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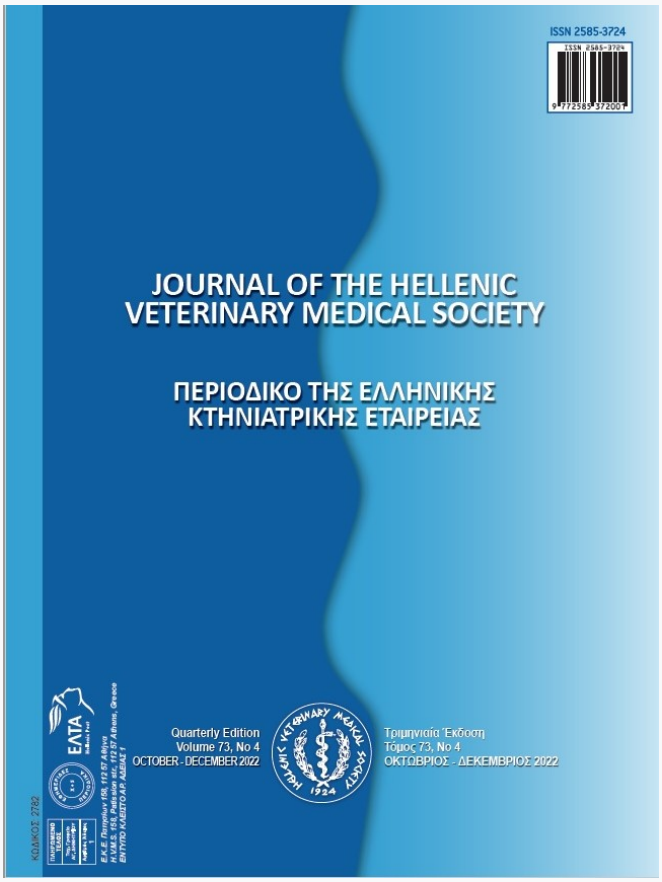

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Phenotypic and genotypic characterization of antimicrobial resistance of *Campylobacter*isolates from poultry meat in Turkey

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ABSTRACT: Phenotypic and genotypic antimicrobial resistances of *Campylobacter* strains from 152 chicken meatsamples were investigated. A total of 19 isolates (*Campylobacter jejuni*=6 and *Campylobacter coli*=13) were identified by multiplex PCR.The *C. jejuni* and *C. coli* isolates were tested for resistance to ciprofloxacin, erythromycin, and tetracycline using the disc diffusion method. In addition, all the strains were analyzed for resistance gene *tet-O* and mutations in the *gyrA* and 23S rRNA genes by PCR.Overall, a high frequency of resistance was detected against tetracycline (14/19, 73.6%), followed by erythromycin (7/19, 36.8%), and ciprofloxacin (6/19, 31.5%). Two of *C. coli* isolates (15.3%) were multidrug resistant, whereas none of the *C. jejuni* isolates were resistant to three antibiotics at the same time.Ten of the 14 *Campylobacter* strains (71.4%) resistant to tetracycline carried *tet-O* gene. Mutations in the 23S rRNA and *gyrA* genes were identified in 57.1% (4/7), 66.6% (4/6) of the isolates resistant to erythromycin and ciprofloxacin, respectively.

Keywords:*Campylobacter*, antimicrobial resistance, *gyrA*, *tet-O, 23S rRNA,* poultry meat

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INTRODUCTION

Campylobacter has been the most commonly reported cause of bacterial human gastroenteritisin the world (WHO, 2020). Campylobacters are usually transmitted to humans from animals or through the consumption of contaminated food of animal origin (especially poultry, also pig and beef meat or raw milk) and contaminated water (Avrain et al., 2003; Cantero et al., 2018; EFSA, 2009; Maćkiw et al., 2012; Obeng et al.,2012;Su et al., 2017; Szczepanska et al., 2017). They cause illness called campylobacteriosis and the most common clinical symptoms of the disease are diarrhea, abdominal pain, fever, headache, and nausea. Although the symptoms usually resolve within a few days, sometimes antibiotic treatment may be required in severe *Campylobacter* infections, particularly in the elderly, very young children and immunocompromised individuals (Obeng et al., 2012;Shobo et al. 2016;Silva et al., 2011;Wieczorek and Osek, 2013;Woźniak-Biel et al., 2018).Because antibiotics such as fluoroquinolones, macrolides, and tetracycline are generally used for the treatment of *Campylobacter* infections in humans, this pathogen has become increasingly resistant to these antibiotics (Engberg et al., 2001; Luangtongkum et al., 2009; Su et al., 2017).Antibiotic resistance in *Campylobacter* is emerging globally and now,antibiotic-resistant *Campylobacter* has become an important public health and food safety problem in both developed and developing countries (D'Lima et al., 2007; EFSA, 2009; Gibreel et al., 2004; Obeng et al., 2012; Mamelli et al., 2003; Shobo et al. 2016; Su et al., 2017; Szczepanska et al., 2017; Zirnstein et al., 1999).

As *Campylobacter* is a zoonotic pathogen and these antibiotics are also used in veterinary medicine, an increased antibiotic resistance has already been observed in *Campylobacter* strains isolated from food producing animals (Engberg et al. 2001; Luangtongkum et al. 2009; Silva et al. 2011). For example,among *Campylobacter* isolates obtained from broilers, the percentage of tetracycline resistancewas 81% in Spain (Cantero et al., 2018), 57% for *C. jejuni* and 70% for *C. coli*in France (Avrain et al.,2003), 64.3% (Maćkiw et al., 2012) and 78.6% (Wozniak-Biel et al., 2018)in Poland. The resistance rate to ciprofloxacin was 62.1% for *C. coli* in Belgium (Van Looveren et al., 2001), 97.9% (Maćkiw et al., 2012) and 100% (Woźniak-Biel et al., 2018) in Poland. It was also stated that multiple antibiotic resistance was more prevalent among animal and meat isolates than human isolates (EFSA, 2009). From this point of view, improper and excessive usage of antibiotics both in human and veterinary medicine, can cause the development of antibiotic-resistant *Campylobacter* strains.Transmission of these resistant strains to humans can be occurred via contaminated food of animal origin, especially poultry meat, which is the usual source of *Campylobacter* infections in humans (Cantero et al., 2018; Engberg et al., 2001; Luangtongkum et al., 2009; Nachamkin et al., 2002; Silva et al., 2011; Woźniak-Biel et al., 2018).

To date, different antibiotic resistance mechanisms have been described in *Campylobacter* and now, new resistance mechanisms have also appeared in this bacterium. However, fluoroquinolone, tetracycline, and macrolide resistance mechanisms have been studied in detail. Resistance of *Campylobacter* to these antimicrobials is a kind of acquired resistance resulting from chromosomal mutations or plasmid-borne (Luangtongkum et al., 2009; Tang et al., 2017; Taylor et al., 1988; Wieczorek and Osek, 2013). Erythromycin and other macrolide antibiotics bind to the 50S ribosomal subunits in bacteria and prevents the elongation of peptide chain and thus, protein synthesis is inhibited. Macrolide resistance in *Campylobacter* is chromosomally mediated and usually appears due to the target modification by mutation on the 23S rRNA gene. So, the drug can not bind to the ribosomal subunits due to the changes in the target site. Besides mutations, efflux system is another common mechanism causing macrolide resistance in *Campylobacter.* Also, this system is responsible for both intrinsic and acquired resistance of *Campylobacter* to different antibiotics (multidrug resistance) (Engberg et al., 2001; Gibreel et al., 2005; Luangtongkum et al., 2009; Mamelli et al., 2003; Su et al., 2017).Recently, *ermB* gene, encoding a rRNA methyltransferase, has been shownas a novel mechanism inmacrolide resistance in *Campylobacter* (Tang et al., 2017).

Quinolones exhibit their antibacterial action by inhibiting two bacterial enzymes, DNA gyrase and topoisomerase IV, which play an important role in DNA replication. Generally, mutations in the *gyrA* gene, encoding part of the GyrA subunit of DNA gyrase, have been reported to be associated with quinolone resistance in *Campylobacter* (Zirnstein et al., 1999; Zirnstein et al., 2000;Wieczorek and Osek, 2013). In addition to this, other mechanisms such as mutations in the *parC* gene (topoisomerase IV) and efflux system have been described for *Campylobacter* resistance to

fluoroquinolones (Engberg et al., 2001;Luangtongkum et al., 2009; Obeng et al., 2012; Su et al., 2017).

Tetracyclines are still widely used due to their broad spectrum of antimicrobial activity. They inhibit the protein synthesis by destroying bacterial membranes or by binding to the ribosomal subunits. Besides macrolides and fluoroquinolones, tetracyclines are alternatively used in the treatment of *Campylobacter* infections (Wieczorek and Osek, 2013). It has been previously reported that resistance to tetracyclines in *Campylobacter* is mediated by a plasmid encoded *tet-O* and *tet-M* genes (Taylor et al., 1988). These genes are termed as ribosomal protection proteins (RPPs) (Connell et al., 2003), provide resistance by displacing or removing tetracycline from its binding site on the ribosome (Gibreel et al., 2004).Multidrug efflux system also contributes to the tetracycline resistance in *Campylobacter* (Su et al., 2017; Zhang and Plummer, 2008).

Antibiotic-resistant *Campylobacter* strains in the food chain havealready threatenedpublic health and food safety.Because of the importance of this organism in food safety and public health, various studies have been conducted to understand the mechanisms of antibiotic resistance in *Campylobacter*. This study was therefore planned to determine both the phenotypic and genotypic antimicrobial susceptibility of *Campylobacter* isolates obtained from poultry meat.

MATERIALS AND METHODS

Sample collection, isolation and identification of *Campylobacter*

A total of 152 raw chicken drumsticks were collected from retail markets in Hatay between November 2019 to April 2021 and transported to the laboratoryunder cold chain. Then, samples were bagged individually and stored in a conventional freezer at -18 °C until testing. Before microbiological analysis,they were thawed at refrigerator temperature (4ºC) for approximately 24 hours. Samples were aseptically transferred to a sterile stomacher bag and 225 ml of sterile *Campylobacter*Enrichment Broth (LAB135, Lab M, UK) supplemented with CAT (Cefoperozone-Amphotericin B-Teicoplanin; Himedia, India) was poured into each of them and rinsed by shaking the bag for 1 min. The rinsed samples were then investigated for *Campylobacter* by using a conventional culture method, according to Hunt et al. (2001). Small, flat, colourless translucent and/or greyish colonies were selected from *Campylobacter* Blood Free Medium Base Bolton (Biolife, Italy) supplemented with CCDA (Cefoperozone-Amphotericin B; Oxoid), and confirmed by multiplex PCR (Wang et al., 2002), by using four pairs of primers (Linton et al., 1996; Linton et al., 1997;Wang et al.,1992; Wang et al., 2002) (Table 1, Fig. 1). All isolates were stored in Brain-HeartInfusion Broth (Merck, Germany) with 15% glycerol at -20 °C.

Table 1. List of primers used for PCR in this study

Gene	Primer	Sequence (5' to 3')	Size (in bp)	Reference
16S rRNA (Genus *Campylobacter*)	Campy-16S	GGATGACACTTTTCGGAGC	816	Linton et al. (1996)
ask	Campy-coli-F	GGTATGATTTCTACAAAGCGAG	502	Linton et al. (1997)
	Campy-coli-R	ATAAAAGACTATCGTCGCGTG		
glyA	Campy-lari-F	TAGAGAGATAGCAAAAGAGA	251	Wang et al. (2002)
	Campy-lari-R	TACACATAATAATCCCACCC		
cj0414	Campy-jejuni-F	CAAATAAAGTTAGAGGTAGAATGT	161	Wang et al. (1992)
	Campy-jejuni-R	CCATAAGCACTAGCTAGCTGAT		
tet-O	Tet-jejuni-coli-F	GGCGTTTTGTTTATGTGCG	559	Gibreel et al. (2004)
	Tet-jejuni-coli-R	ATGGACAACCCGACAGAAGC		
gyrA	Gyr-jejuni-F	ATT TTT AGC AAA GAT TCT GAT	673	Zirnstein et al. (1999)
	Gyr-jejuni-R	CCA TAA ATT ATT CCA CCT GT		
	Gyr-coli-F	TAT GAG CGT TAT TAT CGG TC	505	Zirnstein et al. (2000)
	Gyr-coli-R	GTC CAT CTA CAA GCT CGT TA		
23S rRNA	Ery-jejuni-coli-F	TCAAGCTGGTTAGCTA	300	Gibreel et al. (2005)
	Ery-jejuni-coli-R	ACGGCGGCCGTAACTATA		

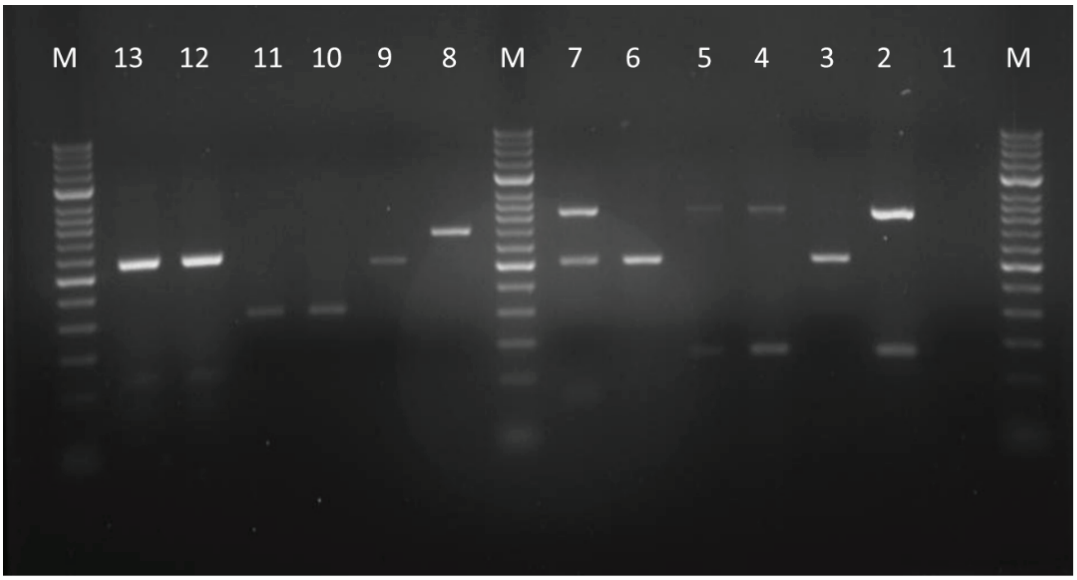

Fig. 1 PCR analysis of isolates and antibiotic resistance genes [M: 100 bp DNA marker, 1. Negative control, 2: Positive control (*C. jejuni* ATCC 29428), 3: Positive control (*C. coli* ATCC 43478); 4-5; *C. jejuni* isolates, 6-7 : *C. coli* isolates, 8: Ciprofloxacin resistance gene (*gyrA*) for *C. jejuni* (673 bp); 9: G*yrA* gene for *C. coli* (505 bp); 10-11: Erythromycin resistance gene (*23S rRNA*) for *C. jejuni* and *C. coli* (300 bp), 12-13: Tetracycline resistance gene (*tet-O*) for *C. jejuni* and *C. coli* (559 bp)].

Reference strains

Campylobacter jejuni ATCC 29428 (Microbiologics, USA) and *Campylobacter coli* ATCC 43478 (Microbiologics, USA) were used as positive controls in microbiological and molecular analysis.

Antimicrobial resistance screening

For culturing *Campylobacter*, the isolates were grown in Brain-Heart Infusion Broth at42°C for 48-72 h under microaerophilic conditions containing 5% O_2, 10% CO_2 and 85% N_2. In the phenotypic screening, suspension was adjusted to 0.5 McFarland standard and Mueller-Hinton agar (M1084, Himedia, India) containing 5% defibrinated horse blood was used for the disc diffusion method. The antibiotic discs included ciprofloxacin (5 µg/disc), erythromycin (15 µg/disc), and tetracycline (30 µg/disc).The EUCAST (2019) recommended zone diameter breakpoints for ciprofloxacin, erythromycin, and tetracycline were usedas follows: <26 mm (ciprofloxacin), <20 mm(erythromycin for *C. jejuni*) and <24 mm (erythromycin for *C. coli*), <30 mm (tetracycline). Genotypically, point mutations in *gyrA* (threonin-86-to-isoleucine;Thr-86-to-Ile) and 23S rRNA (peptidyl transferase region in domain V of the 23S rRNA) genes were used for analyzing resistance to ciprofloxacin and erythromycin respectively, in *Campylobacter* isolates. *Tet-O* gene was also usedfor characterization of tetracycline resistance.These geneswere amplified by PCR using primers (Table 1, Fig. 1) according toGibreel et al. (2004), Zirnstein et al. (1999), Zirnstein et al. (2000), Gibreel et al. (2005). Genomic DNA was extracted using the Bacterial DNAExtraction kit (Nucleic Acid Extraction Kit, GF-1, Vivantis, Malaysia), following the kit manufacturer's instructions. Amplification conditions were carried out as mentioned in Table 3.

RESULTS AND DISCUSSION

Prevalence of *Campylobacter* in poultry meat samples

Campylobacter strains were found in 19 chicken samples out of 152 (12.5%), and *Campylobacter coli* was the most commonly found species with a level of 8.5% (13/152), whereas *Campylobacter jejuni* was isolated from 6 of the samples (3.9%).*Campylobacter lari* was not detected in the study.With regard to the prevalence of *Campylobacter* in poultry meat, Maćkiw et al. (2012) and Lee et al. (1994) found that 60.9% and 95% of the chicken meat samples were contaminated with *Campylobacter* respectively, significantly higher than this study. This difference may be due to the number of samples analyzed,sampling method,sampling parts of poultry carcass, and the sample characteristics (fresh or frozen poultry meat). However, Lee et al. (1994) reported that contamination level was the same on different parts of the poultry carcass. As mentioned before, campylobacters have the ability to form viable but non-culturable cells (VBNC) under stressful conditions such as dry-

Table 3. DNA amplification conditions (Gibreel et al. 2004; Gibreel et al. 2005; Wang et al. 2002;Zirnstein et al. 1999)

PCR conditions	Identification of *Campylobacter* spp., *C. jejuni*, *C. lari*, *C. coli*by multiplex PCR	*23S rRNA*	*tet-O*	*gyrA*
Initial denaturation	95 °C 6 min		95 °C 1 min	94°C 3 min
Denaturation	95 °C 0.5 min	95 °C 0.5 min	95 °C 1 min	94°C 1 min
Primer annealing	59 °C 0.5 min	52 °C 1 min	50 °C 1 min	50 °C 1 min
Extension	72 °C 0.5 min	72 °C 1 min	72 °C 1 min	72 °C 1 min
Final extension	72 °C 0.5 min			72 °C 5 min
Number of cycles	30	30	30	30

ing, low pH, heating, and freezing (Hunt et al.,2001; Silva et al., 2011). This information supports the lower prevalence of *Campylobacter* in our study, since chilling and freezing can inactivate or damage cells. Thus, it could be difficult to culture or convert the VBNC form to a culturable form. Regarding the production types of poultry, the prevalence of *Campylobacter* was 70.3% (Obeng et al., 2012), and 80.0% (Avrain et al., 2003) in free-range broilers, and 89.9% in free-range layer chickens (Obeng et al., 2012). The authors demonstrated that the isolation rates of *C. jejuni* and *C. coli* varied according to production type. Since *Campylobacter* spp.often colonize the intestinal tract of poultry, a high prevalence of this pathogen in poultry is possible and contaminated poultry meat seems to be animportant source of campylobacteriosis in humans.

Screening of phenotypic and genotypic antibiotic resistance of *Campylobacter*

In our study, a total of nineteen (19) isolates were verified as *Campylobacter* by PCR and phenotypically, seventeen (17) of the nineteen (19) isolates (89.5%) were resistant to at least one antimicrobial, whereas only two(2) (10.5%) of them were susceptible to all the antimicrobial agents tested. Overall, a high rate of resistance was detected against tetracycline (14/19, 73.6%), followed by erythromycin (7/19, 36.8%), and ciprofloxacin (6/19, 31.5%). The prevalence of phenotypic resistances to tetracycline, erythromycin, and ciprofloxacin was 66.6, 50, 16.6% for *C. jejuni* and 76.9, 30.7, 38.4% for *C. coli*, respectively. Only two (2) out of the nineteen (19) isolates (10.5%)were resistant to thethree antibiotics at the same time. Moreover, multidrug resistance was common in *C. coli* (2/13, 15.3%) than in *C.jejuni* (0%) (Table 2, Table 4). Resistance to tetracycline and ciprofloxacin was higher in *C. coli* than in *C. jejuni*, whereas erythromycin resistance was found more often in *C. jejuni*.

Similarly, Lee et al. (1994), Avrain et al. (2003) observed widespread resistance to tetracycline.The high rate of phenotypic resistance to tetracycline shows that this antibiotic could be generally used in poultry. Contrary to the above studies, Obeng et al. (2012) detected a lower rate of resistance to tetracycline (5.6-40.7%) in different production types of chicken. Unlike this study, Woźniak-Biel et al. (2018) found the highest rate of resistance against ciprofloxacin. Again, Maćkiw et al. (2012) found high prevalence (97.9%) of ciprofloxacin resistance, followed by tetracycline (64.3%), and erythromycin (9.1%) resistance in *Campylobacter* from chicken meat and giblets. However,in the Obeng et al. (2012) study, no resistance to ciprofloxacin and erythromycin was observed. Multidrug resistance was 7.0% in the study ofMaćkiw et al. (2012), which is lower than this study, but Woźniak-Biel et al. (2018) didn't isolate any multidrug resistant strains from turkey and broilers. Cantero et al. (2018) reported that the prevalence of resistance to quinolones, tetracycline, and erythromycin was 100%, 81%, and 56%, respectively, among their broiler isolates.

In previous studies (Avrain et al., 2003; Bae et al., 2005; D'Lima et al., 2007; Elmalı and Can, 2019; Van Looveren et al., 2001), *C. coli* isolates have been reported to be more frequently resistant to the tested drugs, and multidrug resistance was more common in*C. coli* than in *C. jejuni*, consistent with the results of our study.Avrain et al. (2003) found an important difference among their isolates related to antimicrobial resistance according to the production type of the poultry, while Obeng et al. (2012) didn't detect any difference.

Szczepanska et al. (2017) study from Polandrevealed that surface water, poultry meat and pets were potential sources of children campylobacteriosis. They found much less resistance to erythromycin in both *C. coli* (3.7%) and *C. jejuni* (3.3%) isolates in poultry meat compared with this study. They isolated

Table 2. Phenotypic and genotypic antimicrobial resistance profiles *Campylobacter* strains isolated from poultry meat

Isolates no.	Species	Antimicrobial resistance profile	
		Phenotypic	Genotypic
1	*C. coli*	ER, TR,CS	*23S rRNA*(+), *tet-O* (-), *gyrA* (+)
2	*C. coli*	S	*23S rRNA*(+), *tet-O* (-), *gyrA* (+)
3	*C. coli*	S, TR, CR	*23S rRNA*(-),*tet-O* (+),*gyrA* (+)
4	*C. coli*	R	*23S rRNA*(-),*tet-O* (+),*gyrA*(-)
5	*C. coli*	ES, TR, CS	*23S rRNA*(-),*tet-O* (+),*gyrA*(-)
6	*C. coli*	ES, TR, CS	*23S rRNA*(-),*tet-O* (+),*gyrA*(-)
7	*C. coli*	ES, TR, CS	*23S rRNA*(-),*tet-O* (+),*gyrA*(-)
8	*C. coli*	R	*23S rRNA*(+), *tet-O* (-), *gyrA* (+)
9	*C. coli*	ER, TS, CS	*23S rRNA*(-),*tet-O* (+),*gyrA* (+)
10	*C. coli*	ES,TR,CS	*23S rRNA*(+), *tet-O* (+), *gyrA* (-)
11	*C. coli*	ES, TR, CR	*23S rRNA*(+), *tet-O* (-), *gyrA* (+)
12	*C. coli*	S	*23S rRNA*(+), *tet-O* (-), *gyrA* (+)
13	*C. coli*	ES, TR, CR	*23S rRNA*(-),*tet-O* (+), *gyrA*(-)
14	*C. jejuni*	ER, TS, CS	*23S rRNA*(+),*tet-O* (+),*gyrA* (+)
15	*C. jejuni*	ER,TR,CS	*23S rRNA*(-),*tet-O* (+),*gyrA* (-)
16	*C. jejuni*	ES, TR, CS	*23S rRNA*(+),*tet-O* (+), *gyrA* (+)
17	*C. jejuni*	ES, TR, CR	*23S rRNA*(+),*tet-O* (-), *gyrA* (+)
18	*C. jejuni*	ER, TS, CS	*23S rRNA*(+),*tet-O* (-), *gyrA* (+)
19	*C. jejuni*	ES, TR, CS	*23S rRNA*(+), *tet-O* (+), *gyrA* (+)

S: Susceptible to all antimicrobials tested; R: Resistance to all antimicrobials tested; ES: Susceptible to erythromycin; ER: Resistant to erythromycin; TS: Susceptible to tetracycline; TR: Resistant to tetracycline, CS: Susceptible to ciprofloxacin; CR: Resistant to ciprofloxacin

Table 4. Prevalence of antibiotic resistance genes in *C. jejuni* and *C. coli* isolates

Pattern of resistance genes	Number of isolates with resistance genes (% of isolates)					
	C. jejuni (n=6)			*C. coli* (n=13)		
	23S rRNA	*tet-O*	*gyrA*	*23S rRNA*	*tet-O*	*gyrA*
Resistant with genes	2 (33.3)	3 (50)	1 (16.6)	2 (15.3)	7 (53.8)	3 (23)
Resistant without genes	1 (16.6)	1 (16.6)	0	2 (15.3)	3 (23)	2 (15.3)
Susceptible with genes	3 (50)	1 (16.6)	4 (66.6)	4 (30.7)	1 (7.6)	4 (30.7)
Susceptible without genes	0	1(16.6)	1(16.6)	5 (38.4)	2 (15.3)	4 (30.7)

multidrug-resistant *Campylobacter* from a fountain, and this reflects that fountains can pollute other environments with multidrug-resistant *Campylobacter* strains. These strains may pose difficulty with limiting the choice of antibiotics used in the treatment of campylobacteriosis in humans.

The results of phenotypic and genotypic analyses of resistance to tetracycline revealed that 71.4% of the strains which were phenotypically resistant to tetracycline, carried *tet-O* gene. The prevalence of *tet-O* gene was similar in both *C. jejuni* (66.6%) and *C. coli* (61.5%) isolates. Differently, Shobo et al. (2016), Cantero et al. (2018), Woźniak-Biel et al. (2018) found that the phenotypic and genotypic results of tetracycline resistance were fully compatible, that means all of the strains resistant against tetracycline carried *tet-O* gene. In the study of Lee et al. (1994), 98% of the chicken isolates resistant to tetracycline were positive for *tet-O*, including 87% on plasmids and 11% on the chromosome. The above results show that *tet-O* gene is mostly associated with tetracycline resistance and highly specific for detection of tetracycline resistance in *Campylobacter*.

Interestingly, Obeng et al. (2012) observed that there was a significant difference between different groups of poultry with regard to presence of resistance genes. Resistance to tetracycline encoded by *tet-O* was detected in *C. jejuni* isolates from free

range egg layers (40.7%) and indoor meat chickens (9.1%), whereas any free range meat chicken isolates were found to carry this gene.

Mutation in 23S rRNA genewas detected in 57.1% (4/7) of the isolates showing resistance to erythromycin. Mutation in 23S rRNA gene was more prevalent in *C. jejuni* (5/6, 83.3%) than *C. coli* (6/13, 46.1%) (Table 4). Cantero et al. (2018) found that 75% of *C. coli* isolates had a mutation at position 2075 on the 23S rDNA, associated with high level erythromycin resistance. Also,Maćkiw et al. (2012) detected two point mutations at two positions (2075 and 2074 mutations) in 40% of *C. coli* isolates resistant to erythromycin, whereas none of *C. jejuni* isolates resistant to erythromycin were found to have these mutations. They indicated that other mechanisms can be responsible for erythromycin resistance.In addition to the 23S rRNA mutations, recent studies (Cantero et al., 2018; Obeng et al., 2012) have shown that multidrug efflux pump system (CmeABC) plays an important role for resistance to macrolides.

G*yrA* mutation associated with ciprofloxacin resistance was more common in *C. jejuni* (5/6, 83.3%) than in *C. coli* (7/13, 53.8%) in this study. Six of the 19 isolates (31.5%) were ciprofloxacin resistant phenotypes, but 66.6% of them (4/6) had a mutation in *gyrA*Thr-86,while the rest of them didn't have this mutation. In those strains, other factors and mechanisms such as changes in membrane permeability, efflux system, mutations in other genes (*parC, parE*) or other mutations in the *gyrA* genemay be associated with fluoroquinolone resistance (Engberg et al., 2001; Luangtongkum et al., 2009; Su et al., 2017; Wieczorek and Osek, 2013;Zirnstein et al., 1999). From this point of view, quinolone resistance has a heterogeneous structure as phenotypic and genotypic. However, in the studies of Maćkiw et al. (2012), Shobo et al. (2016), Cantero et al. (2018) and Woźniak-Biel et al. (2018), mutation in the *gyrA* gene at position Thr-86-Ilewas observed in all the ciprofloxacin resistant strains.

CONCLUSION

Our study demonstrate that the prevalence of *Campylobacter* was not very high and the most common *Campylobacter* spp. was *C. coli* in poultry meat in Turkey. With regard to antimicrobial resistance,-*Campylobacter* strains showed the highest resistance to tetracycline and multi-drug resistance has been detected only among the *C.coli*strains. To better understand the antibiotic resistance mechanisms in *Campylobacter*, both phenotypic and genotypic resistance to three antimicrobials was analyzed. Although phenotypic and genotypic results of antibiotic resistance were not fully compatible and also, new resistance mechanisms have emerged in the pathogen recently, our results may provide important information for controlling antibiotic-resistant *Campylobacter* and for antibiotic selection both in human and veterinary medicine.

CONFLICT OF INTEREST

None reported.

REFERENCES

Avrain L, Humbert F, L'Hospitalier R, Sanders P, Vernozy-Rozand C, Kempf I (2003) Antimicrobial resistance in *Campylobacter* from broilers: association with production type and antimicrobial use. Vet Microbiol 96(3):267-76.

BaeW, Kaya KN, Hancock DD, Call DR, Park YH, Besser TE (2005) Prevalence and antimicrobial resistance of thermophilic *Campylobacter* spp. from cattle farms in Washington State. Appl Environ Microbiol71(1):169-74.

Cantero G, Correa-Fiz F, Ronco T, Strube M, Cerdà-Cuéllar M, Pedersen K(2018) Characterization of *Campylobacter jejuni* and *Campylobacter coli* Broiler Isolates by Whole-Genome Sequencing. Foodborne Pathog Dis 15(3): 145-152. http://doi.org/10.1089/fpd.2017.2325.

Connell SR, TraczDM, Nierhaus KH, TaylorDE (2003) Ribosomal protection proteins and their mechanism of tetracycline resistance. Antimicrob Agents Chemother 47(12): 3675-3681.

D'lima, CB, Miller WG, Mandrell RE, Wright SL, Siletzky RM, Carver DK, Kathariou S (2007) Clonal population structure and specific genotypes of multidrug-resistant Campylobacter coli from Turkeys. Appl Environ Microbiol 73(7):2156-64.

EFSA (2009) The community summary report on trends and sources of zoonoses and zoonotic. Agents in the European union in 2007. *EFSA J*, 223, 223-440.

Elmalı M, Can HY (2019)Antimicrobial susceptibility and virulence-associated genes in *Campylobacter* isolates from milk and wastewater in Hatay, Turkey. Cienc Rural49 (5):1-8.

Engberg J, Aarestrup FM, Taylor DE, Gerner-Smidt P, Nachamkin I (2001) Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanisms and trends in human isolates. Emerg Infect Dis 7(1):24-34.

EUCAST(The European Committee on Antimicrobial Susceptibility Testing) (2019) Breakpoint tables for interpretation of MICs and zone diameters. Version 9.0.Available at: http://www.eucast.org. Accessed: September6, 2021.

Gibreel A, Tracz DM, Nonaka L, Ngo TM, Connell SR, Taylor DE (2004) Incidence of Antibiotic resistance in *Campylobacter jejuni*isolated in Alberta, Canada, from 1999 to 2002, with special reference to tet(O)-mediated tetracycline resistance.Antimicrob Agents Chemother 48 (9): 3442-3450.

Gibreel A, Kos VN, Keelan M, Trieber CA, Levesque S, Michaud S, Taylor DE (2005) Macrolide Resistance in *Campylobacter jejuni* and *Campylobacter coli*: Molecular mechanism and stability of the re-

sistance phenotype.Antimicrob Agents Chemother 49 (7):2753-2759.
Hunt JM, Abeyta C, Tran T (2001) Bacteriological Analytical Manual Chapter 7: *Campylobacter*.U.S. Food and Drug Administration. Available at:https://www.fda.gov/food/laboratory-methods-food/bam-chapter-7-campylobacter. Accessed: September 27, 2021.
Lee CY, Tai CL, Lin SC, Chen YT (1994) Occurrence of plasmids and tetracycline resistance among *Campylobacter jejuni* and *Campylobacter coli* isolated from whole market chickens and clinical samples.Int J Food Microbiol 24(1-2):161-170.
Linton D, Owen RJ, Stanley J (1996) Rapid identification by PCR of the genus *Campylobacter* and of five Campylobacter species enteropathogenic for man and animals. Res Microbiol 147 (9): 707-718. https://doi.org/10.1016/S0923-2508(97)85118-2
Linton D, Lawson AJ, Owen RJ, Stanley J (1997) PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. J Clin Microbiol 35(10), 2568-2572.
Luangtongkum T, Jeon B, Han J, Plummer P, Logue CM, Zhang Q (2009) Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence. Future Microbiol 4(2):189-200.
Maćkiw E, Korsaka D, Rzewuska K, Tomczuk K, Rożynek E (2012) Antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli* isolated from food in Poland. Food Control, 23(2): 297-301.
Mamelli L, Amoros JP,Page`s JM, Bolla JM (2003) A phenylalanine-arginineβ-naphthylamide sensitive multidrug effluxpump involved in intrinsic and acquired resistance of *Campylobacter* to macrolides.Int J Antimicrob Agents 22:237-241.
Nachamkin I, Ung H, Li M (2002)Increasing fluoroquinolone resistance in *Campylobacter jejuni*, Pennsylvania, USA,1982-2001.Emerg Infect Dis 8(12):1501-1503.
Obeng AS, Rickard H, Sexton M, Pang Y, Peng H, Barton M (2012) Antimicrobial susceptibilities and resistance genes in *Campylobacter* strains isolated from poultry and pigs in Australia. J Appl Microbiol 113(2):294-307.
Shobo CO, Bester LA, Baijnath S, Somboro AM, Peer AK, Essack SY (2016) Antibiotic resistance profiles of *Campylobacter* species in the South Africa private health care sector. J Infect Dev Ctries 10:1214-1221.
Silva J, Leite D, Fernandes M, Mena C, Gibbs PA, Teixeira P (2011)*Campylobacter* spp. as a foodborne pathogen: a review. Front Microbiol 2:100.
Su CC, Yin L, Kumar N, Dai L, Radhakrishnan A, Bolla JR, Lei HT, Chou TH, Delmar JA, Rajashankar KR, Zhang Q, Shin YK, Yu EW (2017) Structures and transport dynamics of a *Campylobacter jejuni* multidrug efflux pump. Nat Commun, 8(1):171.
Szczepanska B, Andrzejewska M, Spica D, Klawe JJ (2017) Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from children and environmental sources in urban and suburban areas. BMC Microbiol 17(1):80.
Tang Y, Fang L, Xu C, Zhang Q (2017) Antibiotic resistance trends and mechanisms in the foodborne pathogen, *Campylobacter*. Anim Health Res Rev 18(2):87-98.
Taylor DE, Courvalin P (1988) Mechanisms of antibiotic resistance in *Campylobacter* species.Antimicrob Agents Chemother 32(8):1107-12.
Van Looveren M, Daube G, De Zutter L, Dumont JM, Lammens C, Wijdooghe M, Vandamme P, Jouret M, Cornelis M, Goossens H. (2001) Antimicrobial susceptibilities of *Campylobacter* strains isolated from food animals in Belgium. J Antimicrob Chemother 48(2):235-40.
Wang RF, Slavik MF, Cao WW (1992)A rapid PCR methodfor directdetection of low numbers of *Campylobacter jejuni*. J Rapid Meth Aut Mic1(2):101-108.
Wang G, Clark CG, Taylor TM, Pucknell C, Barton C, Price L, Woodward DL, Rodgers FG (2002)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(12):4744-4747.
WHO(World Health Organisation) (2020)*Campylobacter*. Available at: https://www.who.int/news-room/fact-sheets/detail/campylobacter
Wieczorek K, Osek J (2013) Antimicrobial resistance mechanisms among *Campylobacter*. Biomed Res Int 2013:340605.
Woźniak-Biel A, Bugla-Płoskońska G, Kielsznia A, Korzekwa K, Tobiasz A, Korzeniowska-Kowal A, Wieliczko A (2018) High prevalence of resistance to fluoroquinolones and tetracycline *Campylobacter* spp. isolated from poultry in Poland. Microb Drug Resist 24(3): 314-322.
Zhang Q, Plummer PJ (2008) Mechanisms of antibiotic resistance in *Campylobacter*. In Campylobacter (eds I. Nachamkin, C.M. Szymanski and M.J. Blaser).
Zirnstein G, Li Y, Swaminathan B, Angulo F(1999)Ciprofloxacin resistance in *Campylobacter jejuni*isolates: Detection of gyrA resistance mutations by mismatch amplification mutation assay PCR and DNA sequence. J Clin Microbiol37(10):3276-3280.
Zirnstein G, Helsel L, Li Y, Swaminathan B, Besser J (2000) Characterization of *gyrA* mutations associated with fluoroquinolone resistance in *Campylobacter coli* by DNA sequence analysis and MAMA PCR. FEMS Microbiol Lett190:1-7.