Office guards and daily laborers; **cafeteria workers and daily laborers.

Table 2 Behavioral and Clinical Characteristics of the Study Participants at Jimma Medical Center, Southwest Ethiopia (N = 214)

| Variables                               | Categories               | Freq. (%) |
|-----------------------------------------|--------------------------|-----------|
| Child contact with domestic animals     | Yes                      | 76 (35.5) |
|                                         | No                       | 138 (64.5)|
| Caretakers’ handwashing habit           | Before preparation of food | Yes | 147 (68.7)|
|                                         |                           | No       | 67 (31.3) |
|                                         | After toilet              | Yes      | 172 (80.4)|
|                                         |                           | No       | 42 (19.6) |
|                                         | Before breastfeeding      | Yes      | 19 (10.8) |
|                                         |                           | No       | 157 (89.2)|
| The habit of treating water before usage| Yes                      | 143 (66.8)|
|                                         | No                       | 71 (33.2) |
| Chance of consuming raw/undercooked meat| Yes                      | 9 (4.2)  |
|                                         | No                       | 205 (95.8)|
| Consumption of raw dairy products       | Yes                      | 52 (24.3) |
|                                         | No                       | 162 (75.7)|
| Consumption of raw/ Unpeeled fruits     | Yes                      | 24 (11.2)|
|                                         | No                       | 190 (88.8)|
| Consumption of poultry                  | Yes                      | 8 (3.7)  |
|                                         | No                       | 206 (96.3)|
| Nutritional status of the children      | Stunting                | 86 (40.2)|
|                                         | Underweight              | 127 (59.3)|
|                                         | Wasting                  | 60 (28)  |
|                                         | Overweight               | 9 (4.2)  |
| Vomiting                                | Yes                      | 164 (76.6)|
|                                         | No                       | 50 (23.4) |
Prevalence of *Campylobacter* spp

*Campylobacter* spp. were isolated from 19 under-five children with diarrhea, with a prevalence of 8.9% [CI: (5.76, 13.4)]. All isolated spp. were *C. jejuni* and/or *C. coli*. The majority, 16 (84.2%) of the isolated *Campylobacter* spp. were among children aged 6–23 months. About 79% of the spp. were isolated from children living in rural areas.

Factors Associated with *Campylobacter* Infection

In the final logistic regression analysis, the absence of caretakers’ hand washing before preparation of food, child contact with domestic animals, and child consumption of raw dairy products were independent predictors of *Campylobacter* infection among under-five children.

Accordingly, the odds of *Campylobacter* infection among under-five children whose mothers/caretakers do not wash their hands before preparing food were 3.7 times [AOR = 3.7, 95% CI: (1.2, 10.8)] higher compared to their counterparts. Likewise, under-five children who had contact with domestic animals were 3.6 times [AOR = 3.6, 95% CI: (1.0, 12.7)] more likely to be infected by *Campylobacter* spp. On the other hand, the odds of *Campylobacter* infection were 4.5 times higher among under-five children who consumed raw dairy products [AOR = 4.5, 95% CI: (1.4, 13.9)] compared to those who did not consume raw dairy products (Table 3).

Table 2 (Continued).

| Variables          | Categories | Freq. (%) |
|--------------------|------------|-----------|
| Bloody diarrhea    | Yes        | 10 (4.7)  |
|                    | No         | 204 (95.4)|
| HIV status         | Positive   | 3 (1.4%)  |
|                    | Negative   | 211 (98.6%)|

Table 3 Bivariate and Multivariable Logistic Regression Analysis Between Candidate Variables and *Campylobacter* Infection Among Under-Five Children at Jimma Medical Center, Southwest Ethiopia (N = 214)

| Variables                                | Categories | Campylobacter Infection | COR, (95% CI) | AOR, (95% CI) |
|------------------------------------------|------------|-------------------------|---------------|---------------|
|                                         |            | Positive Freq. (%)      | Negative Freq. (%) |               |               |
| Paternal/male caretakers’ occupational status | Govt. Employee         | 2 (2.6)                 | 76 (97.4)      | 1             | 5.6 (1.3, 25.1)| 1.1 (0.1, 9.1) |
|                                          | Others#     | 17 (13.4)               | 110 (86.6)     |               |               |
| Place of residence                       | Urban       | 4 (3.5)                 | 110 (96.5)     | 1             | 4.8 (1.6, 15.2)| 0.4 (0.05, 2.8) |
|                                          | Rural       | 15 (15.0)               | 85 (85.0)      |               |               |
| Handwashing before preparing food        | Yes         | 6 (4.1)                 | 141 (95.9)     | 1             | 5.6 (2.0, 15.5)| 1.1 (0.3, 5.2) |
|                                          | No          | 13 (19.4)               | 54 (80.6)      |               |               |
| Water treatment before child using       | Yes         | 5 (3.5)                 | 138 (96.5)     | 1             | 6.8 (2.3, 19.7)| 1.8 (0.5, 6.7) |
|                                          | No          | 14 (19.4)               | 57 (80.3)      |               |               |
| Presence of domestic animals             | Yes         | 12 (20)                 | 48 (80)        | 5.3 (1.9, 14.1)| 1.1 (0.3, 5.2) |
|                                          | No          | 7 (4.5)                 | 147 (95.5)     |               |               |
| Child contact with domestic animals      | Yes         | 15 (19.7)               | 61 (80.3)      | 8.2 (2.6, 25.9)| 3.6 (1.0, 12.7)*| 1             |
|                                          | No          | 4 (1.9)                 | 134 (98.1)     |               |               |
| Consumption of raw dairy products        | Yes         | 13 (25)                 | 39 (75)        | 8.6 (3.1, 24.3)| 4.5 (1.4, 13.9)*| 1             |
|                                          | No          | 6 (3.7)                 | 156 (96.3)     |               |               |

Notes: *p*<0.05; #Farmers, merchants, office guards, and daily laborers; 1, reference category; Numbers in bold font represent variables significantly associated with *Campylobacter* infection in multivariate logistic regression.

Abbreviations: COR, crude odds ratio; AOR, adjusted odds ratio.
Antimicrobial Susceptibility Pattern of the Isolates

All isolates (100%) of Campylobacter spp. were susceptible to gentamicin and azithromycin. Nearly 95% of the isolates were susceptible to ciprofloxacin and about 79% were susceptible to erythromycin. Of all Campylobacter spp., 94.7% were resistant to ceftriaxone and trimethoprim-sulfamethoxazole. About one-fourth (22%) of the isolated strains were resistant to ampicillin. More than half (57.9) were resistant to chloramphenicol. About 79% of the isolated strains were non-susceptible to at least one agent in three or more antimicrobial categories (MDR). However, neither XDR nor PDR strains were isolated according to the definitions of MDR, XDR, and PDR given through the initiatives of the European Centre for Disease Prevention and Control (ECDC), and the Centers for Disease Control and Prevention (CDC)

Table 4 Antimicrobial Susceptibility Pattern of Campylobacter spp. Isolated from Under-Five Children at Jimma Medical Center, Southwest Ethiopia (N = 19)

| Selected Antibiotics | Disk Concentration (μg) | Susceptibility Pattern |
|----------------------|-------------------------|------------------------|
|                      | S Freq. (%) | I Freq. (%) | R Freq. (%) |
| Quinolone            |             |             |             |
| Ciprofloxacin        | 5           | 18 (94.7)  | -           | 1 (5.3)    |
| Macrolides           |             |             |             |
| Azithromycin         | 15          | 19 (100)   | -           | -          |
| Erythromycin         | 15          | 15 (78.9)  | 2 (10.5)   | 2 (10.5)   |
| Phenicol             |             |             |             |
| Chloramphenicol      | 30          | 8 (42.1)   | 3 (15.8)   | 8 (42.1)   |
| Penicillin           |             |             |             |
| Ampicillin           | 10          | 3 (15.8)   | 1 (5.3)    | 15 (78.9)  |
| Beta-lactam/ beta-lactamase inhibitor | 30 | 16 (84.2) | - | 3 (15.8) |
| Cephalosporin        |             |             |             |
| Ceftriaxone          | 30          | 1 (5.3)    | -           | 18 (94.7)  |
| Carbapenem           |             |             |             |
| Meropenem            | 10          | 16 (84.2)  | 1 (5.3)    | 2 (10.5)   |
| Aminoglycoside       |             |             |             |
| Gentamicin           | 10          | 19 (100)   | -           | -          |
| Folate pathway inhibitor | 25 | 1 (5.3) | - | 18 (94.7) |
| MDR                  |             |             |             |
|                      |             | 15 (78.9)  |             |            |

Note: MDR, non-susceptibility to at least one agent in three or more antimicrobial categories.
Abbreviations: S, susceptible; I, intermediate; R, resistant.

Antimicrobial Susceptibility Pattern of the Isolates

All isolated Campylobacter spp. (100%) were susceptible to gentamicin and azithromycin. Nearly 95% of the isolates were susceptible to ciprofloxacin and about 79% were susceptible to erythromycin. Of all Campylobacter spp., 94.7% were resistant to ceftriaxone and trimethoprim-sulfamethoxazole. About one-fourth (22%) of the isolated strains were resistant to ampicillin. More than half (57.9) were resistant to chloramphenicol. About 79% of the isolated strains were non-susceptible to at least one agent in three or more antimicrobial categories (MDR). However, neither XDR nor PDR strains were isolated according to the definitions of MDR, XDR, and PDR given through the initiatives of the European Centre for Disease Prevention and Control (ECDC), and the Centers for Disease Control and Prevention (CDC)

Discussion

The principal aim of the study was to assess the burden of Campylobacter infection among under-five children. Our study showed that the prevalence of Campylobacter spp. among under-five children was 8.9%. This finding is inline with studies conducted in Poland (9.3%), Egypt (9.37%), Tanzania (9.7%), and Uganda (9.3%). However, a higher prevalence of Campylobacter infections among under-five children was reported in South Africa (25%), India (17.35%), and Kenya (16%). The variation in the isolation rates of Campylobacter spp. could be due to differences in socio-demographic characteristics, study subjects, study periods, and methods employed. However, the finding of this study was lower than the studies conducted in other parts of Ethiopia, where the isolation rates were 15.4%, and 16.7% in Gondar and Jimma, respectively. The major variation observed between this study and the previous studies conducted in Ethiopia could be due to variation in study periods, as seasonality is one of the factors that affect the prevalence of Campylobacteriosis. Additionally, previous studies were conducted on samples taken from health centers where acute diarrhea could be found common.

The odds of Campylobacter infection were higher among under-five children who had contact with domestic animals. This finding is consistent with studies conducted in California, Sweden, Norway, and Jordan. A similar study conducted in Ethiopia also agrees with the finding of an association between Campylobacter infections and the presence and contact of domestic animals in the household. This could be due to the fact that Campylobacter spp. are harbored by most domestic animals as normal gut microbiota. Therefore, the presence and contact with domestic animals increases the possibility of contamination of food and hence increases the rate of infection.
This study has also identified under-five children who did not consume raw dairy products had low odds of infection with *Campylobacter* spp. This finding agrees with the findings of the study conducted in Sweden, Norway, and New Zealand. The possible reason for this finding is due to the probability of contamination of dairy products, mainly raw milk with animals manure.

*Campylobacter* infection was higher among under-five children whose mothers/caretakers do not wash their hands before preparing food. This result is consistent with a study conducted in 8 selected low-income countries. The absence of routine handwashing practices could be the cause of *Campylobacter* infection in the presence of maternal contact with domestic animals.

On the other hand, all isolated strains of *Campylobacter* spp. were found to be susceptible to gentamicin and azithromycin. This finding is supported by studies conducted in Macedonia and Korea. However, it was found to be higher as compared to the previous study conducted in Ethiopia where nearly 87% of the isolates were susceptible to gentamicin. This could be due to the difference in the *Campylobacter* spp. isolated from the participants, where the isolated strains of *Campylobacter* were all *C. jejuni* and/or *C. coli*.

Moreover, nearly 95% of the isolates were susceptible to ciprofloxacin, which is consistent with another study conducted in Ethiopia. Our study also revealed the rate of resistance to erythromycin is comparable with findings from South Africa and other parts of Ethiopia. This study also indicates a high rate of resistance to ceftriaxone (94.7%), trimethoprim-sulfamethoxazole (94.7%), ampicillin (78.5%), and chloramphenicol (42.1%). However, lower rates of resistance were recorded in Macedonia and Korea, Pakistan and Bangladesh. The main reason could be due to inappropriate use of antibiotics in Ethiopia.

**Limitation of the Study**
This study has a limitation as it could not differentiate between *C. jejuni* and *C. coli* due to the absence of hippurate hydrolysis test used for this purpose. The limited sample size could also be a reason for which additional risk factors of infections were not identified.

**Conclusion and Recommendation**
The rate of isolation of *Campylobacter* spp. among under-five children in the study area was noticeable. The magnitude of *Campylobacter* gastroenteritis indicates the need for routine isolation and identification of *Campylobacter* spp. from every under-five child clinically diagnosed with diarrhea. Although appreciable susceptibility was recorded for azithromycin and gentamicin, resistance to the drug of choice for *Campylobacter* infection is also emerging. Health education on the importance of pasteurization of milk and caretakers’ hand washing can mitigate the transmission. The mechanism of handling domestic animals should also be considered to reduce the transmission of zoonotic diseases like Campylobacteriosis. Moreover, having these baseline data, researchers and concerned bodies should undergo further studies to disclose the burden of *Campylobacter* infection in developing countries, which in turn points out the mechanism of dwarfing the burden among under-five children.

**Abbreviations**
DALYs, Disability-Adjusted Life Years; HFA, height-for-age; MDR, multi-drug resistant; XDR, extensively-drug resistant; PDR, pan-drug resistant; JMC, Jimma Medical Center; WFA, Weight-for-Age; WFH, Weight-for-Height.

**Data Sharing Statement**
Data used in the findings of this study are included in the manuscript.

**Ethics Approval and Consent to Participate**
The study was approved and ethical clearance was obtained from the Institutional Review Board of the Institute of Health, Jimma University (Ref. No: IHRPGD/581/18). The study protocol was performed following the Declaration of Helsinki. Overviews of the study were explained for families/caretakers of under-five children. Then, informed consent
was obtained from families/caretakers of under-five children regarding socio-demographic and clinical data collection including sample collection.

**Consent for Publication**
Not applicable – this manuscript does not contain any individual data.

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**Author Contributions**
All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

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All the authors declare no conflicts of interest in this work.

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