Kafkas Univ Vet Fak Derg
24 (5): 701-707, 2018
DOI: 10.9775/kvfd.2018.19741

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://submit.vetdergikafkas.org

Research Article

Prevalence and Antimicrobial Resistance of Thermophilic *Campylobacter* Isolates from Raw Chicken Meats [1] [2]

Ghassan ISSA [1] Beren BASARAN KAHRAMAN [2] Mehmet Cemal ADIGUZEL [3]
Funda YILMAZ EKER [4] Esra AKKAYA [4] Gülay Merve BAYRAKAL [4]
Ahmet KOLUMAN [5] Tolga KAHRAMAN [4]

[1] *Supported by the Scientific Research Projects Coordination Unit of Istanbul University (Project No. 44016)*
[2] *This study was presented in 3rd International VETistanbul Group Congress, 17-20 May 2016, Bosnia and Herzegovina*
1 Avrupa Vocational School, TR-34020 Kazlıçeşme, Istanbul - TURKEY
2 Department of Microbiology, Faculty of Veterinary Medicine, Istanbul University, TR-34320 Avcılar, Istanbul - TURKEY
3 Department of Microbiology, Faculty of Veterinary Medicine, Atatürk University, TR-25240 Yakutiye, Erzurum - TURKEY
4 Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Istanbul University, TR-34320 Avcılar, Istanbul - TURKEY
5 Faculty of Biomedical Engineering, Pamukkale University, TR-20070 Denizli - TURKEY

Article Code: KVFD-2018-19741 Received: 12.03.2018 Accepted: 11.06.2018 Published Online: 11.06.2018

How to Cite This Article

Issa G, Basaran Kahraman B, Adiguzel MC, Yilmaz Eker F, Akkaya E, Bayrakal GM, Koluman A, Kahraman T: Prevalence and antimicrobial resistance of thermophilic *Campylobacter* isolates from raw chicken Meats. *Kafkas Univ Vet Fak Derg,* 24 (5): 701-707, 2018. DOI: 10.9775/kvfd.2018.19741

Abstract

Globally, the spread of antibiotic resistance via chicken meat consumption cause serious public health concerns. With this respect, the current study aimed to investigate the prevalence of thermophilic *Campylobacter* species isolated from raw meat chicken samples and their genetic determinants of resistance to various classes of antibiotics. A total of 540 chicken raw meat samples collected from various supermarkets and slaughterhouses in Istanbul, Turkey were analyzed according to EN ISO 10272-1:2006 standard procedure. For identification of the genus and species of the isolates, multiplex PCR assay was held. Minimum inhibitory concentrations of the antimicrobial agents (nalidixic acid, ciprofloxacin, tetracycline, gentamicin, kanamycin, and erythromycin) were initially determined using the broth microdilution method. In addition, the genetic determinants of antimicrobial resistance were investigated by PCR assays. In total, 357 (66.1%) *Campylobacter* isolates were obtained including 268 *Campylobacter jejuni* and 89 *Campylobacter coli*. Resistance to quinolones (nalidixic acid and ciprofloxacin) was the most common in all strains (80.1%), followed by resistance to tetracycline's (70.3%). The lowest resistance was determined as resistance to kanamycin (4.2%). Gentamicin and erythromycin resistance was not observed in this study. Only five *C. coli* isolate (1.4%) was classified as multidrug resistant. On the basis of these data, execute widely presence of antimicrobial resistance to quinolones and tetracycline's in *C. jejuni* and *C. coli* isolates from chicken raw meat samples and emphasizes that further multidisciplinary studies and novel strategies in the concept of 'One Health' are needed.

Keywords: *Campylobacter, Raw chicken meat, Prevalence, Antimicrobial resistance, PCR*

Çiğ Tavuk Etlerinden İzole Edilen Termofilik *Campylobacter* İzolatlarının Prevalansı ve Antimikrobiyal Direnci

Öz

Dünyada, tavuk eti tüketimi yoluyla antibiyotik direncinin yayılması ciddi halk sağlığı sorunlarına neden olmaktadır. Bu bağlamda, bu çalışmada, çiğ tavuk eti örneklerinden izole edilen termofilik *Campylobacter* türlerinin prevalansını ve çeşitli antibiyotik sınıflarına direnci gösteren genetik belirleyicileri araştırmayı amaçlandı. İstanbul'daki çeşitli süpermarketlerden ve kesimhanelerden toplanan toplam 540 çiğ tavuk eti numunesi, EN ISO 10272-1:2006 standart prosedürüne göre analiz edildi. İzolatların cins ve türlerinin belirlenmesi için multipleks PCR testi yapıldı. Antimikrobiyal ajanların (nalidiksik asit, siprofloksasin, tetrasiklin, gentamisin, kanamisin ve eritromisin) minimum inhibisyon konsantrasyonları sıvı mikrodilüsyon yöntemi kullanılarak tespit edildi. Bununla beraber, antimikrobiyal direncin genetik belirleyicileri de PCR ile araştırıldı. Toplamda 357 (%66.1) *Campylobacter* izolatı, 268 *Campylobacter jejuni* ve 89 *Campylobacter coli* saptandı. Kinolonlara (nalidiksik asit ve siprofloksasin) karşı direnç, tüm suşlarda en sık görülen direnç (%80.1) olarak saptandı, bunu tetrasiklinlere (%70.3) direnç izledi. En düşük direnç, kanamisin direnci (%4.2) olarak belirlendi. Bu çalışmada gentamisin ve eritromisin direnci gözlenmedi. Sadece beş *C. coli* izolatı (%1.4) çok ilaca dirençli olarak sınıflandırıldı. Bu verilere dayanarak, çiğ tavuk eti örneklerinden elde edilen *C. jejuni* ve *C. coli* izolatlarında kinolonlara ve tetrasiklinlere karşı yaygın antimikrobiyal direnç varlığı saptandı ve "Tek Sağlık" konsepti içinde daha fazla disiplinler arası çalışmalara ve yeni stratejilere ihtiyaç duyulduğu vurgulandı.

Anahtar sözcükler: *Campylobacter, Çiğ tavuk eti, Prevalans, Antimikrobiyal direnç, PCR*

İletişim (Correspondence)
+90 212 4737070/17360
beren@istanbul.edu.tr

INTRODUCTION

Poultry is an extremely high nutritive versatile meat, which is of a great importance for human nutrition, so the safety protection measures of poultry meat are very important subject [1]. Thermophilic *Campylobacter*, including *Campylobacter jejuni* and *Campylobacter coli*, is a main bacterial cause of acute gastroenteritis in humans. Raw poultry products are the main reservoir of thermophilic *Campylobacter* infection in particular via consumption of undercooked products or cross-contamination of ready-to-eat products [2,3]. Also, *Campylobacter* infection is associated with the development of Guillain-Barre´ syndrome, a neurological disorder affecting the peripheral nervous system. Particularly, the chicken is a natural host of *C. jejuni* and serves as a major reservoir for this pathogenic organism. Contamination of chicken carcasses often occurs during the slaughtering process and consumption of chicken meat is a significant source of human *Campylobacter* infections [2-4].

The emergence of antimicrobial resistance is not a new phenomenon, nor an unexpected one. Several reports have been published about antibiotic resistance problem and the reasons behind the increasing rates. These reports have highlighted that poultry meat may play a major role in transmission [3-9]. The uncontrolled and excessive use of antibiotics in the treatment of infections in humans and veterinary medicine may be the reason for high rates of resistance, in poultry [1,10]. In Turkey, antibiotics feed additives were widely used for control of the growth in poultry, but in 2006 the usage of antibiotics in broiler flocks were forbidden by the European Union (EU) Council Directive 90/167/EEC [11,12]. All of the countries in EU have been started to investigate the prevalence of *Campylobacter* spp. in broiler carcasses and the antimicrobial resistance in broiler flocks [10]. However, the number of the studies on antibiotic-resistant *Campylobacter*s isolated from poultry meat in Turkey, is rather limited.

This study was aimed to carry out to determine the prevalence and antimicrobial resistance of thermophilic *Campylobacter* species isolated from chicken raw meat samples available in retail trade in İstanbul, Turkey.

MATERIAL and METHODS

Sample Collection

A total of 540 chicken raw meat samples including chicken thigh, breast and wings were collected from various supermarkets and slaughterhouses in Istanbul, Turkey, between January 2015 and March 2016. With this aim each month, 6 thigh, 6 breast, and 6 wings were obtained from slaughterhouse and same amounts were collected from different markets. A sum of 540 samples were analysed for *Campylobacter* contamination.

Isolation and Species Identification

Campylobacter species detection and isolation were performed according to EN ISO 10272-1:2006 standard procedures [13]. A 25 g portion of each sample was homogenized in a stomacher and were enriched in Bolton broth (Oxoid, USA) for 4 h at 37°C and then incubated for up to 44 h at 42°C under microaerophilic conditions created by using a CampyGen gas pack (Oxoid, USA). The enriched samples were subsequently subcultured by spreading 10 µL aliquots on modified Charcoal Cefoperazone Deoxycholate agar (CCDA, Oxoid, USA) and incubated for up to 48 h at 42°C under microaerophilic conditions. Suspected colonies were cultured onto plates of Columbia Blood agar (Oxoid, USA) containing 5% horse blood, and were confirmed by microscopic analysis, oxidase testing (Oxoid, USA), microaerophilic growth at 25°C and aerobic growth at 42°C. The remainder of each plate was harvested and stored in 1 mL of nutrient broth plus 10% glycerol at 80°C. Conventional culture method was verified using ISO 16140 method. According to this method 30 positive and 30 negative samples were analysed using the method. The results obtained showed a specifity and sensitivity of 95%.

For identification of the genus and species of the isolates, multiplex PCR was carried out following the PCR assay method described by Linton et al.[14] and Denis et al.[15]. Simultaneous amplification of 16SrRNA gene fragment (genus-specific), *map*A gene (for *C. jejuni)* and *ceu*E gene (for *C. coli)* was carried using primers and protocol. The details of primers and cycling conditions are given in *Table 1*. Amplified PCR products were visualized by electrophoresis in 1.5% agarose gel stained with ethidium bromide. For quality control, *C. jejuni* ATCC 33291, *C. jejuni* ATCC 33560 and *C. coli* ATCC 33559 strains were used.

Antimicrobial Susceptibility Testing

Minimum inhibitory concentrations (MIC) of antimicrobial agents (ciprofloxacin, erythromycin, gentamicin, kanamycin, nalidixic acid and tetracycline) was determined with a microbroth dilution method [16].

The clinical breakpoints were interpreted according to the EUCAST [16] guidelines for *Campylobacter* as regards erythromycin, nalidixic acid, gentamicin, ciprofloxacin and tetracycline, and to CLSI guidelines for Enterobacteriaceae [17] as regards kanamycin (MIC≤16 susceptibility, MIC=32 intermediate, MIC≥64 resistant), because there was no ECOFFS for *Campylobacter*.

Campylobacter jejuni ATCC 33560 was used as reference strains for quality control assurance in each batch of broth microdilution plates [16].

Detection of Antimicrobial Resistance Genes

All of the phenotypically resistant isolates were analyzed for the presence of *ery*, *tet*(O), *aph*A-3, *gyr*A (Thr-86-Ile

Table 1. *Primer sequences, product sizes and cycling conditions*

Primer Specific For	Target(s)	Primers (5′ to 3′, as synthesized)	Size (bp)	Cycling Conditions
Campylobacter	16SrRNA	ATCTAATGGCTTAACCATTAAAC GGACGGTAACTAGTTTAGTATT	857	95°C 60 s; 95°C 15 s; 59°C 60 s; 72°C 90 s (35 cycles); 72°C 3 min
C. jejuni	*mapA*	CTATTTTATTTTTGAGTGCTTGTG GCTTTATTTGCCATTTGTTTTATTA	589	
C. coli	*ceuE*	AATTGAAAATTGCTCCAACTATG TGATTTTATTATTTGTAGCAGCG	462	

Table 2. *Primer sequences, product sizes and cycling conditions*

Primer Specific For	Target(s)	Primers (5′ to 3′, as synthesized)	Size (bp)	Cycling Conditions
Erythromycin resistance	23S rRNA-F 23S rRNA-R	TTAGCTAATGTTGCCCGTACCG AGCCAACCTTTGTAAGCCTCCG	697	94°C 5 min; 94°C′ 30 s; 59°C′ 30 s; 72°C 45 s; (30 cycles); 72°C 5 min
	ERY2075-R	TAGTAAAGGTCCACGGGGTCGC	485	
	ERY2074-R	AGTAAAGGTCCACGGGGTCTGG	485	
Quinolones resistance	GZgyrA5-F GZgyrA6-R	ATTTTTAGCAAAGATTCTGAT CCATAAATTATTCCACCTGT	673	94°C 3 min; 94°C′ 30 s; 50°C′ 30 s 72°C′ 20 s; (30 cycles); 72°C 5 min
	CampyMAMAgryA-F CampyMAMAgyrA-R	TTTTTAGCAAAGATTCTGAT CAAAGCATCATAAACTGCAA	265	
	CampyMAMAgryA1-F GZgyrA4	TTTTTAGCAAAGATTCTGAT CAGTATAACGCATCGCAGCG	368	
	GZgyrACcoli3F-F CampyMAMAgyrA8-R	TATGAGCGTTATTATCGGTC TAAGGCATCGTAAACAGCCA	192	
	GZgyrACcoli3F-F GZgyrACcoli4R-R	TATGAGCGTTATTATCGGTC GTCCATCTACAAGCTCGTTA	505	
Aminoglycoside resistance	*aph*A-3 F *aph*A-3 R	GGGACCACCTATGATGTGGAACG CAGGCTTGATCCCCAGTAAGTC	600	95°C 30s; 55°C 1 min; 72°C 1 min (30 cycles); 72°C 5 min
Tetracycline resistance	*tet*O F *tet*O R	GGCGTTTTGTTTATGTGCG ATGGACAACCCGACAGAAGC	559	95°C 1 min; 95°C 15 s; 58°C 15 s 72°C 30 s; (30 cycles); 72°C 5 min
cmeABC	*cme*A- F *cme*A- R	TAGCGGCGTAATAGTAAATAAAC ATAAAGAAATCTGCGTAAATAGGA	435	94°C 7 min; 94°C 1 min; for cmeA 49.8°C, for cmeB 50.8°C, for cmeC 52.3°C 90 s; 72°C 2.5 min (31 cycles); 72°C 5 min
	*cme*B- F *cme*B- R	AGGCGGTTTTGAAATGTATGTT TGTGCCGCTGGGAAAAG	444	
	*cme*C- F *cme*C- R	CAAGTTGGCGCTGTAGGTGAA CCCCAATGAAAAATAGGCAGAGTA	431	

mutation), *cme*A, *cme*B and *cme*C genes, representing resistance to erythromycin, tetracycline, aminoglycoside, and quinolones, and CmeABC efflux system components, respectively.

Mismatch Amplification Mutation Assay (MAMA-PCR) for the detection of point mutations at position 2075 and 2074, which present high-level erythromycin resistance, were performed [18]. Genes *tet*(O) and *aph*A-3 were detected by PCR assay as described [19]. Thr-86-Ile mutations in the quinolones resistance determining region (QRDR) of gene *gyr*A were detected by MAMA-PCR [20,21]. The presence of the *cme*A, *cme*B and *cme*C genes were determined by PCR assays [22]. The primers sequences, product sizes and cycling conditions are listed in *Table 2*.

Multi-drug resistance (MDR) was defined as resistance to three or more antimicrobial agents with different mechanisms of action, as previously described [23].

RESULTS

The prevalence rate of *Campylobacter* spp. in chicken raw meat samples were found in 66.1%. Monthly distribution is summarized in *Fig. 1*. Totally 357 *Campylobacter* isolates, whereas *C. jejuni* was identified in the remaining 268 (75.07%) and *C. coli* 89 (24.93%).

Distribution of *C. jejuni* according to tight, breast and wing samples were 73 (27.23%), 106 (39.55%) and 89 (33.22%). Distribution of *C. jejuni* from different parts at slaughterhouse level was not significant with months and no seasonal change was observed. On the contrary, seasonal distribution of *C.jejuni* was observed in market samples. *C. jejuni* was mostly isolated during summer months with a rate of 88.88% (48 of 54 samples) and was lowest during January with a rate of 5.56% (1 of 18 samples).

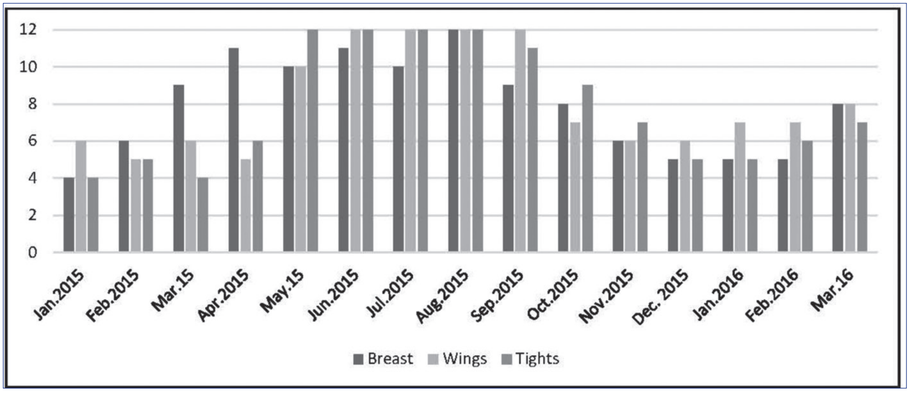

Fig 1. Monthly distribution of *Campylobacter* isolates

Table 3. *Antibacterial resistance profiles and MIC distributions of the isolates*

Antimicrobial	MIC Range (µg/mL)		Number of Isolates According to MIC														
	S ≤	R >	Isolates	0.094	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Erythromycin	4	4	Cj		3	182	53	30									
	2	2	Cc		6	56	13	14									
Gentamicin	2	2	Cj		8	214	34	12									
			Cc			66	15	8									
Kanamycin	4	4	Cj				9	34	197	18	10						
			Cc					4	80						5		
Nalidixic acid	16	16	Cj							16	31	4	100	117			
			Cc						2	6	12		10	59			
Ciprofloxacin	0.5	0.5	Cj							8	52		52	156			
			Cc							10	1		22	56			
Tetracycline	1	1	Cj		80	19						5	109	16	35	4	
	2	2	Cc		3	4						4	56	8	12	2	

MIC: *Minimum Inhibitory Concentration,* ***S:*** *Susceptible,* ***R:*** *Resistant,* ***Cj:*** *C. jejuni,* ***Cc:*** *C. coli*

Table 4. *Comparison of phenotypic and genotypic resistance to antimicrobial agents*

Isolates	Number of Strains Resistant to Antimicrobial Agents							
	Quinolones				Tetracycline		Kanamycin	
	Nalidixic Acid		Ciprofloxacin					
	Broth Microdilution	Mutation Thr86Ile	Broth Microdilution	Mutation Thr86Ile	Broth Microdilution	tet(O) gene	Broth Microdilution	aphA-3 gene
C. jejuni (n=268)	217	217	208	208	169	169	10	10
C. coli (n=89)	69	69	78	78	82	82	5	5
Total (n=357)	286 (80.1%)				251 (70.3%)		15 (4.2%)	

Resistance to quinolones (nalidixic acid and ciprofloxacin) was the most common finding (80.1%), followed by resistance to tetracyclines (70.3%). Conversely, the lowest resistance was recorded against to kanamycin (4.2%). Furthermore, all isolates were detected susceptible to gentamicin and erythromycin. Only five *C. coli* isolates (1.4%) were evaluated as multidrug resistant.

The antibacterial susceptibility testing results of 357 *Campylobacter* isolates against six different antibacterial agents are exhibited in *Table 3*.

The phenotypic and genotypic results were fully concordant. Comparison of phenotypic and genotypic resistance to antimicrobial agents was shown in *Table 4*.

In this study, all isolates were resistant to at least one antibacterial agent, while most of the isolates were resistant to tetracycline, nalidixic acid, and ciprofloxacin. 20% of the isolates were resistant to two antibacterial agents and 1.4% of the isolates to more than two antibiotics.

DISCUSSION

Poultry products are the most important single source of human Campylobacteriosis. The European Food Safety Authority (EFSA) reported 246.307 laboratory confirmed cases in the EU [24]. Turkey was one of the most often reported as the probable country of infection outside EU (5.5%). Han et al.[3] in Korea, Guyard-Nicodème et al.[7] in France, and Maesaar et al.[8] in Estonia were reported *Campylobacter* spp. prevalence from broiler chicken meat 68.3%, 76%, and 88.8%, respectively.

Regarding previous studies in Turkey, Hızlısoy et al.[25] found 100% of the chicken meat samples positive for *Campylobacter* species. Abay et al.[6] reported that among 100 carcass samples examined, 96°*C. jejuni* strains were isolated. In this study, it has been demonstrated that *Campylobacter* spp. are frequently present (66.1%). Withal, when comparing the reported prevalence of *Campylobacter* spp. among our country during recent years, the results of the present study are considerably lower. Seasonal distribution of samples were showing similarity with the results of Koluman [26] and Pamuk [27].

High fluoroquinolones resistance levels among *Campylobacter* poultry meat isolates have been widely stated, in Poland [5,28], Italy [29], Turkey [6], Korea [9] and many other European countries [30]. In the current study, resistance to quinolones (nalidixic acid and ciprofloxacin) was the most common and these results substantiate other authors' findings. The broad use of this class of antibiotics in poultry may be the reason for this crucial problem.

The tetracycline's, being the first major group of antimicrobial agents, are among the most frequently used therapeutics in veterinary medicine. In the current study, the resistance rate to tetracycline was determined as 70.3%. The prevalence was higher in comparison to those detected by Abay et al.[6], Guyard-Nicodème et al.[7], Maesaar et al.[8], Wei et al.[9], Wieczorak et al.[15].

The aminoglycosides are a group of antimicrobials used both in human and veterinary medicine. Gentamicin is the most widely used aminoglycosides in poultry. EFSA [31] reported that the gentamicin resistance was comparatively very low (0.3%) in *C. jejuni* isolates and resistance were not detected in *C. coli* isolates from broiler meat. Wei et al.[9] call attention to the high prevalence of gentamicin-resistant Campylobacter isolated in food-producing animals in China. Moreover, low to moderate resistance ranging from 0 to 27% was observed in various studies [32-34]. Kanamycin is an aminoglycoside antibiotic which is effective in the treatment of severe infections caused by Gram-negative bacteria [5]. In the current study, all isolates were susceptible to gentamicin. Also, kanamycin resistance was determined in 15 strains (4.2%).

Macrolides are still the most effective antibiotics against *Campylobacter* infections. Macrolide resistance in *Campylobacter* spp. has been the result of the point mutation(s) occurring in ribosomal RNA or proteins. The authors reported high resistance to erythromycin in Spain [35]. However, in European countries, low resistance levels were stated from 0 to 8% [32]. According to EU summary report, the variable occurrence of resistance to erythromycin among *Campylobacter* species were reported, depending on the country of isolation [24]. In this study, all of the isolates were susceptible to erythromycin which is the drug of choice for the treatment of human Campylobacteriosis. This result is in agreement with those reported for chicken meat isolates Wieczorak et al.[5] in Poland and Guyard-Nicodème et al.[7] in France. Because of the low level of resistance might be consequences of the ban of macrolides as a growth promoter in broilers.

Otherwise, except these individual resistance mechanisms, multidrug efflux system CmeABC contributes to *Campylobacter* resistance to multiple drugs, including fluoroquinolones, β-lactams, erythromycin, and tetracycline [19,36]. The authors indicated that the effect of CmeABC on aminoglycoside resistance (like gentamicin) was less apparent [36]. In this study, only five *C. coli* isolate (1.4%) was classified as multidrug resistant. Contrary, the authors reported much higher percentages ranged from 44.9 to 86% [3,4,37]. The use of antimicrobial drugs in food animals has been regulated in European countries, the conflicted results may base on the implementation of legislation. In some developing countries, even where legislation does exist and is enforced, their enforcement may be a problem and virtually non-existent [10,37]. The absence and/or weakness of regulations and implementation particularly about usage of antibiotics in the food animals, also inadequate hygiene and sanitation, may have accelerated the emergence and dissemination of antimicrobial resistance.

Over recent decades, antibiotic resistance undoubtedly represents a global public health problem. The global author's highlight that poultry meat is an important risk of human exposure to antimicrobial resistance due to residual resistance of high impact antibiotic application of 20th century or illegal applications as growth promoters [38,39]. In the current study provides baseline information on the highlights the widespread presence of this emerging foodborne pathogen and resistance profiles in poultry meat. These data emphasize that further multidisciplinary studies, surveillance programmes and reports in animals, and humans, as well as food, are important in terms of manifesting the current status of resistance against antimicrobial drugs and emerging health problems. Therewithal, the data acquired here will be useful for risk assessment for public health hazard *C. jejuni*. It is significant that the population and demographic character of Istanbul is highly variable with widespread chicken consuming behaviour. This picture can be generalized with significant variations of other cities to determine a significant hazard map to prevent *C. jejuni* borne infections.

REFERENCES

1. Ljubojević D, Pelić M, Puvača N, Milanov D: Resistance to tetracycline in *Escherichia coli* isolates from poultry meat: Epidemiology, policy and perspective. *Worlds Poult Sci J*, 73 (2): 409-417, 2017. DOI: 10.1017/S0043933917000216

2. Fontanot M, Iacumin L, Cecchini F, Comi G, Manzano M: Rapid detection and differentiation of important *Campylobacter* spp. in poultry samples by dot blot and PCR. *Food Microbiol*, 43, 28-34, 2014. DOI: 10.1016/j.fm.2014.05.001

3. Han K, Jang SS, Choo E, Heu S, Ryu S: Prevalence, genetic diversity, and antibiotic resistance patterns of *Campylobacter jejuni* from retail raw chickens in Korea. *Int J Food Microbiol*, 114 (1): 50-59, 2007. DOI: 10.1016/j.ijfoodmicro.2006.10.042

4. 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 (1-2): 133-139, 2010. DOI: 10.1016/j.vetmic.2009.12.035

5. Wieczorek K, Szewczyk R, Osek J: Prevalence, antimicrobial resistance, and molecular characterization of *Campylobacter jejuni* and *C. coli* isolated from retail raw meat in Poland. *Vet Med Czech*, 57 (6): 293-299, 2012.

6. 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

7. Guyard-Nicodème M, Rivoal K, Houard E, Rose V, Quesne S, Mourand G, Rouxel S, Kempf I, Guillier L, Gauchard F, Chemaly M: Prevalence and characterization of *Campylobacter jejuni* from chicken meat sold in French retail outlets. *Int J Food Microbiol*, 203, 8-14, 2015. DOI: 10.1016/j.ijfoodmicro.2015.02.013

8. Mäesaar M, Kramarenko T, Meremäe K, Sõgel J, Lillenberg M, Häkkinen L, Ivanova M, Kovalenko K, Hörman A, Hänninen ML, Roasto M: Antimicrobial resistance profiles of *Campylobacter* spp. isolated from broiler chicken meat of Estonian, Latvian and Lithuanian origin at estonian retail level and from patients with severe enteric infections in Estonia. *Zoonoses Public Health*, 63 (2): 89-96, 2016. DOI: 10.1111/zph.12208

9. Wei B, Cha SY, Yoon RH, Kang M, Roh JH, Seo HS, Lee JA, Jang HK: Prevalence and antimicrobial resistance of *Campylobacter* spp. isolated from retail chicken and duck meat in South Korea. *Food Control*, 62, 63-68, 2016. DOI: 10.1016/j.foodcont.2015.10.013

10. Maćkiw E, Korsak D, Rzewuska K, Tomczuk K, Rożynek E: Antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli* isolated from food in Poland. *Food Control*, 23 (2): 297-301, 2012. DOI: 10.1016/j.foodcont.2011.08.022

11. EC. Council Directive 90/167/EEC of 26 March 1990 laying down the conditions governing the preparation, placing on the market and use of medicated feeding stuffs in the Community. *Offic J Eur Comm*, L92: 42-48, 1990.

12. Anonymous: Yem katkıları ve premikslerin üretimi, ithalatı, ihracatı, satışı ve kullanımı hakkında tebliğde değişiklik yapılmasına dair tebliğ. Tarım ve Köy İşleri Bakanlığından. *Resmi Gazete.* 21 Ocak 2006; Sayı: 26056 Tebliğ No: 2006/1.

13. Anonymous. Microbiology of food and animal feeding stuffs - horizontal method for detection and enumeration of *Campylobacter* spp. - Part 1: Detection method (EN ISO 10272-1:2006). International Organization for Standardisation, Geneva, Switzerland. 2006.

14. Linton D, Owen RJ, Stanley J: Rapid identification by PCR of the genus *Campylobacter* and of five *Campylobacter* species enteropathogenic for man and animals. *Res Microbiol*, 147, 707-718, 1996. DOI: 10.1016/S0923-2508(97)85118-2

15. Denis M, Petton JR, Laisney MJ, Ermel G, Salvat G: *Campylobacter* contamination in French chicken production from farm to consumers. Use of a PCR assay for detection and identification of *Campylobacter jejuni* and *Campylobacter coli*. *J Appl Microbiol*, 91, 255-267, 2001. DOI: 10.1046/j.1365-2672.2001.01380.x

16. EUCAST (The European Committee on Antimicrobial Susceptibility Testing): Breakpoint tables for interpretation of MICs and zone diameters. version 5.0, 2015.

17. CLSI (Clinical and Laboratory Standards Institute): Performance standards for antimicrobial susceptibility testing. Twenty-second informational supplement. Document M100-S22. Wayne, PA, USA, 2012.

18. Alonso R, Mateo E, Churruca E, Martinez I, Girbau C, Fernández-Astorga A: MAMA-PCR assay for the detection of point mutations associated with high-level erythromycin resistance in *Campylobacter jejuni* and *Campylobacter coli* strains. *J Microbiol Methods*, 63 (1): 99-103, 2005. DOI: 10.1016/j.mimet.2005.03.013

19. Gibreel A, Sköld O, Taylor DE: Characterization of plasmid-mediated *aphA-3* kanamycin resistance in *Campylobacter jejuni*. *Microb Drug Resist,* 10 (2), 98-105, 2004. DOI: 10.1089/1076629041310127

20. Zirnstein, G, Helsel L, Li Y, Swaminathan B, Besser J: Characterization of gyrA mutations associated with fluoroquinolone resistance in *Campylobacter coli* by DNA sequence analysis and MAMA PCR. *FEMS Microbiol Lett*, 190, 1-7, 2000. DOI: 10.1111/j.1574-6968.2000.tb09253.x

21. Zirnstein G, Li Y, Swaminathan B, Angulo F: Ciprofloxacin resistance in *Campylobacter jejuni* isolates: Detection of gyrA resistance mutations by mismatch amplification mutation assay PCR and DNA sequence analysis. *J Clin Microbiol*, 37, 3276-3280, 1999.

22. 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

23. 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 (1): 40-52, 2016. DOI: 10.1089/mdr.2015.0079

24. EFSA (European Food Safety Authority): The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. *EFSA J*, 15 (12): 5077, 2017. DOI: 10.2903/j.efsa.2017.5077

25. Hızlısoy H, Kılıç H: The resistance state of *Campylobacter jejuni* isolated from broiler carcasses against to macrolide, quinolone and tetracycline groups of antibiotics. *Erciyes Univ Vet Fak Derg*, 12 (2): 81-92, 2015.

26. Koluman A: Detection of *Campylobacter jejuni* contamination in poultry houses and slaughterhouses. *Turk Hij Den Biyol Derg*, 67 (2): 57-64, 2010.

27. Pamuk S: Afyon'da paketlenmeden satılan piliç karkaslarında termofilik *Campylobacter* türlerinin saptanması ve *C. jejuni* izolatlarının PCR ile doğrulanması. *Doktora Tezi,* Ankara Üniversitesi Sağlık Bilimleri Enstitüsü, 2010.

28. Rozynek E, Dzierzanowska-Fangrat K, Korsak D, Konieczny P, Wardak S, Szych J, Jarosz M, Dzierzanowska D: Comparison of antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from humans and chicken carcasses in Poland. *J Food Prot*, 71 (3): 602-607, 2008.

29. Di Giannatale E, Di Serafino G, Zilli K, Alessiani A, Sacchini L, Garofolo G, Aprea G, Marotta F: Characterization of antimicrobial resistance patterns and detection of virulence genes in *Campylobacter* isolates in Italy. *Sensors*, 14 (2): 3308-3322, 2014. DOI: 10.3390/s140203308

30. EFSA (European Food Safety Authority): The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2011. *EFSA J*, 11 (5): 3196, 2013. DOI: 10.2903/j.efsa.2013.3196

31. EFSA (European Food Safety Authority): The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2014. *EFSA J*, 14 (2): 4380, 2016. DOI: 10.2903/j.efsa.2016.4380

32. EFSA (European Food Safety Authority): Analysis of the baseline survey on the prevalence of *Campylobacter* on 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

33. EFSA (European Food Safety Authority): The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. *EFSA J*, 10 (3): 2597, 2012. DOI: 10.2903/j.efsa.2012.2597

34. Economou V, Zisides N, Gousia P, Petsios S, Sakkas H, Soultos N, Papadopoulou C: Prevalence and antimicrobial profile of *Campylobacter* isolates from free-range and conventional farming chicken meat during a 6-year survey. *Food Control*, 56, 161-168, 2015. DOI: 10.1016/j.foodcont.2015.03.022

35. Sáenz Y, Zarazaga M, Lantero M, Gastañares MJ, Baquero F, Torres C: Antibiotic resistance in *Campylobacter* strains isolated from animals, foods, and humans in Spain in 1997-1998. *Antimicrob Agents Chemother*, 44 (2): 267-271, 2000. DOI: 10.1128/AAC.44.2.267-271.2000

36. Lin J, Michel LO, Zhang Q: CmeABC functions as a multidrug efflux system in *Campylobacter jejuni*. *Antimicrob Agents Chemother*, 46, 2124-2131, 2002. DOI: 10.1128/AAC.46.7.2124-2131.2002

37. Rodrigo S, Adesiyun A, Asgarali Z, Swanston W: Prevalence of *Campylobacter* spp. on chickens from selected retail processors in Trinidad. *Food Microbiol*, 22, 125-131, 2005. DOI: 10.1016/j.fm.2004.03.008

38. Bilge N, Vatansever L, Sezer Ç: Antibiotic resistance of *Salmonella* spp. isolated from raw chicken wings. *Kafkas Univ Vet Fak Derg*, 24 (3): 431-435, 2018. DOI: 10.9775/kvfd.2017.19134

39. Büyükünal SK: Prevalence and antibiotic resistance of thermotolerant *Campylobacter* spp. in chicken meat sold in İstanbul. *J Fac Vet Med Istanbul Univ*, 43 (2): 98-109, 2017. DOI: 10.16988/iuvfd.282027