Original Article

Isolation, characterization, and antimicrobial resistance profiles of *Campylobacter jejuni* and *Campylobacter coli* from raw meat of large livestock in Shahrekord, Iran

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ABSTRACT

Campylobacter spp. genera is one of the most common causes of microbial enteritis worldwide. This study aimed to find out how common *Campylobacter* organisms were in raw meat from large livestock in Iran, as well as to determine their antibiotic susceptibility profiles. Several 550 fresh, ready-to-eat meat samples were collected from slaughterhouses, butcher shops, and restaurants in the study region. The samples were collected from cattle (n=138), goats (n=102), camels (n=56), and sheep (n=254). *Campylobacter* spp. were isolated and identified using normal bacteriological methods and polymerase chain reaction (PCR). Genotyping was performed using PCR to identify virulence genes. The disc diffusion technique was used to determine antibiotic susceptibility. The two *Campylobacter* spp. were found in 84 (15.27%) of the 550 meat samples tested. Cattle and camel samples accounted for the highest (52.38%) and lowest (3.57%) frequencies of *Campylobacter* spp., respectively. There were significant differences in the prevalence of *Campylobacter* spp. in cattle (2=43.04 or OR=7.68, CI=3.40-17.30, *P*<0.01). *Campylobacter jejuni* and *Campylobacter coli* accounted for 82.14% (n=69) of *Campylobacter* spp. isolated from raw meat. While *C. jejuni* was found in 39.28% of the samples (n=33), *C. coli* was observed in 42.85% (n=36). Other *Campylobacter* spp. formed 17.85 % (n=15) of the samples. The most common genotypes observed in *C. jejuni* bacteria collected from different types of large animal samples were *ciaB* (100%) and *flaA* (100%). On the other hand, *virbll* (7.69%) was the *C. jejuni* strain found with the lowest incidence in different large animal samples. The most frequent genotypes found in *C. coli* bacteria were *ciaB* (100%) and *flaA* (100%). *C. coli* isolates *dnaJ* (0%), *wlaN* (0%), *virbll* (0%), and *ceuE* (0%) were detected with the lowest frequency in several samples from large livestock. *Campylobacter* spp. isolated from different sample types and sources were 100% sensitive to *aphA-3-1* and *GM10*. The isolates were reported to be resistant to *E15* (76.93%), *cmeB* (69.24%), *aadE1* (69.24%), *CIP5* (69.24%), and *AM10* (69.24%). According to this study, *Campylobacter* was found in food from factory farming. Consequently, the disease can be transmitted by eating raw or undercooked meat. Therefore, proper handling and preparation of meat meals, as well as hygiene measures from the slaughterhouse to the retailer, are critical in preventing *Campylobacter* infections.

Keywords: *Campylobacter coli*, *Campylobacter jejuni*, Iran, virulence factors

1. Introduction

Foodborne infections are caused by spoiled meals, especially red meat, including diseased meat or cadavers contaminated with harmful bacteria (1, 2). Numerous foodborne organisms, including, *Campylobacter* spp., *Salmonella enterica* non-Typhi serovars, Shiga toxin-producing *Escherichia coli* isolates, and *Listeria monocytogenes*, have significant sources in food-producing livestock. Pathogenic large animals are responsible for millions of sporadic illnesses, chronic outcomes, and significant and difficult outbreaks in several countries (3). Pathogenic *Campylobacter* organisms are the most common foodborne pathogen, responsible for approximately 400-500 million illnesses per year (4). Various livestock, including camels, cattle, sheep, and goats, as well as wild animals, carry *Campylobacter* organisms in their digestive systems. Fecal matter is an important source of contamination that can enter cadavers through direct deposition (5). Campylobacteriosis can infect their meals when animals are killed and carcasses are dressed. Consumption of undercooked or cleaned meat, manipulation of raw items, cross-contamination of raw food with heated foods, bathing in natural waters, direct contact with contaminated animals or animal carcasses, and travel are ways that people can become ill (6). *Campylobacter jejuni, Campylobacter rectus, Campylobacter hyointestinalis, Campylobacter insulaenigrae, Campylobacter sputorum, Campylobacter helveticus, Campylobacter lari, Campylobacter foetal, Campylobacter mucosalis, Campylobacter coli, Campylobacter upsaliensis,* and *Campylobacter ureolyticus* are dangerous *Campylobacter* spp. associated with human illness. *Campylobacter jejuni* and *C. coli* are the most commonly observed zoonotic agents in humans and the most common agents of gastrointestinal infections worldwide (7).

Campylobacter jejuni is responsible for 90% of campylobacteriosis cases, followed by *C. coli*, accounting for 5-10% of cases (8). In addition, *Campylobacter* with antibiotic resistance has been associated with outbreaks throughout the world (9). The use of antimicrobials in meat animals has led to the establishment and spread of antibiotic-resistant bacteria, such as antimicrobial-resistant *Campylobacter*, which can be detrimental to human and animal health. In underdeveloped countries, where antimicrobial use is widespread and unregulated, the situation appears to be deteriorating even faster (10). In Iran, few studies have been conducted on the incidence and antibacterial tolerance of intestinal campylobacteriosis in humans (11) and animal-derived products. The lack of a national surveillance program, which limits the regular supply of cultures for *Campylobacter* spp. isolation in clinical practice and research, and the need for a selective medium and a specific growth environment make it difficult to accurately assess the impact of the disease in Iran (12).

The virulence of *Campylobacter* spp. depends on their virulome. Although relatively little is known about the virulence of *Campylobacter* spp., these microorganisms possess several virulence factors related to motility, adhesion, invasion, toxin activity, immune evasion, and iron uptake, among others (8).

This indicates that *Campylobacter* as a zoonotic disease is not receiving the attention it deserves, especially in the current research area. Therefore, the present study aimed to investigate the patterns of antimicrobial resistance, virulence genes, and genetic variation of thermophilic *Campylobacter* spp. obtained from a large livestock sample in Iran.

2. Materials and Methods

2.1 Ethical considerations

The Research Ethics Committee of the College of Veterinary Sciences, Islamic Azad University, Shahrekord Branch, Iran, reviewed and approved this work.

2.2. Research area and study design

The study was performed in Shahrekord City, Iran, between October 2020 and May 2021. Shahrekord is the capital of Chaharmahal and Bakhtiari province, where tens of thousands of large livestock from numerous districts of the region and surrounding areas can be slaughtered. The abundance and antibiotic resistance profiles of *C. jejuni* and *C. coli* were isolated, identified, and estimated from meat samples of large animals from slaughterhouses, butcher shops, and restaurants during a cross-sectional survey from October 2020 to May 2021.

2.3. Sample size and collection

A total of 550 fresh, ready-to-eat meat samples were collected from the slaughterhouses, butcher shops, and restaurants in the study region. The samples included meat from cattle (n=138), goats (n=102), camels (n=56), and sheep (n=254). To avoid spillage and cross-contamination, all samples were stored in polyethylene plastic packaging and immediately transferred to the molecular biology laboratory of the College of Veterinary Sciences, Islamic Azad University, Shahrekord Branch, using a refrigerator with ice packs.

2.4. Isolation and identification of *Campylobacter* spp.

The meat was placed on modified charcoal cefoperazone deoxycholate agar (mCCDA) (Oxoid Ltd., Basingstoke, Hampshire, England), including *Campylobacter* mCCDA selective additive, SR155E (Oxoid Ltd., Basingstoke, Hampshire, England) upon

arrival at the laboratory. CampyGen™ gas packets were used to create microaerophilic conditions, which were maintained at 37°C for 48 h (Oxoid, Basingstoke, England, United Kingdom). *Campylobacter* colonies, which are grayish, flat, moist, and readily spread, were subcultured on Mueller-Hinton agar enriched with 5% defibrinated horse blood and cultured for 48 h at 37°C under microaerophilic conditions. *Campylobacter* isolates were stored at 80°C in Mueller-Hinton broth containing 25% glycerol (v/v).

2.5. Extraction of DNA and identification of genus by polymerase chain reaction

The Qiagen QIAamp PowerFecal Kit (Qiagen, Hilden, Germany) was used to extract genomic DNA from pure cultures according to the instructions of the manufacturer. Multiplex polymerase chain reaction (PCR) was then performed using genus-specific primers (C412F and C1228R), *C. jejuni* cj0414 gene primers (C1 and C3), and *C. coli* ask gene primers (CC18F and CC519R) (8). The primers were selected for their ability to discriminate between *Campylobacter* genus and species. To prepare the PCR mixture (25 µl), 12.5 µl of 2X Master Mix (Thermo Fisher Scientific, Seoul, South Korea), 1 µl of primer (10 µM), 1.5 µl of template DNA (20 µg/ml), and 7 µl of sterile deionized water were used. The MiniAmp Plus Thermal Cycler (Applied Biosystems, MA, USA) was utilized to perform one cycle at 95°C for 5 min, 35 cycles at 94°C for 45 s, 55°C for 45 s, and 72°C for 45 s, and a final extension at 72°C for 5 min. The PCR products were stored at 4°C before analysis.

2.6. Antimicrobial susceptibility testing

The isolated *Campylobacter* spp. samples were tested for *in vitro* antibiotic susceptibility on Mueller-Hinton agar supplemented with 5% sheep blood (Oxoid Ltd., Basingstoke, Hampshire, England) using the standard agar disc diffusion method according to the recommendations of the Clinical and Laboratory Standards Institution (CLSI). The following 15 different antibiotic disks were used for antibiotic susceptibility testing with their concentrations indicated in parentheses: *aphA-3*-1, *cmeB*, *tet(O)*, *blaOXA*-61, *aadE1*, *GM10*, *CIP5*, *NA30*, *TE30*, *AM10*, *AMC30*, *E15*, *AZM15*, *CC2*, and *C30* (Oxoid Company, Hampshire, England). The size of the clear zones (zones of inhibition of bacterial growth around antibiotic discs, including the discs) was evaluated for each antibacterial agent and then classified as sensitive, intermediate, and resistant according to the CLSI interpretation table after 48 h of microaerophilic cultivation at 37°C.

2.7. Detection of antimicrobial resistance genes

Genes encoding antimicrobial resistance were determined by PCR experiments using the primers in table 1. The genes studied were: *aphA-3-1* (gentamicin resistance), *cmeB* (efflux pump), *blaOXA-61* (ampicillin (beta-lactam) resistance), *tet(O)* (tetracycline resistance), and *aadE1* (aminoglycoside resistance) (Table 1) (9). After electrophoresis, bands of PCR products were observed under ultraviolet (UV) light using a dual UV transilluminator (Core BioSystem, Huntington Beach, CA, USA). The PCR procedure was performed as described above. After electrophoresis, the bands of PCR products were visualized under UV light using a dual UV transilluminator (Core BioSystem, Huntington Beach, CA, USA). The bands of amplification products were assessed by comparison with a 100-bp DNA ladder (Dyne bio, Seongnam-si, Republic of Korea). The antibiotic resistance gene PCR products underwent purification using AMPure XP beads (Beckman Coulter, Fullerton, CA, USA). These products were then sequenced through the Sanger technique at SolGent (Solutions for Genetic Technologies, Daejeon, Republic of Korea).

2.8. Detection of virulence genes

PCR was performed using specific primers for virulence-related genes (*recR*, *wlaN*, *cdtB*, *cdtA*, *cdtC*, *virbll*, *flaA*, *pidA*, *cadF*, *ciaB*, *ceuE*, *cgtB*, and *dnaJ*). The PCR mix (25 µl) was made by combining 12.5 µl of 2X Master Mix (Thermo Fisher Scientific, Seoul, South Korea), 1 µl of primer (10 µM), 1.5 µl of template DNA (20 µg/ml), and 7 µl of sterile deionized water. The MiniAmp Plus Thermal Cycler was used to run one cycle at 95°C for 5 min, 35 cycles at 94°C for 30 s, 55°C for 45 s, and 72°C for 45 s, and a final extension at 72°C for 5 min (Applied Biosystems, MA, USA).

2.9. Statistical analysis

All data were entered into a Microsoft Excel sheet (Microsoft Corp., Redmond, WA, USA) and analyzed in SPSS software (version 20). Associations were evaluated using the Chi-square test and logistic regression. A p-value of less than 0.05 was statistically significant for all experiments.

Table 1. Primer sequences for the multiplex PCR experiment

Gene	Primer Sequences (5'-3')	Annealing Temperatures (°C)	Product size (bp)
16S rRNA	C412F: GGATGACACTTTTCGGAGC C1228R: CATTGTAGCACGTGTGTC	58	816
cj0414	C-1: CAAATAAAGTTAGAGGTAGAATGT C-3: CCATAAGCACTAGCTAGCTGAT	56	161
ask	CC18F: GGTATGATTTCTACAAAGCGAG CC519R: ATAAAAGACTATCGTCGCGTG	60	502
racR	GATGATCCTGACTTTG TCTCCTATTTTTACCC	45	584
dnaJ	AAGGCTTTGGCTCATC CTTTTTGTTCATCGTT	46	720
wlaN	TTAAGAGCAAGATATGAAGGTG CCATTTGAATTGATATTTTTG	46	672
virbll	TCTTGTGAGTTGCCTTACCCCTTTT CCTGCGTGTCCTGTGTTATTTACCC	53	494
cdtC	CGATGAGTTAAAACAAAAAGATA TTGGCATTATAGAAAATACAGTT	47	182
cdtB	CAGAAAGCAAATGGAGTGTT AGCTAAAAGCGGTGGAGTAT	51	620
cdtA	CCTTGTGATGCAAGCAATC ACACTCCATTTGCTTTCTG	49	370
flaA	AATAAAAATGCTGATAAAACAGGTG TACCGAACCAATGTCTGCTCTGATT	53	585
cadF	TTGAAGGTAATTTAGATATG CTAATACCTAAAGTTGAAAC	45	400
pldA	AAGCTTATGCGTTTTT TATAAGGCTTTCTCCA	45	913
ciaB	TTTTTATCAGTCCTTA TTTCGGTATCATTAGC	42	986
ceuE	CCTGCTACGGTGAAAGTTTTGC GATCTTTTTGTTTTGTGCTGC	48.9	793
cgtB	TAAGAGCAAGATATGAAGGTG GCACATAGAGAACGCTACAA	49.9	561
***tet*(O)**	GCGTTTTGTTTATGTGCG ATGGACAACCCGACAGAAG	54	559
***cme*B**	TCCTAGCAGCACAATATG AGCTTCGATAGCTGCATC	54	241
***bla*$_{OXA-61}$**	AGAGTATAATACAAGCG TAGTGAGTTGTCAAGCC	54	372
aphA-3-1	TGCGTAAAAGATACGGAAG CAATCAGGCTTGATCCCC	54	701

Table 2. *Campylobacter* spp. prevalence in various sample types

Type of meat	*Number of samples*	*Positive number of Campylobacter*	*Positive number of C. jejuni*	*Positive number of C. coli*	*Positive number of other species*
Cattle	138	44	26	13	5
Sheep	254	28	6	16	6
Goat	102	9	1	4	4
Camel	56	3	-	3	-
Collect livestock meat	550	84	33	36	15

3. Results

3.1. Prevalence of *Campylobacter* spp. meat in large cattle

Two *Campylobacter* spp. (i.e., *C. jejuni* and *C. coli*) were found in 84 (15.27%) of the 550 meat samples. Cattle were much more likely to be infected with *Campylobacter* than with the other specimens. Cattle and camel samples were responsible for the highest (52.38%) and lowest (3.57%) frequency rates of infection with *Campylobacter* spp., respectively. As shown in figure 1 and table 2, there were significant differences in the prevalence of *Campylobacter* spp. in cattle compared to others (2=43.04 or odds ratio [OR]=7.68, confidence interval [CI]=3.40-17.30, *P*<0.01).

3.2. Infection rates of *Campylobacter jejuni* and *Campylobacter coli* in different sample types

It was found that *C. jejuni* and *C. coli* accounted for 82.14% (n=69) of *Campylobacter* spp. isolated and characterized in the raw meat from cattle, goats, sheep, and camels. While *C. jejuni* was found in 39.28% of the samples (n=33), *C. coli* was found in 42.85% (n=36). Other *Campylobacter* spp. formed 17.85% (n=15) of the samples.

Based on the results, *C. jejuni* was detected in 59.09% (n=26), 11.11% (n=1), 21.42% (n=6), and 0% (n=0) of cattle, goat, sheep, and camel meat samples, respectively. The presence of *C. coli* was identified in 29.54% (n=13), 44.44% (n=4), 57.14% (n=16), and 100% (n=3) of cattle, goat, sheep, and camel meat samples, respectively (Table 2).

3.3 Polymerase chain reaction amplification results

The PCR products of the 84 samples showed that 39.28% (n=33) of the strains were *C. jejuni* (with a molecular size of 589 bp) and the rest 42.85% (n=36) were *C. coli* (with a molecular size of 462 bp) in terms of gene products. *Campylobacter jejuni* and *C. coli* accounted for 82.14% (n=69) of *Campylobacter* spp. isolated from raw meat. While *C. jejuni* was found in 39.28% of the samples (n=33), *C. coli* was detected in 42.85% (n=36). Other *Campylobacter* spp. accounted for 17.85% (n=15) (Figure 2).

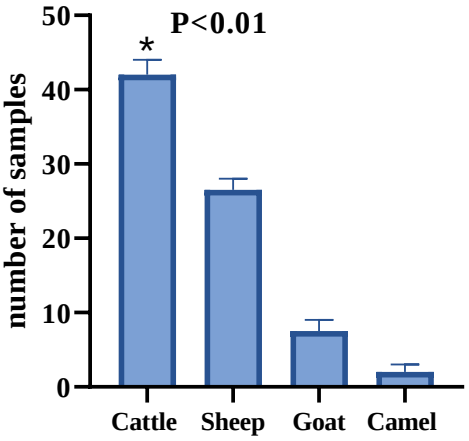

Fig 1. Prevalence of *Campylobacter* spp. among different sample types

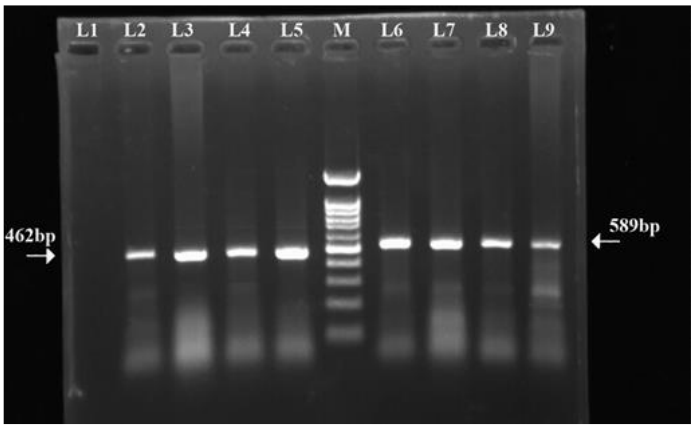

Fig 2. Results of molecular analysis of *Campylobacter* isolates by PCR. L1: Negative control, L2-L5: Some *C. coli samples*; L6-L9: Some examples of *C. jejuni*; M: DNA marker 100 bp.

3.4 Distribution of genotype of *Campylobacter* spp. isolates

Tables 3 and 4 present the genotype pattern of *Campylobacter* spp. strains derived from different types of raw meat from large livestock. The most common genotypes observed in the collected *C. jejuni* bacteria were *ciaB* (100%), *flaA* (100%), *recR* (80.77%), *cadF* (76.93%), and *dnaJ* (76.93%), whereas *wlaN* (7.69%) and *virbll* (7.69%) were the least prevalent. Other genes were also detected in *C. jejuni* isolates from large livestock populations. There was a significant difference between the types of samples and the frequency of genotypes (P<0.05).

The genotyping pattern of the *C. coli* isolates is shown in table 4. Accordingly, *ciaB* (100%), *flaA* (100%), *pidA* (61.54%), and *cadF* (61.54%) were the most frequent genotypes found in *C. coli* bacteria from a series of large animal samples. On the other hand, *dnaJ* (0%), *wlaN* (0%), *virbll* (0%), and *ceuE* (0%) were detected with the lowest frequency in a series of large animal samples. Additional genes were identified in *C. coli* strains from large animal samples. There was a significant difference between the types of specimens and the prevalence of alleles (P<0.05).

3.5 Patterns of *Campylobacter* spp. isolates' susceptibility to antimicrobials

Campylobacter spp. isolated from various sample types and sources were 100% sensitive to *aphA-3-1* and *GM10*. The isolates were found to be resistant to *E15* (76.93%), *cmeB* (69.24%), *aadE1* (69.24%), *CIP5* (69.24%), and *AM10* (69.24%) (Table 5). As tabulated in table 5, 96.8% of the isolates showed resistance to two or more drugs.

According to the antibiogram results, the *C. jejuni* isolates demonstrated the highest sensitivity to *aphA-3-1*. In contrast, based on table 5, *C. jejuni* had the highest resistance to *E15*.

Sensitivity to antimicrobials was the highest in *C. coli* isolates *aphA-3-1* (100%) and *GM10* (100%) in cattle (Table 6). Moreover, *C. coli* isolates had the lowest susceptibility rate to *E15* (23.07%). In addition, resistance to *cmeB* (69.24%), *blaOXA*-6 (69.24%), *aadE1* (69.24%), *CIP5* (69.24%), and *AM10* (69.24%) were common. The results showed that most *C. coli* isolates from the samples of large livestock were resistant to at least three antibiotics. These isolates exhibited multiple drug resistance phenotypes. There was a statistical difference between the specimens and the frequency of antimicrobial resistance (P<0.05).

4. Discussion

In the current study, 15.27% of the 550 meat sample isolates were analyzed positive for *Campylobacter* species. Cattle meat samples were found to have the highest incidence (52.38%). Beef showed a twofold increased risk of *Campylobacter* compared to sheep, goat, and camel meat. The incidence of *Campylobacter* was found to vary significantly among meat samples (2=43.04 or OR=7.68, CI=3.40-17.30, P<0.01). The prevalence of *Campylobacter* spp. in meat samples was 52.38%, which was consistent with the values reported by Dabiri et al. (44%) (13), Rahimi et al. (56.1%) (14), and Habib et al. (48.02%) (15). This was more than the frequency of 1.93% reported by Marinou et al. (16). However, the current finding was lower than the prevalence of *Campylobacter* spp. reported by Rahimi et al. (61.7%) in Ahvaz, Iran, (17) and Pezzotti et al. (81.3%) in northern Italy (18). Large cattle were found to be a significant source of *Campylobacter* compared with other livestock, and cattle were reported to be strong gastrointestinal carriers of *Campylobacter*. In different countries, fresh meat showed a wide range of *Campylobacter* abundance (0-90%). These discrepancies in *Campylobacter* spp. abundance could be attributed to hygienic conditions, cross-contamination from de-feathering and excoriation, or some other environmental components.

In this study, the percentage of *Campylobacter* spp. in cattle meat was obtained at 52.38%. This was more than the results of studies performed in Nigeria (12.9%) (19) and Iran (10%) (20). However, it was more significant than the results in Ethiopia (6.2%), Morogoro, Tanzania (5.6%), and Australia (0.8%) (21). Food derived from animals is considered a major cause of *Campylobacter* infections in humans (22). Because raw beef is commonly used in this country, the presence of *Campylobacter* in meat increases the risk of infection in humans. The current result was lower than those of previous studies, which showed prevalence rates of 69.1% and 22%, respectively (23).

Differences in detection techniques for thermophilic *Campylobacter*, particularly the lack of an enrichment method for the separation of thermophilic *Campylobacter* in the work of Chen et al., are one of the most likely explanations for the discrepancies. These discrepancies could be caused by changes in sample collection methods, isolation and identification procedures, and sample size (24).

Campylobacter spp. was detected in 9.0% of goat meat. This result was consistent with those of previous studies reporting the prevalence of 7.6% and 6.4% (25). However, it was slightly higher than 4.4%, which was reported in the previous study. However, the current study had lower results than earlier studies, which showed 41.2% and 27.5%, respectively (26-28). Microbiological analysis and PCR identification of the isolated *Campylobacter* strains revealed that *C. jejuni* was more prevalent than *C. coli* in the present study. *Campylobacter jejuni* was identified as the most common spp. derived from animal-based foods, particularly beef (29). In previous studies, *C. jejuni* and *C. coli* were detected in 76% and 24% of beef, goat meat, and chicken, respectively (30). These results were consistent with those of a previous study demonstrating the detection of 78% of *C. jejuni* and 18% of *C. coli* (31). The incidence of *C. jejuni* in raw meat was consistent with previous studies from other countries (32).

Campylobacter antibiotic resistance is a worldwide problem that has already been identified by serval researchers and recognized as a public health problem by the World Health Organization. Antibiotic resistance to *Campylobacter* spp. (*C. jejuni* and *C. coli*) can be transmitted to humans in serval ways. This circumstance underscores the need for *Campylobacter* antibiotic susceptibility testing. The drug of choice for the treatment of foodborne campylobacteriosis are mainly macrolides and fluoroquinolones (33, 34). Previous studies in Ethiopia found that 80%-100% of food animal strains were sensitive to antimicrobials. However, data from various parts of the world indicate that antibiotic resistance is increasing in both food animals and human isolates (35, 36). In this study, the antibiotic susceptibility patterns of *C. jejuni* and *C. coli* strains were investigated. The percentage of *Campylobacter* strains with *aphA-3-1* and *GM10* susceptibility was 100%. This was consistent with the 97.2% and 83.3%, respectively, reported in the previous studies. In addition, Toledo et al. observed a *C. coli* resistance level of 100%, and reported that *C. coli* strains were often more resistant than *C. jejuni* strains (37). Despite international promises to reduce antibiotic resistance and ensure antimicrobial efficacy, most countries have failed to implement government policies to decrease the overuse and misuse of antibiotics (38). In countries such as Iran, where there is no uniform regulation or guidelines for therapeutic interventions, antibiotics can be purchased without medication for humans or animals, and antimicrobials are often overprescribed by healthcare workers and veterinarians and overused by the general public (39). In addition, new resistance mechanisms are emerging and spreading worldwide. As a result, antimicrobial resistance is rapidly increasing in all regions of the world.

The virulence of *Campylobacter* spp. depends on their virulome (8). The most common genotypes observed in *C. jejuni* bacteria collected from different types of large animal samples were *ciaB* (100%), *flaA* (100%), *recR* (80.77%), *cadF* (76.93%), and *dnaJ* (76.93%). On the other hand, *wlaN* (7.69%) and *virbll* (7.69%) were the *C. jejuni* strains with the lowest incidence reported in a variety of large livestock specimens. The most common genotypes found in *C. coli* bacteria from a variety of large animal samples were *ciaB* (100%), *flaA* (100%), *pidA* (61.54%), and *cadF* (61.54%). The diagnostic accuracy was consistent with that reported in a recent report from Korea (40), however, higher than that reported in South Africa (41, 42) and Chile (43). The discrepancy may be due to the complexity of the colonization process, which involves several genes, as well as the use of isolates from a single sampling site (43).

In this study, *Campylobacter* isolates were characterized by the detection of specific resistance and virulence factors, which is limited to understanding the mechanisms of resistance and virulence. The results of whole genome sequencing analysis can determine the epidemiology and evolutionary pathways of *Campylobacter* spp. to better tailor measures to reduce campylobacteriosis cases in Iran.

In conclusion, *Campylobacter* spp. collected from raw meat of large livestock in this study showed significant antibiotic resistance and carried various virulence and antimicrobial resistance genes. These strains can pose a public health risk. The intensive use of antibiotics in large livestock farming is responsible for the increase in multidrug-resistant-*Campylobacter* isolates.

Antibiotic resistance in pathogenic bacteria can be reduced by monitoring antibiotic resistance in *Campylobacter* and appropriate administration of antimicrobials in feed production.

Table 3. Distribution of genotypes amongst the *C. jejuni* strains isolated from different types of raw big livestock meat samples

Type of meat	*Sample*	*recR*	*dnaJ*	*wlaN*	*virbll*	*cdtC*	*cdtB*	*cdtA*	*flaA*	*cadF*	*pidA*	*ciaB*	*ceuE*	*cgtB*
	1	+	+	-	-	+	+	+	+	+	-	+	-	-
	2	+	+	-	-	+	+	-	+	+	+	+	+	+
	3	+	+	-	-	+	+	+	+	+	-	+	-	-
	4	+	+	-	-	+	+	+	+	-	+	+	+	+
	5	+	+	-	-	+	+	+	+	+	-	+	-	-
	6	+	-	-	-	-	-	-	+	+	+	+	-	-
	7	+	+	+	+	+	+	+	+	+	+	+	-	-
	8	-	-	-	-	-	-	-	+	+	-	+	+	+
	9	+	+	-	-	+	+	+	+	-	+	+	+	+
	10	+	+	-	-	+	+	+	+	+	-	+	+	+
	11	+	+	-	-	+	+	-	+	+	+	+	-	-
	12	+	+	-	-	+	+	+	+	+	+	+	-	-
Cattle	**13**	+	+	-	-	+	+	+	+	+	-	+	-	-
	14	-	+	-	-	+	+	+	+	-	-	+	-	-
	15	-	+	-	-	+	+	-	+	+	+	+	+	-
	16	+	+	-	-	+	+	+	+	-	+	+	+	-
	17	+	+	+	+	+	+	+	+	+	-	+	+	+
	18	+	+	-	-	+	+	+	+	+	+	+	-	-
	19	+	+	-	-	+	+	+	+	+	+	+	-	-
	20	+	-	-	-	-	-	-	+	-	-	+	-	-
	21	+	-	-	-	-	-	-	+	+	+	+	+	-
	22	-	+	-	-	+	+	+	+	+	+	+	+	+
	23	+	-	-	-	-	-	-	+	+	-	+	-	-
	24	-	+	-	-	+	+	+	+	-	+	+	-	-
	25	+	-	-	-	-	-	-	+	+	+	+	-	-
	26	+	+	-	-	+	+	+	+	+	-	+	-	-
	1	+	-	-	-	-	-	-	+	+	+	+	-	-
	2	+	+	-	-	+	+	+	+	+	-	+	-	-
Sheep	**3**	+	+	-	-	+	+	+	+	-	+	+	+	-
	4	+	+	-	-	+	+	+	+	+	-	+	+	+
	5	+	+	-	-	+	+	-	+	+	+	+	-	-
	6	+	+	+	+	+	+	+	+	-	+	+	+	+
Goat	**1**	+	+	-	-	+	+	+	+	+	-	+	-	-
Camel	**0**	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4. Distribution of genotypes amongst the *C. coli* strains isolated from different types of raw big livestock meat samples

Type of meat	*Sample*	*recR*	*dnaJ*	*wlaN*	*virbll*	*cdtC*	*cdtB*	*cdtA*	*flaA*	*cadF*	*pidA*	*ciaB*	*ceuE*	*cgtB*
Cattle	**1**	-	-	-	-	-	-	+	+	+	-	+	-	-
	2	-	-	-	-	-	-	-	+	-	+	+	-	-
	3	-	-	-	-	-	-	-	+	+	-	+	-	+
	4	-	-	-	-	-	-	-	+	+	+	+	-	-
	5	+	-	-	-	+	-	+	+	+	-	+	-	+
	6	-	-	-	-	-	+	-	+	-	+	+	-	-
	7	-	-	-	-	-	-	-	+	+	+	+	-	+
	8	-	-	-	-	-	-	-	+	-	+	+	-	+
	9	+	-	-	-	-	-	-	+	+	-	+	-	-
	10	-	-	-	-	-	+	-	+	+	+	+	-	+
	11	-	-	-	-	+	-	-	+	-	+	+	-	-
	12	+	-	-	-	+	-	+	+	+	-	+	-	+
	13	-	-	-	-	-	+	-	+	-	+	+	-	-
Sheep	**1**	-	-	-	-	-	-	+	+	+	-	+	-	-
	2	-	-	-	-	-	-	-	+	-	+	+	-	-
	3	-	-	-	-	-	-	-	+	+	-	+	-	+
	4	-	-	-	-	-	-	-	+	+	+	+	-	-
	5	+	-	-	-	+	-	+	+	+	-	+	-	+
	6	-	-	-	-	-	+	-	+	-	+	+	-	-
	7	-	-	-	-	-	-	-	+	+	+	+	-	+
	8	-	-	-	-	-	-	-	+	-	+	+	-	+
	9	+	-	-	-	-	-	-	+	+	-	+	-	-
	10	-	-	-	-	-	+	-	+	+	+	+	-	+
	11	-	-	-	-	+	-	-	+	-	+	+	-	-
	12	+	-	-	-	+	-	+	+	+	-	+	-	+
	13	-	-	-	-	-	+	-	+	-	+	+	-	-
	14	+	-	-	-	-	-	-	+	+	-	+	-	-
	15	-	-	-	-	-	+	-	+	+	+	+	-	+
	16	-	-	-	-	+	-	-	+	-	+	+	-	-
Goat	**1**	-	-	-	-	-	-	+	+	+	-	+	-	-
	2	-	-	-	-	-	-	-	+	-	+	+	-	-
	3	-	-	-	-	-	-	-	+	+	-	+	-	+
	4	-	-	-	-	-	-	-	+	+	+	+	-	-
Camel	**1**	-	-	-	-	-	+	-	+	+	+	+	-	+
	2	-	-	-	-	+	-	-	+	-	+	+	-	-
	3	+	-	-	-	+	-	+	+	+	-	+	-	+

Table 5. Antibacterial sensitivity profile of *C. jejuni* isolates

Type of meat	C. Jejuni	aphA-3-1	cmeB	Tet) O(	blaOXA-61	aadE1	GM10	CIP5	NA30	TE30	AM10	AMC30	E15	AZM15	CC2	C30
	1	-	-	+	-	+	-	-	-	+	-	-	+	+	-	+
	2	-	-	+	-	+	-	+	-	+	-	-	+	-	-	+
	3	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
	4	-	-	-	+	+	-	-	-	-	-	+	+	+	-	-
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	6	-	-	+	+	+	-	-	-	+	+	-	+	-	+	-
	7	-	+	+	+	+	-	+	+	+	+	-	+	-	+	+
	8	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-
	9	-	-	+	-	+	-	+	-	+	-	-	+	+	-	+
	10	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
	11	-	-	+	-	+	-	-	-	+	-	-	+	-	-	+
	12	-	-	+	+	-	-	-	-	+	+	+	-	-	+	-
Cattle	**13**	-	-	+	-	+	-	+	+	+	-	-	+	+	-	+
	14	-	-	-	+	+	-	-	-	-	+	+	+	+	-	-
	15	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-
	16	-	-	-	+	+	-	-	-	-	+	+	+	-	-	+
	17	-	+	+	+	-	-	+	+	+	+	+	-	-	+	+
	18	-	-	+	+	+	-	-	-	+	-	+	+	+	-	-
	19	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-
	20	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
	21	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
	22	+	-	-	+	+	+	-	-	-	+	+	+	+	-	-
	23	-	-	-	+	-	-	-	+	-	+	+	-	-	+	+
	24	-	-	-	+	-	-	-	-	-	+	+	-	+	-	-
	25	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
	26	+	-	+	+	-	+	-	-	+	+	-	-	-	-	-
	1	-	-	+	-	+	-	-	+	+	-	-	+	+	-	-
	16	-	-	-	+	+	-	-	-	-	+	+	+	-	-	+
Sheep	**17**	-	+	+	+	-	-	+	+	+	+	+	-	-	+	+
	18	-	-	+	+	+	-	-	-	+	-	+	+	+	-	-
	19	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-
	20	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Goat	**1**	-	-	+	-	+	-	-	+	+	-	-	+	+	-	-
Camel	**0**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 6. Antibacterial sensitivity profile of *C. coli* isolates

Type of meat	*C. coli*	*aphA-3-1*	*cmeB*	*Tet) O(*	*blaOXA-61*	*aadE1*	*GM10*	*CIP5*	*NA30*	*TE30*	*AM10*	*AMC30*	*E15*	*AZM15*	*CC2*	*C30*
Cattle	1	-	-	+	+	+	-	-	-	-	+	-	+	+	+	-
	2	-	+	-	+	+	-	+	-	-	+	+	+	-	-	-
	3	-	+	+	+	+	-	+	+	+	+	+	+	+	-	+
	4	-	+	-	-	+	-	+	-	-	-	-	+	+	+	-
	5	-	-	+	+	+	-	-	-	+	+	-	+	-	-	+
	6	-	+	+	-	-	-	+	+	+	-	-	+	-	-	-
	7	-	+	-	+	+	-	+	-	-	+	+	+	+	+	-
	8	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	9	-	+	-	+	+	-	+	+	-	+	-	-	-	-	+
	10	-	+	+	+	-	-	+	+	+	+	-	-	-	-	-
	11	-	+	-	+	+	-	+	+	-	+	-	+	-	+	+
	12	-	-	+	+	+	-	-	-	+	+	-	+	-	-	+
	13	-	+	+	-	-	-	+	+	+	-	-	+	-	-	-
Sheep	1	-	-	+	+	+	-	-	-	-	+	-	+	+	+	-
	2	-	+	-	+	+	-	+	-	-	+	+	+	-	-	-
	3	-	+	+	+	+	-	+	+	+	+	+	+	+	-	+
	4	-	+	-	-	+	-	+	-	-	-	-	+	+	+	-
	5	-	-	+	+	+	-	-	-	+	+	-	+	-	-	+
	6	-	+	+	-	-	-	+	+	+	-	-	+	-	-	-
	7	-	+	-	+	+	-	+	-	-	+	+	+	+	+	-
	8	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	9	-	+	-	+	+	-	+	+	-	+	-	-	-	-	+
	10	-	+	+	+	-	-	+	+	+	+	-	-	-	-	-
	11	-	+	-	+	+	-	+	+	-	+	-	+	-	+	+
	12	-	-	+	+	+	-	-	-	+	+	-	+	-	-	+
	13	-	+	+	-	-	-	+	+	+	-	-	+	-	-	-
	14	-	+	-	-	+	-	+	-	-	-	-	+	+	+	-
	15	-	-	+	+	+	-	-	-	+	+	-	+	-	-	+
	16	-	+	+	-	-	-	+	+	+	-	-	+	-	-	-
Goat	1	-	+	+	-	-	-	+	+	+	-	-	+	-	-	-
	2	-	+	-	-	+	-	+	-	-	-	-	+	+	+	-
	3	-	-	+	+	+	-	-	-	+	+	-	+	-	-	+
	4	-	+	+	-	-	-	+	+	+	-	-	+	-	-	-
Camel	1	-	-	+	+	+	-	-	-	-	+	-	+	+	+	-
	2	-	+	-	+	+	-	+	-	-	+	+	+	-	-	-
	3	-	+	+	+	+	-	+	+	+	+	+	+	+	-	+

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Authors' Contribution

ER and BM performed the sampling and culture method, carried out the molecular genetic studies, participated in primer sequence alignment, and drafted the manuscript. ER and AS participated in the design of the study, performed the statistical analysis, and wrote the manuscript. All authors read and approved the final manuscript.

Ethics

This research was conducted as part of a PhD thesis in food hygiene and was ethically approved by the Research Council of the Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran. The review of this research project and licenses for sampling were approved by Professor Ebrahim Rahimi (Approval Ref Number MIC19817).

Conflict of Interest

The authors declare that they have no competing interests.

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