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Research Article

# Genotypic, Antimicrobial Resistance and Virulence Profiles of Thermophilic *Campylobacter* Isolates in Broilers [1]

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## Abstract

The objective of this study was to investigate the prevalence, antimicrobial resistance and genetic determinants of resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from commercial broiler farms in Adana and Hatay provinces, Turkey. The assessment of the genetic diversity among the isolates was determined by *fla*A based RFLP-Polymerase Chain Reaction (PCR) with restriction enzyme *Dde*I and sequence analysis of short variable regions (SVRs) of *fla*A and *fla*B-SVR genes. Antimicrobial susceptibility of the isolates was performed by disk diffusion method and tetracycline *(tet*O), ampicillin *(bla*$_{OXA-61}$), aminoglycoside *(aph-3-1)* and multidrug efflux pump *(cme*B) resistance genes were investigated by PCR as well. The genes conferring resistance to ciprofloxacin was screened by PCR and following DNA sequencing. The presence of ten virulence *(fla*A, *vir*B11, *rac*R, *cad*F, *cia*B, *dnaJ* and *pld*A) and toxin genes *(cdt*A, *dt*B, *cdt*C) among the isolates was also investigated using PCR. Out of 220 cloacal swabs, 218 (99.1%) *Campylobacter* spp. including *C. jejuni* (n=194; 89%) and *C. coli* (n=24; 11%) were isolated. While all the isolates were susceptible to chloramphenicol, gentamicin and erythromycin, resistance rates for *C. jejuni* and *C. coli* isolates to nalidixic acid, ciprofloxacin, ampicillin, tetracycline and amoxicillin-clavulanic acid were determined as 86.6-100%, 86.6-100%, 45.9-45.8%, 43.3-50% and 2.6-0.0%, respectively. The *tet*O, *bla*$_{OXA-61}$, *cme*B and *aph-3-1* genes detected in *C. jejuni* and *C. coli* isolates were 45.3-54.2%, 36.1-75%, 1.5-83.3% and 0.5-0%, respectively. The prevalence of *fla*A, *cdt*A, *cdt*B, *cdt*C, *rac*R, *cad*F, *cia*B, *dnaJ* and *pld*A in *C. jejuni* and *C. coli* isolates was 100-100%, 95.4-100%, 94.3-100%, 89.7-54.2%, 89.2-79.2%, 92.8-100%, 67-16.7%, 85.6-75% and 80-66.7%, respectively. *vir*B11 gene was not detected in any of *Campylobacter* isolates. All *fla*A and *fla*B-SVR alleles displayed same PCR-RFLP patterns. The results of this study revealed high prevalence, pathogenic potantial, genetic diversity and antimicrobial resistance of *Campylobacter* spp. in broiler flocks, which highlights urgent need for implementing effective contol measures to reduce emergence and spread of antimicrobial resistant *Campylobacters*.

***Keywords:*** *Thermophilic Campylobacter, Antimicrobial Resistance, Virulence, Genotyping*

# Broilerlerden İzole Edilen Termofilik *Campylabacter*'lerin Genotipik, Antimikrobiyal Direnç ve Virulens Profilleri

## Özet

Bu çalışmanın amacı, Adana ve Hatay illerindeki ticari broyler çiftliklerinde *Campylobacter jejuni* ve *Campylobacter coli*'nin prevalansını, antimikrobiyal direnç ve genetik belirleyicilerini araştırmaktır. İzolatlar arasındaki genetik çeşitliliğin değerlendirilmesi, *fla*A geninin *Dde*I restriksiyon endonükleaz enzimi ile kesilmesine dayalı RFLP-Polimeraz Zincir Reaksiyonu (PZR) ve *fla*A ve *fla*B-SVR genlerinin kısa değişken bölgelerinin (SVR'ler) dizi analizi ile belirlendi. Ayrıca, izolatların antimikrobiyal duyarlılıkları disk difüzyon yöntemi ile yapıldı ve tetrasiklin *(tet*O), ampisilin *(bla*$_{OXA-61}$), aminoglikozid *(aph-3-1)* ve çoklu dirence aracılık eden efluks pompası *(cme*B) direnç genlerinin varlığı ise PZR ile araştırıldı. Siprofloksasine dirence neden olan mekanizmalar PZR ve takiben DNA dizi analizi ile araştırıldı. İzolatlar arasında 10 virulans *(fla*A, *vir*B11, *rac*R, *cad*F, *cia*B, *dnaJ* and *pld*A) ve toksin geninin *(cdt*A, *cdt*B, *cdt*C) varlığı PZR ile incelendi. İncelenen 220 kloakal sıvabtan 194'ü *C. jejuni* (%89) ve 24'ü *C. coli* (%11) olmak üzere 218 (%99.1) *Campylobacter* spp. izole edildi. Tüm izolatlar kloramfenikol, gentamisin ve eritromisin'e duyarlı iken; *C. jejuni* ve *C. coli* izolatlarının nalidiksik asit, siprofloksasin, ampisilin, tetrasiklin ve amoksisilin-klavulanik asite direnç oranları sırasıyla %86.6-100, %86.6-100, %45.9-45.8, %43.3-50 ve %2.6-0.0 olarak belirlendi. *C. jejuni* ve *C. coli* izolatlarında *tet*O, *bla*$_{OXA-61}$, *cme*B ve *aph-3-1* genlerinin prevalansı sırasıyla %45.3-54.4, %36.1-75, %1.5-83.3 ve %0.5-0 tespit edildi. *C. jejuni* ve *C. coli* izolatlarında *fla*A, *cdt*A, *cdt*B, *cdt*C, *rac*R, *cad*F, *cia*B, *dnaJ* ve *pld*A genlerinin prevalansı ise sırasıyla %100-100, %95.4-100, %94.3-100, %89.7-54.2, %89.2-79.2, %92.8-100, %67-16.7, %85.6-75 ve %80-66.7 olarak belirlendi. *vir*B11 geni *Campylobacter* izolatlarının hiçbirinde tespit edilmedi. Tüm *fla*A ve *fla*B -SVR allelleri aynı PCR-RFLP patternleri gösterdi. Bu çalışmanın sonuçları broyler sürülerinde *Campylobacter* spp. prevalansının, patojenik potansiyelinin, genetik çeşitliliğinin ve antimikrobiyal direnç oranlarının yüksek olduğunu göstermektedir. Bu durum antimikrobiyallere dirençli *Campylobacter*'lerin ortaya çıkışını ve yayılımını azaltmak için etkili kontol önlemlerinin acilen uygulanması gerektiğini vurgulamaktadır.

***Anahtar sözcükler:*** *Termofilik Campylobacter, Antimikrobiyal direnç, Virulens, Genotiplendirme*

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# INTRODUCTION

Of the 25 *Campylobacter* species described to date, *Campylobacter jejuni* and *Campylobacter coli* are the most frequently reported foodborne pathogens responsible for human gastroenteritis in both developing and developed countries [1]. Although thermophilic *Campylobacters* can colonize on the intestinal flora of many animal species, poultry (especially commercial chicken) are the main reservoir of *Campylobacter* spp. and responsible for most of human campylobacteriosis cases [2] through improper handling and consumption of raw chicken meat [3]. One of the important vehicles for the transmission of *campylobacter* to humans is improper handling and consumption of chicken meat [3].

Human campylobacteriosis cases are mostly self-limiting, however, in some cases may result in severe health consequences such as Guillain-Barre syndrome, reactive arthritis and irritable bowel syndrome [4]. In severe cases, fluoroquinolones, macrolides and tetracyclines are the drugs of choise. However, over the years, increasing trend of resistance to these antimicrobials in *C. jejuni* and *C. coli* have been reported by various studies in Turkey [5-7] and in the world [8-11].

In addition to antimicrobial resistance, *Campylobacter* spp. produces several virulence factors playing an important role in the pathogenesis of infection. The most important virulence genes of campylobacteriosis were: *fla*A, *cad*F, *rac*R and *dna*J genes responsible for adherence and colonization; *vir*B11, *cia*B and *pld*A genes responsible for invasion and survival within the host cells and *cdt*A, *cdt*B and *cdt*C genes responsible for the expression of cytolethal distending toxins [12]. Studies regarding the prevalence of virulence genes in *Campylobacter* obtained from broiler are very scarce in Turkey, and there have been only a study in order to determine *cdt* toxin genes in limited number of *C. jejuni* isolates of broiler origin [13].

Many molecular methods have been used for molecular characterization of *C. jejuni* and *C. coli* strains. But, each molecular method has different discriminatory power to determine the genetic relatedness of the *campylobacter* isolates [14]. Of these methods, *fla*A gene based molecular techniques are widely used for epidemiological studies of thermophilic *Campylobacter* spp. due to its rapidity, relative simplicity, low cost and easy method with an acceptable discriminatory power [15]. One of these methods, *fla*A gene based PCR-RFLP is one of the widely used method for discrimination of *C. jejuni* and *C. coli* isolates [16]. Sequence analysis of short variable regions (SVRs) of *fla*A and *fla*B genes is another widely used method for genotyping of *Campylobacter* isolates, particularly in short-term and localized epidemiological studies [17-19].

The aims of this study were to (i) determine the prevalence and antimicrobial resistance of thermophilic *Campylobacter* spp. in commercial broiler flocks in Adana and Hatay provinces and its resistance mechanisms, (ii) to investigate genetic diversity of all *C. coli* and representative *C. jejuni* isolates using *fla*A based PCR-RFLP and *fla*A-SVR and *fla*B-SVR sequence-based typing and (iii) to determine the presence and frequency of ten virulence genes.

# MATERIAL and METHODS

### *Study Area and Sample Collection*

A cross-sectional study was conducted to determine the prevalence of *Campylobacter* spp. from May 2015 through September 2015. A total of 220 cloacal swabs from 11 commercial broiler farms were collected from Hatay and Adana provinces. The study was approved by Mustafa Kemal University Animal Ethic Commitee (2014-8/5).

### *Bacterial Isolation*

The cloacal swabs were taken using Amies Transport Medium with charcoal (LP Italiana, 118598, Italy) and transported to laboratory in a cold chain for further analysis. The cloacal swabs were directly streaked on modified charcoal cefoperazone deoxycholate agar (mCCDA) (Oxoid, CM0739, England) containing CDDA selective supplement (Oxoid, SR0155, England) for primary isolation. The plates were incubated at 41.5°C for 36-48 h under microaerophilic conditions. One presumptive colony from each mCCDA plate and subcultured onto blood agar (Merck, 110886, Germany) supplemented with 5% defibrinated sheep blood to obtain pure culture. The isolates, microscobically curved Gram negative rods with characteristic seagull-winged morphology, catalase and oxidase positive were accepted as *Campylobacter* spp. and stored within cryobeads (Biomériuex, France) in deep freeze (-80°C) until use.

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

Chromosomal DNA was obtained by boiling method as previously described [20]. Briefly, one colony was suspended in 200 µL RNase and DNase free water and heated 100°C for 10 min and centrifuged at 10.000 g for 10 min. Supernatant was transferred to another sterile eppendorf tube and used as template DNA.

The isolates were identified to genus/species level by multiplex polymerase chain reaction (mPCR) as described by Wang et al.[20] using primers spesific for *Campylobacter* spp., *C. jejuni* and *C. coli*.

### *Antimicrobial Susceptibility Testing*

Antimicrobial susceptibility of *C. jejuni* and *C. coli* isolates was determined by disk diffusion method according to Clinical Laboratory Standards Institute guidelines (CLSI, 2008) [21]. Following antimicrobial disks (Bioanalyse, Turkey)

were used: ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), chloramphenicol (30 µg), tetracycline (30 µg), gentamicin (10 µg), streptomycin (10 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg) and erythromycin (15 µg). The inocula was prepared from colonies of overnight agar plates and suspended within steril 0.85% sodium chloride to obtain MacFarland 0.5 turbity. The bacterial suspension was inoculated on Mueller-Hinton agar (Merck, Germany) plates containing 5% (v/v) defibrinated sheep blood. Antimicrobial disks were placed on the dry medium and incubated at 37°C for 24 h in anaerobic jar under microaerophilic condition. Inhibition zones were recorded and evaluated following CLSI (2008) criteria. *C. jejuni* (NCTC 12500) and *C. coli* (NCTC 12525) were used as control strains for antimicrobial susceptibility testing.

### Detection of Antimicrobial Resistant 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.[22].

### Analysis of the Molecular Mechanisms of Fluoroquinolone Resistance

Mutations in the quinolone resistance determining region (QRDR) of *gyr*A gene was determined by PCR amplification of *gyr*A gene and sequencing, using primers as described by Gibreel et al.[23].

### Genotyping by *fla*A-SVR and *fla*B-SVR Sequencing

PCR amplification of fragments of *fla*A and *fla*B genes comprising the SVRs were performed following the procedures described by Lévesque et al.[24] and Korczak et al.[25], respectively. Primers described by those authors were used, amplifying regions of 641 base pairs (bp) and 602 bp of the *fla*A and *fla*B genes, respectively. The *fla*A and *fla*B allele types were determined by comparing the nucleotide sequences with those in the PubMLST *Campylobacter* database *(http://pubmlst.org/campylobacter/)*.

### *fla*A-RFLP Assay

*fla*A-RFLP analysis of the isolates (24 *C. coli* and 55 *C. jejuni*, 5 strain from each flock) were performed as described elsewhere by Nachamkin et al.[6]. The *fla*A amplicons were digested for 18 h at 37°C with *Dde*I (Fermentas, *Lithuania*). Then, DNA fragments were separated using 2.5% agarose gels in TBE buffer at 200 V for 1 h, and visualized under UV light.

### Detection of Virulence Genes

Presence of virulence genes *(fla*A, virB11, *cad*F, *dna*J, *cia*A, *fla*A, and *pld*A) and cytolethal distending toxin genes *(cdt*A, *cdt*B and c*dt*C) were investigated by PCR as previously described by Nachamkin et al.[16], Bang et al.[26], Konkel et al.[27], Bacon et al.[28] and Datta et al.[29].

## RESULTS

### Occurence and Distribution of Campylobacter spp.

Out of 11 broiler flocks examined, all were positive for thermophilic *Campylobacter* spp. *(Table 1)*. Two hundred and eighteen (99.1%) *Campylobacter* spp. was isolated from cloacal swabs taken. Of these isolates, 194 (89.1%) were *C. jejuni* and 24 (10.9%) were *C. coli* by PCR analysis.

### Antimicrobial Susceptibility Testing

The results of antimicrobial susceptibility of *C. jejuni* and *C. coli* isolates are given in *Table 2*. All isolates were found to be susceptible to erythromycin, chloramphenicol and gentamicin. *Campylobacter* isolates showed highest resistance to ciprofloxacin and nalidixic acid at the rate of 86.6-86.6% and 100-100%, respectively. High resistance rates were also detected to tetracycline (43.3% for *C. jejuni* and 50% for *C. coli*) and ampicillin (45.9% for *C. jejuni* and 45.8% for *C. coli*). Low resistance rate for amoxicillin/

**Table 1.** *Distribution of Campylobacter spp. in broiler flocks*

| Flocks | Sampling Location | Broilers' Age (days) | Numbers of Samples | Species | |
|---|---|---|---|---|---|
| | | | | *C. jejuni* (%) | *C. coli* (%) |
| I | Adana | 33 | 20 | 19 (95) | 1 (5) |
| II | Adana | 35 | 20 | 14 (70) | 5 (25) |
| III | Hatay | 42 | 20 | 20 (100) | 0 (0) |
| IV | Hatay | 40 | 20 | 20 (100) | 0 (0) |
| V | Adana | 38 | 20 | 20 (100) | 0 (0) |
| VI | Adana | 36 | 20 | 19 (95) | 0 (0) |
| VII | Hatay | 37 | 20 | 20 (100) | 0 (0) |
| VIII | Hatay | 41 | 20 | 20 (100) | 0 (0) |
| IX | Hatay | 40 | 20 | 20 (100) | 0 (0) |
| X | Adana | 35 | 20 | 14 (70) | 6 (30) |
| XI | Adana | 37 | 20 | 8 (40) | 12 (60) |
| **Total** | | | 220 | 194 (88.2) | 24 (10.9) |

**Table 2.** *Antimicrobial resistance of C. jejuni and C. coli isolates*

| Antimicrobial | No of Isolates (%) | |
|---|---|---|
| | *C. jejuni* (n=194) | *C. coli* (n=24) |
| Nalidixic acid | 168 (86.6) | 24 (100) |
| Ciprofloxacin | 168 (86.6) | 24 (100) |
| Ampicillin | 89 (45.9) | 11 (45.8) |
| Tetracycline | 84 (43.3) | 12 (50) |
| Amoxicillin/clavulanic acid | 5 (2.6) | 0 (0) |
| Chloramphenicol | 0 (0) | 0 (0) |
| Gentamicin | 0 (0) | 0 (0) |
| Erythromycin | 0 (0) | 0 (0) |

clavulanic acid was only detected in *C. jejuni* as being 2.6% *(Table 2)*. Multidrug resistance (MDR) phenotype was observed in 70 (36.1%) *C. jejuni* and in 8 (33.3%) *C. coli* isolates, repectively. The most common MDR phenotype encountered among isolates were NA/CIP/TE/AM *(Table 3)*.

**Table 3.** *Antimicrobial resistance profile of C. jejuni and C. coli isolates*

| Resistance Profile* | No. of Resistant Isolates (%) | No. of Resistant Isolates (%) | |
|---|---|---|---|
| | | *C. jejuni* | *C. coli* |
| Pan-susceptible | 2 (0.92) | 2 (1.03) | 0 |
| TE | 6 (2.8) | 6 (3.1) | 0 |
| AM, TE | 1 (0.5) | 1 (0.5) | 0 |
| NA, CIP | 78 (35.8) | 69 (35.6) | 9 (37.5) |
| NA, CIP, TE | 18 (8.3) | 14 (7.2) | 4 (16.7) |
| NA, CIP, AM | 32 (14.7) | 29 (14.9) | 3 (12.5) |
| NA, CIP, TE, AM | 76 (34.9) | 68 (35.1) | 8 (33.3) |
| NA, CIP, AM, AMC | 3 (1.4) | 3 (1.5) | 0 |
| NA, CIP, TE, AM, AMC | 2 (0.9) | 2 (1.0) | 0 |

** TE: tetracycline, AM: ampicillin, CIP: ciprofloxacin, NA: nalidixic acid, AMC: amoxicillin-clavulanic acid*

**Table 4.** *Distribution of antimicrobial resistance genes in C. jejuni and C. coli isolates*

| Species | Resistance Phenotype and the Occurence of Related Gene | No of Isolates (%) | | | |
|---|---|---|---|---|---|
| | | *tet*O | $bla_{OXA-61}$ | *aph-3-1* | *cme*B |
| *C. jejuni* (n=194 ) | Resistant with genes | 85 (43.8) | 41 (21.1) | 0 | 3 (1.0) |
| | Resistant without genes | 11 (5.7) | 44 (22.7) | 0 | 0 |
| | Susceptible with genes | 3 (1.5) | 29 (14.9) | 1 (0.5) | 0 |
| *C. coli* (n= 24) | Resistant with genes | 12 (50.0) | 9 (37.5) | 0 | 20 (83.3) |
| | Resistant without genes | 0 | 2 (8.3) | 0 | 0 |
| | Susceptible with genes | 1 (4.2) | 9 (37.5) | 0 | 0 |

### Antimicrobial Resistance Genes

Of the 96 tetracycline resistant *C. jejuni* isolates, 85 were found to carry *tet*O gene, whereas *tet*O gene was found in all tetracycline resistant *C. coli* isolates. However, three *C. jejuni* and one *C. coli*, despite carrying *tet*O gene, were found to be susceptible to tetracycline. Similarly *aph-3-1* gene was detected in one phenotypically susceptible *C. jejuni* isolate. Among the ampicillin resistant 85 *C. jejuni*, 41 was found to carry $bla_{OXA-61}$, 29 of the ampicillin susceptible isolates were found to encode $bla_{OXA-61}$. While $bla_{OXA-61}$ was found in 11 ampicillin resistant *C. coli* isolates, 9 isolates that harbored $bla_{OXA-61}$ were susceptible to ampicillin. Although *cme*B gene was found in nearly all of *C. coli* (n=20) isolates, *cme*B was found in low number of *C. jejuni* (n= 3) isolates *(Table 4)*.

### Fluoroquinolone Resistance Mechanism of *Campylobacter* spp.

Sequence analysis of 219 bp amplicon of *gyr*A gene revealed Thr86Ile mutation in all ciprofloxacin resistant isolates.

### *fla*A Gene PCR-RFLP Analysis

Restriction analysis of 1.7 kb *fla*A PCR amplicons with *Dde*I revealed two-five bands ranging from 100 to 1000 bp. Two different band profiles of 24 *C. coli* isolates were observed. Analysis of 55 *C. jejuni* isolates selected randomly from the flocks (five isolates from each flock) gave 11 band profiles *(Fig. 1)*.

### *fla*A-SVR and *fla*B-SVR Typing

The results of *fla*A-SVR sequence typing of the 24 *C. coli* and 55 *C. jejuni* isolates are given in *Table 5* and *Table 6*. Ten *fla*A alleles and 8 *fla*B alleles were detected among *C. jejuni* isolates, two *fla*A and *fla*B alleles were detected among *C. coli* isolates. In *C. coli* isolates, three different

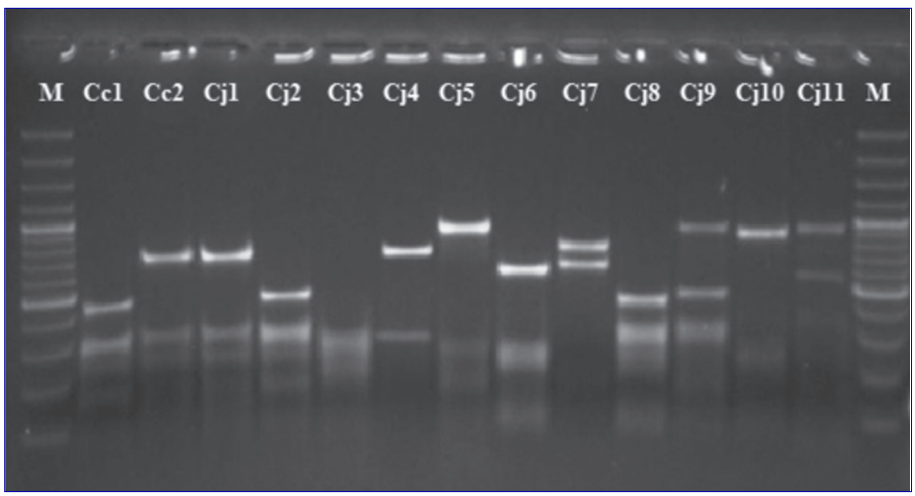


**Fig 1.** RFLP-PCR profiles obtained from *C. coli* and *C. jejuni* isolates. Lane M. 100 bp molecular marker, Lane Cc1-Cc2: RFLP profiles determined in *C. coli* isolates, Lane Cj1-Cj11: RFLP profiles determined in *C. jejuni* isolates

nucleotide sequences (allele numbers) for *fla*A and three for *fla*B were identified, corresponding to a total of two genotypes. *fla*A and *fla*B sequences were identical in four isolates (allele 116). The most common *fla*A alleles were 61 with 20 isolates (83.3%) and 116 with four isolates (16.7%). *fla*B allele 107 and 116 were detected in 20 isolates (83.3%) and in 4 isolate (16.7%). In *C. jejuni* isolates, 11 different nucleotide sequences (allele number) for *fla*A and eight different nucleotide sequences for *fla*B were identified, corresponding to a total of 12 genotypes. *fla*A and *fla*B sequences were identical in 11 isolates (allele 116). The most common *fla*A alleles were 116 with 12 isolates (21.8%), 67 with 16 isolates (29.1%), 36 with 10 isolates (18.2%), 44 with 5 isolates (9.1%), 42 with 5 isolates (9.1%), 67 with 5 isolates (9.1%). Among *fla*B alleles, 116 (17 isolates, 30.9%), 2 (11 isolates, 20%), 87 (6 isolates, 10.9%), 6 (10 isolates, 18.2%), 65 (5 isolates, 9.1%) were the most frequently detected.

**Table 5.** *fla-SVR alleles and PCR-RFLP profiles determined in C. coli isolates according to flocks*

| No of isolates | Species | Flock | *fla*A-SVR Alleles | *fla*B-SVR Alleles | RFLP Profile |
|---|---|---|---|---|---|
| 1 | *C. coli* | I | 116 | 116 | Cc2 |
| 2 | *C. coli* | II | 61 | 107 | Cc1 |
| 3 | *C. coli* | | 116 | 116 | Cc2 |
| 6 | *C. coli* | X | 61 | 107 | Cc1 |
| 12 | *C. coli* | XI | 61 | 107 | Cc1 |

**Table 6.** *fla-SVR alleles and PCR-RFLP profiles determined in C. coli isolates according to flocks*

| No of Isolates | Species | Flock | *fla*A-SVR Alleles | *fla*B-SVR Alleles | RFLP Profile |
|---|---|---|---|---|---|
| 1 | *C. jejuni* | I | 61 | 107 | Cj1 |
| 4 | *C. jejuni* | | 116 | 116 | Cj2 |
| 3 | *C. jejuni* | II | 116 | 116 | Cj2 |
| 2 | *C. jejuni* | | 44 | 119 | Cj3 |
| 5 | *C. jejuni* | III | 44 | 65 | Cj4 |
| 5 | *C. jejuni* | IV | 67 | 2 | Cj5 |
| 5 | *C. jejuni* | V | 36 | 6 | Cj6 |
| 5 | *C. jejuni* | VI | 36 | 6 | Cj6 |
| 5 | *C. jejuni* | VII | 42 | 87 | Cj7 |
| 5 | *C. jejuni* | VIII | 116 | 116 | Cj2 |
| 4 | *C. jejuni* | IX | 67 | 116 | Cj8 |
| 1 | *C. jejuni* | | 54 | 121 | Cj9 |
| 2 | *C. jejuni* | X | 63 | 121 | Cj10 |
| 1 | *C. jejuni* | | 67 | 116 | Cj8 |
| 2 | *C. jejuni* | | 67 | 2 | Cj5 |
| 4 | *C. jejuni* | XI | 67 | 2 | Cj5 |
| 1 | *C. jejuni* | | 31 | 87 | Cj11 |

### *Virulence Gene Patterns*

Prevalence of virulence genes detected in the *Campylobacter* isolates is given in *Table 7*. All isolates had at least two virulence genes investigated and in varying prevalence rates between 16.7% and 100%. *vir*B11 gene was not present in any of the isolates. The prevalence of *fla*A, *vir*B11, *cdt*A, *cdt*B, *cdt*C, *rac*R, *cad*F, *cia*B, *dna*J and *pld*A in *C. jejuni* and *C. coli* isolates was 100-100%, 0-0%, 95.4-100%, 94.3-100%, 89.7-54.2%, 89.2-79.2%, 92.8-100%, 67-16.7%, 85.6-75% and 80-66.7%, respectively.

## DISCUSSION

This study has shown that broiler flocks are colonized with *Campylobacter* spp. at the high rate (99.1%). In Turkey, studies on the prevalence of *Campylobacter* spp. in broiler flocks are very limited. In these studies, samples were taken from different slaughterhouses and the prevalence of thermophilic of *Campylobacter* spp. was reported as 91.8% [7] and 52.5% [6]. Variations in the prevalence rates can be attributed to the sample type, sampling procedures, isolation methods, lack of biosecurity measures applied on farms, presence of other animals around poultry houses, seasonal and climate changes, fly population, use of ventilators, slaughter age, stock density, age and number of houses on a farm, use of old litter, farm equipment and farm workers [4,6].

*C. jejuni* has been reported as the most prevalent species colonizing broiler flocks [4]. Similarly, *C. jejuni* was the dominant species isolated in this study. This finding is consisted with previous studies that *C. jejuni* is the predominant species isolated from broilers [6,7]. Although reasons for the variations are not exactly known, variations seen between prevalence of *C. jejuni* and *C. coli* can be attributed to different factors such as season, production practices and environment [4].

**Table 7.** *Prevalence of virulence genes detected in C. jejuni and C. coli isolates*

| Strain | No of Isolates | Number of Virulence Genes (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *fla*A | *vir*B11 | *cdt*A | *cdt*B | *cdt*C | *rac*R | *cad*F | *cia*B | *dna*J | *pld*A |
| *C. jejuni* | 194 | 194 (100) | 0 (0) | 185 (95.4) | 183 (94.3) | 174 (89.7) | 173 (89.2) | 180 (92.8) | 130 (67) | 166 (85.6) | 156 (80) |
| *C. coli* | 24 | 24 (100) | 0 (0) | 24 (100) | 24 (100) | 13 (54.2) | 19 (79.2) | 24 (100) | 4 (16.7) | 18 (75) | 16 (66.7) |
| Total | 218 | 218 (100) | 0 (0) | 209 (95.9) | 207 (96.7) | 187 (85.8) | 192 (89.7) | 204 (93.6) | 134 (61.5) | 184 (84.4) | 172 (78.9) |

Increasing resistance rates observed in *Campylobacter* isolates to antimicrobial agents has been reported in all over the world [30]. In this study, nearly all isolates (99.1%, 216/218) showed resistance to one or more of antimicrobial agents tested. Particularly, high percentage of resistance to ciprofloxacin is alarming, given the fact that resistant *Campylobacters* can be transmitted to humans via contaminated poultry meat. No resistance fluoroquinolones was determined in *campylobacter* isolates of broiler origin untill 1992 [31]. First fluoroquinolone resistance was observed in strains isolated in 1992 (1.4% for enrofloxacin and 1.2% for ciprofloxacin), and resistance of *Campylobacter* spp. to fluoroquinolones significantly increased in 2000 (75.5% for enrofloxacin and 73% for ciprofloxacin) [32]. In this study, 86.6% of *C. jejuni* and 100% of *C. coli* isolates were resistant to ciprofloxacin. Our results was comparable with findings of Abay et al.[5], but higher than findings of Cokal et al.[6]. The high fluoroquinolone resistance rate may be attributed to unrestricted use of flouroquinolones in poultry production, as it was highly used as a growth promoting agent between 1989 and 2006 in Turkey.

The resistance of *Campylobacter* to fluoroquinolones is mainly associated with point mutations in the QRDR of DNA gyrase *(gyr*A). The most encountered mutation in fluoroquinolone resistant *Campylobacter* isolates are Thr86Ile substitution, which confer resistance to both ciprofloxacin and nalidixic acid [30]. In this study, all ciprofloxacin resistant *Campylobacters* have Thr86Ile substitution in the QRDR of *gyr*A. Recently, Kurekci and Pehlivanlar Önen [33] have also identified the *gyr*A mutation of Thr86Ile among all *Campylobacter* spp. isolated from chicken meat sold in Turkey. Authors also identified Ala40Ser mutation in a *C. jejuni* strain [33].

In this study, all isolates were susceptible to erythromycin, chloramphenicol and gentamicin. Similar results were also reported by previous studies indicating that *Campylobacter* isolates are still sensitive or less resistant to these antimicrobials in Turkey [5-7].

Resistance to ampicillin and other β-lactams have widely been reported among *Campylobacter* isolates from humans, poultry and food of animal origins [5,7,22,33]. A novel class D β-lactamase gene, $bla_{OXA-61}$, described by Alfredson and Korolik [34] has been held responsible for resistance to the β-lactams including ampicillin, piperacillin, and carbenicillin. In this study, *C. jejuni* (45.9%) and *C. coli* (45.8%) isolates showed higher resistance rates to ampicillin. Resistance rate to ampicillin was higher than those findings of Abay et al.[5], but lower than findings of Yıldırım et al.[7]. In contrast to these findings, Cokal et al.[6] found all *C. jejuni* and *C. coli* isolates susceptible to ampicillin. This result might be attributed to the frequent use of β-lactams.

Resistance rate to tetracycline was slightly higher in *C. coli* (50%) than in *C. jejuni* (43.3%). Our findings is consistent with previous studies [5,7]. But, higher tetracycline resistance rate was reported by Cokal et al.[6].

In this study, 97 out of 108 (89.8%) tetracycline-resistant isolates were found to carry *tet*O and 23 (29.5%) of the isolates with MDR phenotype (n=78) had *cme*B, and 51.5% of 97 ampicillin resistant isolates carried $bla_{OXA-61}$. The higher presence of *tet*O in tetracycline resistant isolates was consisted with previous studies conducted by Obeng et al.[22], Abdi-Hachesoo et al.[35], Pratt and Korolik [36]. However, *tet*O was not detected in 11 tetracycline resistant *C. jejuni* isolates in this study. Recently, Abdi-Hachesoo et al.[35] reported presence of *tet*A gene in 18% (15/83) of *Campylobacter* spp. from poultry carcasses in Iran. Also, Guevremont et al.[37] reported that detection of *tet*O gene in tetracycline resistant isolates might vary according to primer sets used. The prevalence of *cme*B in *C. jejuni* isolates showing MDR phenotype was found to be low. Similar results have been previously reported by Obeng et al.[22] and Kashoma et al.[38]. Cagliero et al.[39] indicated that higher sequence variation can exist in *cme*B gene, could lead false negative results when presence of *cme*B gene is examined by PCR. As reported by Olah et al.[40], primers used in the study are probably located in regions subjected to modification. Similarly, in this study, most of ampicillin resistant *Campylobacter* isolates did not harbour $bla_{OXA-61}$ gene, which is chromosomally encoded beta-lactamase. This could be explained by presence of other resistance mechanisms such as reduced uptake due to alterations in outer membrane porins and efflux pump system [41].

Investigation of *Campylobacter* isolates for the presence of ten virulence genes revealed high prevalence of virulence genes. Although numerous studies are available on the investigation of virulence genes in *Campylobacter* spp. isolated from broiler and chicken carcasses, so far, there was a only one study to examine *cdt* genes in a small number (23 isolates) of *C. jejuni* isolates of broiler origin in Turkey by Fındık et al.[13], who reported prevalence of *cdt*A, *cdt*B and *cdt*C genes as 95%, 100% and 90%, respectively. The prevalence of *cdt*A, *cdt*B and *cdt*C genes obtained in this study were comparable to findings of Fındık et al.[13], except prevelance rate of *cdt*C gene (54.2%) in *C. coli* isolates.

*fla*A gene are one of important virulence genes involved in colonization, which was detected in all *Campylobacter* isolates in present study. This result is in agreement with the study of Datta et al.[29], who reported this gene in 100% of *C. jejuni* isolates.

The *rac*R, *dna*J, *cad*F, *pld*A and *cia*B genes have important role in invasion and colonization of *Campylobacters*. Each of these genes were found in 16.7-100% of *Campylobacter* isolates in this study. Chansiripornchai and Sasipreeyajan [42] reported *dna*J, *cad*F, *pld*A and *cia*B virulence genes as 100,

76, 31 and 41% in *C. jejuni* isolates from broilers. In contrast to this study, higher prevalence rate for *rac*R, *dna*J, *cad*F, *pld*A and *cia*B was reported by Datta et al.[29] as 85.7%, 100%, 100%, 100% and 100%, respectively.

To the best of my knowledge, this is the first study to investigate the genetic diversity of *C. jejuni* and *C. coli* isolates in broiler flocks using fla-SVR analysis and compare its results with PCR-RFLP in Turkey. In this study, the results showed a distinct association between *fla*-SVR alleles and RFLP-PCR profiles for both *C. jejuni* and *C. coli* isolates. It has been reported that *fla*A gene based typing methods are not suitable for long-term epidemiological studies due to possible intra- and inter-genomic recombination between flagellin genes, but is a useful tool for *Campylobacter* genotyping, particularly initial screening of *Campylobacter* isolates [15,17,18,43]. Similarly, the results indicated that *fla*-SVR typing alone or in combination with other PCR-based methods can be used preliminary screening of *campylobacter* isolates in epidemiological studies.

In conclusion, the current study revealed that fla-SVR typing could be used successfully for the discrimination of *C. jejuni* and *C. coli* strains. The high prevalence of virulence and toxin genes among *Campylobacter* isolates of broiler origin suggests potential virulence for humans. In addition, high prevalence of ciprofloxacin resistant *Campylobacter* in broilers, which is accepted as one of the drugs of choise for the treatment of human campylobacteriosis, is major concern due to foodborne transmission of antimicrobial resistant *Campylobacter* to humans. Thus, this study highlights the need of establishment of prudent measures and continous efforts to reduce colonization and spread of antimicrobial resistant *Campylobacter*.

## REFERENCES

**1. Skarp CP, Hänninen ML, Rautelin HI:** Campylobacteriosis: The role of poultry meat. *Clin Microbiol Infect,* 22, 103-109, 2016. DOI: 10.1016/j.cmi.2015.11.019

**2. European Food Safety Authority:** Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and Salmonella on broiler carcasses in the EU, 2008 - Part A: *Campylobacter* and Salmonella prevalence estimates. *EFSA J,* 8, 1503, 2010. DOI: 10.2903/j.efsa.2010.1503

**3. Humphrey T, O'Brien S, Madsen M:** *Campylobacters* as zoonotic pathogens: A food production perspective. *Int J Food Microbiol,* 117, 237-257, 2007. DOI: 10.1016/j.ijfoodmicro.2007.01.006

**4. Sahin O, Kassem II, Shen Z, Lin J, Rajashekara G, Zhang Q:** *Campylobacter* in poultry: Ecology and potential interventions. *Avian Dis,* 59, 185-200, 2015. DOI: 10.1637/11072-032315-Review

**5. Abay S, Kayman T, Otlu B, Hizlisoy H, Aydin F, Ertas N:** Genetic diversity and antibiotic resistance profiles of *Campylobacter jejuni* isolates from poultry and humans in Turkey. *Int J Food Microbiol*, 178, 29-38, 2014. DOI: 10.1016/j.ijfoodmicro.2014.03.003

**6. Cokal Y, Caner V, Sen A, Cetin C, Karagenc N:** *Campylobacter* spp. and their antimicrobial resistance patterns in poultry: An epidemiological survey study in Turkey. *Zoonoses Public Health*, 56, 105-110, 2009. DOI: 10.1111/j.1863-2378.2008.01155.x

**7. Yıldırım M, Istanbulluoglu E, Ayvalı B:** Prevalence and antibiotic susceptibility of thermophilic *Campylobacter* species in broiler chickens. *Turk J Vet Anim Sci,* 29, 655-660, 2005.

**8. Luangtongkum T, Morishita TY, Ison AJ, Huang S, McDermott PF, Zhang Q:** Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of *Campylobacter* spp. in poultry. *Appl Environ Microbiol,* 72, 3600-3607, 2006. DOI: 10.1128/AEM.72.5.3600-3607.2006

**9. Wieczorek K, Osek J:** A five-year study on prevalence and antimicrobial resistance of *Campylobacter* from poultry carcasses in Poland. *Food Microbiol,* 49, 161-165, 2015. DOI: 10.1016/j.fm.2015.02.006

**10. Chen X, Naren GW, Wu CM, Wang Y, Dai L, Xia LN, Luo PJ, Zhang Q, Shen JZ:** Prevalence and antimicrobial resistance of *Campylobacter* isolates in broilers from China. *Vet Microbiol,* 144, 133-139, 2010. DOI: 10.1086/513780

**11. Chokboonmongkol C, Patchanee P, Gölz G, Zessin KH, Alter T:** Prevalence, quantitative load, and antimicrobial resistance of *Campylobacter* spp. from broiler ceca and broiler skin samples in Thailand. *Poult Sci*, 92, 462-467, 2013. DOI: 10.3382/ps.2012-02599

**12. Rozynek E, Dzierzanowska-Fangrat K, Jozwiak P, Popowski J, Korsak D, Dzierzanowska D:** Prevalence of potential virulence markers in Polish *Campylobacter jejuni* and *Campylobacter coli* isolates obtained from hospitalized children and from chicken carcasses. *J Med Microbiol,* 54, 615-619, 2005. DOI: 10.1099/jmm.0.45988-0

**13. Findik A, Ica T, Onuk EE, Percin D, Kevenk TO, Ciftci A:** Molecular typing and cdt genes prevalence of *Campylobacter jejuni* isolates from various sources. *Trop Anim Health Prod,* 43, 711-719, 2011. DOI: 10.1007/s11250-010-9758-0

**14. Wassenaar TM, Newell DG:** Genotyping of *Campylobacter* spp. *Appl Environ Microbiol,* 76, 6377-6386, 2000.

**15. Petersen L, On SLW:** Efficacy of flagellin gene typing for epidemiological studies of *Campylobacter jejuni* in poultry estimated by comparison with macrorestriction profiling. *Lett Appl Microbiol,* 31, 14-19, 2000. DOI: 10.1046/j.1472-765x.2000.00757.x

**16. Nachamkin I, Bohachick K, Patton CM:** Flagellin gene typing of *Campylobacter jejuni* by restriction fragment length polymorphism analysis. *J Clin Microbiol,* 31, 1531-1536, 1993.

**17. Wassenaar TM, Fernández-Astorga A, Alonso R, Marteinsson VT, Magnússon SH, Kristoffersen AB, Hofshagen M:** Comparison of *Campylobacter* fla-SVR genotypes isolated from humans and poultry in three European regions. *Lett Appl Microbiol*, 49, 388-395, 2009. DOI: 10.1111/j.1472-765X.2009.02678.x

**18. Wirz SE, Overesch G, Kuhnert P, Korczak BM:** Genotype and antibiotic resistance analyses of *Campylobacter* isolates from ceca and carcasses of slaughtered broiler flocks. *Appl Environ Microbiol,* 76, 6377-6386, 2010. DOI: 10.1128/AEM.00813-10

**19. Giacomelli M, Andrighetto C, Rossi F, Lombardi A, Rizzotti L, Martini M, Piccirillo A:** Molecular characterization and genotypic antimicrobial resistance analysis of *Campylobacter jejuni* and *Campylobacter coli* isolated from broiler flocks in northern Italy. *Avian Pathol*, 41, 579-588, 2012. DOI: 10.1080/03079457.2012.734915

**20. Wang G, Clark CG, Taylor TM, Pucknell C, Barton C, Price L, Woodward DL, Rodgers FG:** Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C.upsaliensis*, and *C. fetus* subsp. *fetus*. *J Clin Microbiol,* 40, 4744-4747, 2002. DOI: 10.1128/JCM.40.12.4744-4747.2002

**21. Clinical and Laboratory Standards Institute:** Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Informational Supplement. CLSI Document M31-A3. Clinical and Laboratory Standards Institute, Wayne, PA, 2008.

**22. Obeng AS, Rickard H, Sexton M, Pang Y, Peng H, Barton M:** Antimicrobial susceptibilities and resistance genes in *Campylobacter* strains isolated from poultry and pigs in Australia. *J Appl Microbiol,* 113, 294-307, 2012. DOI: 10.1111/j.1365-2672.2012.05354.x

**23. Gibreel A, Sjögren E, Kaijser B, Wretlind B, Sköld O:** Rapid emergence of high-level resistance to quinolones in *Campylobacter jejuni* associated with mutational changes in *gyr*A and *par*C. *Antimicrob Agents Chemother,* 42, 3276-3278, 1998.

**24. Lévesque S, Frost E, Arbeit RD, Michaud S:** Multilocus sequence typing of *Campylobacter jejuni* isolates from humans, chickens, raw milk, and environmental water in Quebec, Canada. *J Clin Microbiol,* 46, 3404-3411, 2008. DOI: 10.1128/JCM.00042-08

**25. Korczak BM, Zurfluh M, Emler S, Kuhn-Oertli J, Kuhnert P:** Multiplex strategy for multilocus sequence typing, *fla* Typing, and genetic determination of antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolates collected in Switzerland. *J Clin Microbiol*, 47, 1996-2007, 2009. DOI: 10.1128/JCM.00237-09

**26. 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,* 94, 1003-1014, 2003. DOI: 10.1046/j.1365-2672.2003.01926.x

**27. Konkel ME, Garvis SG, Tipton SL, Anderson DE Jr, Cieplak W Jr:** Identification and molecular cloning of a gene encoding a fibronectin-binding protein (CadF) from *Campylobacter jejuni*. *Mol Microbiol*, 24, 953-963, 1997.

**28. Bacon DJ, Alm RA, Burr DH, Hu L, Kopecko DJ, Ewing CP, Trust TJ, Guerry P:** Involvement of a plasmid in virulence of *Campylobacter jejuni* 81-176. *Infect Immun*, 68, 4384-4390, 2000. DOI: 10.1128/JB.186.19.6508-6514.2004

**29. Datta S, Niwa H, Itoh K:** Prevalence of 11 pathogenic genes of *Campylobacter jejuni* by PCR in strains isolated from humans, poultry meat and broiler and bovine faeces. *J Med Microbiol,* 52, 345-348, 2003. DOI: 10.1099/jmm.0.05056-0

**30. Luangtongkum T, Jeon B, Han J, Plummer P, Logue CM, Zhang Q:** Antibiotic resistance in *Campylobacter*: Emergence, transmission and persistence. *Future Microbiol*, 4, 189-200, 2009. DOI: 10.1128/JB.186.19.6508-6514.2004

**31. Diker KS:** *In vitro* susceptibility of *campylobacters* isolated from poultry to enrofloxacin and ciprofloxacin. *Acta Gastro-Ent Belg*, 56 (Suppl.): 36, 1993.

**32. Savaşan S, Çiftçi A, Diker KS:** Emergence of quinolone resistance among chicken isolates of *Campylobacter* in Turkey. *Turk J Vet Anim Sci*, 28, 391-397, 2004.

**33. Kurekci C, Pehlivanlar Önen S:** Characteristics of ciprofloxacin resistant *Campylobacter* spp. isolated from chicken meat in Turkey. *Food Measure*, 2016. DOI: 10.1007/s11694-016-9426-9

**34. Alfredson DA, Korolik V:** Antibiotic resistance and resistance mechanisms in *Campylobacter jejuni* and *Campylobacter coli*. *FEMS Microbiol Lett,* 277, 123-132, 2007. DOI: 10.1111/j.1574-6968.2007.00935.x

**35. Abdi-Hachesoo B, Khoshbakht R, Sharifiyazdi H, Tabatabaei M, Hosseinzadeh S, Asasi K:** Tetracycline resistance genes in *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry carcasses. *Jundishapur J Microbiol*, 7, e12129, 2014. DOI: 10.5812/jjm.12129

**36. Pratt A, Korolik V:** Tetracycline resistance of Australian *Campylobacter jejuni* and *Campylobacter coli* isolates. *J Antimicrob Chemother*, 55, 452-460, 2005. DOI: 10.1093/jac/dki040

**37. Guévremont E, Nadeau E, Sirois M, Quessy S:** Antimicrobial susceptibilities of thermophilic *Campylobacter* from humans, swine, and chicken broilers. *Can J Vet Res,* 70, 81-86, 2006.

**38. Kashoma IP, Kassem II, John J, Kessy BM, Gebreyes W, Kazwala RR, Rajashekara G:** prevalence and antimicrobial resistance of *campylobacter* isolated from dressed beef carcasses and raw milk in Tanzania. *Microb Drug Resist,* 22, 40-52, 2016. DOI: 10.1089/mdr.2015.0079

**39. Cagliero C, Cloix L, Cloeckaert A, Payot S:** High genetic variation in the multidrug transporter *cme*B gene in *Campylobacter jejuni* and *Campylobacter coli*. *J Antimicrob Chemother,* 58, 168-172, 2006. DOI: 10.1093/jac/dkl212

**40. Olah PA, Doetkott C, Fakhr MK, Logue CM:** Prevalence of the *Campylobacter* multi-drug efflux pump (cmeABC) in *Campylobacter* spp. isolated from freshly processed turkeys. *Food Microbiol*, 23, 453-460, 2006. DOI: 10.1016/j.fm.2005.06.004

**41. Iovine NM:** Resistance mechanisms in *Campylobacter jejuni*. *Virulence,* 4, 230-240, 2013. DOI: 10.4161/viru.23753

**42. Chansiripornchai N, Sasipreeyajan J:** PCR detection of four virulence-associated genes of *Campylobacter jejuni* isolates from Thai broilers and their abilities of adhesion to and invasion of INT-407 cells. *J Vet Med Sci,* 71, 839-844, 2009. DOI: 10.1292/jvms.71.839

**43. Ahmed MU, Dunn L, Ivanova EP:** Evaluation of current molecular approaches for genotyping of *Campylobacter jejuni* strains. *Foodborne Pathog Dis*, 9, 375-385, 2012. DOI: 10.1089/fpd.2011.0988.