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ORIGINAL ARTICLE

Prevalence, species diversity, and antimicrobial susceptibility of *Campylobacter* strains in patients with diarrhea and poultry meat samples: one-year prospective study

Atena Sadeghi[1], Leila Ganji[2], Fereshteh Fani[3], Gholamreza Pouladfar[3], Parisa Eslami[4], Fatemeh Doregiraee[5], Parviz Owlia[6*], Masoud Alebouyeh[7*]

[1]Department of Microbiology, Faculty of Medicine, Shahed University, Tehran, Iran
[2]Department of Microbiology, Iranian Reference Health Laboratory Research Center, Ministry of Health and Medical Education, Tehran, Iran
[3]Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[4]Department of Microbiology, Central Laboratory, Milad Hospital, Tehran, Iran
[5]Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[6]Molecular Microbiology Research Center, Faculty of Medicine, Shahed University, Tehran, Iran
[7]Pediatric Infections Research Center, Research Institute for Children's Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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ABSTRACT

Background and Objectives: Source tracking of antimicrobial resistance in *Campylobacter* is useful for control measures. In this study, *Campylobacter*-associated diarrhea and homology in antimicrobial resistance of humans and poultry meat isolates were investigated.

Materials and Methods: A total of 400 stools of patients and 100 poultry meat samples were analyzed. Susceptibility of the isolates was detected by disk diffusion, Etest, and agar dilution methods. Mismatch amplification mutation assay was used for the detection of mutations in the *gyrA* quinolone resistance determining region (QRDR).

Results: *Campylobacter* spp., including *C. jejuni*, *C. coli*, and *C. lari*, were detected in 35% of the chicken meat and 6.75% of the stool samples, respectively. The QRDR mutation was detected in most of the stool and chicken meat samples. Although the frequency of resistance to tetracycline (53.5% and 62.8%), erythromycin (39.2% and 37.1%), and gentamicin (32.1% and 31.4%) was relatively similar, higher frequency of resistance to ciprofloxacin (51.4% vs 28.6%) and nalidixic acid (42.15% vs 28.6%) among the chicken meat, and ampicillin (50% and 17.1%) among the human stool was detected.

Conclusion: High percentage of poultry meat samples is contaminated with different *Campylobacter* species, which shows homology with the patients' isolates in Tehran.

Keywords: Foodborne diseases; *Campylobacter*; Drug resistance; Diarrhea; Poultry

*Corresponding author: Parviz Owlia, Ph.D, Molecular Microbiology Research Center, Faculty of Medicine, Shahed University, Tehran, Iran. Tel: +98-9121948112 Fax: +98-21-66498482 Email: powlia@gmail.com

*Corresponding author: Masoud Alebouyeh, Ph.D, Pediatric Infections Research Center, Research Institute for Children's Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Tel: +98-9113548284 Fax: +98-21-22226941 Email: masoud.alebouyeh@gmail.com

INTRODUCTION

Foodborne diseases are caused by consuming contaminated water, beverages, and foods (1). These diseases could occur in all seasons, but some types of the etiological agents may exhibit well-defined seasonal patterns (2). Increased tendency to consume ready-to-eat foods, usage of contaminated irrigating water in agriculture, usage of freshly prepared raw products, and weakness in the management of contamination along the food chain are among the main risk factors for transmission of enteric pathogens (3). Detection of sources of these pathogens and their transmission routes could be useful for the control of foodborne diseases.

Centers for Disease Control and Prevention reported that diarrheal diseases account for 10% of child deaths worldwide, which makes diarrhea the second leading cause of death among children under the age of 5 (4). *Campylobacter* is one of the major causative agents that accounts for serious diseases in humans. It can transmit through the consumption of contaminated meat, poultry, dairy, and ready-to-eat products (5). This bacterium can cause inflammatory diarrhea, enteritis, bloody diarrhea, and some types of extraintestinal and systematic diseases, such as Guillain-Barre syndrome, Miller Fisher syndrome, cholecystitis, septic arthritis, osteomyelitis, endocarditis, and meningitis (6). Its prevalence varies based on the geographic regions but could be higher than *Salmonella* and *Shigella* in some countries (7).

Campylobacter frequency in poultry, especially among broilers, is high (8). *Campylobacter* can grow in the caeca of poultry in very high numbers, which assume as the main cause of the contamination of the poultry meat during the processing phase. European Food Safety Authority (EFSA) encountered chicken meat consumption for 20%-30% of campylobacteriosis in the EU (9). To reduce the risk of *Campylobacter* transmission to consumers, interventions were taken into account to reach the contamination rate of chicken meat to less than 1000 colony-forming units per gram. However, variations in contamination rates among different countries and increased rate of antimicrobial resistance among *Campylobacter* strains are currently considered leading worldwide concerns. World Health Organization reported the emergence of resistant strains of *Campylobacter* to common antibiotics, such as fluoroquinolones, macrolides, and tetracyclines which are prescribing for the treatment of severe infections in humans (10). Resistance to these antibiotics has increased significantly over the past two decades, which is associated with their administration in farm animals and the frequency of mutations in genes that are linked to resistance phenotypes (11). While recent studies on *C. jejuni* isolates from chicken showed that resistance to at least one antibiotic is seen in a high percentage of them (12), the link between these isolates with those detected in human samples remains controversial.

To find the risk of infection with *Campylobacter* among the consumers in a community, a surveillance study should be done. A study of the burden of *Campylobacter* infection in symptomatic patients with diarrhea and its link with the contamination rate of poultry meat distributed in the shopping centers at the end of the food chain could provide valuable data for designing more appropriate control programs. In this study, we aimed to investigate the frequency of *Campylobacter* infection among diarrheal patients who were referred to four hospitals in Tehran and its link with the contamination rate of *Campylobacter* in poultry meat samples among distinct shopping centers of the 22 regions in Tehran. Furthermore, source tracking of the strains was done based on the predicted similarity of resistance and molecular patterns among the human feces and chicken meat isolates.

MATERIALS AND METHODS

Ethical approval. This study was approved by the ethical committee of the National Institute for Medical Research Development, Islamic Republic of Iran (IR.NIMAD.REC.1396.105). All subjects gave written informed consent to the Iran National Committee for Ethics in Biomedical Research.

Sample collection. In this study, a total of 500 samples, including 100 fresh poultry meat samples from authorized distribution centers in 22 districts of Tehran and 400 stool samples from patients with community-acquired diarrhea who were referred to one pediatric and three general hospitals in Tehran were collected. Demographic data of the samples were provided by a questionnaire. Admitted patients and those who received antibiotics were excluded from the study. Freshly prepared stool and chicken meat specimens were transferred to the laboratory at 4°C within 2 hours. Amies transport medium was used

for transport of the stool samples, when sent to the laboratory with delay.

***Campylobacter* culture.** In the case of chicken meat, 25 g from different parts of each carcass was prepared and placed in a 225 ml flask containing Preston medium supplemented with CCDA-selective-supplement. The inoculated culture medium was incubated in a shaker incubator for 48 hours at 42°C. Isolation of the bacterium was done by culture of 20 µl of the enrichment medium on CCD-agar medium which was incubated for 1 to 5 days at microaerophilic conditions at 42°C. The formation of colonies was followed daily, and suspected ones were further examined with microscopic analysis and biochemical tests. The confirmed isolates were passaged on Columbia agar medium (Liofilchem-Italy) containing 10% sheep blood and incubated at 42°C for 24 h under microaerophilic conditions. In the cases of stool samples, to enrich fresh stool specimens, a mixture of 1 g stool in 1 ml normal saline was filtered on a CCD-agar medium using 0.65 µm cellulose acetate membrane. The swab samples in the Amies transport medium were cultured on CCD-agar medium directly with similar incubation conditions mentioned previously for 1-5 days. All identified isolates were stocked at -70°C for further characterization.

Molecular characterization of *Campylobacter* species. Single and multiplex PCR methods were used to confirm the identity of *Campylobacter* isolates in terms of genus and species, respectively. For DNA extraction, the boiling method was used and the extracts were stored at -20°C until use. The reaction solution consisted of a volume of 25 µl containing 10 µL of master mix solution (Ampliqon, Denmark), and 0.5 µL of $MgCl_2$ at 1.5 mM concentration (Cinnaclon, Iran), 1 mM of forward and reverse primers, and 4 ng of genomic DNA. The amplification circumstance and sequences of the primers have demonstrated in Table 1.

Mismatch amplification mutation assay (MAMA) PCR for detection of *gyrA* mutations. MAMA PCR was employed to discern the frequency of point mutations in the quinolone resistance determining region (QRDR) of *C. jejuni* isolates (13). CampyMAMAgryA1 and GZgyrA4 primers were used for the detection of the *gyrA* gene which serves as a control region in all the reactions. *gyrA* mutation in the QRDR region was detected using the primers

Table 1. Primers used in this study for characterization of *Campylobacter* isolates at the genus and species levels and *gyrA* mutation Thr-86-Ile.

	PCR products	Gene	Primers 5′ to 3′	Ref	Amplification program
Campylobacter Genus	812 bp	16S rRNA	F: GGATGACACTTTCGGAGC R: CATTGTAGCACGTGTGTC	(41)	5 min. 94°C 1 cycle
C. coli	364 bp	Ask	F: AGGCAAGGGAGCCTTTAATC R: TATCCCTATCTACAAATTCGC	(42)	1 min. 94°C, 1 min. 64-56°C, 1 min 72°C 2 cycles at each 2°C intervals
C. jejuni	773 bp	cj0414	F: CATCTTCCCTAGTCAAGCCT R: AAGATATGGCACTAGCAAGAC		1 min. 94°C, 1 min,.54°C, 1 min 72°C 30 cycles
C. upsaliensis	86 bp	LpxA	F: CGATGATGTGCAAATTGAAGC R: TTCTAGCCCCTTGCTTGATG	(43)	15 min, 95°C, 1 cycle
C. lari	251 bp	GlyA	F: TAGAGAGATAGCAAAAGAGA R: TACACATAATAATCCCACCC	(44)	30 sec. 95°C, 90 sec, 58°C, 30 sec.72°C, 30 cycles
gyrA positive control	368 bp with CampyMAMAgyrA1 256 bp with CampyMAMAgyrA5	GZgyrA4	R: CAG TAT AAC GCA TCG CAG CG		5 min. 94°C, 1 cycle
Thr-86-Ile (ACA→ATA) mutation		CampyMAMAgyrA5	R: CAAAGCATCATAAAACTGCAA	(45)	1 min. 94°C, 1 min. 55-52°C, 1 min 72°C 2 cycles at each 2°C intervals
		CampyMAMAgyrA1	F: TTTTTAGCAAAGATTCTGA		1 min, 94°C, 1 min. 51°C, 1 min 72°C 30 Cycles

CampyMAMAgryA1 and CampyMAMA (Table 2). The final extension for all the reactions was done at 72°C for 10 min. All the PCR products were visualized in 1.2% agarose gel.

Antimicrobial susceptibility testing. Susceptibility of the *Campylobacter* isolates to 7 antibiotics was analyzed using disk diffusion, E-test, and agar dilution methods. The tested antibiotics were included: ampicillin (Germany, Biochemica), gentamicin (Canada-BioBasic), nalidixic acid (E-test, AB BIO DISK, Sweden), tetracycline (Merck, Germany; 30 mg), erythromycin (Merck, Germany; 15 mg), ciprofloxacin (Merck, Germany; 5 mg) and clindamycin (E-test, AB BIO DISK, Sweden). Disk diffusion was done on Mueller-Hinton agar plates (Merck, Germany) containing 5% sheep blood. The inoculated plates were incubated at 42°C for 24 hours under microaerophilic conditions. *E. coli* strain ATCC 25922 and *S. aureus* ATCC 29213 were used for quality control of the antibiotic discs. Resistance to clindamycin, nalidixic acid, and erythromycin was defined when the zone of growth was lower than 8 mm, 32 mm, and 12 mm, respectively. Sensitivity to gentamicin (Canada-BioBasic) and ampicillin (Germany-Biochemica) was determined by the agar dilution method. Accordingly, the supplemented Mueller Hinton Agar medium with 5% sheep blood and sequential concentrations of gentamicin (2-32 μg/ml) and ampicillin (4-64 μg/ml) was inoculated with equivalent to 0.5 MacFarland of freshly grown isolates. According to the CLSI guidelines, strains with growth at concentrations ≥16 μg/ml and ≥32 μg/ml were considered resistant to gentamicin and ampicillin antibiotics, respectively (14).

Statistical analysis. Correlation between the human and chicken meat isolates was determined

Table 2. Frequency of antimicrobial resistance patterns among different strains of *Campylobacter* in chicken meat and human stool samples.

MDR phenotypes	In chicken meat N=35 (%)					MDR phenotypes	In patients' stool N=28 (%)				
	1 DR (%)	2 DR (%)	3 DR (%)	4 DR (%)	5 DR (%)		1 DR (%)	2 DR (%)	3 DR (%)	4 DR (%)	5 DR (%)
Amp/Nal/Tet/Cip			3 (8.7)			Amp/Cm		1 (3.5)			
Amp/Tet/Ery			1 (2.8)			Amp/Nal/Cip		1 (3.5)			
Amp/Cm/Tet/Ery				1 (2.8)		Amp/Tet		4			
Amp/Nal/ERY/Cip			1 (2.8)			Amp/Ery		(14.2)			
Nal/Tet		3 (8.7)				Amp/Tet/Cm		2 (7.1)	2 (7.1)		
Nal/Cip	2 (5.7)					Amp/Nal/Tet/Cip			1 (3.5)		
Cip	1 (2.8)					Amp/Gen					
Ery	1 (2.8)					Amp/Gen/Tet/Ery		1 (3.5)		1 (3.5)	
Tet/Ery		1 (2.8)				Amp/Gen/Tet			1 (3.5)		
Tet/Ery/Cip			2 (5.7)			Nal/Cip/Ery					
Nal/Tet/Cip		2 (5.7)				Nal/Cip	1 (3.5)	2 (7.1)			
Ery/Cip		1 (2.8)				Cm/Ery					
Cm/Tet/Ery			1 (2.8)			Cm/Tet/Ery		3	1 (3.5)		
Tet/Cip		1 (2.8)				Tet/Ery		(10.7)			
Tet	1 (2.8)					Nal/Cm/Gen/Cip			3		
Gen/Cm/Tet			1 (2.8)			Cm/Gen/Tet		2 (7.1)	(10.7)		
Gen/Ery		1 (2.8)				Gen/Tet			1 (3.5)		
Gen/Cm		1 (2.8)				Nal/Gen/Ery/Cip					
Gen/Nal/Tet/Ery/Cip				2 (5.7)		Ery	1 (3.5)	1 (3.5)	1 (3.5)		
Gen/Nal/Ery/Cip			1 (2.8)			Susceptible	2 (7)				
Gen/Cm/Tet/Cip				1 (2.8)							
Gen/Nal/Tet/Cip			1 (2.8)								
Gen	2 (5.7)										
Gen/Tet		1 (2.8)									
Susceptible	7 (19.8)										

statistically based on data on their antimicrobial resistance phenotypes, minimum inhibitory concentration levels of the antibiotics, and the existence of QRDR mutation. A Chi-square test using Graphpad Prism version 5.03 was used to estimate this correlation. Association between *Campylobacter* infection or contamination rate and age or gender of the patients and weight or brands of chicken meat was determined similarly. The p-value ≤0.05 was considered significant. Homology in resistance phenotypes among the isolates was analyzed by NTSYS 2.02 software.

RESULTS

Prevalence of *Campylobacter* infection and its frequency among chicken meat samples. In the present study, 400 stool specimens from patients who were referred to Tehran hospitals and 100 chicken meat specimens from urban distribution markets were analyzed. Nearly, 41% of the patients' samples were female (164/400) and 59% (236/400) were male. The patients were in different age groups, including children (1 month to 9 years old), adolescents (10-19 years old), and adults (≥20 years old). Most of the stool samples showed loose morphology in macroscopic analysis (329/400, 82.2%), while watery (62/400, 15.6%) and dysenteric (9/400, 2.2%) forms were also detected. The chicken meat samples belonged to 41 different brands, in ranges of 0.76 Kg to 2.71 Kg (mean 1.76 ± 0.46 Kg).

Campylobacter spp. was characterized in 35 chicken meat (35%) and 27 stool samples (6.75%). *C. jejuni* (65.7%, 23/35, and 88.9% (24/27), *C. coli* (2.86%, 1/35, and 7.4%, 2/27), and *C. lari*, 5.7%, 2/35, and 3.7%, 1/27) were among the characterized species in the chicken meat and stool isolates, respectively. One of the stool isolates showed infection with other members of the *Campylobacter* spp. The highest rate of infection was detected in patients between 2-9 years of age (13.8%, 5/36), while it was also present in other age groups (<1 year, 5.6%, 3/53; 10-19 years, 11.7%, 2/17; and ≥20 years, 6.1%, 18/294). In children, *Campylobacter* infection was detected mainly among cases ≤5 years of age.

Statistical analysis showed no significant difference in the isolation rate of *Campylobacter*, concerning forms of the stool and brands of the chicken samples (p value= 0.28). Moreover, no significant difference was detected in the prevalence of *Campylobacter* infection in patients with exudative diarrhea (11/167, 6.6%), based on the presence of mucus, leukocyte, red blood cell, and epithelial cells in direct microscopic smears, compared with those who presented secretory diarrhea (16/233, 6.9%), and between males (6.4%) and females (7.3%).

Antimicrobial resistance phenotypes and MIC values among the *Campylobacter* isolates. High rates of resistance to antibiotics were detected among both types of the *Campylobacter* isolates. Although the frequency of resistance to tetracycline (53.5% and 62.8%), erythromycin (39.2% and 37.1%), clindamycin (39.2% and 17.1%), and gentamicin (32.1% and 31.4%) was relatively similar among the meat and stool isolates, higher frequency of resistance to ciprofloxacin (51.4% vs 28.6%) and nalidixic acid (42.15% vs 28.6%) among the chicken meat, and ampicillin (50% and 17.1%) among the human stool, was detected. The diversity of MIC values and minimum inhibitory concentrations of antimicrobials required to inhibit 50% (MIC_{50}) and 90% (MIC_{90}) of the isolates were examined in this study. MIC_{50} and MIC_{90} values for the chicken meat and human feces isolates were as follows, respectively: clindamycin (4 and 32 vs 6 and 16 µg/ml), gentamicin (16 and 32 vs 4 and 32 µg/ml), ampicillin (16 and 64 vs 16 and 64 µg/ml), nalidixic acid (32 and 128 vs 12 and 64 µg/ml), tetracycline (15 and 27 vs 19 and 27 µg/ml), erythromycin (15 and 25 vs 16 and 27 µg/ml), and ciprofloxacin (19 and 27 vs 22 and 27 µg/ml), respectively.

The *gyrA* (Thr-86-to-Ile) mutation and its frequency among the chicken meat and human isolates. PCR was used for the detection of *gyrA* mutation (Thr-86-to-Ile) among the ciprofloxacin-resistant *C. jejuni* isolates. The results showed the existence of this mutation in all the stools (77.7%, 14/18) and 75% (6/8) of the chicken meat and stool isolates, respectively. PCR result for the susceptible strains was negative for the QRDR locus (Fig. 1).

Investigation of the relationship between the human and chicken meat isolates based on the phenotypic resistance patterns. The similarity of resistance phenotypes among different meat and stool isolates was measured using NTSYS software. Accordingly, 16 pairs of the chicken meat and human feces isolates showed identical resistance patterns; where some of them showed similar dates and places of collection (Fig. 2). Homology of the chicken meat and stool

isolates at distinct clusters was supported moreover through similarity in MIC values and the presence of *gyrA* mutation (Fig. 2).

DISCUSSION

The level of contamination with *Campylobacter* in poultry meat varies from region to region and this contamination can cause human campylobacteriosis upon transmission through the food chain (15). Studies and results of surveys of infections caused by campylobacteriosis indicated a growing trend in the number of disease cases over the past decade in North America, Europe, and Australia. Although epidemiological data from Africa and Asia are incomplete, current evidence suggests that infection with *Campylobacter* spp. is endemic in these areas (16, 17). Among the known sources of this infection, chicken meat is recognized as an important source of *Campylobacter* transmission to humans (18). The infection is mainly limited to children under 2 years

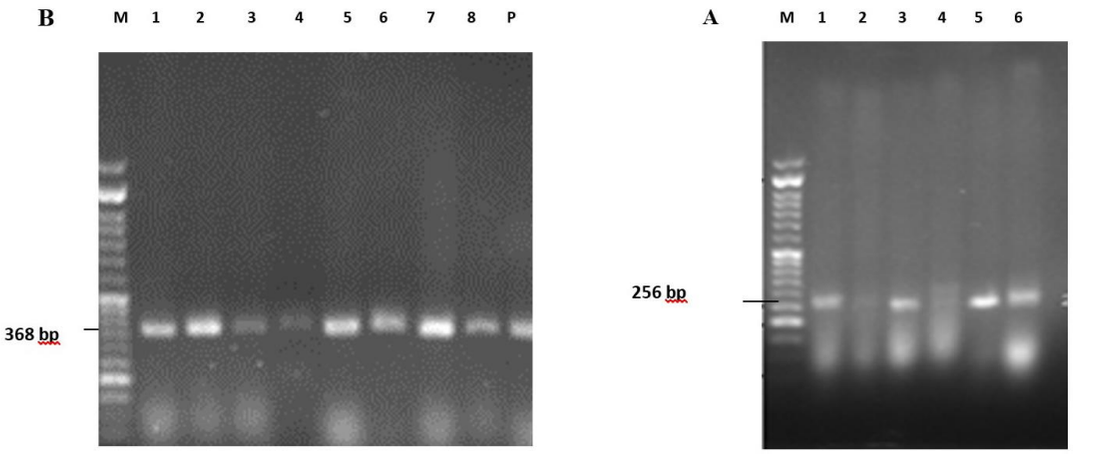

Fig. 1. (A) Detection of the ACA -> ATA mutation was done using MAMA-PCR using A1-A5 primers. (B) PCR using CampyMAMAgyrA1-A4 primers was done as control.

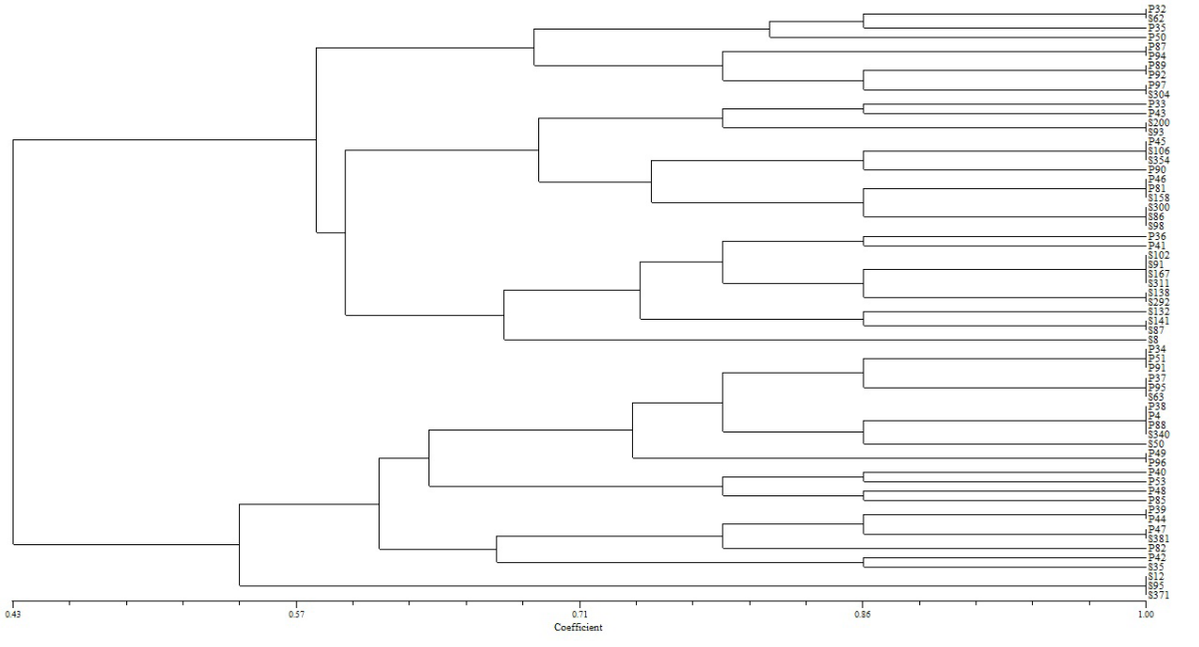

Fig. 2. Phenetic dendrogram for *Campylobacter* strains isolated from chicken meat and human stool samples in Tehran. The S and P codes represent ID related to stool and poultry meat samples, respectively. Homology of the strains was shown as percentage of coefficient.

of age; however, other age groups may also be affected (19). In this study, the prevalence of *Campylobacter* infection among children <9 years was higher than in other age groups. Perhaps one reason for the high prevalence of *Campylobacter* infection among children and adolescent is their relatively immature immune defenses, higher attack rate, and favorable conditions for the *Campylobacter* infection (20). The high prevalence of *Campylobacter* spp. in raw chicken meat samples proposed this product as a major source of the infection in humans in Tehran, which was consistent with other studies. In a study by Divsalar et al. they reported a frequency of 34.33% among the food samples, including chicken meat, in the north of Iran (21). A similar rate of contamination (40.8%) was detected among the chicken meat samples in Gorgan by Raeisi et al. (22). By contrast, in a study by Maktabi et al. in Ahvaz, 0% frequency was reported for contamination of fresh packed chicken meat samples (23).

Molecular analysis of *Campylobacter* species revealed an overall prevalence of 6.7% in symptomatic patients with community-acquired gastroenteritis. This frequency was similar to other reports from southern Ireland (4.7%) (24), India (7.0%) (25), Australia (6%) (26), and Kenya (5.8%) (27). Among *Campylobacter* species, *C. jejuni* showed the highest level of contamination in the chicken meat as well as the patients' stool samples. Although *C. jejuni* followed by *C. coli* is the predominant species in most studies in patients with gastroenteritis, diversity in the frequency of other species could be seen in different countries. In our study, *C. jejuni* and *C. coli* account for 88.9% and 7.4% of all *Campylobacter* species detected, while they showed a frequency of 66% and 6.7% among the isolates in Southern Ireland, respectively, where *C. ureolyticus* (22.3%) showed high frequency (24). We also found *C. lari* and uncommon species of *Campylobacter* in the meat and stool samples, which show their role in the etiology of the infection in Iran. The involvement of other *Campylobacter* species, such as *C. fetus, C. upsaliensis, C. rectus, C. concisus, C. gracilis, C. troglodytis, C. hyointestinalis, C. curvus, C. showae, C. hominis,* in human intestinal and extraintestinal infections was similarly described by other studies (28). In a study in Poland, *Campylobacter* was isolated from 38.6% of poultry meat and 9.6% of 0-4-year-old children's stool samples (32). In our study, infection among the patients in the same age group was similar (8.1% (6/74)); however, we detected a higher amount of contamination among the poultry meat samples.

Comparison of results of antimicrobial resistance tests among the chicken meat and patients isolates showed higher frequencies of resistance rates in our study, except for ciprofloxacin which was higher in Poland (71.4% and 74.1% vs 51.4% and 28.6% among the chicken meat and patients isolates in Poland and Iran, respectively). Our isolates showed also higher resistance rates to erythromycin, gentamicin, and tetracycline in the age group 0-4 years; however, resistance to ciprofloxacin was lower among the patients, similarly. Multi-drug resistance phenotype was characterized only in poultry meat samples in Poland, while it was present in both the chicken meat and the stool samples in Iran. Resistance phenotype Cip+Tet+Ery was the highest MDR pattern detected in the poultry meat samples in Iran and Poland (29).

In a study in India that was done on admitted patients with diarrhea, *Campylobacter* spp. was isolated from ~7% of the patients with no trend in seasonality (27). *C. jejuni* was predominant (75%), while *C. coli, C. lari, C. upsaliensis,* and *C. fetus* were also detected in these patients. The infection was significantly higher in children <5 years of age (10%) compared with those in other age groups (3.7%). While resistance to nalidixic acid and fluoroquinolones (97% vs 28.6%) and ampicillin (26.1% vs 17.1%) was higher among the Indian isolates, resistance to tetracycline (17.6% vs 62.8%), erythromycin and gentamicin (<3% vs 37.1% and 31.4, respectively) were higher among the Iranian isolates. MDR phenotype was detected in 36% of the Indian isolates which was similar to our results. Similarly, most *C. jejuni* isolates had a mutation in the QRDR region of *gyrA* (Thr-86 to Ile) (27).

In a study that was conducted by Aksomaitiene et al. in Lithuania in 2019 antibiotic resistance in poultry and human fecal isolates was detected. Although resistance to ciprofloxacin (91.5%), ceftriaxone (60.4%), and tetracycline (37.8%) were observed, only three species of *C. jejuni* were resistant to erythromycin (0.9%) and all species of *Campylobacter* were sensitive to gentamicin (33). In another study, a high frequency of antibiotic resistance among *Campylobacter* isolates from chicken meat and human feces was detected, especially to ciprofloxacin (92.5%), nalidixic acid (88.9%), tetracycline (68.4%), but it was lower for erythromycin (0.8%) (34). The presence of a 100% resistance rate to nalidixic acid vs a 0%

rate to erythromycin was shown by Rawat et al. (30).

Antimicrobial resistance of *Campylobacter* isolates among chickens and human feces was studied in Poland in 2008, when the highest resistance in the two groups was related to ciprofloxacin (> 40%), followed by ampicillin (13-21%) and tetracycline (8-29%). Resistance to tetracycline was nearly two times higher in our study (62.8% and 53.5% in poultry and fecal isolates, respectively) which could be due to the excessive use of antibiotics. In a recent five-year study, it was shown that 70% of *Campylobacter* isolates from children with and without symptoms of diarrhea were resistant to ciprofloxacin (35). In a similar study in 2017, all isolates (100%) were resistant to ciprofloxacin and nalidixic acid, while 77.4% were resistant to tetracycline, 35.5% to gentamicin, 25.8% to clindamycin, and 9.9% to erythromycin and azithromycin (31). In our study, resistance to clindamycin in fecal samples (39.2%) and erythromycin in both types of the samples (39.2% and 37.1%) was higher.

The basic antibiotics for the treatment of human campylobacteriosis are fluoroquinolones (e.g. ciprofloxacin) and macrolides (e.g. erythromycin). Resistance to fluoroquinolones was observed around 1903 through point mutations in the *gyrA* gene. Increasing resistance to fluoroquinolones has recently been expressed as a major concern. Various mutations in the *gyrA* gene have also been reported to induce resistance to fluoroquinolones in *C. jejuni* isolates. The-86-to-Ile (ACA-> ATA) mutation at codon 86 of the *gyrA* gene in *C. jejuni* is the most common mutation leading to fluoroquinolone resistance (32). In our study resistance to ciprofloxacin was detected among both human and chicken meat samples despite its limitation for usage in poultry. Similarly, the use of fluoroquinolones has been banned in poultry farms in many countries, including Australia and Northern Europe (33). In 2005, the United States prohibited the use of fluoroquinolone and enrofloxacin in poultry farms, while 22% resistance to ciprofloxacin was reported among *C. jejuni* isolates from poultry and humans in 2013 (5). Similarly, a high rate of resistance to ciprofloxacin was detected among human isolates in Denmark (23%) and Spain (23% to 92%) in 2013; while no resistance was observed in Finland (34).

Macrolides are alternative medication for complicated patients who experience an infection with the fluoroquinolones resistant strains (35). Erythromycin resistance was reported as low as 1.1% in a study among Finnish patients from 2003 to 2005 (14), compared with 38.1% in isolates from avian and human samples in our study. Resistance to macrolides was reported in different *Campylobacter* species isolated from humans (Ranges from 0.8% in South Korea and 2.2% in the United Kingdom up to 12.5% in Thailand, 22.2% in India, and 21.8% in China (36), which showed relatively lower frequency compared with our results (37.1%). In the case of clindamycin, a study showed that only 2% of *Campylobacter* isolates were resistant to clindamycin (37), whereas, in our study, 17.1% and 39.2% of chicken and human isolates were resistant, respectively, indicating an upward trend.

In the current study, the MDR phenotype was detected in 42.8% of the *Campylobacter* isolates from the chicken meat and 51.8% of the stool samples, respectively. The resistance patterns were not linked to specific brands of the chicken products, the production date, and city-regions. Previous studies showed the high frequency of MDR patterns in *Campylobacter* spp. could cause through the application of low doses of antibiotics in broiler herds that are prescribed for the prevention of infections during the rearing period. The dominance of this phenotype in food animals and its spread to humans is responsible for treatment failure, which is a concern (38).

Alteration of the nucleotide at codon 86 (ACA to ATA) of the *gyrA* is the main mechanism of resistance to fluoroquinolones in *C. jejuni* (32). Our study confirms the existence of this mutation among the resistant strains. Researchers showed that resistance to fluoroquinolones can occur very quickly, leading to failure in treatment and also relapse of symptoms (39). In one study on ciprofloxacin resistance in the United States, changes in resistance rate were reported from 57% to 96% over just two years, which shows the importance of control programs for the prevention of any further increase (40).

Children are at higher risk of infection with *Campylobacter* compared with adults. The inclusion of a low number of children's stool samples compared to older age groups was the main limitation of our study.

CONCLUSION

The results obtained in the present study showed infection with different species of *Campylobacter* among symptomatic patients with community-acquired diarrhea. The infection was characterized in all age groups, with the highest frequency among

children (2-9 years). A high percentage of *Campylobacter* contamination in various brands of distributed poultry meat samples proposed the role of this product in human infection. This contamination was largely caused by *Campylobacter jejuni*, although other species, such as *C. coli* and *C. lari*, were also identified. Homology of the antibiotic resistance patterns observed between human and poultry meat isolates confirmed the possible link; however, further studies are needed to demonstrate this relatedness at the genomic level. The high resistance rate to fluoroquinolones, gentamicin, and tetracycline, and the development of MDR patterns among chicken and human isolates signified these isolates as an important risk factor for treatment failure upon the occurrence of invasive infections.

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