

## Putative virulence genes and antibiotic resistance profiles of *Campylobacter jejuni* isolated from cats<sup>#</sup>

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### ABSTRACT

Daily contact with cats is an important risk factor for human campylobacteriosis. The main goal of this study was to investigate the virulence genes and antibiotic resistances of *C. jejuni* isolated from the stools of cats brought to Erciyes University, Faculty of Veterinary Medicine, Training and Research Hospital Clinics. In the study, feces taken from different breeds and ages (1 month to 9 years) of 200 (116 female, 84 male) cats were examined between May 2017 and April 2018. *Campylobacter* spp. isolates were identified at genus and species level using genus specific multiplex PCR (mPCR), and the existence of *iam*, *cadF*, *cdtA*, *flaA*, *ceuE*, *cdtC*, *cdtB* and *virB11* genes were found by PCR. The antimicrobial resistance and multidrug resistance (MDR) of the isolates were determined by disc diffusion test. By means of Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR), the genetic relatedness of the isolates were revealed. In this study, 41 (20.5%) of 200 stool samples were shown to be positive for *Campylobacter* spp. in the isolation of *Campylobacter* spp. from stool samples. In the positive samples, 14 of 22 (63.6%) were from diarrhoeic cats and 27 of 178 (15.1%) were non-diarrhoeic cats. Also, in total 71 *Campylobacter* spp. suspicious isolates were recovered from the 41 positive samples obtained. Phenotypic tests and PCR revealed that 65 isolates (20 from diarrhoeic and 45 from non-diarrhoeic cat isolates) were identified as *C. jejuni*. The differences in isolation rate in relation to sex or age were not statistically significant ( $P>0.05$ ). In terms of virulence genes, all *C. jejuni* isolates harbored at least five virulence genes. All isolates were positive for the *cadF*, *cdtC* and *ceuE* genes, respectively. *C. jejuni* isolates contained *iam*, *cadF*, *cdtA*, *flaA*, *ceuE*, *cdtC*, *cdtB* and *virB11*, at the rate of 19 (29.2%), 65 (100%), 64 (98.4%), 54 (83%), 65 (100%), 65 (100%), 64 (98.4%) and 22 (33.8%) respectively. Moreover, *cdtA* and *cdtB* toxin genes were found in most of the isolates analysed. All *C. jejuni* isolates were susceptible to amoxicillin clavulanic acid. The highest resistances of the isolates were found as follows; 64 (98.4%), 63 (96.9%), 62 (95.3%) for ciprofloxacin (CIP), trimethoprim sulfamethoxazole (SXT), nalidixic acid (NA) antibiotics, respectively. Multiple resistance of isolates was detected in the present study. Sixty-one (93.8%) out of 65 isolates were resistant to three or more antibiotics, and the highest resistance levels to three and seven antimicrobials were observed in 23.1% and 15.3% of the isolates respectively. In the study, *C. jejuni* isolates were resistant to most antibiotics currently used, and had extraordinary virulence traits in cats which may constitute a non-negligible risk for public health.

**Key words:** *Campylobacter jejuni*; virulence; antibiotic resistance; ERIC-PCR

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## Introduction

*Campylobacter* spp. is Gram negative, spiral, microaerophilic bacteria in the gastrointestinal tract in humans and animals (VANDAMME and DE LEY, 1991). The genus *Campylobacter* consists of 26 species and nine subtypes (KAAKOUSH et al., 2015; HEREDIA and GARCIA, 2018). In addition to cats and dogs, *Campylobacter* spp. is found as commensal in cattle, sheep, pigs and birds (HEREDIA and GARCIA, 2018). In humans, in addition to gastrointestinal disorders, *Campylobacter* spp. (especially *C. jejuni* and *C. coli*) can cause extra-intestinal findings, such as bacteremia, brain abscesses, meningitis, Guillain-Barre Syndrome and reactive arthritis (KAAKOUSH et al., 2015). *Campylobacter* spp. is also found in the gastrointestinal tracts of dogs and cats (BOJANIC et al., 2017). Daily contact with domestic cats is an important risk factor for human campylobacteriosis (LINDMARK et al., 2009). *Campylobacter* spp. is commonly isolated from feces of infected cats (ACKE, 2018). *C. jejuni* was first isolated from cats in England in 1977 and in the USA in 1980 (KAAKOUSH et al., 2015).

The poor identification of *Campylobacter* spp. as animal pathogens may result from the simultaneous presence of multiple strains or species with various pathogenetic characteristics. In routine diagnostic laboratories, typing of *Campylobacter* spp. is usually restricted to one colony per stool sample (KOENE et al., 2004). More than one subtype of *Campylobacter* spp. within the same species may be simultaneously detected as a causative agent in human and animal campylobacteriosis cases (KAYMAN et al., 2015).

Some authors reported that *Campylobacter* spp. isolates demonstrated resistances to the antibiotics currently used (including macrolides, fluoroquinolones and others) (KULKARNI et al., 2002; SANDBERG et al., 2002; GUPTA et al., 2004; ENGBERG et al., 2004; ANDRZEJEWSKA et al., 2013, SZCZEPANSKA et al., 2017; KUMAR et al., 2012).

In recent years, one of the significant threats to public health has been found to be multidrug resistant microorganisms (MDR) (MAGIORAKOS et al., 2012). MDR reported as a single isolate, was resistant to three or more antimicrobial classes (ABAY et al., 2012). Bacteria can develop multidrug resistance via the gathering of genes encoded for resistance to a single drug, or by increased expression of genes encoding multiple drug delivery pumps in a single cell (NIKAIDO, 2009).

In cats, few documents were found on the clinical symptoms of campylobacteriosis. In some studies, cats infected with *Campylobacter* spp. were clinically healthy, lacking other pathogens (FOX, 2012). Moreover, in the studies carried out on the pathogenesis of campylobacteriosis, motility, adhesion to intestinal epithelial cells, invasion to host cells, and cytotoxin production are mentioned as important virulence factors of *Campylobacter* spp. (CASABONNE et al., 2016). Various genes have been identified with regard to the virulence of campylobacters: *flaA* (Flagellin A), *CadF* (*Campylobacter* adhesion to fibronectin), *dnaJ*, *racR*, *cj0588* genes are present for adherence and colonization; *virB11*, *ciaB* (*Campylobacter* invasion antigen B), *pldA*, *iam* (invasion-associated marker) genes are responsible for invasion; *cdtA*, *cdtB*, *cdtC* (cytolethal distending toxin A, B and C) genes are accountable for cytotoxin production; and *wlaN* and *cgtB* genes are related to Guillain-Barre syndrome (DATTA et al., 2003; MARTINEZ et al., 2006; KHOSHBAKHT et al., 2013; QUETZ et al., 2012; LARSON et al., 2008; KOOLMAN et al., 2016).

Due to the limited data about the virulence factors, antibiotic resistance and multiple drug resistance profiles of *Campylobacter* spp. in cats, we aimed to investigate the virulence genes and antibiotic resistance profiles of *C. jejuni* isolated from stool samples of 200 cats brought to Erciyes University, Faculty of Veterinary Medicine, Training and Research Hospital Clinics.

## Materials and methods

*Animals.* The stool samples taken from different breeds and ages (1 month to 9 years) of 200 (116

female, 84 male) cats, brought to Erciyes University, Faculty of Veterinary Medicine, Training-Research and Practice Hospital Clinics, between May 2017 and April 2018 in for the sake of a year-long study, with the permission of the pet owners. The samples were divided into two groups on the basis of history. One group comprised 22 animals out of the 200 cats identified as having diarrhea, and 178 cats in the other group did not show these symptoms, they were non-diarhoeic, and they did not have any infectious diseases or antimicrobial treatments (cats brought to the clinics for routine examinations and vaccination services). In this study, the cats were also divided into two groups as “younger than six months and older than six months” according to the literature (ACKE et al., 2006; 2009a). Regarding gender/sex, there were no established criteria, only the animal owners' declarations were taken into consideration.

Table 1. Age, gender, non-diarhoeic and diarrhoeic status of cats in the study

	Diarrhoeic cats		Non-diarhoeic cats	
	<i>C. jejuni</i> Positive	<i>C. jejuni</i> Negative	<i>C. jejuni</i> Positive	<i>C. jejuni</i> Negative
<6 months (n = 125)	<b>8</b>	<b>4</b>	<b>19</b>	<b>94</b>
Female (n = 76)	7	3	12	54
Male (n = 49)	1	1	7	40
>6 months (n = 75)	<b>6</b>	<b>4</b>	<b>8</b>	<b>57</b>
Female (n = 40)	4	2	6	28
Male (n = 35)	2	2	2	29
Total (n = 200)	<b>14</b>	<b>8</b>	<b>27</b>	<b>151</b>

*Reference strain.* *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Campylobacter jejuni* ATCC 700819 were used as control strains.

*Isolation, identification and molecular detection of virulence genes.* Fecal swabs were kept in Cary-Blair medium (Oxoid, CM0519, UK) no more than three hours before culturing. The stool samples were inoculated onto modified charcoal cefoperazone

deoxycholate (mCCD) agar (Oxoid, CM739, UK), additionally supplemented with cefoperazone, amphotericin B and teicoplanin (CAT supplement, Oxoid, SR174, UK), and incubated at 42 °C for 48-72 h under microaerobic conditions.

Presumed colonies were confirmed as *Campylobacter* spp. on the basis of cell morphology (Gray to white or metallic-highlighted colonies growth on a mCCD agar) after the incubation period, by phenotypic (colony morphology, Gram staining, oxidase test, catalase test and motion test) tests. Thereafter, all *Campylobacter* spp. isolates were identified at genus and species level using genus specific mPCR assay (WANG et al., 2002). For this purpose, template DNA was initially extracted using the InstaGene™ Matrix (Bio-Rad, USA) kit. Concentrations of the DNA samples (µg/µL) were measured by Qubit 3.0 fluorometer (Thermo Fisher, USA) and stored at -20 °C until analysis. mPCR was carried out in a reaction mixture for each sample; 2.5 µL DNA sample, 2.5 µL 10x PCR buffer (Thermo Scientific, USA), 20 mM MgCl<sub>2</sub> (Vivantis, Malaysia), 0.2 mM dNTP mixture (Thermo Scientific, USA), 0.5 µM *C. jejuni* and *C. lari*; 1 µM *C. coli* and *C. fetus*, 2 µM *C. upsaliensis*, 0.2 µM *Campylobacter* 23S rRNA primers and 1.25 U Taq polymerase (Thermo Scientific, USA) were prepared by adjusting the final concentration to 25 µL with sterile distilled water. DNA amplification was carried out including an initial denaturation step at 95 °C for 6 min, followed by 30 cycles, denaturation at 95 °C for 0.5 min, annealing at 59 °C for 0.5 min, and extension at 72 °C for 0.5 min, ending with a final extension at 72 °C for 7 min. in a thermocycler (Arctic™ Thermal Cycler; Thermo Fisher, USA). As a result of amplification, the PCR products obtained were visualized using a UVP gel documentation system (Vilber Lourmat, France) after electrophoresis (Thermo EC 330, USA) for 90 minutes at 90 V in a 1.5% agarose gel. *Campylobacter* species were identified according to the band sizes (Table 2).

The presence of virulence genes, including *iam*, *cadF*, *cdtA*, *cdtB*, *cdtC*, *flaA*, *ceuE*, and *virB11*, were determined by PCR, as described in earlier studies (KRUTKIEWICZ and KLIMUSZKO, 2010; CHANSIRIPORNCHAI and SASIPREEYAJAN,

2009; DATTA et al., 2003; RIPABELLI et al., 2010) with modifications. PCRs for virulence genes were carried out in a reaction mixture: 4 µL DNA sample, 10x PCR buffer with KCl (Thermo Scientific, USA), 1.5 mM MgCl<sub>2</sub> (Vivantis, Malaysia), 250 mM dNTP mixture (Thermo Scientific, USA), 1 µM each primers and 0.5 U Taq polymerase (Thermo Scientific, USA) to adjust the final concentration to 40 µL with sterile distilled water. The PCR protocol included denaturation at 94 °C for 1 min., annealing

at 52 °C for 1 min. and extension at 72 °C for 1 min. followed by 30 cycles, and a final extension at 72 °C for 5 min. Negative and positive controls were used. Further, all the negative samples were tested. The amplified products were electrophoresed in a 1.5% (w/v) TAE agarose gel at 100 V for 1 h. The gel was visualized under UV light. The investigated primers and expected band size (bp) for mPCR and PCRs for virulence genes are summarized in Table 2.

Table 2. The Oligonucleotide Sequence and Predicted Sizes Used in the PCR

Name	Sequence	Band size (bp)
Prier-I (CJF)	5'- ACT TCT TTA TTG CTT GCT GC - 3'	323
Primer-II (CJR)	5'- GCC ACA ACA AGT AAA GAA GC - 3'	
Primer-I (CCF)	5'- GTAAAACCAAAGCTTATCGTG -3'	126
Primer-II (CCR)	5'- TCCAGCAATGTGTGCAATG -3'	
Primer-I (CLF)	5'- TAGAGAGATAGCAAAAGAGA -3'	251
Primer-II (CLR)	5'- TACACATAATAATCCACCC -3'	
Primer-I (CUF)	5'- AATTGAAACTCTTGCTATCC -3'	204
Primer-II (CUR)	5'- TCATACATTTTACCCGAGCT -3'	
Primer-I (CFF)	5'- GCAAATATAAATGTAAGCGGAGAG-3'	435
Primer-II (CFR)	5'- TGCAGCGGCCCCACCTAT-3'	
23SrRNA-F	5'- TATACCGGTAAGGAGTGCTGGAG-3'	650
23SrRNA-R	5'- ATCAATTAACCTTCGAGCACCG -3'	
<i>fla</i> AF	5'-ATGGGATTTTCGTATTAACAC-3'	1700
<i>fla</i> AR	5'-CTGTAGTAAATCTTAAAACATTTTG-3'	
<i>cdt</i> AF	5'- GGAAATTGGATTTGGGGCTATACT -3'	165
<i>cdt</i> AR	5'- ATCACAAGGATAATGGACAAT -3'	
<i>cdt</i> BF	5'- GTTAAAATCCCTGCTATCAACCA -3'	495
<i>cdt</i> BR	5'- GTTGGCACTTGGAATTTGCAAGGC -3'	
<i>cdt</i> CF	5'- TGGATGATAGCAGGGGATTTTAAC -3'	555
<i>cdt</i> CR	5'- TTGCACATAACCAAAAGGAAG -3'	
<i>vir</i> B11F	5'- TCTTGTGAGTTGCCTTACCCCTTTT -3'	494
<i>vir</i> B11R	5'- CCTGCGTGTCTGTGTTATTTACCC -3'	
<i>ceu</i> EF	5'-CCTGCTCGGTGAAAGTTTTG-3'	794
<i>ceu</i> ER	5'-GATCTTTTTGTTTTGTGCTGC-3'	
<i>cad</i> FR1BF	5'- TTGAAGGTAATTTAGATATG-3'	400
<i>cad</i> FR1BR	5'- CTAATACCTAAAGTTGAAAC -3'	
<i>iam</i> F	5'-GCGCAAAATATTATCACCC-3	518
<i>iam</i> R	5'-TTCACGACTACTATGCGG-3'	

**Antibiotic susceptibility testing.** The antibiotic susceptibilities of the isolates identified as *C. jejuni* were investigated by the disc diffusion method and the results were evaluated according to CLSI recommendations (CLSI, 2014). The following 16 antibiotics were used in the study: amoxicillin clavulanic acid (AMC, 30 µg), ciprofloxacin (CIP, 5 µg), trimethoprim sulfamethoxazole (SXT, 25 µg), enrofloxacin (ENR, 5 µg), clindamycin (DA, 2 µg), nalidixic acid (NA, 30 µg), streptomycin (S, 10 µg), tetracycline (TE, 30 µg), doxycycline (DO, 30 µg), ampicillin (AMP, 10 µg), erythromycin (E, 15 µg), streptomycin (S, 10 µg), amikacin (AK, 30 µg), gentamycin (CN, 10 µg), cefotaxime (CTX, 30 µg), and piperacillin-tazobactam (TZP, 110 µg), (Oxoid, UK). Multidrug resistance was evaluated as to whether a single isolate was resistant to three or more antibacterial classes.

**Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR).** For molecular typing of the isolates, Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) was utilized (HOUF et al., 2002). In the test, ERIC1 and ERIC2 primers were used. Briefly, the PCR mixture was 50 µL in total, composed of 5 µL 10×PCR buffer, 4 mM MgCl<sub>2</sub>, 5 U Taq DNA polymerase, the final concentration for the dNTP mixture was 0.2 mM, 25 pmol for each primer and 1 µL for template DNA. DNA amplification consisted of an initial denaturation at 94 °C and 5 min, followed by 40 cycles of 94 °C for 1 min, 25 °C for 1 min and 72 °C for 2 min. The amplified products were resolved by electrophoresis in a 2% (w/v) TAE agarose gel at 100 V for 1 h. The gel was tested and evaluated by visual inspection under UV light.

**Statistical analysis.** Chi-square tests were used for the statistical analysis in the study, in order to compare the *C. jejuni* presence and gender, age and diarrhoea status of the cats, using the SPSS (Statistical Package for Social Sciences for Windows) 23.0 (Armonk, NY: IBM Corp.) program.

## Results

In the study, 41 (20.5%) of 200 stool swab samples from cats were positive for the presence of *Campylobacter* spp. In the positive samples, 14 of

22 (63.6%) were from diarrhoeic cats, and 27 of 178 (15.2%) were non-diarrhoeic cats. The difference in isolation rate between the diarrhoeic cats or non-diarrhoeic cats was statistically significant ( $P < 0.001$ ,  $\chi^2: 28.22$ ). Also, in total 71 *Campylobacter* spp. suspicious isolates were recovered from the 41 positive samples obtained. Phenotypic tests and PCR revealed that 65 isolates (20 of them diarrhoeic and 45 non-diarrhoeic cat isolates) were identified as *C. jejuni*, whereas the six remaining isolates were positive at genus but negative at species level. The age, gender, non-diarrhoea and diarrhoea status of *C. jejuni* positive cats are detailed in Table 1. The differences in isolation rate in relation to sex ( $P > 0.05$ ,  $P = 0.064$ ,  $\chi^2: 3.432$ ) or age ( $P > 0.05$ ,  $P = 0.619$ ,  $\chi^2: 0.247$ ) in diarrhoeic and non-diarrhoeic cats were not statistically significant. In 19 cat samples, more than one subtype of *C. jejuni* was also identified from each sample according to ERIC-PCR results. It was included that two subtypes of *C. jejuni* isolates were simultaneously found in 14 cat samples (three of them were diarrhoeic and 11 of them were non-diarrhoeic cats) and three subtypes of *C. jejuni* isolates in five positive cat samples (two of them were diarrhoeic and three of them were non-diarrhoeic cats).

In terms of virulence genes, all the *C. jejuni* isolates harbored at least five virulence genes. All isolates were positive for the *cadF*, *cdtC* and *ceuE* genes, respectively. The *C. jejuni* isolates contained *iam*, *cadF*, *cdtA*, *flaA*, *ceuE*, *cdtC*, *cdtB* and *virB11*, at the rate of 19 (29.2%), 65 (100%), 64 (98.4%), 54 (83%), 65 (100%), 65 (100%), 64 (98.4%) and 22 (33.8%) respectively. Moreover, the *cdtA* and *cdtB* toxin genes were found in most of the isolates analyzed (Table 3). The *iam* gene was found in 7.7% and 21.5% of the diarrhoeic and non-diarrhoeic cat isolates, respectively. The *cadF*, *ceuE* and *cdtC* genes were found in 30.7% and 69.2% of diarrhoeic and non-diarrhoeic cat isolates, respectively. The *cdtA* and *cdtB* genes were found in 29.2% and 69.2% of the diarrhoeic and non-diarrhoeic cat isolates, respectively. The *flaA* gene was found in 26.1% and 56.9% of the diarrhoeic and non-diarrhoeic cat isolates, respectively. The *virB11* gene was found in 13.8% and 20% of the diarrhoeic and non-diarrhoeic cat isolates, respectively.

In two of the diarrhoeic and four of the non-diarhoeic cat isolates, all virulence genes were positive. In addition, the combination and distribution of virulence genes in diarrhoeic and non-diarhoeic cat isolates are presented in Table 4.

All *C. jejuni* isolates were susceptible to amoxicillin clavulanic acid. The highest resistances of the isolates were found as follows: 64 (98.4%), 63 (96.9%), 62 (95.3%) for ciprofloxacin (CIP), trimethoprim sulfamethoxazole (SXT), nalidixic acid (NA) antibiotics, respectively (Table 5). In 64 ciprofloxacin resistant isolates, 20 (30.7%) were diarrhoeic cat isolates and 44 (67.6%) were non-diarhoeic cat isolates. In 63 trimethoprim sulfamethoxazole resistant isolates, 20 (30.7%) were diarrhoeic cat isolates and 43 (66.1%) of them were non-diarhoeic cat isolates. In 62 nalidixic acid resistant isolates, 20 (30.7%) were diarrhoeic cat isolates and 42 (64.6%) of them were non-diarhoeic cat isolates (Table 5).

Multiple resistance of isolates was detected in the present study. Sixty-one (93.8%) out of 65 isolates were resistant to three or more antibiotics, where the highest resistance levels to antimicrobials three and seven were observed in 23.1% and 15.3% of the isolates, respectively. Thirteen point eight per cent were resistant to antimicrobials five and eight, 10.7% to antimicrobials four and six and 3.1% to antimicrobials nine and ten. In total, 15 isolates were resistant to three antimicrobials. Four of them were diarrhoeic and 11 of them were non-diarhoeic cat isolates. MDR levels are presented in Table 6 in terms of diarrhoeic and non-diarhoeic cat isolates. The lowest MDR levels were against 9 and 10 antibiotics. This was equally distributed to both diarrhea and non-diarrhea animals. In addition, the highest MDR levels were found to three antibiotics in 15 isolates (23.1%), including 4 and 11, from diarrhea and non-diarrhea cats, respectively.

Table 3. Virulence Gene Profiles of *C. jejuni* Isolates

iam	CadF		CdtA		FlaA		CeuE		CdtC		CdtB		virB11	
	DC	NDC	DC	NDC	DC	NDC	DC	NDC	DC	NDC	DC	NDC	DC	NDC
5 (7.7%)	20 (30.7%)	45 (69.2%)	19 (29.2%)	45 (69.2%)	17 (26.1%)	37 (56.9%)	20 (30.7%)	45 (69.2%)	20 (30.7%)	45 (69.2%)	19 (29.2%)	45 (69.2%)	9 (13.8%)	13 (20%)
19 (29.2%)	65 (100%)	64 (98.4%)	54 (83%)	65 (100%)	54 (83%)	65 (100%)	65 (100%)	65 (100%)	65 (100%)	64 (98.4%)	22 (33.8%)	22 (33.8%)		

DC: Diarrhoeic cat isolates; NDC: non-diarhoeic cat isolates

Table 4. The combination and distribution of Virulence Genes in Diarrhoeic and Non-diarhoeic Cat Isolates

N° of virulence genes combinations	Genes	DC	NDC
8	ABCDEFGH	2	4
7	BCDEFGH	5	7
7	ABCDEFG	2	8
7	ABCEFGH	-	1
6	BCDEFG	7	18
6	BCEFGH	1	1
6	ABCEFG	1	1
5	BCEFG	1	5
5	BDEFH	1	-

A: *iam*; B: *CadF*; C: *CdtA*; D: *FlaA*; E: *CeuE*; F: *CdtC*; G: *CdtB*; H: *VirB11*; DC: Number of diarrhoeic cat isolates; NDC: Number of non-diarhoeic cat isolates

Table 5. Antibiotic Resistance Profiles of *C. jejuni* Isolates

Antibiotics	S (%)			I (%)			R (%)		
	N	DC	NDC	N	DC	NDC	N	DC	NDC
Nalidixic acid (NA)	1 (1. 5)	-	1 (1. 5)	2 (3. 1)	-	2 (3. 1)	62 (95. 4)	20 (30. 7)	42 (64. 6)
Erythromycin ( E )	64 (98. 4)	64 (98. 4)	-	-	-	-	1 (1. 5)	1 (1. 5)	
Doxycycline (DO)	43 (66. 1)	15 (23. 1)	28 (43. 1)	12 (18. 5)	2 (3. 1)	10 (15. 3)	10 (15. 3)	5 (7. 7)	5 (7. 7)
Amoxicillin clavulanic acid (AMC)	65 (100)	20 (30. 7)	45 (69. 2)	-	-	-	-	-	-
Trimethoprim sulfamethoxazole (SXT)	2 (3. 1)	-	2 (3. 1)	-	-	-	63 (96. 9)	20 (30. 7)	43 (66. 1)
Ciprofloxacin (CIP)	1 (1. 5)	-	1 (1. 5)	-	-	-	64 (98. 4)	20 (30. 7)	44 (67. 6)
Ampicillin ( AMP)	28 (43. 1)	9 (13. 8)	19 (29. 2)	-	-	-	37 (56. 9)	12 (18. 5)	25 (38. 5)
Enrofloxacin (ENR)	5 (7. 7)	-	5 (7. 7)	29 (44. 7)	13 (20)	16 (24. 6)	31 (47. 7)	7 (10. 7)	24 (36. 9)
Clindamycin (DA)	63 (96. 9)	20 (30. 7)	43 (66. 1)	2 (3. 1)	-	2 (3. 1)	-	-	-
Tetracycline (TE)	18 (27. 7)	5 (7. 7)	13 (20)	5 (7. 7)	2 (3. 1)	3 (4. 6)	42 (64. 6)	13 (20)	29 (44. 7)
Piperacillin tazobactam (TZP)	36 (55. 4)	10 (15. 3)	26 (40)	16 (24. 6)	7 (10. 7)	9 (13. 8)	13 (20)	4 (6. 2)	9 (13. 8)
Cefotaxime (CTX)	43 (66. 1)	14 (21. 5)	29 (44. 7)	11 (16. 9)	4 (6. 2)	7 (10. 7)	11 (16. 9)	3 (4. 6)	8 (12. 3)
Streptomycin (S)	46 (70. 8)	14 (21. 5)	32 (49. 2)	1 (1. 5)		1 (1. 5)	18 (27. 7)	7 (10. 7)	11 (16. 9)
Amikacin (AK)	64 (98. 4)	20 (30. 7)	44 (67. 6)	-		1 (1. 5)	-	-	
Gentamicin (CN)	64 (98. 4)	20 (30. 7)	44 (67. 6)	-	-		1 (1. 5)		1 (1. 5)
Azythromycine	64 (98. 4)	20 (30. 7)	44 (67. 6)	-	-		1 (1. 5)		1 (1. 5)

S - Susceptible; I - Intermediar; R - Resistant; N: Number of isolates; DC - Diarrhoeic cat isolates; NDC: - Non-diarhoeic cat isolates.

Table 6. Multidrug Resistance Profiles of *C. jejuni* Isolates

Antibiotic numbers	Antibiotic Combinations	N	DC	NDC	%
3	NA, SXT, CIP	13	4	9	23.1
	SXT, CIP, AMP	2	-	2	
4	NA, SXT, CIP, AMP	2	2		10.7
	NA, SXT, CIP, TE	2		2	
	NA, SXT, CIP, CTX	1	-	1	
	NA, SXT, CIP, S	2	2	-	
5	NA, SXT, CIP, TE, CTX	2	2	-	13.8
	NA, SXT, CIP, ENR, TE	3	1	2	
	NA, SXT, CIP, AMP, TE	4	2	2	
6	NA, SXT, CIP, AMP, ENR, CTX	1		1	10.7
	NA, SXT, CIP, AMP, ENR, TE	2	-	2	
	NA, DO, SXT, CIP, ENR, TE	2	-	2	
	NA, SXT, CIP, AMP, TE, S	1	1	-	
	NA, SXT, CIP, ENR, TE, TZP	1	-	1	
7	NA, SXT, CIP, AMP, ENR, TE, S	5	-	5	15.3
	NA, SXT, CIP, AMP, ENR, TE, TZP	3	-	3	
	NA, E, SXT, CIP, AMP, TE, AZM	1	1	-	
	NA, DO, SXT, CIP, AMP, ENR, TE	1	-	1	
8	NA, DO, SXT, CIP, AMP, ENR, TE, TZP	1	1	-	13.8
	NA, SXT, CIP, AMP, ENR, TE, CTX, S	2	-	2	
	NA, SXT, CIP, AMP, ENR, TE, TZP, S	1	1	-	
	NA, DO, SXT, CIP, AMP, ENR, TE, CN	1	1	-	
	NA, SXT, CIP, AMP, ENR, TE, TZP, CTX	2	-	2	
	NA, DO, SXT, CIP, AMP, ENR, TE, S	2	1	1	
9	NA, DO, SXT, CIP, AMP, ENR, TE, TZP, S	1	1	-	3.1
	NA, SXT, CIP, AMP, ENR, TE, TZP, CTX, S	1	-	1	
10	NA, DO, SXT, CIP, AMP, ENR, TE, TZP, CTX, S	2	1	1	3.1

N - Number of resistance patterns of isolates; DC - number of diarrhoeic cat isolates; NDC - number of non-diarrhoeic cat isolates

## Discussion

In the present study, the prevalence of *Campylobacter* spp. was 20.5% in the cats examined. The frequency of isolation of *Campylobacter* spp. in cats ranges from 8% to 47.8% globally (SANDBERG et al., 2002; ENGVALL et al., 2003; ACKE et al., 2009a; SALIHU et al., 2010).

Similar to our study, ABAY et al. (2014), SALIHU et al. (2010) and SANDBERG et al. (2002), found prevalences of *Campylobacter* spp. of 26.7%, 18.3%

and 18% in cats, respectively. However, LAZOU et al. (2017), SZCZEPANSKA et al. (2017) and ANDRZEJEWSKA et al. (2013) found relatively low levels of campylobacters at 15%, 12.1%, 10.5% and 9.8% from cats, respectively. The differences in the isolation and identification methods (filtration, using different media, etc.), sample, regional and geographical varieties could be the causes of the low level results. In a study by BAKER et al.



(1999) in Southern Australia, the filtration method was used for isolation and samples were of different origins. LAZOU et al. (2017), conducted a study in Greece and used mCCD agar and Karmali agar for isolation. In ANDRZEJEWSKA et al. (2013) samples were collected from Poland and were initially inoculated to Preston broth. Later, CCD agar was used for isolation of campylobacters.

Relatively higher results than ours were reported by ACKE et al. (2006; 2009b), who reported *Campylobacter* species in 75% and 42.9% from cat fecal swab samples. In addition, ACKE et al. (2009a) isolated *Campylobacter* spp. from both healthy and diarrhoeic animals at a rate of 45.2% in dogs and cats in Ireland.

In terms of the concurrent presence of *C. jejuni*, in the study, different *C. jejuni* isolates were simultaneously found in 19 (46%) cats, similar to SHEN et al. (2001) who reported that 16 (34%) of 47 *Campylobacter* spp. samples were positive for more than one species of *Campylobacter* spp. in cat samples. Also, BANG et al. (2001) detected that three chickens with two different *C. jejuni* isolates, and one chicken carrying a *C. jejuni* and a *C. coli* isolate concurrently in Denmark. Also, multiple *Campylobacter* species were found in 6 (26%) of 23 positive stool samples in dogs (KOENE et al., 2004). In another study conducted by RICHARDSON et al. (2001), in 7.5% of patients, the presence of concomitant infection with two different types of *C. jejuni* was determined by molecular typing methods, in humans. According to our literature review, this study may be the first report of the simultaneous presence of different *C. jejuni* strains in cats in Turkey. Our data clearly demonstrated that the synchronous existence of different *C. jejuni* strains is quite frequent in cats. It might be of utmost importance for epidemiological studies that different strains are found within a single species, including the commensal presence, for finding the sources of infections, and studies on the pathogenicity of *Campylobacter* spp. in cats. Moreover, cases of infection with subtypes of one species or more than one species are thought to be important in terms of both the antibacterial susceptibility and the epidemiology of human diseases (KAYMAN et al., 2015).

In our study, there was a statistically significant difference in isolation rate between non-diarrhoeic or diarrhoeic cats ( $P < 0.05$ ), but no statistically significant difference between sex ( $P > 0.05$ ) and age status in the cats used as subjects ( $P > 0.05$ ). However, previous studies have reported difference in relation to age and gender in diarrhoeic and healthy animals (BAKER et al., 1999; SANDBERG et al., 2002; ENGVALL et al., 2003).

Since *Campylobacter* spp. (including *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis*) is naturally found in the intestinal biota of dogs and cats, cats and dogs exhibit a significant hazard for human campylobacteriosis (OLSON and SANDSTEDT, 1987; NEWTON et al., 1988). *Campylobacter* spp. might be a primary or secondary pathogen which is related to other predisposing factors, including viral infections or opportunistic agents in pets with other disorders (ACKE et al., 2009a).

In the present study, the *cadF*, *cdtC* and *ceuE* genes were found in all *C. jejuni* isolates recovered from the cats. Moreover, in this study, all isolates contained at least five virulence genes. Similar results were described by previous studies of animals, and they reported that a high ratio of *flaA*, *cadF* and *cdtB* genes was found in the analyzed isolates from domestic animals (ANDRZEJEWSKA et al., 2011; KRUTKIEWICZ and KLIMUSZKO, 2010; ANDRZEJEWSKA et al., 2013). Also, RODRIGUES et al. (2015), among the strains of *C. jejuni* isolates detected in dogs, found that two samples (*flaA*, *cadF* and *ciaB*, or *flaA*, *pldA* and *cadF*) and two isolates contained the *flaA* gene or *cadF* and *ciaB* genes, while two isolates contained only one virulence gene (*cadF* or *flaA*). BISWAS et al. (2011), in *C. jejuni* isolates from cattle and humans, 67% of the isolates contained all of the genes except *virB11*. The *cadF* gene was found in 100% of the isolates tested.

The present study on the antimicrobial susceptibility of strains isolated from cats confirmed a high level of amoxicillin clavulanic acid. The highest level of resistance was to ciprofloxacin (CIP), trimethoprim sulfamethoxazole (SXT), nalidixic acid (NA), and streptomycin (S). Similar results were described in a study conducted by ANDRZEJEWSKA et al. (2013), SANDBERG

et al. (2002) and SZCZEPANSKA et al. (2017). Due to the use of antimicrobial agents in veterinary practice, resistant *Campylobacter* isolates from pets and humans have been revealed (GUPTA et al., 2004; ENGBERG et al., 2004). Also, a study conducted in India on the prevalence of antibiotic resistance in dogs showed that isolates had a high rate of resistance (97.3%) to cefotaxim, chloramphenicol, floxacın, ciprofloxacin, cefaclor, nitrofurazone, norfloxacin, gentamicin, amikacin and enrofloxacin (KULKARNI et al., 2002). The multiple resistance of isolates was detected in 61 (93.8%) out of 65 isolates that were resistant to three or more antibiotics, whereas resistance to three and seven antimicrobials was observed in 23.1% and 15.3% of the isolates, respectively (Table 3). Our results were relatively compatible with those of SZCZEPANSKA et al. (2017) and GONI et al. (2018), who detected multidrug resistance rates of 40% and 50% in *Campylobacter* spp. isolates, respectively. In addition MDR was also reported by KUMAR et al. (2012).

### Conclusion

In conclusion, the data obtained in this study reveal that the high prevalence of virulence-associated genes among *C. jejuni* isolates obtained from cats suggests their crucial role in the pathogenesis of campylobacteriosis infection among cats, and their close contact with humans.

Moreover, the presence of multidrug resistant *C. jejuni* isolates indicates a potential transmission risk from cats to humans.

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### Conflicts of interest

The authors declare no conflict of interest.

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#### **SAŽETAK**

Svakodnevni kontakt s mačkama znatan je čimbenik rizika za kampilobakteriozu u ljudi. Glavni je cilj ovoga rada bio istražiti gene virulencije i antibiotsku rezistenciju bakterije *C. jejuni* izolirane iz izmeta mačaka dovedenih na Erciyes University, Faculty of Veterinary Medicine, Training and Research Hospital Clinics. Izmet je uzet od 200 mačaka (116 ženki i 84 mužjaka) različitih pasmina i dobi (1 mjesec do 9 godina), od svibnja 2017. do travnja 2018. Izolati *Campylobacter* spp. identificirani su na razini roda i vrste primjenom genski specifičnog multipleks PCR-a (mPCR) kojim je ustanovljena prisutnost gena *iam*, *cadF*, *cdtA*, *flaA*, *ceuE*, *cdtC*, *cdtB* i *virB11*. Antimikrobna rezistencija i otpornost na širok spektar lijekova (MDR) izolata određeni su disk-difuzijskim testom. Primjenom *Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction* (ERIC-PCR) otkriveni su genski srodnici izolata. U ovom je istraživanju 41 (20,5 %) od 200 uzoraka izmeta bio pozitivan na *Campylobacter* spp. Među pozitivnim uzorcima njih 14 od 22 (63,6 %) potjecalo je od mačaka koje su imale proljev, a 27 od 178 (15,1 %) uzoraka bilo je od mačaka koje nisu imale proljev. Fenotipski testovi i PCR pokazali su da je 65 izolata (20 od njih potjecalo je od mačaka koje su imale proljev i 45 od mačaka koje nisu imale proljev) identificirano kao *C. jejuni*. Razlike u broju izolata s obzirom na spol i dob nisu bile statistički znakovite ( $P > 0,05$ ). Svi izolati *C. jejuni* imali su barem pet gena virulencije i bili pozitivni na gene *cadF*, *cdtC* i *ceuE*. Gen *iam* sadržavalo je 19 izolata *C. jejuni* (29,2 %), gen *cadF* 65 izolata (100 %), gen *cdtA* 64 izolata (98,4 %), gen *flaA* 54 izolata (83 %), gen *ceuE* 65 izolata (100 %), gen *cdtC* 65 izolata (100 %), gen *cdtB* 64 izolata (98,4 %) i gen *virB11* 22 izolata (33,8 %). Geni toksina *cdtA* i *cdtB* pronađeni su u većini analiziranih izolata. Svi su izolati *C. jejuni* bili osjetljivi na amoksicilin-klavulansku kiselinu. Najveća rezistencija bila je: 64 izolata (98,4 %) na ciprofloksacin (CIP), 63 izolata (96,9 %) na trimetoprim-sulfametoksazol (SXT) i 62 izolata (95,3 %) na nalidiksičnu kiselinu (NA). U ovom je istraživanju ustanovljena višestruka rezistencija izolata. 61 od 65 izolata (93,8 %) bilo je otporno na tri ili više antibiotika, a najveća je otpornost utvrđena na tri antimikrobna lijeka u 23,1 % izolata i na sedam antimikrobnih lijekova u 15,3 % izolata. Istraživanjem je ustanovljena otpornost izolata *C. jejuni* na većinu antibiotika koji se trenutačno primjenjuju te iznimna svojstva virulencije u mačaka, koja mogu imati određeni rizik za javno zdravstvo.

**Ključne riječi:** *Campylobacter jejuni*; virulencija; antibiotska rezistencija; ERIC-PCR

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