

## RESEARCH ARTICLE

# Isolation, identification, and antimicrobial susceptibility pattern of *Campylobacter jejuni* and *Campylobacter coli* from cattle, goat, and chicken meats in Mekelle, Ethiopia

Yohans Hagos<sup>1</sup>, Getachew Gugsag<sup>2</sup>\*, Nesibu Awol<sup>2</sup>, Meselu Ahmed<sup>2</sup>, Yisehak Tsegaye<sup>3</sup>, Nigus Abebe<sup>3</sup>, Abrha Bsrat<sup>3</sup>

**1** Shire Agricultural Technical Vocational and Education Training College, Shire, Tigray, Ethiopia,

**2** Department of Veterinary Medicine, School of Veterinary Medicine, Wollo University, Dessie, Ethiopia,

**3** Department of Veterinary Medicine, College of Veterinary Sciences, Mekelle University, Mekelle, Ethiopia

✉ These authors contributed equally to this work.

\* [gugsag@yahoo.com](mailto:gugsag@yahoo.com)



## OPEN ACCESS

**Citation:** Hagos Y, Gugsag G, Awol N, Ahmed M, Tsegaye Y, Abebe N, et al. (2021) Isolation, identification, and antimicrobial susceptibility pattern of *Campylobacter jejuni* and *Campylobacter coli* from cattle, goat, and chicken meats in Mekelle, Ethiopia. PLoS ONE 16(2): e0246755. <https://doi.org/10.1371/journal.pone.0246755>

**Editor:** Kumar Venkitanarayanan, University of Connecticut, UNITED STATES

**Received:** October 3, 2020

**Accepted:** January 25, 2021

**Published:** February 10, 2021

**Peer Review History:** PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: <https://doi.org/10.1371/journal.pone.0246755>

**Copyright:** © 2021 Hagos et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** All relevant data are within the paper.

## Abstract

*Campylobacter jejuni* and *Campylobacter coli* are globally recognized as a major cause of bacterial foodborne gastroenteritis. A cross-sectional study was conducted from October 2015 to May 2016 in Mekelle city to isolate, identify, and estimate the prevalence of *C. jejuni* and *C. coli* in raw meat samples and to determine their antibiotic susceptibility pattern. A total of 384 raw meat samples were randomly collected from bovine (n = 210), goat (n = 108), and chicken (n = 66), and isolation and identification of *Campylobacter spp.* were performed using standard bacteriological techniques and PCR. Antibiotic susceptibility test was performed using disc diffusion method. Of the total 384 raw meat samples, 64 (16.67%) were found positive for *Campylobacter spp.* The highest prevalence of *Campylobacter spp.* was found in chicken meat (43.93%) followed by bovine meat (11.90%) and goat meat (9.25%). The most prevalent *Campylobacter spp.* isolated from meat samples was *C. jejuni* (81.25%). The overall prevalence of *Campylobacter* in restaurants, butcher shops, and abattoir was 43.93%, 18.30%, and 9.30%, respectively. 96.8%, 81.25%, 75%, and 71% of the *Campylobacter spp.* isolates were sensitive to norfloxacin, erythromycin, chloramphenicol, and sulphamethoxazole-trimethoprim, respectively. However, 96.9%, 85.9%, and 50% of the isolates were resistant to ampicillin, amoxicillin, and streptomycin, respectively. Strains that developed multi-drug resistant were 68.7%. The result of this study revealed the occurrence of *Campylobacter* in bovine, goat, and chicken meats. Hence, there is a chance of acquiring infection via consumption of raw or undercooked meat. Thus, implementation of hygienic practices from a slaughterhouse to the retailers, proper handling and cooking of foods of meat are very important in preventing *Campylobacter* infection.

**Funding:** This research work was funded by College of Veterinary Sciences, Mekelle University.

**Competing interests:** The authors have declared that no competing interests exist.

## Introduction

Foodborne diseases occur as a result of the consumption of contaminated foodstuffs especially from animal products such as meat from infected animals or carcasses contaminated with pathogenic bacteria [1, 2]. Food-producing animals 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*. Foodborne pathogens cause millions of cases of sporadic illness and chronic complications, as well as large and challenging outbreaks in many countries and between countries [3].

Worldwide, pathogenic *Campylobacter species* are the leading cause of bacterial-derived foodborne disease and are responsible for the cause of over 400–500 million infections cases each year [4–6]. *Campylobacter species* are normally carried in the intestinal tracts of many domestic livestock such as poultry, cattle, sheep, goat, pigs, as well as wild animals and birds [7–10]. Fecal matter is a major source of contamination and could reach carcasses through direct deposition [11]. Animal food products can become contaminated by this pathogen during slaughtering and carcass dressing [12]. Humans are infected by ingestion of undercooked or decontaminated meat, or handling of raw products or cross-contamination of raw to cooked foods, swimming in natural waters, direct contact with contaminated animals or animal carcasses, and traveling [13–15].

Pathogenic *Campylobacter spp.* known to be implicated in human infections include *C. jejuni*, *C. concisus*, *C. rectus*, *C. hyointestinalis*, *C. insulaenigrae*, *C. sputorum*, *C. helveticus*, *C. lari*, *C. fetus*, *C. mucosalis*, *C. coli*, *C. upsaliensis*, and *C. ureolyticus* [6]. Of these, *C. jejuni* and *C. coli* are considered the most commonly reported zoonosis in humans and recognized as the most common causative agents of bacterial gastroenteritis in the world [16–19].

Moreover, *Campylobacter* with resistance to antimicrobial agents has also been implicated worldwide [4, 20, 21]. The use of antimicrobial agents in food animals 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. The situation seems to deteriorate more rapidly in developing countries where there is widespread and uncontrolled use of antibiotics [22].

In Ethiopia, few studies were conducted on the prevalence and antimicrobial susceptibility of enteric *Campylobacteriosis* of human beings [23–25] and food of animal origins [9, 20, 26–28]. The absence of a national surveillance program, limited routine culture availability for the isolation of *Campylobacter spp.* in clinical and research settings, and the need for selective media and unique growth atmosphere make it difficult to give an accurate picture of the burden of the disease in Ethiopia. This fact indicates that *Campylobacter* as a zoonosis is not given appropriate weight and consideration, particularly in the current study area. As a result, the objectives of this study were to isolate and identify *C. jejuni* and *C. coli* from the meat of cattle, goat, and chicken collected from an abattoir, butcher shops, and restaurants in Mekelle City, estimate their prevalence and determine the antibiotic susceptibility pattern of *C. jejuni* and *C. coli* isolates.

## Material and methods

### Ethics approval

This study was reviewed and approved by the Research Ethics Committee of the College of Veterinary Sciences, Mekelle University.

### Study area

The study was conducted from October 2015 to May 2016 at an abattoir, butcher shops, and restaurants of Mekelle City. Mekelle is the capital city of Tigray National Regional State of

Ethiopia where thousands of cattle and goats are accessible from different districts of the region and the neighboring regions of the country for slaughter. Mekelle is found at 39° 29' East and 13° 30' North of the equator which is 783 kilometers away from Addis Ababa, which is the capital city of Ethiopia. The altitude of the area ranges from 2000–2200 meters above sea level. The mean annual rainfall of the area is 628.8 mm and an annual average temperature of 21°C. The city has seven sub-cities and a total population of 215,546 [29], 308 cafeterias, 292 restaurants, 258 supermarkets, and an active urban-rural exchange of goods which has 30000 micro-and small enterprises [30].

### Study design

A cross-sectional study was employed from October 2015 to May 2016 to isolate, identify, and estimate the prevalence and antibiotic susceptibility patterns of *C. jejuni* and *C. coli* from bovine, goat, and chicken meat samples collected from the abattoir, butcher shops, and restaurants.

### Sample size and sample collection

A total of 384 raw ready-to-eat meat samples comprising of cattle (n = 210), goat (n = 108), and chicken (n = 66) meats were collected from the abattoir (n = 258), butcher shops (n = 60), and restaurants (n = 66) of the study area. All samples were placed in polyethylene plastic bags to prevent spilling and cross-contamination and immediately transported to the Molecular Biology Laboratory of the College of Veterinary Sciences, Mekelle University using an icebox with ice packs.

### Bacteriological isolation and identification of *Campylobacter* species

Approximately 10 grams of raw meat sample was aseptically collected using sterile forceps and scissor and placed into 90ml of buffered peptone water in a sterile plastic bag and homogenized for 1 minute using a stomacher (Lab Blender 400, Seward Medical, London, England) and incubated at 37°C for 48h in the microaerophilic atmosphere (gas mix of 5% O<sub>2</sub>, 10% CO<sub>2</sub>, and 85% N<sub>2</sub>). Then a 0.1ml of the enriched sample was streaked onto Karmali *Campylobacter* Agar Base (HiMedia Laboratories, Mumbai, India)(Blood free *Campylobacter* selective agar base medium containing *Campylobacter* selective supplement comprising cefoperazone, amphotericin B (CCDA selective supplement SR0155E)) [31] and kept in a gas jar containing *Campylobacter* gas pack systems to maintain the microaerophilic condition and was incubated at a temperature of 37°C for 48h. The colonies were provisionally identified based on staining reaction with Gram's stain, cellular morphology [32], catalase test, and oxidase test [33], and growth appearance on 5% sheep blood agar (Oxoid Ltd., Basingstoke, Hampshire, England) at 37°C after 24 h [34].

All the thermophilic *Campylobacter* isolates were tested for Hippurate hydrolysis, H<sub>2</sub>S production, and susceptibility to Nalidixic acid and Cephalothin as proposed by [34]. Susceptibility tests to Nalidixic acid (30 µg) and Cephalothin (30 µg) were performed using the standard agar disc diffusion method as recommended by Clinical and Laboratory Standards Institutions (CLSI) and isolates were categorized as sensitive and/or resistant according to the interpretation table of the [35]. The presumed *Campylobacter* isolates were preserved in brain heart infusion broth supplemented with 15% glycerol in Eppendorf tubes at -20°C for further analysis. Bacterial strains that were used as quality control organisms in this study were standard strains of *S. aureus*, *S. agalactiae*, and *E. coli* obtained from the National Veterinary Institute (NVI), Debre-Zeit.

## Polymerase chain reaction for detection of *mapA* and *ceuE* genes

The genomic DNA of the pheno typically resembled isolates of *Campylobacter* was extracted using the Phenol Chloroform method (Phenol: Chloroform: Isoamyl alcohol mixture (24:25:1)) according to [36]. Then 20 $\mu$ l of each extracted genomic DNA sample was run in an agarose gel electrophoresis and visualized under UV-light gel doc. Then after, a genome-based polymerase chain reaction (PCR) was done as described by [37] using the following species-specific primers: F-5' CTA TTT TAT TTT TGA GTG CTT GTG3' and R-5' GCT TTA TTT GCC ATTT GTT TTA TTA3' was used to amplify the *mapA* gene of *C. jejuni*(589 bp), and F-5' ATT TGA AAA TTG CTC CAA CTA TG3' and R-5' TGA TTT TAT TAT TTG TAG CAG CG3' were used to amplify the *ceuE* gene of *C. coli*(462 bp). Each PCR reaction mixture was performed in a 50 $\mu$ l total volume containing 10 $\mu$ l of template DNA, 5 $\mu$ l of 5X PC buffer, 5 $\mu$ l of MgCl<sub>2</sub>, 1 $\mu$ l of each of the primers, 0.75 $\mu$ l of 10 mM of each dNTPs, 0.15 $\mu$ l of Taq DNA polymerase, and 27.1 $\mu$ l nuclease-free distilled water. Amplification was carried out with thermal cycling conditions of an initial denaturation at 95°C for 5 min followed by 45 cycles of denaturation at 94°C for 35s, annealing at 54°C for 35s and extension at 72°C for 35s, and with a final extension at 72°C for 6 min. Finally, the PCR products were separated by running on a 1.5% (w/v) agarose gel containing 0.3mg/ml ethidium bromide. Electrophoresis was conducted in a horizontal equipment system for 120 min at 90 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 doc and their molecular weights were estimated by comparing with 100bp DNA molecular weight marker (Solis BioDyne, Tartu, Estonia).

## 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 supplemented with 5% sheep blood (Oxoid Ltd., Basingstoke, Hampshire, England). The following nine different antibiotic discs, with their concentrations given in parentheses, were used in the antibiogram testing: Amoxicillin (AML)(10 $\mu$ g), Ampicillin (AMP)(10 $\mu$ g), Chloramphenicol (C)(30 $\mu$ g), Erythromycin (E)(15 $\mu$ g), Gentamycin (CN)(10 $\mu$ g), Norfloxacin (NOR)(10 $\mu$ g), Streptomycin (S)(10 $\mu$ g), Tetracycline (TE)(30 $\mu$ g), and Sulfamethoxazole-trimethoprim (SXT)(25 $\mu$ g) (Oxoid Company, Hampshire, England). After 48h of microaerophilic incubation at 37°C, the clear zones (inhibition zones of bacterial growth around the antibiotic discs(including the discs)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 Clinical and Laboratory Standard Institute [35].

## Data storage and statistical analysis

All collected data were entered into Microsoft Excel Sheet (Microsoft Corp., Redmond, WA, USA) and analyzed using SPSS version 20 statistical computer software program. Chi-square ( $\chi^2$ ) test and Logistic regression were applied to assess the associations. For all tests, a *p-value* of less than 0.05 was considered statistically significant.

## Results

### The overall prevalence of *Campylobacter* species

Out of the total of 384 collected meat samples, 64(16.67%) were positive for the two *Campylobacter spp.* The highest (43.93%) and lowest (9.25%) prevalence of *Campylobacter spp.* were

**Table 1. Prevalence of *Campylobacter spp.* among different sample types and sources.**

Risk Factors	No. of Examined	No. of Positive (%)	$\chi^2$	P-value
<b>Type of meat</b>				
Goat meat	108	10(9.25)	43.04	0.000
Cattle meat	210	25(11.90)		
Chicken meat	66	29(43.93)		
<b>Total</b>	<b>384</b>	<b>64(16.67)</b>		
<b>Sources of meat</b>				
Abattoir	258	24(9.30)	45.53	0.000
Butcher	60	11(18.33)		
Restaurants	66	29(43.93)		
<b>Total</b>	<b>384</b>	<b>64(16.67)</b>		

<https://doi.org/10.1371/journal.pone.0246755.t001>

recorded in samples taken from chicken (found to be 7.68 times more likely to have *Campylobacter* contamination compared to other sample types) and goat, respectively. Whereas, the highest (43.93%) and lowest (9.25%) prevalence were recorded in meat samples collected from restaurants (found to be four times more likely to have *Campylobacter* contamination compared to other sample sources) and abattoir, respectively. Both sample types and sources had significant differences ( $p = 0.00$ ;  $\chi^2 = 43.04$  or  $OR = 7.68$ ,  $CI = 3.40-17.30$ , and  $p = 0.00$ ;  $\chi^2 = 45.53$  or  $OR = 7.64$ ,  $CI = 4.01-14.52$ , respectively) in the prevalence of the two *Campylobacter spp.* as it is shown in Tables 1 and 2 below.

### Contamination rate of *C.jejuni* and *C. coli* in the different sample types

Of the two *Campylobacter spp.* isolated and identified from cattle, goat, and chicken meat samples *C. jejuni* and *C. coli* accounted for 81.25% and 18.75%, respectively. The prevalence of *C. jejuni* and *C.coli* in cattle, goat, and chicken meat samples were found to be 76% and 24%, 80% and 20%, and 86.21% and 13.79%, respectively (Table 3).

### PCR amplification results of *Campylobacter spp.* isolates

Besides the phenotypic characterization, PCR amplification of the 64 samples revealed that 52(81.25%) of the isolates were *C.jejuni*(having a molecular weight of 589 bp) and the remaining 12(18.75%) isolates were *C.coli* (having a molecular weight of 462 bp) to the targeted genes.

**Table 2. Logistic regression analysis results of sample types and sample sources.**

Types of sample	No of examined	No of positive (%)	OR(95%CI)	P-value
Goat meat*	108	10(9.25)	1	
Cattle meat	210	25(11.90)	1.32(0.61-2.86)	0.476
Chicken meat	66	29(43.93)	7.68(3.40-17.30)	0.000
<b>Total</b>	<b>384</b>	<b>64(16.67)</b>		
Abattoir*	258	24(9.30)	1	
Butcher	60	11(18.33)	2.18(1.00-4.76)	0.048
Restaurants	66	29(43.93)	7.64(4.01-14.52)	0.000
<b>Total</b>	<b>384</b>	<b>64(16.67)</b>		

OR = Odd ratio; CI = Confidence interval.

<https://doi.org/10.1371/journal.pone.0246755.t002>

**Table 3. The contamination rate of *C. jejuni* and *C. coli* among different sample types.**

Sample Type Prevalence	<i>Campylobacter spp.</i>	
	<i>C.jejuni</i>	<i>C.coli</i>
Cattle meat (n = 25)	19(76%)	6(24%)
Goat meat (n = 10)	8(80%)	2(20%)
Chicken meat (n = 29)	25(86.21%)	4(13.79%)
<b>Total (n = 64)</b>	<b>52(81.25%)</b>	<b>12(18.75%)</b>

<https://doi.org/10.1371/journal.pone.0246755.t003>

### Antimicrobial susceptibility pattern of *Campylobacter spp.* isolates

*Campylobacter spp.* isolated from the different sample types and sources were susceptible to Norfloxacin (96.8%), Erythromycin (81.25%), Chloramphenicol (75%), and Gentamycin (75%). However, the isolates had shown resistance to Ampicillin (96.9%) and Amoxicillin (85.9%) (Table 4). Moreover, 96.8% of the isolates developed resistance for two or more than two drugs as it is shown in Fig 1.

Species based antibiogram result of the isolates revealed that the highest level of sensitivity of both *C. jejuni* (98.1%) and *C. coli* (91.7%) was observed against Norfloxacin. Whereas both *C.jejuni*(96.2%) and *C. coli*(100%) were showed the highest level of resistance against Ampicillin as it is shown in Table 5.

### Discussion

In the current study, the overall prevalence of *Campylobacter spp.* was found to be 16.67%. The highest prevalence was found in chicken meat samples (43.93%). Chicken meats were found to be 7.68 times more likely to have *Campylobacter* when compared to goat and cattle meat. The difference in the prevalence of *Campylobacter* between different types of meat samples was found to be statistically significant ( $p < 0.05$ ) (OR = 7.68, CI = 3.40–17.30). The prevalence of *Campylobacter spp.* in chicken meat samples was 43.93% which was comparable with those reported by [38], 44%, [39], 56.1%, and [40], 48.02% in Iran. This was higher than the report of [41] who reported a prevalence of 1.93%. However, the present finding was lower than studies conducted by [42–44], and [45] who reported the prevalence of 61.7% and 70.7% and 65% and 81.3% of *Campylobacter spp.* in Ahvaz, Iran; Washington; Northern Ireland, and Northern Italy, respectively. It is a well-known fact that poultry appeared to be a significant source of *Campylobacter* and chicken was found to be heavily intestinal carriers of *Campylobacter* when compared with other food animals [8]. Wide variation (0–90%) in the prevalence of

**Table 4. In vitro antimicrobial sensitivity pattern of *Campylobacter spp.* isolates.**

Type of antibiotics	Interpretations		
	Susceptible (%)	Intermediate (%)	Resistant (%)
Ampicillin	0(0)	2(3.1)	62(96.9)
Amoxicillin	3(4.7)	6(9.4)	55(85.9)
Chloramphenicol	48(75)	5(7.8)	11(17.2)
Erythromycin	52(81.25)	1(1.6)	11(17.2)
Gentamycin	48(75)	8(12.5)	8(12.5)
Norfloxacin	62(96.8)	1(1.6)	1(1.6)
Streptomycin	25(39.06)	7(10.9)	32(50)
Tetracycline	42(65.6)	6(9.4)	16(25)
Sulfamethoxazole-Trimethoprim	46(71.8)	4(6.25)	14(21.9)

<https://doi.org/10.1371/journal.pone.0246755.t004>



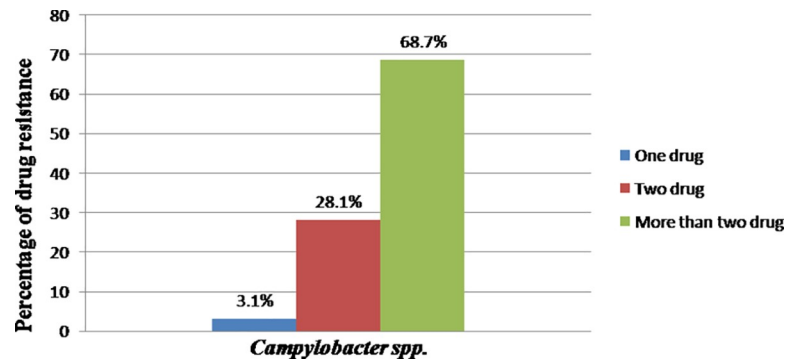


Fig 1. Percentage of drug resistance of *Campylobacter spp.*

<https://doi.org/10.1371/journal.pone.0246755.g001>

*Campylobacter* in fresh poultry meat had been reported in different countries [46–48]. These variations in *Campylobacter spp.* prevalence might be due to differences in hygienic conditions, cross-contamination that may occur during de-feathering, eviscerating, and some other environmental factor such as the temperature of water in the scalding tank.

In this study, the prevalence of *Campylobacter spp.* in bovine meat was 11.90%. This was comparable to the finding reported from a previous study done by [10] (12.9%) in Nigeria, and [49] (10%) in Iran. However, it was higher than the findings reported by [9] (6.2%) in Ethiopia [50]; (5.6%) in Morogoro, Tanzania; and [51] (0.8%) in Australia. Food of animal origin has been incriminated for being the main source of *Campylobacter* infection in humans [52]. Since raw meat from beef is widely consumed in the country; the occurrence of *Campylobacter* in meat increases the likelihood of the pathogen transmission to humans. The present finding was lower than studies conducted by [10] and [53] who reported a prevalence of 69.1% and 22%, respectively. One of the most likely hypotheses to explain the discrepancies is the differences in protocols used for the detection of thermophilic *Campylobacter*, and especially the absence of an enrichment step for the isolation of thermophilic *Campylobacter* in [54] work. In general, these variations might be due to approaches of sample collection, the difference in isolation and identification techniques, and differences in sample size.

The prevalence of *Campylobacter spp.* in goat meat was found to be 9.25%. This finding was in agreement with the reports of [9] and [55], who reported 7.6% and 6.4%, respectively. But it

Table 5. In vitro antimicrobial sensitivity pattern of *C. jejuni* and *C. coli* isolates.

Antibiotic Discs	Interpretations					
	<i>C. jejuni</i> (N = 52)			<i>C. coli</i> (N = 12)		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Ampicillin	-	2(3.8)	50(96.2)	-	-	12(100)
Amoxicillin	3(5.8)	4(7.7)	45(86.5)	-	2(16.7)	10(83.3)
Chloramphenicol	40(76.9)	4(7.7)	8(15.4)	8(66.7)	1(8.3)	3(25)
Erythromycin	43(82.7)	-	9(17.3)	9(75)	1(8.3)	2(16.7)
Gentamycin	41(78.8)	6(11.5)	5(9.6)	7(58.3)	2(16.7)	3(25)
Norfloxacine	51(98.1)	-	1(1.9)	11(91.7)	1(8.3)	-
Streptomycin	22(42.5)	7(13.5)	23(44.2)	3(33.3)	-	9(66.7)
Tetracycline	38(73.1)	6(11.5)	8(15.4)	4(41.7)	-	8(58.3)
Sulfamethoxazole- Trimethoprim	39(75.5)	3(5.8)	10(19.2)	7(58.3)	1(8.3)	4(33.3)

S = Susceptible, I = Intermediate, R = Resistant.

<https://doi.org/10.1371/journal.pone.0246755.t005>

slightly higher than the report of [42] who reported 4.4%. However, the present study was lower than the findings of [5] and [26] who reported 41.2% and 27.5%, respectively.

The meat samples collected from restaurants had the highest prevalence (43.93%). The meat samples collected from restaurants were found to be four times more likely to have *Campylobacter* compared to meat collected from Butcher and eight times more likely to have *Campylobacter* compared to meat collected from the abattoir. The difference in the prevalence of *Campylobacter* between sources of meat samples was found to be statistically significant ( $P < 0.05$ ) (OR = 7.64, CI = 4.01–14.52). This might be due to an extra chance of acquiring contamination from individuals who are working in restaurants during handling or cross-contamination among different carcasses.

In the current study, the bacteriological and PCR characterization of *Campylobacter* isolates revealed that the prevalence of *C. jejuni* was higher than *C. coli*. *C. jejuni* has been reported to be the most frequent species recovered from the food of animal origin especially chicken meat [48, 56–58]. The prevalence of *C. jejuni* and *C. coli* in bovine, goat, and chicken meat were found to be 76% and 24%; 80% and 20%; and 86.3% and 13.7%, respectively. These findings were in agreement with the findings of [9] who reported 78% *C. jejuni* and 18% *C. coli* [28]; who reported 78% *C. jejuni* and 22% *C. coli* [27]; who reported 93.3% *C. jejuni* and 6.7% *C. coli* [26]; who reported 72.5% *C. jejuni* and 27.5% *C. coli* in Ethiopia. The prevalence of *C. jejuni* in raw meat was in agreement with the reports from other countries [48, 57, 59, 60].

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 [61–63]. *Campylobacter* spp. resistance to antibiotics (*C. jejuni* and *C. coli*) can be transferred from different sources to humans. This situation, alarmingly, announces the need to perform an antimicrobial sensitivity test for *Campylobacter*. Macrolides and Fluoroquinolones are usually considered the drugs of choice for the treatment of foodborne *Campylobacteriosis* [64–66]. Antibiotic susceptibility patterns have been determined in previous studies conducted in Ethiopia where the 80%–100% of isolates from food animals were sensitive to these antimicrobial agents [9, 20]. However, there are pieces of evidence from different parts of the world that antimicrobial resistance in food animals and human isolates is increasing.

In the current study, 52 *C. jejuni* and 12 *C. coli* isolates were investigated for their antimicrobial susceptibility pattern. The percentage of ampicillin and amoxicillin resistant *Campylobacter* isolates were 96.9% and 85.9%, respectively. This was in agreement with the report of [28], who reported 97.2% and 83.3% for ampicillin and amoxicillin, respectively. Moreover, [67] reported a resistance level of 100% for *C. coli*. On the other hand, *C. coli* isolates are generally more resistant than *C. jejuni* strains [68]. In general, several studies have reported resistance to beta-lactam antibiotics is high in food animals [69–71]. The resistance rate of *Campylobacter* isolates (25%) to tetracycline in the present study was comparable with the findings of [27] (20.8%) but higher than that of [9] (10%) and [72] (6%). However, it was lower than the report of [73] (77.94%). The resistance level to streptomycin in the current study was 50%, which was higher than reports from Ireland and Thailand by [73] and [74].

Multi-drug resistance isolates always remained susceptible to norfloxacin and erythromycin and chloramphenicol. In the present study, multi-drug resistance to more than two antimicrobial agents was 68.7% which was comparable to the findings of [69] in Belgium, 60% [75]; in Estonia, 60% [49]; in Iran, 75%; and [76] in Korea, 93.4%. However, the current multidrug resistance finding was higher than the report from Addis Ababa and Debre Zeit, Ethiopia, by [9] (20%). Despite global commitments to reduce antimicrobial resistance and protect the effectiveness of antimicrobials, most countries have not yet started implementing government policies to reduce their overuse and misuse of antimicrobials [77]. Hence, the current multi-drug resistance finding might be since 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 over-prescribed by health workers and veterinarians and over-used by the public. Moreover, new resistance mechanisms are emerging and spreading globally. Hence, antibiotic resistance is rising to dangerously high levels in all parts of the world.

## Conclusion and recommendations

The present study revealed the occurrence of *Campylobacter* in bovine, goat, and chicken raw meat samples collected from different sites of the study area. Hence, they can serve as a potential vehicle for transmitting *Campylobacter spp.* and risk of infection to humans through the consumption of raw or undercooked meat. Therefore, retailers can act as a major source of cross-contamination. Moreover, the current study was revealed the development of antimicrobial resistance by the isolated *Campylobacter spp.* for certain drugs which is an alert for the concerned bodies. Hence, coordinated actions are needed to reduce or eliminate the risks posed by these pathogens at various stages in the food chain. Moreover, controlled and careful use of antibiotics, both in veterinary and human treatment regimes should be practiced. Finally, further nationwide molecular epidemiology and phenotypic and molecular characterization of the disease should be undertaken.

## Acknowledgments

The authors acknowledged owners, managers, and workers of the different abattoir, butcher shops, and restaurants of the study site for their keen interest and cooperation during the collection of the meat samples. We would like to extend our acknowledgement to the College of Veterinary Science Staff members' who were directly or indirectly helping us during the research period.

## Author Contributions

**Conceptualization:** Yohans Hagos, Getachew Gugsu, Nesibu Awol, Meselu Ahmed, Yisehak Tsegaye, Nigus Abebe, Abrha Bsrat.

**Data curation:** Yohans Hagos, Getachew Gugsu, Nesibu Awol, Meselu Ahmed, Yisehak Tsegaye, Nigus Abebe, Abrha Bsrat.

**Formal analysis:** Yohans Hagos, Getachew Gugsu, Nesibu Awol, Meselu Ahmed, Yisehak Tsegaye, Nigus Abebe, Abrha Bsrat.

**Funding acquisition:** Yohans Hagos, Getachew Gugsu, Nesibu Awol, Meselu Ahmed, Yisehak Tsegaye, Nigus Abebe, Abrha Bsrat.

**Investigation:** Yohans Hagos, Getachew Gugsu, Nesibu Awol, Meselu Ahmed, Yisehak Tsegaye, Nigus Abebe, Abrha Bsrat.

**Methodology:** Yohans Hagos, Getachew Gugsu, Nesibu Awol, Meselu Ahmed, Yisehak Tsegaye, Nigus Abebe, Abrha Bsrat.

**Project administration:** Yohans Hagos, Getachew Gugsu, Nesibu Awol, Meselu Ahmed, Yisehak Tsegaye, Nigus Abebe, Abrha Bsrat.

**Resources:** Yohans Hagos, Getachew Gugsu, Nesibu Awol, Meselu Ahmed, Yisehak Tsegaye, Nigus Abebe, Abrha Bsrat.

**Software:** Yohans Hagos, Getachew Gugsu, Nesibu Awol, Meselu Ahmed, Yisehak Tsegaye, Nigus Abebe, Abrha Bsrat.

**Supervision:** Yohans Hagos, Getachew Gugsu, Nesibu Awol, Meselu Ahmed, Yisehak Tsegaye, Nigus Abebe, Abrha Bsrat.

**Validation:** Yohans Hagos, Getachew Gugsu, Nesibu Awol, Meselu Ahmed, Yisehak Tsegaye, Nigus Abebe, Abrha Bsrat.

**Visualization:** Yohans Hagos, Getachew Gugsu, Nesibu Awol, Meselu Ahmed, Yisehak Tsegaye, Nigus Abebe, Abrha Bsrat.

**Writing – original draft:** Yohans Hagos, Getachew Gugsu, Nesibu Awol, Meselu Ahmed, Yisehak Tsegaye, Nigus Abebe, Abrha Bsrat.

**Writing – review & editing:** Yohans Hagos, Getachew Gugsu, Nesibu Awol, Meselu Ahmed, Yisehak Tsegaye, Nigus Abebe, Abrha Bsrat.

## References

1. Rosef O, Johnsen G, Stølan A, Klæboe H. Similarity of *Campylobacter lari* among human, animal, and water isolates in Norway. *Foodborne Pathog Dis.* 2008; 5:33–93. <https://doi.org/10.1089/fpd.2007.0027> PMID: 18260813
2. Wong JSJ, Anderson TP, Chambers ST, On SL, Murdoch DR. *Campylobacter fetus*-associated epidural abscess and bacteremia. *J Clin Microbiol.* 2009; 47:857–8. <https://doi.org/10.1128/JCM.00725-08> PMID: 19144805
3. EFSA-ECDC European food safety authority and European Centre for disease prevention and control. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *EFSA J.* 2016;14.
4. Nawal AH. Antimicrobial resistant *C.jejuni* isolated from humans and animals in Egypt. *Glob Vet.* 2011; 6:195–200.
5. Mpalang RK, Boreux R, Pierrette M, Ni Bitiang K, Daube G, Mol PD. Prevalence of *Campylobacter* among goats in Congo. *J Infect Dev Ctries.* 2014; 8:168–75. <https://doi.org/10.3855/jdc.3199> PMID: 24518626
6. Heredia N, García S. Animals as sources of food-borne pathogens: A review. *Animal Nutr.* 2018; 4 (2018); 250e255. <https://doi.org/10.1016/j.aninu.2018.04.006> PMID: 30175252
7. Ertaş HB, Cetinkaya B, Muz A., Ongor H. Genotyping of broiler-originated *C. jejuni* and *C. coli* isolates using *fla* typing and random amplified polymorphic DNA methods. *Int J Food Microbiol.* 2004; 94:203–9.
8. Humphery T, O'Brien S, Madsen M. *Campylobacters* as zoonotic pathogens: A food production perspective. *Int J of Food Microbiol.* 2007; 117:237–57.
9. Dadi L, Asrat D. Prevalence and antimicrobial susceptibility of thermotolerant *Campylobacter* strains in retail raw meat products in Ethiopia. *Ethiop J Health Dev.* 2008; 22:195–6.
10. Salihu MD, Junaidu AU, Oboegbulem SI, Egbu GO. Prevalence and biotypes of *Campylobacter spp.* isolated from sheep in Sokoto state, Nigeria. *Int J Anim Vet Ad.* 2009; 1:6–9.
11. Abdalla M, Siham A, Suliman E, YYH, Alian A. Microbial Contamination of Sheep Carcasses at El Kadero Slaughterhouse Khartoum State. *Sudan. J Vet Sci Anim Husband.* 2009; 48:1–2.
12. Caprioli A, Morabito S, Brugère H, Oswald E. Enterohemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. *Vet Res.* 2005; 36:289–311. <https://doi.org/10.1051/vetres:2005002> PMID: 15845227
13. Wingstrand A, Neimann J, Engber J, Nielsen EM, Gerner-Smidt P, Wegener HC, et al. Fresh chicken as main risk factor for *Campylobacteriosis*, Denmark. *Emerg Infect Dis.* 2006; 12: 280–5. <https://doi.org/10.3201/eid1202.050936> PMID: 16494755
14. Heuvelink AE, Van heerwaarden C, Zwartkruis-Nahuis A, Tilburg JJ, Bos MH, Heilmann FG, et al. Two outbreaks of *Campylobacteriosis* associated with the consumption of raw cows' milk. *Int J of Food Microbiol.* 2009; 134:70–4. <https://doi.org/10.1016/j.ijfoodmicro.2008.12.026> PMID: 19167125
15. Ricotta EE, Palmer A, Wymore K, Clogher P, Oosmanally N, Robinson T, et al. Epidemiology and antimicrobial resistance of international travel-associated *Campylobacter* infections in the United States, 2005–2011. *Ame J of Public Health.* 2014; 104:108–14. <https://doi.org/10.2105/AJPH.2013.301867> PMID: 24832415

16. Altekruze SF, Stern NJ, Fields PI, Swerdlow DL. *C. jejuni* emerging foodborne pathogen. Emerg Infect Dis. 1999; 5:28–35. <https://doi.org/10.3201/eid0501.990104> PMID: 10081669
17. Rautelin H, Hanninen ML. *Campylobacters*: the most common bacterial enteropathogens in the Nordic countries. Ann of Med. 2000; 32:440–5. <https://doi.org/10.3109/07853890009002018> PMID: 11087163
18. Wesley I. Public health impact of foodborne illness: impetus for the international food safety effort. In: Heredia N., Wesley I., Garcia S.(Eds.), Microbiologically Safe Foods. Wiley, John Wiley & Sons, Inc., USA; 2009,pp. 3–13.
19. EFSA (European Food Safety Authority). The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Foodborne Outbreaks in 2013. EFSA J. 2015; 13(1):3991.
20. Kassa T, Gebre-selassie S, Asrat D. The prevalence of thermotolerant *Campylobacter* species in food animals in Jimma Zone, southwest Ethiopia. Ethiop J Health Dev. 2005; 9:225–9.
21. Ekin IH, Gurturk JK, Boynukara B. Prevalence and characteristic of *Campylobacter* spp isolated from gallbladder of slaughter sheep in Van, (Eastern) (Turkey). Acta Vet Brno. 2006; 75:145–9.
22. Ebrahim R. Occurrence and resistance to antibiotics of *Campylobacter* spp. in retail raw sheep and goat meat in ShahreKord, Iran. GlobVet. 2010; 4:504–9.
23. Gedlu E, Aseffa A. *Campylobacter* enteritis among children in northwest Ethiopia: A 1-year prospective study. Ann Trop Paediatr. 1996; 16:207–12. <https://doi.org/10.1080/02724936.1996.11747828> PMID: 8893950
24. Asrat D, Hathaway A, Ekwall E. Studies on enteric Campylobacteriosis in TikurAnbessa and Ethio-Swedish children's Hospital, Addis Ababa. Ethiopia. Ethiop Med J. 1999; 37:71–84. PMID: 11957308
25. Tafa B, Sawunet T, Tassew H, Asrat D. Isolation and Antimicrobial Susceptibility Patterns of *Campylobacter*spp among Diarrheic Children at Jimma, Ethiopia. Int J Bacteriol. 2014; 19:16:3–26.
26. Tefera W, Daniel A, Girma Z. Prevalence of Thermophilic *Campylobacter* species in carcasses from sheep and goats in an abattoir in Debre Zeit area, Ethiopia. Ethiop J Health Dev. 2009; 23(3):229–33.
27. Yeshimebet C, Daniel A, Patamapom A, Winya L. Prevalence and Antimicrobial susceptibility of thermophilic *Campylobacter* isolated from sheep at Debre Birhan, North-Shoa, Ethiopia. Kasetsart J (NatSci). 2013; 47:551–60.
28. Faris G. Identification of *Campylobacter* spp. and their Antibiotic resistance pattern from raw bovine meat in Addis Ababa, Ethiopia. IJMIR. 2015; 4(1):001–5.
29. CSA (Central statistic Authority CSA). Federal Democratic Republic of Ethiopia Population Census Commission. Summary and Statistical Report of population and Housing. 2007.
30. Bryant C. Investment opportunities in Mekelle, Tigray state, Ethiopia [online]. 2016. Available from: <https://www.ciaonet.org/attachments/15494>. Accessed on 27th May 2016.
31. Bolton FJ, Hutchinson DN, Coates D. Blood-free selective medium for isolation of *C. jejuni* from feces. J Clin Microbiol. 1984; 19:169–71. <https://doi.org/10.1128/JCM.19.2.169-171.1984> PMID: 6699146
32. Nachamkin I. *Campylobacter* and *Arcobacter*. In: Murray P.R. and Baron E.G.O., Manual of Clinical Microbiology 7th Eds. ASM press; Washington DC. America. Society of Microbiology. 1999,pp. 716–26.
33. Elmer WK, Stephen DA, Janda PhD, William M, Paul CS, Winn, MDWC Jr. Color Atlas and Text book of Diagnosis Microbiology. 5<sup>th</sup> Edition. 1998, pp.322-6.
34. On SLW. Identification methods for *Campylobacters*, *Helicobacters*, and related organisms. Clin Microbiol Rev. 1996; 9(3):405–22. <https://doi.org/10.1128/CMR.9.3.405-422.1996> PMID: 8809468
35. CLSI (Clinical and Laboratory Standard Institute) Performance standard for antimicrobial susceptibility testing; twenty-fourth informational supplement. CLSI document M100-S24. CLSI, Wayne, PA; 2014.
36. Sambrook J, Russel WD. Molecular cloning a laboratory manual 3<sup>rd</sup> ed. Cold Spring Harbour, NY: Cold Spring Harbour Laboratory Press; 2007.
37. Denis M, Soumet C, Rivoal K, Ermel G, Blivet D, Salvat G, et al. Development of a m-PCR for simultaneous identification of *C. jejuni* and *C. coli*. Lett Appl Microbio. 1999; 29:406–10.
38. Hossein D, Shadi A, Hossein G, Maryam N, Manouchehr AH, Seyed C. Prevalence and Antibiotic susceptibility of *Campylobacter* spp. isolated from chicken and beef meat. Int Enteric Pathog. 2014; 2(2): e17087.
39. Rahimi E, Tajbakhsh E. Prevalence of *Campylobacter* spp in poultry meat in the Esfahan city, Iran. Bul Vet Med. 2008; 11:257–62.
40. Habib I, Sampers I, Utyyendaele M, Berkvens D, De Zutte, L. Base line Data from a Belgium-Wide Survey of *Campylobacter* Spp. Contamination in Chicken Meat preparation and Consideration for a Reliable Monitoring Program. Appl Environ Microbiol. 2008; 72(17):5483–9.

41. Marinou I, Bersimis S, Ioannidis A, Nicolaou C, Mitroussia-Ziouva A, Legakis NJ, et al. Identification and antimicrobial resistance of *Campylobacter species* isolated from animal sources. *Front Microbiol.* 2012; 3:58. <https://doi.org/10.3389/fmicb.2012.00058> PMID: 22375138
42. Rahimi E, Kazemeini HR, Safaei S, Allahbakhshi K, Momeni M, Riahi M. Detection and identification of *Campylobacter spp* from retail raw chicken, turkey, sheep and goat meat in Ahvaz, Iran. *Afr J Microbiol Res.* 2010; 4(15):1620–3.
43. Zhao C, Ge B, De Villena J, Sudler R, Yeh E, Zhao S, et al. Prevalence of *Campylobacter spp.*, *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, D.C., area. *Appl Environ Microbiol.* 2001; 67:431–6.
44. Orla MC, Geraldine D, Sheridan JJ, Blair DA, McDowell DA. A survey on the incidence of *Campylobacter spp.* and the development of a surface adhesion polymerase chain reaction (SA-PCR) assay for the detection of *C. jejuni* in retail product. *Food Microbiol.* 2001; 18:287–8.
45. Pezzotti G, Serafin A, Luzzi I, Mioni R, Milan M, Perin R. Occurrence and resistance to antibiotics of *C. jejuni* and *C. coli* in animals and meat in northeastern Italy. *Int J Food Microbiol.* 2003; 82:281–7. [https://doi.org/10.1016/s0168-1605\(02\)00314-8](https://doi.org/10.1016/s0168-1605(02)00314-8) PMID: 12593931
46. Jorgensen F, Bailey R, Williams S, Henderson P, Wareing DRA, Bolton FJ, et al. Prevalence and numbers of *Salmonella* and *Campylobacter spp.* on raw, whole chickens in relation to sampling methods. *Int J Food Microbiol.* 2002; 76:151–64. [https://doi.org/10.1016/s0168-1605\(02\)00027-2](https://doi.org/10.1016/s0168-1605(02)00027-2) PMID: 12038572
47. Mayrhofer S, Paulsen P, Smulders FJM, Hilbert H. Antimicrobial resistance profile of five major food-borne pathogens isolated from beef, pork and poultry. *Int J Food Microbiol.* 2004; 97:23–9. <https://doi.org/10.1016/j.ijfoodmicro.2004.04.006> PMID: 15527915
48. Hussain I, Mahmood MS, Akhtar M, Khan A. Prevalence of *Campylobacter spp.* in meat, milk and other food commodities in Pakistan. *Food Microbiol.* 2007; 24:219–22. <https://doi.org/10.1016/j.fm.2006.06.001> PMID: 17188200
49. Taremi M, Soltan-Dallal MM, Gachkar L, MoezArdalan S, Zolfagharian K, Zali MR. Prevalence and antimicrobial resistance of *Campylobacter* isolated from retail raw chicken and beef meat, Tehran, Iran. *Int J Food Microbiol.* 2006; 108:401–3. <https://doi.org/10.1016/j.ijfoodmicro.2005.12.010> PMID: 16481059
50. Nonga HE, Sells P, Karimuribo ED. Occurrences of thermophilic *Campylobacter* in cattle slaughtered at Morogoro municipal abattoir, Tanzania. *Tropi Animal Heal Productio.* 2010; 42:73–8. <https://doi.org/10.1007/s11250-009-9387-7> PMID: 19551483
51. Vanderlinde PB, Shay B, Murray J. Microbiological quality of Australian beef carcass meat and frozen bulk packed beef. *J Food Prot.* 1998; 61:437–43. <https://doi.org/10.4315/0362-028x-61.4.437> PMID: 9709207
52. Oberhelman RA, Taylor DN. *Campylobacter* infections in developing countries. In: *Campylobacter*, 2nd ed.; Nachamkin I., Blaser M.J., Eds.; Washington DC: American Society of Microbiology (SAM) Press; 2000, pp. 139–53. [https://doi.org/10.1016/s0360-3016\(00\)00774-4](https://doi.org/10.1016/s0360-3016(00)00774-4) PMID: 11121652
53. Thépault A, Poezevara T1, Quesne S, Rose V, Chemaly M, Rivoal K. Prevalence of Thermophilic *Campylobacter* in Cattle Production at Slaughterhouse Level in France and Link Between *C. jejuni* Bovine Strains and *Campylobacteriosis*. *Front Microbiol.* 2018; 9:471. <https://doi.org/10.3389/fmicb.2018.00471> PMID: 29615999
54. Châtre P, Haenni M, Meunier D, Botrel MA, Calavas D, Madec JY. Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from cattle between 2002 and 2006 in France. *J Food Prot.* 2010; 73:825–31. <https://doi.org/10.4315/0362-028x-73.5.825> PMID: 20501032
55. Rahimi E. Occurrence and Resistance to Antibiotics of *Campylobacter Species* in Retail Raw Sheep and Goat Meat in Shahr-e Kord, Iran. *GlobVet.* 2010; 4(5):504–9.
56. Zanetti F, Varoli O, Stampi S. Prevalence of thermophilic *Campylobacter* and *Arcobacter butzleri* in food of animal origin. *Int J Food Microbiol.* 1996; 33:315–21. [https://doi.org/10.1016/0168-1605\(96\)01166-x](https://doi.org/10.1016/0168-1605(96)01166-x) PMID: 8930716
57. Ghafir Y, China B, Dierick K, DeZutter L, Daube G. A seven year survey of *Campylobacter* contamination in meat at different production stages in Belgium. *Int J Food Microbiol.* 2007; 116:111–20. <https://doi.org/10.1016/j.ijfoodmicro.2006.12.012> PMID: 17321622
58. Son I, Englen MD, Berrang ME, Fedorka-Cray PJ, Harrison MA. Prevalence of *Arcobacter* and *Campylobacter* on broiler carcasses during processing. *Int J Food Microbiol.* 2007; 113:16–22. <https://doi.org/10.1016/j.ijfoodmicro.2006.06.033> PMID: 16979251
59. Whyte P, McGill K, Cowley D, Madden RH, Moran L, Scates P, et al. Occurrence of *Campylobacter* in retail foods in Ireland. *Int J Food Microbiol.* 2004; 95:111–8. <https://doi.org/10.1016/j.ijfoodmicro.2003.10.018> PMID: 15282123

60. Suzuki H, Yamamoto S. *Campylobacter* contamination in retail poultry meats and by-products in Japan: A literature Survey. *J Vet Med Sci.* 2009; 71(3):255–61. <https://doi.org/10.1292/jvms.71.255> PMID: 19346690
61. WHO. World Health Organization / Department of Communicable Disease Surveillance and Response. Increasing incidence of human *Campylobacteriosis*. Report and Proceeding of a WHO Consultation of Experts. 2001.
62. Elhelberg S, Simonsen J, Gerner-Smid P, Olsen KE, Mølbak K. Spatial distribution and registry-based case-control analysis of *Campylobacter* infections in Denmark, 1991–2001. *Ame J Epidemiol.* 2005; 162:1008–15.
63. CDC (Centre for Disease Control). Preliminary food net data on the incidence of infection with pathogens transmitted commonly through food-10states, 2009. *Morb Mortal Wkl Rep.* 2010; 59:418–22.
64. Blaser M., Taylor DN, Feldman RA. Feldman, Epidemiology of *C. jejuni* infections. *Epidemiol Rev.* 1983; 5:157–76.
65. Allos BM. *C. jejuni* infections: update on emerging issues and trends. *Clin Infect Dis.* 2001; 32:1201–6. <https://doi.org/10.1086/319760> PMID: 11283810
66. Butzler JP. *Campylobacter*, from obscurity to celebrity. *Clin Microbiol Infect.* 2004; 10(10):868–76. <https://doi.org/10.1111/j.1469-0691.2004.00983.x> PMID: 15373879
67. Toledo Z, Simaluiza RJ, Fernández H. Occurrence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from domestic animals from Southern Ecuador. *Cienc Rural.* 2018; 48(11):e20180003.
68. Gallay A, Prouzet-Mauleon V, Kempf I, Lehours P, Labadi L, Camou C, et al. *Campylobacter* antimicrobial drug resistance among humans, broiler chickens, and pigs, France. *Emerging Infect Dis.* 2007; 13:259–66.
69. Van Looveren M, Daube G, Zutter L, Dumont JM, Lammens C, Wijdooghe M, et al. Antimicrobial Susceptibilities of *Campylobacter* strains isolated from food animals in Belgium. *J Antimicrob Chemother.* 2001; 48:235–40. <https://doi.org/10.1093/jac/48.2.235> PMID: 11481294
70. Tajada P, Gomez-Garces JL, Alos JI, Balas D, Cogollos R. Antimicrobial susceptibilities of *C. jejuni* and *C. coli* to 12 beta-lactam agents and combinations with beta-lactamase inhibitors. *Antimicrob Agents Chemother.* 1996; 40:1924–5. <https://doi.org/10.1128/AAC.40.8.1924> PMID: 8843305
71. Nachamkin I, Engberg J, Aarestrup FM. Diagnosis and antimicrobial susceptibility of *Campylobacter Spp.* in: *Campylobacter*. 2nd ed., Nachamkin I. and Blaser M.J. (Eds). Washington, DC: ASM Press; 2000, pp. 45–66.
72. Tan YF, Haresh KK, Chai LC, Son R. Antibiotic susceptibility and genotyping by RAPD of *C. jejuni* isolated from retailed ready to eat sushi. *Int J Food Res.* 2009; 16:31–8.
73. Sukhapesna J, Amavisit P, Wajjwalku W, Thamchaipenet A, Ukpuaram T. Antimicrobial resistance of *C. jejuni* isolated from chicken in Nakhon Pathom province Thailand. *Kasetsart J (Nat Sci).* 2005; 39(2):240–6.
74. Fallon R, Sullivan NO, Mahe M, Carroll C. Antimicrobial resistance of *C. jejuni* and *C. coli* isolates from broiler chickens isolated at an Irish poultry processing plant. *Lett Appl Microbiol.* 2003; 36:277–81. <https://doi.org/10.1046/j.1472-765x.2003.01308.x> PMID: 12680938
75. Praakle-Amin K, Roasto M, Korkeala H, Hänninen ML. PFGE genotyping and antimicrobial susceptibility of *Campylobacter* in retail poultry meat in Estonia. *Int J Food Microbiol.* 2007; 114:105–12. <https://doi.org/10.1016/j.ijfoodmicro.2006.10.034> PMID: 17182145
76. Hong J, Kim JM, Jung WK, Kim SH, Bae W, Koo HC, et al. Prevalence and antibiotic resistance of *Campylobacter spp.* isolated from chicken meat, pork, and beef in Korea, from 2001 to 2006. *J Food Prot.* 2007; 70:860–6. <https://doi.org/10.4315/0362-028x-70.4.860> PMID: 17477253
77. Rogers Van Katwyk S, Grimshaw JM, Nkangu M, Nagi R, Mendelson M, Taljaard M, et al. Government policy interventions to reduce human antimicrobial use: A systematic review and evidence map. *PLoS Med.* 2019; 16(6): e1002819. <https://doi.org/10.1371/journal.pmed.1002819> PMID: 31185011