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Antibiotic resistance patterns of chicken and human origin *Campylobacter* spp. in Turkey

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Summary

Campylobacteriosis is of great importance for both human and chicken populations. Unconcious and overuse of antibiotics in chickens has led to the transmission of antibiotic resistance patterns to humans. The purpose of this study was to determine the prevalence of thermophilic *Campylobacter* species from the cecal samples at slaughter houses, and also common antibiotic resistance patterns shared between chicken origin and human origin thermophilic *Campylobacter* species. Isolation and identification was performed according to EN ISO 10272-1: 2017 and Real-Time Multiplex qPCR, respectively. Antibiotic susceptibility test was performed by using the Kirby Bauer Disk Diffusion Method. Of the examined randomly collected 180 cecal samples at evisceration stage in slaughterhouses, 19 (10.5%), 17 (9.44%) and 2 (1.11%) were found to harbour *Campylobacter* spp., *C. jejuni* and *C. coli*, respectively. The highest resistance was determined against quinolones (86.04%) and fluoroquinolones (86.04%) among the tested 43 *Campylobacter* spp., comprising 19 chicken origin and 24 human origin. Except for erythromycin and gentamicin, all *C. jejuni* isolates from chickens and humans were found to be resistant to two or three of the antibiotics tested. The same multidrug resistance profiles observed in chicken origin *C. jejuni* isolates for TET/CIP/NA (70.58%) and CIP/NA (29.41%) were also determined in human origin *C. jejuni* isolates with the rate of 25% and 50%, respectively for each. To sum up, the same resistance patterns against common antibiotics shared in both human and chicken origin *C. jejuni* has pose a significant public health problem.

Keywords: *C. jejuni*, *C. coli*, cecal samples, human stool samples, antibiotic resistance pattern

Campylobacteriosis has been the most commonly reported zoonosis, with 220,639 confirmed human cases in 2019 in the EU, and it caused a significant economic burden, surpassing that of Salmonellosis in developed countries. Campylobacteriosis is subject to compulsory annual monitoring in the EU according to List A of the Annex I of the Zoonoses Directive 2003/99/EC (15). Although water, milk and red meat are among the important risk factors for *Campylobacter* infection in humans, it is reported that the primary source is poultry meat. Infections caused by consumption of poultry meat account for around 50-70% of all *Campylobacter* infections worldwide (34). Contaminated poultry products, in particular raw, undercooked chicken and raw meat processing, pose a risk for human campylobacteriosis (3). According to EFSA and ECDC (18) data, 37.4% and 31.5% of fresh meat from broilers and turkeys, respectively, harbor *Campylobacter* spp. *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*), the most common thermophilic *Campylobacter* species, are responsible for the most infections, and account for 20.8% and 21.7% of confirmed human infections, respectively (16). Thermophilic *Campylobacter* spp. are the commensals of the gastrointestinal tract of domestic and wild animals, in particular chickens (8). Chickens are a natural reservoir of thermophilic *Campylobacter* because of their internal body temperature, 41°C, is favorable for *Campylobacter* growth (38). Thermophilic *Campylobacter* spp. colonize the colon and ceca of chickens, and the contamination of chicken meat occurs when the bacteria in the intestine contaminate the carcass during slaughter (2).

Increased antimicrobial resistance has been reported in the treatment of human campylobacteriosis in recent years (17). However, it has also been reported that the

frequent use of the antibiotics in farm animals causes the development of resistance and that animal origin foodstuffs are an important source of transmission of resistant *Campylobacter* spp. to humans (6). That is why the reporting of resistant *Campylobacter* spp. from poultry is necessary. The first preferred antibiotic groups in the treatment of *Campylobacter* infections are quinolones and macrolides. Tetracyclines can be preferred in clinical cases, albeit rarely. In severe cases such as bacteremia and other systemic infections, intravenous aminoglycoside therapy is applied. Therefore, antibiotic resistance that are requested to be reported for *C. jejuni* and *C. coli* resistance include ciprofloxacin, erythromycin, gentamicin, nalidixic acid, tetracycline and on an optional basis, streptomycin (16).

This study aimed to determine the prevalence of thermophilic *Campylobacter* species from the cecal contents of broilers at the evisceration stage in slaughter houses and to also evaluate the antibiotic resistance patterns of thermophilic *Campylobacter* spp. recovered from the ceca of broiler poultry and from the stools of hospital patients who have complaints of diarrhea, abdominal pain, cramps, and fever symptoms in Turkey.

Material and methods

Sample collection. A total of 180 cecal samples were collected from two different slaughterhouses located in Bolu, Turkey on June-July, 2019. One hundred cecal samples were collected from one slaughterhouse and 80 cecal samples from the other, and all samples were obtained during the evisceration stage of broiler. Each of the cecal samples was taken into tubes containing sterile peptone water (CM0009, Oxoid) and transported to the laboratory under cold chain within 3 hours, and examined immediately. Twenty-four human origin *Campylobacter* isolates were kindly obtained from Mennenmen State Hospital, Microbiology Laboratory culture collection. Briefly, human stool samples were collected from the patients who came to clinic with the suspicion of acute gastroenteritis between June-September 2019. The collected stool samples were transferred to Cary Blair Transport Medium (BD, 211102) and sent to the Microbiology Laboratory of Mennenemen State Hospital, and analysed immediately for the presence of thermophilic *Campylobacter* spp.

***Campylobacter* culture.** All samples were examined for the presence of thermophilic *Campylobacter* spp. by direct plating according to EN ISO 10272-1: 2017 Microbiology of food chain-Horizontal method for detection and enumaration of *Campylobacter* spp. – Part 1: Detection Method (13). Briefly, all samples were homogonized and cultured on modified charcoal cefaperazone deoxycholate agar (CM739, Oxoid) with selective supplement (SR155, Oxoid). All plates were incubated under microaerobic conditions for 48 h at 42°C. Small, curved, gray, catalase- and oxidase-positive, Gram-negative bacilli were suspected as *Campylobacter* spp. Suspected colonies were identified to genus level by real-time multiplex qPCR. All isolates were transferred to *Brucella* broth (BD, 296185) with 7% lysed horse blood and 10% glycerol, and stored at –80°C for antimicrobial susceptibility testing.

Molecular detection

DNA extraction. DNA extraction was performed by a commercial DNA extraction kit according to the manufacturer's instructions (DNeasy Blood & Tissue Kits, Qiagen, Cat. No. 69504). All DNAs were stored at –20°C.

Identification of *Campylobacter* species by real-time multiplex qPCR. After extraction, identification of thermophilic *Campylobacter* species was performed by using a commercial kit (Biocheck, Multispecies *Campylobacter* qPCR Multiplex Test Kit, MP103). According to the manufacturer's instructions, the amplification of target genes was observed in FAM channel for *C. jejuni*, Texas Red channel for *C. coli*, HEX channel for internal control. In this study, the observation of CY-5 channel for *C. lari* was omitted because *C. jejuni* and *C. coli* were inquired in the current study. Real-time multiplex qPCR was performed on a Rotor-Gene Q thermal cycler (Qiaqen, Germany). Positive and negative controls were in the same kit.

Interpretation of sample test results. Assessment of the sample test results was performed after the negative and positive controls had been examined and determined to be valid according to the manufacturer's instructions. The *C. jejuni*-positive control in the FAM channel had been accepted for a value of $22.0 < Cq < 33.0$. If the *C. jejuni*-positive control had a value of $Cq < 22.0$ or $Cq > 33.0$, the test was invalid for *C. jejuni*. *C. coli*-positive control in the Texas Red Channel had been accepted for a value of $22.0 < Cq < 33.0$. If the *C. coli* positive control had a Cq value < 22.0 or > 33.0, the test was invalid for *C. coli*. In the negative control well the signal for *C. jejuni*, *C. coli* had been accepted as N/A or $Cq > 40.0$. In the negative control well, a Cq value for internal control in the HEX channel had been accepted for $26.0 < Cq < 34.0$.

Real-time multiplex qPCR conditions. Thermal cycling conditions were as follows: 1 cycle at 95°C for 3 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 60 sec.

Real-time multiplex qPCR reaction volumes. The real-time multiplex PCR was carried out in 25 µl volume using 12.5 µl master mix, 7.5 µl primer/probe mix with internal control, 5 µl template DNA.

Antimicrobial susceptibility test. All *Campylobacter* isolates were tested for antimicrobial susceptibility using the Kirby Bauer Disk Diffusion Method according the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (1, 5). A suspension of 0.5 MacFarland standard prepared in Mueller-Hinton broth (Oxoid) was inoculated into Mueller Hinton agar (Oxoid) plates containing 5% (v/v) defibrinated sheep blood and incubated at 37°C for 48 h under microaerobic conditions. Inhibition zones recorded and interpreted according to CLSI (5). All the isolates were screened for resistance by using antibiotic discs obtained from Oxoid. The following antibiotics were used: gentamicin (10 µg), tetracycline (10 µg), erythromycin (5 µg), nalidixic acid (30 µg), ciprofloxacin (10 µg). *C. jejuni* (ATCC-33560), *C. coli* (ATCC 33559) were used as positive controls and *Escherichia coli* ATCC25922 was used as negative control.

Results and discussion

Campylobacter spp., *C. jejuni*, and *C. coli* were detected in 19 (10.55%), 17 (9.44%), and 2 (1.11%) of the 180 cecal samples from broilers, respectively. (Tab. 1). According to reports, the prevalence of *Campylobacter* within positive flocks at slaughter was reported to be as high as 80% (9). In a study conducted to identify risk factors for *Campylobacter* contamination levels on broiler carcasses during the slaughter process, Seliwiorstow et al. (34) demonstrated that *Campylobacter* counts on carcasses collected after plucking, evisceration, washing, and chilling were related to the level of colonization in the ceca. In our study, we chose to examine cecal samples collected during evisceration according to EFSA and ECDC (14). When Berrang et al. (2) evaluated *Campylobacter* contamination in the ceca and outer liver of each broiler carcass in an experimental model, the authors reported 25.7% and 58.57% positive in two organs, respectively. The same authors declared the likelihood of *Campylobacter* detection on the liver surface was higher when the ceca was positive.

Tab. 1. Number of *C. jejuni* and *C. coli* isolated from cecal samples in the slaughterhouses

Place where caecum samples collected	Number of collected cecal samples	Number of isolated *Campylobacter* spp. (%)	
		C. jejuni	*C. coli*
Slaughterhouse A	100	12	2
Slaughterhouse B	80	5	0
Total	180	17 (9.44%)	2 (1.11%)
General total		19 (10.55%)	

Emanowicz et al. (12) found a positive correlation between *Campylobacter* counts in ceca and on carcasses, which was consistent with the findings of Hue et al. (23) of *Campylobacter* prevalence of 77.2 percent in ceca and 87.5 percent on carcasses. In the current study, the percentages of 10.5 *Campylobacter* spp., comprising 9.44 *C. jejuni* and 1.11 *C. coli* from the cecal samples at the evisceration process could define the first introduction of internal *Campylobacter* carriage to slaughterhouses, most likely from *Campylobacter* positive flocks at the farm level. A similiar comment by Rafei et al. (33) reported that the result of 41% *Campylobacter* spp. prevalence in cecal samples found in their study may implicitly reflect the level of colonisation occurring in broilers at pre-slaughter level. When viscera are ruptured during the evisceration stage of processing in a slaughterhouse, cecal contents have been reported to be a good possibility for cause of contamination (30). *C. jejuni* has been reported more frequently than *C. coli* from poultry at the flock, slaughter, and raw-meat levels in several studies paralleling our *C. jejuni* isolation rate (2, 7, 10, 22, 23, 31). In contrast to the high isolation rate of *C. jejuni*, predominance of *C. coli* from neck skin and cecal samples in Italy (32), slaughterhouses and farms in Lebanon (33, 35), and broilers at slaughter age in Ecuador was reported (42).

Human and chicken origin *Campylobacter* spp. was determined to be clonally related by PFGE, MLST analysis in different countries (36, 45). Among the different genotypes found by the combination of PFGE and *fla*A-RFLP in northern Spain, Naffarate et al. (29) reported 4 genotypes of *Campylobacter* spp., that included isolates of different origins, overlapping human clinical isolates, and those from broiler faeces and meat. Also from Turkey, Abay et al. (1) revealed a major significance of poultry meat in human campylobacteriosis in Turkey, with 54.3 percent clonaly linked PFGE profiles in *C. jejuni* isolates from both human and chicken origins. The results indicated that transmission of isolates from farm and broiler meat suggests a possible source of human infection. Morover, particularly, an increased resistance rate in *C. jejuni* isolates against a wide range of antibiotics in both human and food specimens was also reported in the United States by Antimicrobial Resistance Monitoring System (NARMS) (4).

In one health approach, antibiotic resistance patterns transmitted to humans via consumption of raw and undercooked poultry meat originated from flocks treated with the same antibiotics used to treat human campylobacteriosis (21, 38). Therefore, resistance to the common antibiotics used in both human campylobacterosis and chicken was also investigated in the current study. It was found that all *C. jejuni* isolates from chickens were found to be resistant to two or three of the antibiotics examined, except for erythromycin and gentamicin. Among the antibiotics tested, fluoroquinolones, ciprofloxacin, and quinolones, nalidixic acid had the highest resistance rates, with 100 percent for each. *C. coli* isolates demonstrated the highest resistantance to fluoroquinolones and quinolones with 100% for each. Tetracycline was shown to be the second most resistant antibiotic in *C. jejuni* isolates, with a rate of 70.58 percent, while *C. coli* isolates had 100% tetracycline sensitivity. *C. jejuni* isolates were shown to be 100 percent sensitive to macrolides (erythromycin), just like *C. coli* isolates. None of the *C. jejuni* isolates exhibited resistance to aminoglicosides (gentamicin), half of the *C. coli* isolates were found to be resistant to gentamicin (Tab. 2). Human *C. jejuni* isolates were shown to be 100 percent sensitive to erythromycin and gentamicin, while resistant to ciprofloxacin and nalidixic acid at a rate of 75 percent for each. Tetracycline resistance was determined in 25 percent of human *C. jejuni* isolates. (Tab. 2). Except for erythromycin, 43 *Campylobacter* spp. including 19 from chickens and 24 from humans were shown to be resistant to at least two antibiotics. Resistance to gentamicin, tetracycline, ciprofloxacin, and nalidixic acid was determined to be

Tab. 2. Antibiotic resistance and sensitivity of *C. jejuni* and *C. coli* isolated from chicken cecal samples and human origin *C. jejuni* against tested antibiotics

Number of resistant (%)	Antibiotics	Chicken origin *Campylobacter* spp.				Human origin *Campylobacter* spp.	
		C. jejuni		*C. coli*		*C. jejuni*	
		N of resistant (%)	N of sensitive (%)	N of resistant (%)	N of sensitive (%)	N of resistant (%)	N of sensitive (%)
0 (0.00)	E	0 (0.00)	17 (100)	0 (0.00)	2 (100)	0 (0.00)	24 (100)
20 (46.51)	TET	12 (70.58)	5 (29.41)	0 (0.00)	2 (100)	8 (25)	18 (75)
1 (2.32)	GEN	0 (0.00)	17 (100)	1 (50)	1 (50)	0 (0.00)	24 (100)
37 (86.04)	CIP	17 (100)	0 (0.00)	2 (100)	0 (0.00)	18 (75)	6 (25)
37 (86.04)	NA	17 (100)	0 (0.00)	0 (0.00)	2 (100)	18 (75)	6 (25)

Explanations: N – number; E – erythromycin; TET – tetracycline; GEN – gentamicin; CIP – ciprofloxacin; NA – nalidixic acid

Tab. 3. Resistance patterns of chicken and human origin *Campylobacter* spp. isolates

MDR patterns	MDR prevalences of *C. jejuni* isolates by origin		MDR prevalences of *C. coli* isolates by origin		General total (%)
	N of human isolates (%)	N of chicken isolates (%)	N of human isolates (%)	N of chicken isolates (%)	
TET/CIP/NA	6/24 (25)	12/17 (70.58)	0/0	0/2 (0.0)	18/43 (41.86)
CIP/NA	12/24 (50)	5/17 (29.41)	0/0	1/2 (50)	18/43 (41.86)
CIP/NA/GEN	0/24 (0.00)	0/17 (0.00)	0/0	1/2 (50)	1/43 (2.32)
Total	18/24 (75)	17/17 (100)	0/0	2/2 (100)	37/43 (86.04)

Explanations: MDR – multidrug resistance; N – number; TET – tetracycline; GEN – gentamicin; CIP – ciprofloxacin; NA – nalidixic acid

2.32% (N = 1), 46.51% (N = 20), 86.04% (N = 37), and 86.04% (N = 37), respectively (Tab. 2). *C. jejuni* isolates recovered from chickens and humans were shown to be resistant to two or three antibiotics. Combined resistance patterns in *C. jejuni* from cecal samples of chickens were as follows: TET/CIP/NA (70.58%), CIP/NA (29.41%). The same multi-drug resistance patterns observed in chicken origin *C. jejuni* isolates were also determined in human origin *C. jejuni* isolates for TET/CIP/NA and CIP/NA with the rates of 25% and 50%, respectively (Tab. 3). CIP/NA/GEN (50%) and CIP/NA (50%) resistance patterns were determined in chicken origin *C. coli* isolates (Tab. 3).

Common custom, curing with macrolides and fluoroquinolones is of great importance to public health for the treatment of human campylobacteriosis (38). A combined resistance pattern, CIP/E were reported as an alarming issue due to being recognised in two antimicrobial agents as CIAs for treatment of human campylobacteriosis (16). Neither human nor chicken origin *Campylobacter* isolates exhibited that pattern in the current study. The highest resistance to fluoroquinolones and quinolones in thermophilic *Campylobacter* isolated from chicken cecal samples at slaughter line, evisceration step in our study was consistent with the findings of the studies conducted on the chicken samples at various slaughter lines, retail meat by Zhang et al. (46) in China, chicken thigh samples by Kouglenou et al. (26) in West Africa, raw chicken retail meat samples by Deckert et al. (10) in Ontorio, cecal and neck samples by Rafei et al. (33) in Lebanon, and cecal samples by Cokal et al. (7) in Turkey. CIP and NA resistance in both *C. jejuni* and *C. coli* isolates recovered from Ecudarion and Lebanon broilers at slaughter was declared to be in the range of 97-100 percent by Vinueza-Burgos et al. (42), 87.5-95 percent by Rafei et al. (33). Although the results of two previous studies were consistent with 100 percent resistance to both CIP and NA in chicken origin *C. jejuni* and *C. coli* isolates in our study, we thought that interpreting resistance patterns of *C. coli* depending on solely 2 *C. coli* isolates would be insufficient, but not for *C. jejuni* isolates. Also, similiar rates of CIP and NA resistance against thermophilic *Campylobacter* species were declared to be in the range of 95-100% from chicken carcasses by Hungaro et al. (24), Moura et al. (28), Sierra-Arguello et al. (37) in Brazil. The results of our study demonstrated that the high quionolone and fluroquinolone resistance rates for *C. jejuni* (100%) isolates derived from chicken origin, as well as *C. jejuni* (75%) isolates from human origin were found to be similar to those reported by EU member state countries (16). Morover, that the isolates being resistant to nalidixic acid were determined to be resistant to ciprofloxacin was not astonishing. Furthermore, the high frequency of fluoroquinolone resistant strains has been reported in humans and animals, including chickens in several Latin American countries (19), humans and food-producing animals, including chickens in Iran (11), chickens and humans in Turkey (1). Following the detection that ciprofloxacin causes rising rates of resistance in humans and poultry, the drug's use has been prohibited in a number of countries. However, ciprofloxacin resistance has emerged worldwide (39). The relatively high fluoroquinolone resistance rates among *C. jejuni* recovered from the human stool samples in

the current study were attributed to the development of resistance due to widespread unconscious usage of these antimicrobials in poultry production, and the poultry products can be a source of the transmission of resistant *C. jejuni* isolates to humans.

None of the *Campylobacter* isolates of both human and chicken origin exhibited resistance against erythromycin in the current study. In parallel manner to our study, Szczepanska et al. (40), reported that *Campylobacter* spp. were resistant to ciprofloxacin and tetracycline but were susceptible to erythromycin in Poland. In a study conducted in Estonia, erythromycin resistance was found in none of the human origin *Campylobacter* isolates, but only in one chicken origin *Campylobacter* isolate from the Baltic countries (27), while none of the chicken origin *C. coli* and *C. jejuni* in Poland were reported to be resistant to erythromycin (44). However 74 percentages erythromycin resistance in *C. coli* isolates was reported in Lebanon (33). In contrast to our study results, Tang et al. (41) and Zhang et al. (46) reported the rate of 71.1% and 80% erythromycin resistance, respectively in *C. jejuni* and in *C. coli*, recovered from chicken cloacal swabs in China.

The second highest rate of resistance to tetracycline was found in *C. jejuni* isolates, with 25% and 70.58% for human and chicken origins, respectively, and also 2 *C. coli* isolates recovered from cecal samples were found to be resistant to tetracycline. The tetracycline resistance rate in human isolates in the current study was found to be lower than high proportions of resistance to tetracycline with the rate of 47.2% in human origin *C. jejuni* reported by member state of EU countries (16). Zhang et al. (46) and Wozniak-Biel et al. (44) reported the high resistance rates of tetracycline in chicken origin isolates is compatible with our results. Tetracycline resistance were attributed to common usage for treatment in both humans and chickens. In the current study, resistance to gentamicin was found in neither *C. jejuni* isolates recovered from human nor chicken isolates, but only in *C. coli* isolates recovered from cecal samples, which was consistent with a study conducted in Poland that found 0.4 percent resistance in *C. jejuni*, which was hardly any (43). In contrast to our findings, Kim et al. (25) found gentamicin resistance in *C. jejuni* isolates from humans and poultry, with rates of 15.4% and 8.3%, respectively.

In the present study, 3 multi-drug resistance patterns were identified for examined *Campylobacter* isolates. One of three was observed against tetracycline, ciprofloxacin and nalidixic acid (TET/CIP/NA), the other resistance pattern was determined to ciprofloxacin and nalidixic acid (CIP/NA), and the last one was ciprofloxacin, nalidixic acid and gentamicin (CIP/NA/GEN). TET/CIP/NA resistance pattern was observed both in human and chicken origin *C. jejuni* in the current study and was consistent with previous findings in Ecudarian broilers at slaughter age (42), in poultry and human origin isolates from China (45), in human origin isolates from the Baltic countries (26), in poultry origin isolates from Poland (44), as wel as in poultry and human origin isolates from Turkey (1). In a study conducted in New Zealand, transmission of fluoroquinolon and tetracycline resistant clone of *C. jejuni* ST6964 between the poultry and humans via the food chain was attributed to evidence of fresh poultry supply as a source of human campylobacteriosis (20). The other resistance pattern was determined as CIP/NA in both human and chicken origin *C. jejuni* isolates with the rate of 50% and 29.41%. Wieczorek et al. (43) found the same resistance pattern with a rate of 52.5% in chicken origin *C. jejuni* isolates which was was higher than our result. When the resistance pattern of CIP/NA/GEN in chicken origin *C. coli* isolates was compared with the other studies, common resistance patterns shared by CIP/NA/GEN, CIP/GEN with additional antimicrobials in chicken origin *Campylobacter* isolates were declared (26, 27).

In conclusion, the current study demonstrated that *Campylobacter* spp., especially *C. jejuni* strains, are common in chickens and humans. In addition, it was found that antimicrobial resistant *C. jejuni* strains were circulating in both species and that some of these strains were multidrug resistant. These pose a potential risk to public health. Perhaps one of the main strategies for controlling C*ampylobacter* infections in humans should be to completely eliminate or reduce carcass contamination with *C. jejuni*, the main causative agent. The first approach towards this goal is to prevent *Campylobacter* colonization in broiler flocks and thus to deliver flocks without *Campylobacter* to slaughterhouses.

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