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Isolation and Molecular Characterization of Thermophilic *Campylobacter* spp. in Dogs and Cats [1]

Özkan ASLANTAŞ [1,a]

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[1] Department of Microbiology, Faculty of Veterinary Medicine, Hatay Mustafa Kemal University, TR-31060 Hatay - TURKEY
[a] ORCID: 0000-0003-0407-8633

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Abstract

This study aimed to evaluate the occurence, virulence properties, genetic diversity, antimicrobial susceptibilities and genetic determinants of resistance of thermophilic *Campylobacters* from dogs and cats under different housing conditions. Rectal swabs were taken from 136 dogs (household dogs, n=56; shelter-housed dogs, n=80), and 14 shelter-housed cats. Antimicrobial susceptibilities of the isolates were performed by disc diffusion method. Tetracycline *(tet*O), ampicillin *(bla$_{OXA}$-61),* aminoglycoside (*aph-3-1)* resistance and multidrug efflux pump *(cme*B) genes were investigated by PCR. The genetic diversity among the isolates was determined by sequence analysis of short variable regions (SVRs) of *fla*A gene. The presence of virulence and toxin genes was also investigated by PCR. *Campylobacter* were isolated from 33.8% of dogs and 28.6% of cats. *C. jejuni* was the most common species in both dogs (52.2%) and cats (100%), followed by *C. coli,* which was isolated from 41.3% of dogs. High rates of resistance against nalidixic acid (78.7%), ciprofloxacin (74.5%), ampicillin (68.1%), tetracycline (53.2%) were observed. The frequency of *fla*A, *vir*B11, *cdt*A, *cdt*B, *cdt*C, *rac*R, *cad*F, *cia*B, *dnaJ* and *pld*A genes was 100%, 2.1%, 83%, 72.3%, 72.3%, 57.4%, 93.6%, 12.8%, 53.2% and 44.7%, respectively. Based on *fla*A-SVR typing, 17 different alleles were determined among the isolates. The results of this study suggested that pet animals were colonized with antimicrobial resistant thermophilic *Campylobacter* spp. having high pathogenic potential and genetic diversity.

Keywords: *Thermophilic campylobacter, Antimicrobial resistance, Virulence, flaA-SVR typing*

Köpek ve Kedilerden Thermofilik *Campylobacter* İzolasyonu ve Moleküler Karakterizasyonu

Öz

Bu çalışmada, farklı koşullarda barındırılan köpekler ve kedilerde termofilik *Campylobacter* türlerinin varlığı, virülans özellikleri, genetik çeşitliliği, antimikrobiyal duyarlılık ve direnç genlerinin belirlenmesi amaçlandı. Rektal svab örnekleri 136 köpekten (sahipli, n=56; barınak, n=80) ve 14 barınak kedisinden alındı. İzolatların antimikrobiyallere olan duyarlılıkları disk difüzyon metodu ile belirlendi. Tetrasiklin *(tet*O), ampisilin *(bla$_{OXA}$-61)*, aminoglikozid *(aph-3-1)* direnç ve multidrug efluks pompası *(cme*B) genleri PZR ile araştırıldı. İzolatlar arasındaki genetik farklılık, *fla*A geninin kısa değişken bölgelerinin (SVR'ler) dizi analizi ile belirlendi. İzolatlar arasında virülans ve toksin geninin varlığı ise PZR ile araştırıldı. *Campylobacter*, köpeklerin %33.8'inden ve kedilerin %28.6'sından izole edildi. *C. jejuni* hem köpeklerde (%52.2) hem de kedilerde (%100) en sık izole edilen tür iken; *C. coli* sadece köpeklerin %41.3'ünden izole edildi. Nalidiksik asit (%78.7), siprofloksasin (%74.5), ampisilin (%68.1) ve tetrasikline (%53.2) karşı yüksek oranlarda direnç gözlendi. *fla*A, *vir*B11, *cdt*A, *cdt*B, *cdt*C, *rac*R, *cad*F, *cia*B, *dnaJ* ve *pld*A virulens genleri sırasıyla %100, %2.1, %83, %72.3, %72.3, %57.4, %93.6, %12.8, %53.2 ve %44.7 oranlarında saptandı. *fla*A-SVR tiplendirme metodu ile *C. jejuni* ve *C. coli* izolatları arasında 17 farklı allel belirlendi. Bu çalışmanın sonuçları, pet hayvanlarının antimikrobiyal dirençli, yüksek patojenik potansiyele ve genetik çeşitliliğe sahip termofilik *Campylobacter* ile kolonize olduğunu göstermektedir.

Anahtar sözcükler: *Termofilik campylobacter, Antimikrobiyal direnç, Virulens, flaA-SVR tiplendirme*

INTRODUCTION

Campylobacter spp. are among the most frequently reported causes of foodborne gastroenteritis in the world. The vast majority of human infections were attributed to consumption of contaminated poultry meat [1]. However, repeated contact with dogs and cats has also been identified as an important source of *Campylobacter* infection to their

İletişim (Correspondence)
+90 3262458545/1523 Fax: +90 326 2455704
ozkanaslantas@yahoo.com

owners [2-4], and human infections originating from pet animals have been reported [5]. Dogs and cats are mostly subclinical hosts of *Campylobacter* spp., infected mainly by *C. helveticus*, *C. upsaliensis*, *C. jejuni* and *C. coli* [6,7]. However, gastroenteritis cases related with these agents have also been reported in dogs and cats [4]. The most of *Campylobacter* infections are self-limited and do not require antimicrobial treatment, however, in severe cases, fluoroquinolones (FQ) and macrolides are drug of choise used for the treatment of clinical campylobacteriosis. However, increasing prevalence of antibiotic-resistant *Campylobacter* from various sources such as humans, animals and food, especially FQ, became as serious threat to public health [8-10].

Campylobacter produce a number of virulence factors playing important role in their pathogenesis. The factors involved in pathogenesis of *Campylobacter* include flagella mediated motility, chemotaxis, adhesion to intestinal mucosa, invasion, translocation and production of toxin and secreted proteins [11].

Many molecular methods have been developed to investigate the diversity within *C. jejuni* and *C. coli* isolates. Each molecular method has advantages and disadvantages to determine the genetic relatedness of the *Campylobacter* isolates [12]. Of these methods, sequence analysis of short variable regions (SVRs) of *fla*A gene is widely used method for genotyping of *Campylobacter* isolates [13,14]. This method was reported as one of the effective and reliable methods for typing of *Campylobacter* spp. and has discriminatory power comparable to Pulsed Field Gel Electrophoresis (PFGE) and Multilocus Sequence Typing (MLST) [15]. In addition, the depositions of the *fla*A-SVR nucleotide alleles in a central web site (http://pubmlst.org/) make access to the *fla*A-SVR allele types of *Campylobacter* spp. possible.

The studies on the occurence of *Campylobacter* spp. in pets in Turkey are scarce, and have mainly focused on poultry [9,16,17]. Investigation of the prevalence and other characteristics of *Campylobacter* in cats and dogs is an important step to assess their role as a potential source of human infections. Therefore, the present study aimed (i) to determine the prevalence and antimicrobial susceptibilities of thermophilic *Campylobacter* spp. in stray and household pets and its resistance mechanisms, (ii) to investigate genetic diversity of *C. coli* and *C. jejuni* isolates using *fla*A-SVR sequence-based typing and (iii) to determine the presence and frequency of these virulence genes.

MATERIAL and METHODS

Ethical Statement

The study was approved by Mustafa Kemal University Animal Ethic Committee (2016-2/3).

Study Area and Sample Collection

From March 2016 to June 2016, individual rectal swab specimens were taken from owned household pets (dogs=56), unowned pets (dogs, n=80; cats, n=14). Unowned pets were housed at Hatay Metropolitan Municipality Kennel. Age and sex distribution of dogs and cats were recorded during the sampling. Age proportion of male/female dogs and cats were 63/73 and 9/5, respectively. Immediately after sampling, the swabs were placed in Amies Transport Medium with charcoal (LP Italiana, 11898, Italy) and transported to the laboratory and processed immediately upon arrival.

Isolation of Campylobacter spp.

The rectal swabs were directly streaked on modified charcoal cefoperazone deoxycholate agar (mCCDA), containing CCDA selective supplement for primary isolation. The plates were incubated at 41.5ºC for 36-48 h under microaerophilic conditions. One presumptive colony from each mCCDA plate was subcultured onto blood agar supplemented with 5% defibrinated sheep blood. The isolates, microscopically curved Gram negative rods with characteristic seagull-winged morphology, catalase and oxidase positive were accepted as *Campylobacter* spp. and stored within cryobeads in deep freeze (-80ºC) untill use.

DNA Extraction and PCR Analysis for Identification of Genus/Species Level

Chromosomal DNA was obtained by boiling method as previously described Wang et al.[18]. Briefly, one colony was suspended in 200 µL RNase and DNase free water and heated at 100ºC for 10 min and centrifuged at 10.000 g for 10 min. Supernatant was transferred to another steril eppendorf tube and used as template DNA.

For genus confirmation and species determination, a multiplex polymerase chain reaction (mPCR) assay targeting *Campylobacter* genus, *C. jejuni* and *C. coli* was performed using primers and reaction conditions described by Wang et al.[18].

Antimicrobial Susceptibility Testing

Antimicrobial susceptibilities of the isolates were determined by disc diffusion method according to Clinical Laboratory Standards Institute (CLSI, 2008) guidelines [19]. Following antimicrobial discs were used: nalidixic acid (NA, 30 µg), ciprofloxacin (CIP, 5 µg), ampicillin (AM, 10 µg), tetracycline (TE, 30 µg), chloramphenicol (C, 30 µg), gentamicin (CN, 10 µg), and erythromycin (E, 15 µg). *C jejuni* (NCTC 12500) and *C. coli* (NCTC 12525) were used as control strains for antimicrobial susceptibility testing.

Detection of Antimicrobial Resistance Genes

All *Campylobacter* spp. were tested for the presence of *tet*O (tetracycline), *aph-3-1* (aminoglycoside), bla_{OXA-61} (ampicillin)

and *cme*B (multi-drug efflux pump) genes by mPCR as previously reported by Obeng et al.[20].

Genotyping by flaA-SVR

PCR amplification of a fragments of 641 bp of the *fla*A gene comprising the SVRs were performed following the procedures described by Lévesque et al.[21]. The *fla*A types were determined by comparing the nucleotide sequences with those in the PubMLST *Campylobacter* database (http://pubmlst.org/campylobacter/).

Detection of Virulence Genes

Presence of putative virulence genes responsible for adhesion, colonization, invasion and toxin production were investigated by PCR as previously described by Bang et al.[22], Konkel et al.[23], Bacon et al.[24], Datta et al.[25], and Nachamkin et al.[26].

Statistical Analysis

Differences in frequencies of isolation rates according to age groups and genders were evaluated using Pearson's chi-square test. SPSS 14.01 was used for statistical analysis. Any P value equal to/or less than <0.05 was accepted as statistically significant.

RESULTS

Overall, 33% (50/150) of the samples tested were positive for *Campylobacter* spp. including 33.8% (46/136) of dog samples and 28.6% (4/14) of cat samples. Of the *Campylobacter* isolates, 56% (28/50) were identified as *C. jejuni*, and 38% (19/50) were determined to be *C. coli* by PCR. The remaining three isolates (6%) were different than *C. jejuni* and *C. coli* and were not characterized further to species level.

The results of antimicrobial susceptibilities of *Campylobacter* isolates are given in *Table 1*. *C. jejuni* isolates from dogs showed high resistance rate to nalidixic acid (79.2%), ciprofloxacin (75%), tetracycline (66.7%) and ampicillin (62.5%), while low resistance were observed to erythromycin (12.5%), gentamicin (12.5%) and chloramphenicol (4.2%). Considering *C. coli* isolates from dogs, similarly high resistance rates to nalidixic acid (78.9%), ciprofloxacin (73.7%), ampicillin (68.4%) and tetracycline (31.6%), but low resistance rates to erythromycin (21.1%), gentamicin (15.8%) and chloramphenicol (5.3%) were recorded. *C. jejuni* isolates from cats were resistant to ampicillin (100%), nalidixic acid (75%), ciprofloxacin (75%), tetracycline (75%), gentamicin (25%), and erythromycin (25%), except chloramphenicol.

Multi drug resistance (MDR) was frequently observed in *C. jejuni* and *C. coli* isolates. The most common multidrug pattern detected among *C. jejuni* isolates was nalidixic acid, ciprofloxacin, ampicillin and tetracycline, which was observed in 35.7% of the isolates, whereas the most common MDR pattern among *C. coli* isolates was nalidixic acid, ciprofloxacin and ampicillin, which was observed in 26.3% of *C. coli* isolates *(Table 2)*.

Of the 19 tetracycline resistant *C. jejuni* isolates, 16 carried *tet*O, and two *C. jejuni* isolates, despite carrying *tet*O gene, were susceptible to tetracycline. *aph-3-1* gene was detected in one phenotypically resistant *C. coli* isolates. Among the ampicillin resistant 19 *C. jejuni* isolates, 14 were found to carry $bla_{\text{OXA-61}}$. While $bla_{\text{OXA-61}}$ was found in 8 ampicillin resistant *C. coli* isolates, one isolate that harbored $bla_{\text{OXA-61}}$ was susceptible to ampicillin. Four of the ampicillin resistant isolates did not carry $bla_{\text{OXA-61}}$. *cme*B gene was only detected in *C. coli* (89.5%, 17/19) isolates *(Table 3) (Fig. 1)*.

The results of *fla*A-SVR sequence typing of the 28 *C. jejuni* and 19 *C. coli* isolates are given in *Table 4*. Among *C. jejuni* isolates, nine alleles were detected. In *C. coli* isolates, eight alleles were identified. Two *fla*A alleles (alleles 23 and 120) were identical in both *C. jejuni* and *C. coli* isolates.

The frequency of virulence genes detected in the isolates is given in *Table 5*. Among 28 *C. jejuni* isolates, 12 virulence associated gene profile was detected. Whereas 10 virulence associated gene profiles were detected among 19 *C. coli* isolates. The frequency of *fla*A, *vir*B11, *cdt*A, *cdt*B, *cdt*C,

Table 1. *Antimicrobial resistance of C. jejuni and C. coli isolates from dogs and cats*

Antimicrobial	Cats	Shelter Dogs		Household Dogs	
	***C. jejuni* (n=4)**	***C. jejuni* (n=18)**	***C. coli* (n=15)**	***C. jejuni* (n=6)**	***C. coli* (n=4)**
Nalidixic Acid	3 (75)	14 (77.8)	13 (6.7)	5 (83.3)	2 (50)
Ciprofloxcin	3 (75)	13 (72.2)	11 (73.3)	5 (83.3)	3 (75)
Ampicillin	4 (100)	13 (72.2)	9 (60)	2 (33.3)	4 (100)
Tetracycline	3 (75)	11 (61.1)	5 (33.3)	5 (83.3)	1 (25)
Chlorampenicol	0 (0)	1 (5.6)	1 (6.7)	0 (0)	0 (0)
Gentamicin	1 (25)	2 (11.1)	2 (13.3)	1 (16.7)	1 (25)
Erythromycin	1 (25)	3 (16.7)	2 (13.3)	0 (0)	2 (50)

Table 2. *Multidrug resistance patterns determined among C. jejuni and C. coli isolates from dogs and cats*

Resistance Profile	Shelter			Household	
	Dogs		Cats	Dogs	
	***C. jejuni* (n=18)**	***C. coli* (n=15)**	***C. jejuni* (n=4)**	***C. jejuni* (n=6)**	***C. coli* (n=4)**
NA, CIP, AM, TE, CN, E	1	1	1	-	-
NA, CIP, AM, CN, E	-	-	-	-	1
AM, TE, CN, C, E	1	1	-	-	-
NA, CIP, TE, CN	-	-	-	1	-
NA, CIP, AM, TE	6	-	2	2	1
NA, CIP, TE	1	2	-	2	-
NA, CIP, AM	3	5	-	-	1
NA, AM, TE	1	1	-	-	-
NA, CIP	1	4	1	-	-
AM, E	1	-	-	-	1
NA	1	1	-	-	-
TE	1	-	-	-	-
Susceptible	1	-	-	1	-

Table 3. *Distribution of resistance genes among the isolates*

Source	Species	Resistance Phenotype and the Occurence of Related Gene	No of The Isolates			
			***tet*O**	***bla*$_{OXA-61}$**	***aph-3-1***	***cme*B**
Household	*C. jejuni* (n=6)	Resistant with genes	5	3	-	-
		Resistant without genes	1	1	-	-
		Susceptible with genes	-	1	-	-
	C. coli (n=4)	Resistant with genes	1	2	1	4
		Resistant without genes	-	2	-	-
		Susceptible with genes	-	-	-	-
Shelter	*C. jejuni* (n=22)	Resistant with genes	11	11	-	-
		Resistant without genes	-	2	-	-
		Susceptible with genes	2	1	-	-
	C. coli (n=15)	Resistant with genes	3	6	-	13
		Resistant without genes	-	2	-	-
		Susceptible with genes	2	1	-	-

*rac*R, *cad*F, *cia*B, *dna*J and *pld*A was 100%, 2.1%, 83%, 72.3%, 72.3%, 57.4%, 93.6%, 12.8%, 53.2% and 44.7%, respectively.

DISCUSSION

The findings from this study revealed that 19.6% of household dogs, 43.8% of stray dogs and 28.6% of stray cats were colonized with *Campylobacter* spp. Such a high level resistance rates were not reported in earlier studies conducted in different countries. In Italy, Giacomelli et al.[27] reported a prevalence of *Campylobacter* spp. of 11% in household dogs, 26% in shelter dogs and shelter cats in Italy. Another study carried out in Italy, Gargiulo et al.[28] isolated *C. jejuni* with a prevalence rate of 16.8% in stray cats. In Taiwan, Tsai et al.[29] found that 2.7% of household dogs and 23.8% of stray dogs were positive for *Campylobacter* spp. In New Zealand, Bojanić et al.[30] reported prevalence of *Campylobacter* spp. in household dogs and cats as 36% and 16%, respectively. In Korea, Cho et al.[31] reported prevalence of thermophilic *Campylobacter* spp. in stray, breeding and household dogs as 25.2%, 12% and 8.8%, respectively. In Malaysia, Goni et al.[6] reported frequency of *Campylobacter* in stray dogs and cats as 16.3% and 32.6% respectively, while in household dogs and cats as 12.5% each. These findings clearly indicate that dogs and cats were imporant reservoirs of *Campylobacter* spp. in Turkey.

The species distribution of *Campylobacter* from dogs and cats differs considerably according to populations studied,

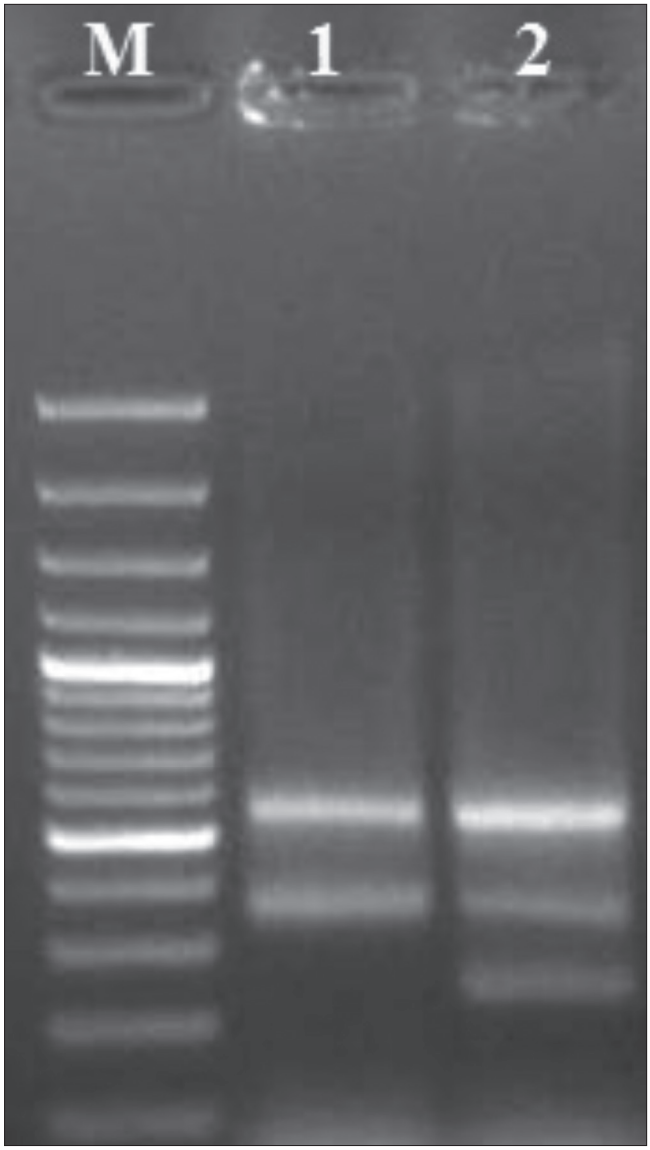

Fig. 1. Agarose gel electrophoresis showing antibiotic resistance genes. Lane M: 100 bp plus molecular marker, Lane 1: *tet*O (559 bp)-bla_{OXA-61}; Lane 2: *tet*O (559 bp)-bla_{OXA-61}-*cme*B (241 bp)

Table 4. *flaA alleles detected in C. jejuni and C. coli isolates*

Source	Animal	Species	*fla*A-SVR Allel	Number of the Isolates
Shelter	Dog	*C. jejuni*	120	4
			85	4
			82	4
			43	3
			80	1
			23	11
			41	1
	Cat	*C. jejuni*	41	1
			44	1
			80	1
			82	1
		C. coli	90	5
			51	3
			62	2
			84	1
			120	1
			23	1
			61	1
			118	1
Household	Dog	*C. jejuni*	82	2
			36	1
			23	1
			41	1
			43	1
		C. coli	118	2
			51	1
			90	1

isolation protocols, region, fastidious character of the agent and years [6,32]. In different studies, *C. upsaliensis* [6,30,33] and *C. jejuni* [27,29] have been reported to be most prevalent species in dogs and cats. In this study, *C. jejuni* was found as the most prevalent species among *Campylobacter*-positive dogs and cats. This is an important finding from public health point of view, since *C. jejuni* is the species most frequently associated with human gastroenteritis [34].

Housing conditions were defined as a risk factor for *Campylobacter* infection in dogs and cats. Unsanitary conditions observed in shelters may increase the spread of infection among sheltered dogs [27]. Acke et al.[35] reported that close contact between shelter-housed animals facilitates infection transmission. Humprey [36] suggested that animals under stresfull conditions could produce noradrenaline, leading to increased susceptibility to infectious agents. Abovementioned factors could explain the higher prevalence of *Campylobacter* infection in shelter-housed dogs compared to household dogs.

The ages of pets animals have been reported to be a risk factor and association between age and *Campylobacter* carriage [6,37]. Similarly, in this study, significantly higher carriage rate found in younger dogs compared with older dogs (P<0.0001). However, a conradictory finding reported by Rahimi et al.[3], who found no significant influence of the age of dogs and cats on *Campylobacter* infection.

Rising trend of antimicrobial resistance have been observed in *Campylobacter* isolates [38,39]. FQs (danofloxacin and enrofloxacin) are frequently used drugs in veterinary field for the treatment and control of infectious diseases of pets and food-producing animals in Turkey. In this study, high resistance rate was determined against ciprofloxacin (73.7% in *C. coli* and 76.9% in *C. jejuni*). Higher resistance rate to ciprofloxacine is highly important, because the FQs are drug of choise for the treatment of *Campylobacter*

Table 5. *The frequency of virulence genes detected among the isolates*

Virulence Gene Patterns	No of The Isolates	Species	
		C. jejuni	*C. coli*
*fla*A, *dna*J, *cad*F, *pld*A, *rac*R, *cdt*A, *cdt*B, *cdt*C, *vir*B11	1	1	-
*fla*A, *dna*J, *cad*F, *pld*A, *rac*R, *cdt*A, *cdt*B, *cdt*C, *cia*B	3	2	1
*fla*A, *dna*J, *cad*F, *pld*A, *rac*R, *cdt*A, *cdt*B, *cdt*C	13	10	3
*fla*A, dnaJ, *cad*F, *pld*A, *rac*R, *cdt*A, *cdt*C, *cia*B	1	1	-
*fla*A, *dna*J, *cadF*, *racR*, *cdt*A, *cdt*B, *cdt*C, *cia*B	2	1	1
*fla*A, *dna*J, *cad*F, *rac*R, *cdt*A, *cdt*B, *cdt*C	5	4	1
*fla*A, *cad*F, *pld*A, *rac*R, *cdt*A, *cdt*B, *cdt*C	2	1	1
*fla*A, *cad*F, *rac*R, *cdt*A, *cdt*B, *cdt*C	1	1	-
*fla*A, *dna*J, *cad*F, *cdt*A, *cdt*B, *cdt*C	1	-	1
*fla*A, *cad*F, *cdt*A, *cdt*B, *cdt*C	6	1	5
*fla*A, *cad*F, *cdt*A, *cdt*B, *cdt*C	3	-	3
*fla*A, *cad*F, *cdt*A	4	4	-
*fla*A, *cad*F, *cdt*C	2	-	2
*fla*A, *cad*F, *pld*A	1	1	-
*fla*A, *cdt*C	2	1	1

infections. These findings are almost similar to previous studies in broilers [9], chicken meat [8] and humans [10] in Turkey.

Resistance to ampicillin in *Campylobacters* are mainly due to synthesis of beta-lactamases, low affinty binding of the beta-lactams to the target [penicillin binding proteins (PBP)] or reduced permeability of outer membran porins [40]. In this study, high level ampicillin resistance observed in *Campylobacters* might be due to the wide-spread use of beta-lactams or combination of beta-lactams with other antimicrobials for the treatment of infections in pet animals. Besides, resistance to ampicillin in 68.8% (22/32) of the isolates were found to be associated with enzymatic inactivation by $bla_{\text{OXA-61}}$. Therefore, it should be noted that *Campylobacter* resistance to ampicillin is not only associated with enzymatic inactivation by $bla_{\text{OXA-61}}$, but also other resistance mechanisms mentioned above.

In this study, 20 out of 25 (80%) tetracycline-resistant isolates were found to possess *tet*O. The frequent detection of *tet*O in tetracycline-resistant isolates is also reported previous studies (Aslantaş, Obeng). However, *tet*O was also detected in four tetracycline-susceptible *Campylobacter* (two *C. jejuni* and two *C. coli*), and the gene was not detected in one tetracycline-resistant *C. coli* isolate. This finding is not surprising because similar findings have already been reported by some investigators [9,20]. Guévremont et al.[41] reported that *tet*O might be present in tetracycline resistant isolates but might be detected by primers used. Another study conducted by Abdi-Hachesoo et al.[42], presence of tetA gene was reported in some tetracycline resistant *Campylobacter* isolates.

In this study, low levels of resistane were observed for chloramphenicol (4.3%), gentamicin (14.9%), and eryhromycin (14.9%) in *Campylobacter* spp. isolates. These findings are also similar to previous studies carried out in Iran [3,38].

Several virulence factors have been documented for *Campylobacter* spp. contributing its pathogenicity. Of these virulence factors, *fla*A gene is necessary for colonization [43], which was detected in all *Campylobacter* isolates in present study. Similarly, Cho et al.[31] reported this gene in 100% of *C. jejuni* and *C. coli* isolates. Other virulence genes responsible for adherence and colonization *(cad*F, *rac*R and *dnaJ*) and invasion *(pld*A, *cia*B and *vir*B11) were found in at varying rates. Frequency of *cad*F, *rac*R, *dnaJ*, *pld*A and *cia*B genes in *C. jejuni* and *C. coli* isolates were 91.9%-94.7%, 75%-36.8%, 64.3%-31.6%, 57.126.3%, 14.3%-10.5%, respectively. In contrast, Cho et al.[31] reported higher prevalence rate for *rac*R, *dnaJ*, *cad*F, *pld*A and *cia*B genes in *C. jejuni* and *C. coli* isolates as 73.2%-0%, 100%-100%, 100%-100%, 78%-0%, and 73.2%-0%, respectively. The *vir*B11 gene was only detected in one (2.1%) *C. jejuni* isolate from shelter-housed of dog.

CDT is a bacterial protein toxin consisting of three subunits encoded by the *cdt*A, *cdt*B and *cdt*C genes that products of all three gene are required for functionally active toxin. The toxin exerts its effect by inhibiting transition of the cell from G-2 phase-mitosis [44]. Cho et al.[31] detected *cdt* genes in 100% of the isolates. However, the authors found that only some of these isolates show CDT production in the HEp-2 cell cytotoxin assay. Similar observation was also reported by Açık et al.[45]. Since cytotoxity assays are

influenced by *in vitro* factors such as repeated subcultures of isolates, cell types, therefore, it has been suggested that more sensitive methods should be applied to cytotoxity assays for accurate determination cytotoxic activity of isolates [31].

*fla*A gene-based typing methods have been used for genotyping of *Campylobacter* for a long time. Of these methods, *fla*A-SVR typing has been reported as reliable method, giving reliable and reproducible results comparable to PFGE analysis [46]. In this study, discriminatory power (DI) of *fla*A-SVR analysis for *C. jejuni* isolates were 0.845 and 0.8538 for *C. coli* isolates.

In conclusion, to the author's knowledge, the study is the first to investigate the occurence, antimicrobial susceptibility, virulence properties and *fla*A-SVR typing of *C. jejuni* and *C. coli* in dogs and cats under different housing conditions in Turkey. Regardless of their origin, dogs and cats was found a significant source of *Campylobacter* infection in humans. The high antimicrobial resistance to some antimicrobials, particularly FQ, is another striking finding, making treatment options of *Campylobacter* infections very limited. Therefore, continuous surveillance is needed to determine the emergence and dissemination of resistant *Campylobacter* in different origin. Occurence of high rate of virulence genes observed in this study indicate potential pathogenicity of the isolates. Given cohabitation of dogs and cats with humans, good hygiene paratices should be promoted, contact with stray pet animals should be reduced to minimise the risk of transmission.

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