



Prevalence, Virulence, and Antimicrobial Resistance of *Campylobacter* Species Isolated From Carcasses of Camels Slaughtered in Slaughterhouses of Chaharmahal and Bakhtiari Province, 2018-2019

Amir Shafiei¹ , Ebrahim Rahimi^{1*} , Amir Shakerian²

¹Department of Food Hygiene, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

²Research Center of Nutrition and Organic Products (R.C.N.O.P), Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

Abstract

Background and aims: Gastritis is basically caused by *Campylobacter coli* and *jejuni*, and usually occurs after the consumption of raw animal products.

Methods: This study investigated the prevalence, virulence, and antimicrobial resistance of *Campylobacter* species isolated from slaughtered animals in Juneqan, Farokhshahr, Saman, and Lordegan slaughterhouses in Chaharmahal and Bakhtiari Province of Iran. From 40 camels, 5 samples of liver, neck meat, kidney, heart, and rectal contents were taken from each carcass. The obtained samples were cultured and then the PCR was performed for them and, finally, the toxin genes of virulence and resistance against antibiotics were examined.

Results: Out of 19 *Campylobacter* specimens isolated, 8 specimens were *coli* and 11 ones were *jejuni*. It was also found that the infection with *Campylobacter* in the carcasses was the highest in warmer seasons.

Conclusion: The carcasses of slaughtered animals in slaughterhouses were likely a potential reservoir for *coli* and *jejuni* species, and their viscera and meat could have transmitted these bacteria to humans and animals.

Keywords: *Campylobacter coli*, *Campylobacter jejuni*, Slaughterhouse, Antimicrobial resistance, Virulence genes

*Corresponding Author:

Ebrahim Rahimi,
Department of Food Hygiene,
Faculty of Veterinary Medicine,
Shahrekord Branch, Islamic
Azad University, Shahrekord,
Iran
Tel: +989133278377,
Email: ebrahimrahimi55@
yahoo.com

Received: 16 Feb. 2021
Accepted: 20 June 2021
ePublished: 29 Sep. 2021



Introduction

Foodborne illnesses are important public health problems that cause considerable economic damages.^{1,2} *Campylobacter* is a rod-shaped, Gram-negative, and non-spore-forming bacterium belonging to the Enterobacteriaceae family. It has various hosts, and constitutes a common zoonotic pathogen.^{3,4} The two important species *C. jejuni* and *C. coli* are the causal agents of most *Campylobacter* infections that are transmitted to humans by animal vectors.^{5,6} *Campylobacter* is responsible for 2%-35% of bacterial diarrhea in human.⁷⁻¹¹ The infective dose of *Campylobacter* is very low.^{10,11} The bacterium affects livestock and results in economic loss, and is the causative agent of food poisoning in humans.^{12,13} These infections are clinically manifested as gastroenteritis, typhoid fever, and septicemia with local lesions. Arbitrarily use of antibiotics in the livestock and poultry industry as well as in human communities have become troublesome, leading to the emergence of *Campylobacter* resistance to various antibiotics.⁸ Consumption of raw and undercooked meat is the main transmission route of

Campylobacter to humans, but unpasteurized milk and raw vegetables can also be a source of these bacteria and cause diseases in humans. *Campylobacter* infections often occur during traveling, which they are also referred to as traveler's diarrhea.

The rate of *Campylobacter* infections is 380 per 100 000 population.¹⁴ *Campylobacteriosis* is the dominant bacterial infection in food materials and is considered the major public health problem in Europe and many other countries worldwide.¹²

Campylobacter has been isolated from intestinal contents, liver, gallbladder, and feces of livestock. Also, in a few cases, it has been isolated from carcasses in slaughterhouse cold rooms.¹

In a study conducted in Egypt, *Campylobacter* was found in 20% of the fecal and up to 33% of the meat samples.¹⁴ The infection with *Staphylococcus aureus* and *Campylobacter* spp in camels is of the highest importance.^{15,16}

This study aimed to determine the prevalence, intensity, and antimicrobial resistance of *Campylobacter* isolated from carcasses of slaughtered camels in Chaharmahal and

Bakhtiari province, Iran.

Materials and Methods

Our study samples were obtained from the liver, neck meat, kidney, heart, and rectal contents of the carcasses of 40 camels slaughtered in Juneqan, Farrokhshahr, Saman, and Lordegan slaughterhouses from September 2018 to September 2019. The samples were cultured and polymerase chain reaction (PCR) was performed. As for the isolation of campylobacter species: first, all samples were homogenized, and in each one, 10 g was added to 90 mL Campylobacter Enriched Broth (Preston enrichment broth base, Himedia, Mumbai, India, M899); then the selected Campylobacter supplement (Himedia, Mombia, India, FD042) and 25mL defibrinated sheep blood were added to each 475 mL of the medium. After 24 hours of incubation, 0.1 mL of it added to the selective Campylobacter media (Himedia, Mumbai, India, M994) was enriched with antibiotic supplements (Himedia, Mumbai, India, FD006) and 5% sheep's defibrinated blood, and incubated for 48 hours at 42°C. Single-growing colonies were studied to confirm and separate Campylobacter species in terms of warm staining, catalase production, oxidase, hydrolysis of hippurate, and resistance to cephalothin.

DNA Extraction and PCR Test

DNA of the confirmed colonies was extracted using the DNA extraction kit (Cinna Gen, Iran). The PCR was performed as described by Denis et al¹⁷. To conduct the PCR reaction, the final reaction volume was considered 25 µL, including 20 ng of template DNA, 2 mM MgCl₂, 25 pmol of each primer, one Taq polymerase enzyme unit, and 200 µM dNTP mixture. Table 1 shows the size of the PCR product for each sample. To confirm the presence of amplified fragment, 20 µL of the PCR product was electrophoresed on 1.5% agarose gel containing ethidium bromide in the presence of 100 bp DNA marker at a constant voltage of 80 V. Other primers are listed in Table 2. The main method of this study was PCR Test.

Antimicrobial susceptibility test was performed using the disk diffusion method on Muller Hinton medium (HiMedia, Laboratories, Mumbai, India) enriched with

5% sheep defibrinated blood, according to the method proposed by CLSI (Clinical and Laboratory Standards Institute, 2006). The antibiotic discs used in this study were manufactured by Indian HiMedia companies (HiMedia, Laboratories, Mumbai, India). The type and concentration of each antibiotic used were as follow: Nalidixic acid (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), tetracycline (15 µg), streptomycin (30 µg), ampicillin (10 µg), amoxicillin (30 µg), gentamicin (10 µg), and chloramphenicol (30 µg). After culturing and disking at 42°C under microaerophilic conditions for 48 hours, the plates were incubated. After incubation, non-growth areas around antibiotic discs were measured by a KT model caliper made in China. The sensitivity of Campylobacter strains to each antibiotic was then compared to the pattern presented by CLSI. The PCR method was used to trace the virulence genes of Campylobacter isolated from the studied samples based on the study conducted by Bang et al.⁵

The samples were taken from neck muscles in the slaughterhouse after being washed. Campylobacter species as the pathogen-dependent variable were isolated by the culture method and biochemical tests, confirmed by these methods, and examined in terms of presence of virulence genes. Antimicrobial resistance of Campylobacter species was evaluated by the disk diffusion method.

As for the livestock slaughtered in Chaharmahal and Bakhtiari province's slaughterhouses, sampling was conducted from neck, heart, liver, kidney, and intestine contents. Statistical analyses were conducted using SPSS software 16.0 (SPSS Inc, Chicago, IL.), and chi-square test as well as Fisher's exact two-tailed test analysis were performed.

Results

Out of 40 meat samples, 5 samples were infected with *Campylobacter* (1 with *C. coli* and 4 with *C. jejuni*); out of 40 liver samples, 6 ones were *Campylobacter* positive (4 with *C. coli* and 2 with *C. jejuni*); as for 80 kidney and heart samples, no *Campylobacter* (*C. jejuni* or *C. coli*) was isolated; and out of 40 rectal content samples, 8 ones had *Campylobacter* (3 with *C. coli* and 5 ones with *C. jejuni*).

Table 1. Sequences of Primers Used to Detect *Campylobacter* Genus and *Campylobacter* Species: *jejuni* and *coli*

| Gene | Primer sequence | Product size | Reference |
|----------------|--|---------------------------------------|-----------|
| <i>16SrRNA</i> | MD16S1 upper primer 5'-ATC TAA TGG CTT AAC CAT TAA AC-3' MD16S1 lower primer 5'-GGA CCG TAA CTA GTT TAG TAT T-3' | 857 bp for <i>Campylobacter</i> genus | 5 |
| <i>mapA</i> | MDmapA1 upper primer 5'-CTA TTT TAT TTT TGA GTG CTT GTG-3' MDmapA2 lower primer 5'-GCT TTA TTT GCC ATT TGT TTT ATT A-3' | 589 bp for <i>C. jejuni</i> | 5 |
| <i>ceuE</i> | COL3 upper primer 5'-AAT TGA AAA TTG CTC CAA CTA TG-3' MDCOL2 lower primer 5'-TGA TTT TAT TAT TTG TAG CAG CG-3' | 462 bp for <i>C. coli</i> | 5 |

Overall, 19 samples out of the 200 ones were found positive for *Campylobacter* (8 with *C. coli*, and 11 with *C. jejuni*). In other words, 4% of the total infected samples contained *C. coli* and 5.5% of them contained *C. jejuni* (see Table 3 and Figure 1).

Out of the 5 *Campylobacter*-infected camel meat samples, *C. coli* was found in 20% of the samples and *C. jejuni* was observed in 80% of them. Out of 6 *Campylobacter*-infected camel liver samples, *C. coli* was found in 66.6% of them and *C. jejuni* was detected in 33.3% of them. Neither *C. coli* nor *C. jejuni* was found in the samples from camels' kidney and heart (0%). As for the *Campylobacter*-infected camel

rectal content samples, *C. coli* and *C. jejuni* were found in 37.5% and 62.5% of them, respectively. Out of the total 19 infected camel samples, *C. coli* was found in 42.1% of the samples and *C. jejuni* was observed in 57.8% of them ($P=0.04$; Table 4).

Presences of the genes effective in motility (*flaA*), adhesion (*cadF*), and cytotoxin production (*cdtB*, *cdtA*) were confirmed. The ability of *Campylobacter* strains in producing toxins is also important in the infection process. In *Campylobacter* infections, the cytolethal distending toxin consisting of the subunits CdtA and CdtB is the most important characteristic showing the presence of

Table 2. Sequences of Primers Used to Trace *Campylobacter* Virulence Genes and *Campylobacter* Species: *jejuni* and *coli*

| Primers | Sequences (Amplicon Sizes) | PCR Conditions |
|---|--|--|
| <i>cadF</i> gene | F2B: 5'-TGGAGGGTAATTTAGATATG-3' RIB: 5'-CTAATACCTAAAGTTGAAAC-3' (Amplicon: 400 bp) | 94°C 1 min (30 cycles) 45°C 1 min 72°C 3 min |
| <i>ceuE</i> gene (for <i>C. jejuni</i>) | JeJt: 5'-CCTGCTCGGTGAAAGTTTTG-3' JeJ2: 5'-GATCTTTTGTGGTGCTGC-3' (Amplicon: 794 bp) | 93°C 3 min 93°C 3 min |
| <i>ceuE</i> gene (for <i>C. coli</i>) | COL1: 5ATGAAAAATATTTAGTTTTTGGGA3' COL2: 5'-ATTTTATTTGTAGC.AGCG-3' (Amplicon: 894 bp) | 95°C 30 s 57°C 30 s (30 cycles) 72°C 1 min |
| <i>flaA</i> gene | fla A-F: 5'-GGAAATTGGATTTGGGGCTATACT-3' fla A-R: 5'-CTGTAGTAATCTTAAACATTTTG-3' (Amplicon: 1728 bp) | 94°C 1 min 45°C 1 min (30 cycles) 72°C 3 min |
| <i>Cdt A</i> gene | GNW: 5'-GGAAATTGGATTTGGGGCTATACT-3' IVH: 5'-ATCACAAAGGATAATGGACAAT-3' (Amplicon: 165 bp) | |
| <i>cdtB</i> gene | VAT2I: 5' GTTAAATCCCCTGCTATCAACCA 3' WMI-R 5' GTTGGCACTTGGAAATTTGCAAGGC3' (Amplicon: 555bp) | |
| <i>Cdt</i> genes cluster | GNW and LPF-X) (Amplicon: 1215 bp) | |
| <i>Cdt</i> genes | LYA-f: 5'-CTTTATGCATGTTCTTCTAAATTT-3' MII-R: 5'GTAAAGGTGGGGTTATAATCATT-3' (Amplicon: 2212 bp) | |

Table 3. Frequency of *Campylobacter* spp. Isolated From Different Samples From Camels

| Sample | No. of Samples | No. of Positive Samples (%) | <i>C. coli</i> Positive (%) | <i>C. jejuni</i> Positive (%) |
|---------------------|----------------|-----------------------------|-----------------------------|-------------------------------|
| Meat | 40 | 5 (12.5) | 1 (2.5) | 4 (10) |
| Liver | 40 | 6 (15) | 4 (10) | 2 (10) |
| Kidney | 40 | 0 (0) | 0 (0) | 0 (0) |
| Heart | 40 | 0 (0) | 0 (0) | 0 (0) |
| Intestinal contents | 40 | 8 (20) | 3 (7.5) | 5 (12.5) |

Table 4. Percentage of *Campylobacter* Isolated From Different Samples From Camels

| Sample | No. of Positive Samples (%) | No. of <i>C. coli</i> Positive Samples (%) | No. of <i>C. jejuni</i> Positive Samples |
|-----------------|-----------------------------|--|--|
| Meat | 5 (100) | 1 (20) | 4 (80) |
| Liver | 6 (100) | 4 (66.6) | 2 (33.3) |
| Kidney | 0 (0) | 0 (0) | 0 (0) |
| Heart | 0 (0) | 0 (0) | 0 (0) |
| Rectal contents | 8 (100) | 3 (37.5) | 5 (62.5) |
| Total | 19 (100) | 8 (42.1) | 11 (57.8) |

Campylobacter toxins (Figure 2).

Campylobacter isolated from liver samples indicated that *cadF* and *flaA* were the most frequent genes followed by *cdtA* gene (83.3%). The *cdtA* gene was found in 75% of the rectal content samples. These genes were least frequently detected in kidney and heart samples containing *Campylobacter* ($P= 0.03$; Table 5).

As shown in Tables 5 and 6, the frequencies for the *cadF* and *flaA* genes in the camels were both 100% (Figure 3), whereas those for the *cdtA* and *cdtB* genes were 73.6%,

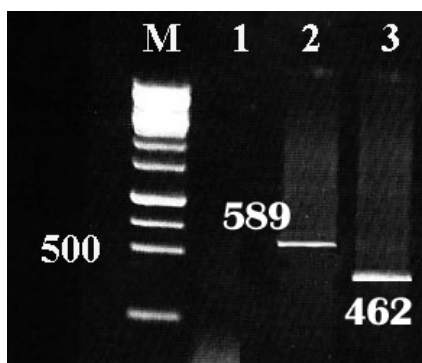


Figure 1. Specificity of PCR Assay for the Detection of *Campylobacter jejuni* and *Campylobacter coli*. M: Ladder (lane 3: 462 bp for *C. coli*) (lane 2: 589 bp for *C. jejuni*) (lane 1: Negative Control)

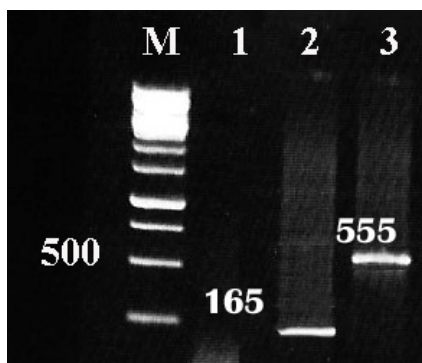


Figure 2. Detection of CDT Toxin Genes and *cadF* Gene in *Campylobacter* spp. Strains Isolated From Camel Carcasses. PCR amplification of *cdt* genes (lane 2: *cdtA* 165 bp, lane 3: *cdtB* 555 bp), M: Ladder, (lane 1: Negative Control).

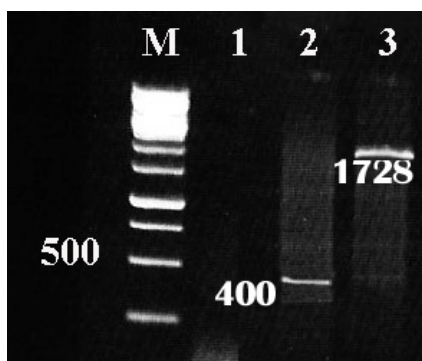


Figure 3. *flaA* (1728 bp) Gene and *cadF*(400 bp) in *Campylobacter* PCR. PCR amplification of *flaA* gene (lane 3: *flaA* 1728 bp, lane 2: *cadF* 555 bp), M: Ladder, (lane 1: Negative Control).

Table 5. Prevalence of the Virulence Genes in *Campylobacters* Isolated From Camels

| Sample | No. of Isolated Genes | <i>cadF</i> | <i>flaA</i> | <i>cdtA</i> | <i>cdtB</i> |
|-----------------|-----------------------|-------------|-------------|-------------|-------------|
| Meat | 5 | 5 (100) | 5 (100) | 3 (60) | 2 (40) |
| Liver | 6 | 6 (100) | 6 (100) | 5 (83.3) | 3 (50) |
| Kidney | 0 | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Heart | 0 | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Rectal contents | 8 | 8 (100) | 8 (100) | 6 (75) | 4 (50) |

47.3%, respectively ($P=0.03$; Table 5). The most isolated genes of *Campylobacter* detected from rectal contacts was 100%, and the least isolated genes of *Campylobacter* detected from both kidneys and hearts were both 0% (Table 6).

As shown in Table 7, the rates of antimicrobial resistance of *Campylobacter* were 42.1% for erythromycin, 15.78% for meropenem, 5.26% for imipenem, 47.36% for amoxicillin and streptomycin, 73.68% for ciprofloxacin, 26.31% for norfloxacin, 21.05% for amikacin, 42.01% for gentamicin, 89.47% for tetracycline, 10.52% for nalidixic acid, 84.21% for chloramphenicol, and 63.15% for ampicillin. The highest antibiotic resistance rates were found for chloramphenicol, tetracycline, and ciprofloxacin, and the lowest ones were detected for imipenem (5.26%), nalidixic acid (10.52%), and meropenem (15.78%).

The rates of antimicrobial resistance in *C. jejuni* were 54.54% for erythromycin, 18.18% for meropenem, 9.09% for imipenem and nalidixic acid, 45.45% for amoxicillin and streptomycin, 81.81% for ciprofloxacin, 27.27% for norfloxacin and amikacin, 36.36% for gentamicin, 90.9% for tetracycline, 81.8% for chloramphenicol, and 54.54% for ampicillin. The highest antibiotic resistance rate of *C. jejuni* was recorded for tetracycline (90.9%), and the lowest one was recorded for nalidixic acid and imipenem (9.09%).

The rate of antimicrobial resistance in *C. coli* isolated from the samples of camels slaughtered in Chaharmahal and Bakhtiari province was 25% for erythromycin and norfloxacin, 12.5% for meropenem, amikacin, and nalidixic acid, 0% for imipenem, 50% for amoxicillin, gentamicin, and streptomycin, 62.5% for ciprofloxacin, 87.5% for tetracycline, and 75% for ampicillin. The highest resistance rate was observed for tetracycline (87.5%) and the lowest one was found for imipenem (0%).

Several studies have already investigated the contamination of camel carcasses with this pathogen in slaughterhouses in different seasons; therefore, our study results only covered the contamination in different seasons as follows:

Spring: The highest and lowest percentages of infection with *Campylobacter* were observed at the slaughterhouse in Lordegan (14.4%) and Juneqan (4.8%), respectively, which were significantly different. In this season, the largest and smallest percentages of infection with *C. coli* were found in Saman (62.5%) and in Juneqan (33.3%),

Table 6. Frequencies of Virulence Genes Identified in *Campylobacters* Isolated From Camels

| Sample | <i>Campylobacter</i> Strains | No. of Isolated Samples | <i>cadF</i> | <i>flaA</i> | <i>cdtA</i> | <i>cdtB</i> |
|-----------------|------------------------------|-------------------------|-------------|-------------|-------------|-------------|
| Meat | <i>C. jejuni</i> | 1 | 1 | 1 | 1 | 1 |
| | <i>C. coli</i> | 4 | 4 | 4 | 2 | 1 |
| Liver | <i>C. jejuni</i> | 2 | 2 | 2 | 2 | 2 |
| | <i>C. coli</i> | 4 | 4 | 4 | 3 | 1 |
| Kidney | <i>C. jejuni</i> | 0 | 0 | 0 | 0 | 0 |
| | <i>C. coli</i> | 0 | 0 | 0 | 0 | 0 |
| Heart | <i>C. jejuni</i> | 0 | 0 | 0 | 0 | 0 |
| | <i>C. coli</i> | 0 | 0 | 0 | 0 | 0 |
| Rectal contents | <i>C. jejuni</i> | 4 | 4 | 4 | 3 | 2 |
| | <i>C. coli</i> | 4 | 4 | 4 | 3 | 2 |

Table 7. Antimicrobial Resistance of *Campylobacter* Isolated From Samples From Slaughtered Camels

| Antibiotic | <i>Campylobacter</i> Positive (%) | <i>Campylobacter jejuni</i> Positive (%) | <i>Campylobacter coli</i> Positive (%) |
|-----------------|-----------------------------------|--|--|
| Erythromycin | 8 (42.1) | 6 (54.54) | 2 (25) |
| Meropenem | 3 (15.78) | 2 (18.18) | 1 (12.5) |
| Imipenem | 1 (5.26) | 1 (9.09) | 0 (0) |
| Amoxicillin | 9 (47.36) | 5 (45.45) | 4 (50) |
| Ciprofloxacin | 14 (73.68) | 9 (80.81) | 5 (62.2) |
| Norfloxacin | 5 (26.31) | 3 (27.27) | 2 (25) |
| Amikacin | 4 (21.05) | 3 (27.27) | 1 (12.5) |
| Gentamycin | 8 (42.01) | 4 (36.6) | 4 (50) |
| Tetracycline | 17 (89.47) | 10 (90.9) | 7 (87.5) |
| Nalidixic acid | 2 (10.52) | 1 (9.09) | 1 (12.5) |
| Chloramphenicol | 16 (84.21) | 11 (81.8) | 7 (87.5) |
| Ampicillin | 12 (63.15) | 6 (54.54) | 6 (75) |
| Streptomycin | 9 (47.36) | 5 (45.45) | 4 (50) |

respectively, which were also significantly different. As for *C. jejuni*, the highest and lowest percentages of infection were recorded in Juneqan (66.6%) and Saman (37.5%), respectively, which were significantly different.

Summer: The largest and smallest percentages of infection with *Campylobacter* were observed in Lordegan (18.4%), and Saman & Juneqan (0.8%), respectively, which were significantly different. The highest and lowest percentages of infection with *C. coli* were detected in Lordegan (56.5%) and Saman (20%), respectively. As for *C. jejuni*, the largest and smallest percentages of infection were recorded in Saman (80%) and Lordegan (43.4%), respectively, which were significantly different.

Autumn: The smallest and largest percentages of *Campylobacter* infection were discovered in Juneqan (0%) and Lordegan (88%), respectively, which were significantly different. The highest and lowest percentages of infection with *C. coli* were found in Farrokhshahr (57.1%) and Juneqan, which were significantly different. The lowest and highest percentages of infection with *C. jejuni* were recorded in Juneqan (0%) and (54.5%) Lordegan, respectively.

Winter: The smallest and the largest percentages of

Campylobacter infection were recorded in Juneqan and Lordegan, respectively, which were significantly. The lowest percentages of *C. coli* and *C. jejuni* infections (0%) were those for Juneqan, and the highest percentages of *C. coli* and *C. jejuni* infections (75% and 50%, respectively) were those for Farrokhshahr and Saman, respectively, which were significantly different.

Discussion

According to Table 4 and out of 5 *Campylobacter*-infected camel meat samples, *C. coli* was found in 20% of the samples and *C. jejuni* was detected in 80% of them. Out of 6 *Campylobacter*-infected camel liver samples, *C. coli* was found in 66.6% of the samples and *C. jejuni* was detected in 33.3% of them. Neither *C. coli* nor *C. jejuni* was found in the camels' kidney and heart samples (0%). In the *Campylobacter*-infected camel rectal content samples, *C. coli* was discovered in 37.5% of the samples and *C. jejuni* was observed in 62.5% of them. Out of 19 infected camel samples, *C. coli* was found in 42.1% of the samples and *C. jejuni* was detected in 57.8% of them ($P=0.04$).

A study in Egypt indicated that 20% of the fecal samples were infected with *Campylobacter*, whereas

up to 33% of camel meat samples were infected with *Campylobacter*; and 15% of the liver samples were infected with *Campylobacter*. Moreover, *C. jejuni* was detected in 26.3% of the infected meat samples, and *C. coli* and *C. lari* were observed in 57.9% and 15.7% of them, respectively.¹⁴ In the present study, 42% and 57% of the *Campylobacter*-positive samples were infected with *C. coli* and *C. jejuni*, respectively. Out of 19 *Campylobacter*-infected samples, 5 were from meat (one sample with *C. coli* and 4 samples with *C. jejuni*). In other words, 20% and 80% of meat samples were infected with *C. coli* and *C. jejuni*, respectively, indicating a significant difference between the two *Campylobacter* spp. and concerning their presence in *Campylobacter*-infected camel meat ($P=0.03$). *C. coli* was detected in a higher percentage of the infected meat samples in this study compared to the study conducted in Egypt, demonstrating a difference between Iran and Egypt regarding the prevalence of *Campylobacter* spp.

Distribution of the Virulence Genes in the *Campylobacter* Isolates

In a study by Casabonne et al in 2016, *cdtA*, *flaA*, and *cadF* genes were found in 100% of the isolates (the survey was done in Poland).⁷ Bang et al reported that *cdtA* genes were detected in 90% of *Campylobacter* isolates (the survey was done in Denmark).⁵ In the present study, these genes were detected in 19 *Campylobacter*-infected samples. All 19 samples contained the *cadF* and *flaA* genes, whereas the frequencies of *cdtA* and *cdtB* genes were 79.3% and 61.3%, respectively. Therefore, there were no significant differences between the *cadF* and *flaA* genes concerning their presences in the infected samples. However, there was a significant difference between *cdtA* and *cdtB* genes in terms of their frequencies ($P=0.03$).

Antimicrobial Resistance Among *Campylobacter* Isolated From the Infected Samples

Erythromycin: In their study on slaughtered camel samples in Egypt in 2019, Gwida et al¹⁵ reported that *C. coli* exhibited 100% resistance to erythromycin in slaughtered camel samples; however, this resistance was 42.1% ($P=0.03$) in the present study. This indicated that these microorganisms were more resistant to this antibiotic in Egypt compared to Iran.

Meropenem: In Finland, Lehtopolku et al reported that *Campylobacter* was completely susceptible to this antibiotic under *in vivo* conditions, and detected no resistance to it.¹⁶ However, the resistance rate to this antibiotic was 15.78% ($P:0.04$) in our study.

Imipenem: In Finland, Lehtopolku et al¹⁶ showed that *Campylobacter* was completely susceptible to imipenem under *in vitro* conditions, whereas in the present study, it was 5.26%.

Amoxicillin: The study on samples from camels in Iran by Rahimi et al⁸ showed that resistance to this antibiotic was 6.5%. However, in this study it was 47.36% (45.45% in *C. jejuni* and 50% in *C. coli*), which suggested a significant

difference between the two reports.

Norfloxacin: Rahimi et al⁸ demonstrated that the resistance rate to norfloxacin in *Campylobacter*-positive samples was 32.7%. It was 26.31% in the present study, which indicated the increased resistance of *Campylobacter* to this antibiotic.

Amikacin: In the study by Gwida et al,¹⁵ *Campylobacter* was 100% resistant to amikacin. In the present study, however, the resistance was 21.05%, which indicated the higher resistance of microorganisms to amikacin in Egypt compared to Iran.

Gentamicin: Rahimi et al⁸ showed that the resistance rate to this antibiotic in *Campylobacter*-positive camel samples was 3.2%, while it was 42.01% in the present study. These findings showed a considerable increase in the resistance of *Campylobacter* to this antibiotic.

Tetracycline: The study by Rahimi et al⁸ determined that the resistance rate to this antibiotic was 75%. In our study, however, it was 89.47% indicating the increased resistance of *Campylobacter* to this antibiotic in various geographic regions.

Nalidixic acid: Gwida et al showed that the resistance rate to this antibiotic in *Campylobacter*-positive samples was 75%,¹⁵ while it was 10.52% in our study.

Chloramphenicol: Rahimi et al⁸ in Iran revealed that the resistance rate to this antibiotic in *Campylobacter*-positive samples was 6.5%. In the present study, however, it was higher (84.21%) indicating a higher resistance of this microorganism to this antibiotic.

Ampicillin: Gwida et al¹⁵ reported that the resistance rate to this antibiotic in *Campylobacter*-positive camel samples was 90%. In our study, however, it was 63.15% suggesting a higher resistance of this microorganism to this antibiotic in Egypt compared to Iran.

Streptomycin: Gwida et al¹⁵ reported that the resistance rate to Amikacin in *Campylobacter*-positive samples was 100%, whereas the resistance rates to streptomycin in these samples were 0.9% and 0% in *C. jejuni* and *C. coli*, respectively.¹¹ In the present study, the resistance rate to streptomycin in *Campylobacter*-positive samples was 47.36%, which suggested that this microorganism was more resistant to these antibiotic studies in Egypt compared to Iran.

Ciprofloxacin: Lehtopolku et al¹⁶ reported that the resistance rate of *Campylobacter* isolates to this antibiotic was 73.68%, which indicated a higher resistance.

Conclusion

Our study results highlighted the necessity of performing further researches on how to combat the summer peak of *Campylobacter* in camels to improve the safety of the human food supply. *Campylobacter* is difficult to isolate, grow, and identify. Public health reference laboratories can play a key role in standardizing, validating and disseminating methods for clinical diagnosis, as well as in supporting periodic or sentinel targeted surveillance studies. This study showed the importance of the camel

meat products as potential sources of *Campylobacter* spp. infection in people who had consumed the given products. It was recommended that coordinated measures be urgently adopted in order for reducing or eliminating the risks posed by this organism at a number of stages in the food chain. These included good agricultural practice, sound manufacturing practice, and hazard analysis in slaughters.

Conflict of Interest Disclosures

The authors declare that there is no conflict of interests.

Ethical Approval

All stages of this research have been performed on carcasses slaughtered legally in the mentioned slaughterhouses in Chaharmahal and Bakhtiari province and are in accordance with ethical principles. It should be noted that the present study was performed on carcasses, not living organisms.

References

- Esfandiari Z, Weese JS, Ezzatpanah H, Chamani M, Shoaei P, Yaran M, et al. Isolation and characterization of *Clostridium difficile* in farm animals from slaughterhouse to retail stage in Isfahan, Iran. *Foodborne Pathog Dis*. 2015;12(10):864-6. doi: 10.1089/fpd.2014.1910.
- Róžańska H, Lewtak-Piłat A, Osek J. Antimicrobial resistance of *Enterococcus faecalis* isolated from meat. *Bull Vet Inst Pulawy*. 2015;59(2):229-33. doi: 10.1515/bvip-2015-0034.
- Little CL, Richardson JF, Owen RJ, de Pinna E, Threlfall EJ. Prevalence, characterisation and antimicrobial resistance of *Campylobacter* and *Salmonella* in raw poultrymeat in the UK, 2003-2005. *Int J Environ Health Res*. 2008;18(6):403-14. doi: 10.1080/09603120802100220.
- Rahimi E, Ameri M, Kazemeini HR. Prevalence and antimicrobial resistance of *Campylobacter* species isolated from raw camel, beef, lamb, and goat meat in Iran. *Foodborne Pathog Dis*. 2010;7(4):443-7. doi: 10.1089/fpd.2009.0421.
- Bang DD, Nielsen EM, Scheutz F, Pedersen K, Handberg K, Madsen M. PCR detection of seven virulence and toxin genes of *Campylobacter jejuni* and *Campylobacter coli* isolates from Danish pigs and cattle and cytolethal distending toxin production of the isolates. *J Appl Microbiol*. 2003;94(6):1003-14. doi: 10.1046/j.1365-2672.2003.01926.x.
- Grant A, Hashem F, Parveen S. *Salmonella* and *Campylobacter*: antimicrobial resistance and bacteriophage control in poultry. *Food Microbiol*. 2016;53(Pt B):104-9. doi: 10.1016/j.fm.2015.09.008.
- Casabonne C, Gonzalez A, Aquili V, Subils T, Balague C. Prevalence of seven virulence genes of *Campylobacter jejuni* isolated from patients with diarrhea in Rosario, Argentina. *Int J Infect*. 2016;3(4):e37727. doi: 10.17795/iji-37727.
- Rahimi E, Ameri M, Alimoradi M, Chakeri A, Bahrami AR. Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from raw camel, beef, and water buffalo meat in Iran. *Comp Clin Path*. 2013;22(3):467-73. doi: 10.1007/s00580-012-1434-5.
- Redondo N, Carroll A, McNamara E. Molecular characterization of *Campylobacter* causing human clinical infection using whole-genome sequencing: virulence, antimicrobial resistance and phylogeny in Ireland. *PLoS One*. 2019;14(7):e0219088. doi: 10.1371/journal.pone.0219088.
- Wieczorek K, Osek J. A five-year study on prevalence and antimicrobial resistance of *Campylobacter* from poultry carcasses in Poland. *Food Microbiol*. 2015;49:161-5. doi: 10.1016/j.fm.2015.02.006.
- Wieczorek K, Wolkowicz T, Osek J. Antimicrobial resistance and virulence-associated traits of *Campylobacter jejuni* isolated from poultry food chain and humans with diarrhea. *Front Microbiol*. 2018;9:1508. doi: 10.3389/fmicb.2018.01508.
- Friedrich A, Marshall JC, Biggs PJ, Midwinter AC, French NP. Seasonality of *Campylobacter jejuni* isolates associated with human campylobacteriosis in the Manawatu region, New Zealand. *Epidemiol Infect*. 2016;144(4):820-8. doi: 10.1017/s0950268815002009.
- Raji MA, Garaween G, Ehricht R, Monecke S, Shibl AM, Senok A. Genetic characterization of *Staphylococcus aureus* isolated from retail meat in Riyadh, Saudi Arabia. *Front Microbiol*. 2016;7:911. doi: 10.3389/fmicb.2016.00911.
- El-Badawi AY. The present situation of animal protein in Egypt and the role of camels in providing cheap and healthy meat for people in poor greenery lands. *Int J Avian Wildl Biol*. 2018;3(4):319-22. doi: 10.15406/ijawb.2018.03.000113.
- Gwida MA, Zakaria A, El-Sherbiny H, Elkenany R, Elsayed MO. Prevalence of *Campylobacter*, *Enterococcus* and *Staphylococcus aureus* in slaughtered camels. *Vet Med*. 2019;64(12):521-30. doi: 10.17221/104/2019-VETMED.
- Lehtopolku M, Nakari UM, Kotilainen P, Huovinen P, Siitonen A, Hakanen AJ. Antimicrobial susceptibilities of multidrug-resistant *Campylobacter jejuni* and *C. coli* strains: in vitro activities of 20 antimicrobial agents. *Antimicrob Agents Chemother*. 2010;54(3):1232-6. doi: 10.1128/aac.00898-09.
- Denis M, Soumet C, Rivoal K, et al. Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. *Lett Appl Microbiol*. 1999;29(6):406-410. doi:10.1046/j.1472-765x.1999.00658.x.