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Article

Investigating the Significance of Non-Jejuni/Coli *Campylobacter* Strains in Patients with Diarrhea

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Abstract: *Campylobacter* is one of the most commonly reported foodborne bacteria worldwide. Although *C. jejuni* and *C. coli* have been reported to be responsible for the great majority of campylobacteriosis, the burden of infections by species other than *C. jejuni* and *C. coli* have been increasing as a result of a transition to diagnostic test methods that enable the isolation of emerging species. The aim of the present study was to recover *C. jejuni*, *C. coli*, and emerging species from the stool samples of 500 patients with gastroenteritis and 100 healthy subjects by use of a filtration method and culture techniques using Butzler agar and mCCDA under microaerophilic or hydrogen enriched atmosphere, identify the species by multiplex PCR methods and assess the significance of emerging species in enteric diseases. Thirty-one (6.2%) *Campylobacter* spp. were isolated from the stool samples of diarrheic patients but none from healthy individuals. Of 31 isolates, 21 (67.8%), 9 (29%) and 1 (3.2%) were identified as *C. jejuni*, *C. coli* and *C. concisus* by multiplex PCR, respectively. Filtration method was superior to the culture technique using mCCDA under microaerophilic atmosphere. *C. concisus* was evaluated as the etiology of gastroenteritis as a result of laboratory and clinical evaluations. The present study was the first to indicate that emerging *Campylobacter* species are rarely detected and *C. concisus* is linked to acute gastroenteritis in Turkey where additional studies are warranted to clarify the significance of emerging species in gastroenteritis.

Keywords: *Campylobacter* spp.; *C. concisus*; gastroenteritis; multiplex PCR; non-jejuni/coli

1. Introduction

Campylobacter is a Gram negative, spiral shaped bacterium that is responsible for foodborne infections which transmit to human mainly by consumption of undercooked broiler meat. Campylobacteriosis was the most frequently reported bacterial foodborne infection in the United States of America and the European Union (EU) affecting 7,208 and 127,840 individuals in 2020 and 2021, respectively [1,2]. *Campylobacter* infections are also endemic with the pooled prevalence ranging from 8 to 10% in Africa and Asia [3–5].

Campylobacter infections are characterized by mild to moderate diarrhea that is generally self-limited. However, enteritis can be life threatening due to severe dehydration especially among neonates, elderly individuals, and immunosuppressed patients. Campylobacteriosis is an important public health concern of global importance because of the post infectious complications, including Guillain-Barré syndrome, reactive arthritis, irritable bowel syndrome and various systemic infections such as bacteremia, especially among the elderly and immunosuppressed individuals together with the concern for the treatment of the infections due to increased antibiotic resistance [6].

C. jejuni and *C. coli* have been reported to account for the great majority of the infections worldwide, although *C. fetus*, *C. upsaliensis*, and *C. lari* were also clearly linked to human infections [7]. However, the tendency for higher recovery rates of *C. jejuni* and *C. coli* can be due to bias that results from the preference of the diagnostic techniques (using antibiotic-containing selective media,

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microaerophilic atmosphere without H_2 enrichment, incubation at 42 °C, and shorter incubation time) that are inappropriate for the detection of non-jejuni/coli species. By using a filtration method performed on non-specific media at 37 °C under a hydrogen enriched microaerophilic atmosphere or a PCR technique performed directly on fecal samples, non-jejuni/coli species were reported to account for as high as 50% of *Campylobacter* species isolated from human [8,9]. Although there has recently been an increase in the detection of emerging species, healthy subjects were not included in most of the studies that reported the isolation of the strains from patients. Moreover, in a limited number of studies that investigated the presence of the strains both in patients with acute gastroenteritis and in a control group, the role of less commonly detected species, especially those that are human hosted such as *C. concisus*, in gastroenteritis was controversial. Thus, the role of *C. concisus*, if any, in gastroenteritis remains to be elucidated [10]. On the other hand, together with the implementation of molecular biology and sequencing techniques, accumulating data indicate that *C. concisus* may be associated with inflammatory bowel diseases such as active Crohn's disease [11].

Campylobacter spp. is one of the two most frequently detected bacteria among patients with acute gastroenteritis in Turkey [12]. Although *C. jejuni* and *C. coli* are the most frequent causal agents of campylobacteriosis, little is known about the occurrence of species other than *C. jejuni* and *C. coli* and their role in gastroenteritis in Turkey because culture methods used for the recovery of *Campylobacter* species are biased towards the detection of *C. jejuni* and *C. coli*.

In this study, it was aimed to investigate the presence of *Campylobacter* species both in patients with acute gastroenteritis and healthy individuals, identify the species using the multiplex PCR (mPCR) method, determine the utility of phenotypical tests in identification and assess the significance of emerging species in gastroenteritis.

2. Materials and Methods

2.1. Study Population

The sample size (n) of the study was determined as 500 according to the formula:

$n = t^2 . p . (1-p)/d^2$; where; t = z-score value at 95% confidence level (standard value of 1.96), p= prevalence of non-jejuni/coli *Campylobacter* spp. [estimated to be 6.4% a result of the literature review [13–19]; d = margin of error (2%).

Stool samples were collected from the patients and 100 healthy individuals between December 2016 and January 2018. Only one sample from each individual was tested. The research was approved by Istanbul University Istanbul Faculty of Medicine Ethics Committee (protocol code 2015/1246 and date of approval: 26 June 2015).

2.2. Campylobacter Isolation

2.2.1. Culture Method

Stool samples were directly inoculated on two modified charcoal cefaperazone deoxycolate agar (mCCDA, Oxoid, England) and a Butzler agar (Oxoid, England). Butzler agar and one of the mCCDA were incubated at 37 °C for 72 hours under the classical microaerophilic atmosphere (5% O_2, 10% CO_2, 85% N_2) generated using gaspak kits (Becton Dickinson, USA). The remaining mCCDA was incubated for six days at 37 °C under H_2-enriched microaerophilic atmosphere consisting a gas mixture of 1.5% O_2, 7% H_2, 10% CO_2 and 81.5% N_2 created by a gas delivery system.

2.2.2. Filtration Technique

Ten drops of a stool suspension prepared in sterile saline were deposited on a 0.6-µm-pore-size membrane filter which was applied on the center of tryptose agar (Oxoid, England) supplemented with 5% unlysed horse blood. Following a 15–20-minute filtration process, the filter was removed under aseptic conditions and the medium was incubated at 37°C under microaerophilic environment enriched with H_2 for six days [20].

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2.3. Identification of Campylobacter Species

2.3.1. Phenotypical Identification

Oxidase positive, L-alanine negative, Gram negative bacteria with the characteristic gull-wing microscopical morphology were identified as *Campylobacter* spp. Strains that hydrolyzed hippurate was identified as *C. jejuni*. Catalase production, indoxyl acetate, pyrazinamide and urea hydrolysis, nitrate reduction, H_2S production using a strip test and triple sugar iron (TSI) agar, ability to grow at 25 °C and 42 °C, in 1% glycine, on Mac Conkey agar, in the presence of hydrogen enriched atmosphere and cephalotin and nalidixic acid sensitivities of the strains were investigated [20–22].

2.3.2. Molecular Identification

DNAs of the strains were extracted from the colonies using a commercially available DNA extraction kit (Invitrogen; PureLink, USA). Genus level identification of the isolates was confirmed by using C412F and C1288K primer pairs that were specific to 16 S rRNA gene (Table 1). PCR mixture consisted of 2.5 mM $MgCl_2$, 200 µM dNTP, 0.4 µM primer pairs, 0.625 U Taq DNA polymerase, 1X Taq buffer ve 5 µl target DNA at the final volume of 50 µl. Amplification program was adjusted as follows: 94 °C 1 min, 55 °C 1 min and 72 °C 1 min for 25 cycles [23].

C. jejuni, *C. coli*, *C. lari*, and *C. upsaliensis* were identified by mPCR test-1 and *C. fetus, C. sputorum, C. curvus, C. helveticus*, and *C. mucosalis* by mPCR test-2 using primers specific to *lpxA* (Table 1). 50 µl PCR reaction mixture included 2.5 U Taq DNA polymerase, 1× Taq buffer, 200 µm dNTP, 10 pmol/µl each of forward primers, 30 pmol/µl reverse primer and 3µl target DNA [24]. The amplification programs of both of the tests were similar to the genus identification program except elongation step that was performed at 60 °C for 1 min [25]. Inconsistent results between phenotypical and mPCR tests for the identification of *C. jejuni* were confirmed by a PCR test using the primers Hip400F and Hip1134R (Table 1) which were specific to *hipO* gene as described previously [23].

For the differentiation of *C. concisus* and *C. mucosalis*, mPCR test-3 including Con1, Con2 and Muc1 primers that were specific to 23S rDNA was performed (Table 1). The reaction mixture at the final volume of 50 µl was as follows: 2.5 U Taq DNA polymerase, 1× PCR buffer, 200µm dNTP, 5 pmol/µl of Con1 and Con2 primers each, 10 pmol/µl Muc1 primer, 1.2 mM $MgCl_2$, and 3µl target DNA. Amplification program was similar to that of mPCR test-1 and -2 [24,26]. Negative control that consisted distilled water instead of target DNA and positive control that included DNAs extracted from the quality control strains (Table 2) were used for each run. PCR products were visualized by ethidium bromide following gel electrophoresis using 1% and 2% agarose for genus and species identification, respectively.

2.4. Antibiotic Susceptibility Testing

Ciprofloxacin, erythromycin, and tetracycline susceptibilities of the isolates were investigated by disk diffusion method as suggested by European Committee on Antimicrobial Susceptibility Testing (EUCAST) [27]. Because EUCAST did not provide clinical breakpoints of the antibiotics for non-jejuni/coli strains, antibiotic susceptibility testing of the strains was interpreted according to breakpoints provided for *C. jejuni* and *C. coli* strains.

2.5. Statistical Analysis

Statistical analysis of the data was carried out by Fischer's exact test. A p value less than 0.05 was considered as statistically significant.

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Table 1. Sequences of the primers used for the genus and species level identification.

Gene	Primer sequence	Amplicon size (bp)	Reference
***Campylobacter* genus**			
C412 F	5'GGA TGA CAC TTT TCG GAG C 3'	816	[23]
C1288 R	5'CAT TGT AGC ACG TGT GTC 3'		[23]
***Campylobacter* spp.**			
IpxAC. *coli*	5'AGA CAA ATA AGA GAG AAT CAG 3'	391	[25]
IpxAC. *jejuni*	5'ACA ACT TGG TGA CGA TGT TGT A 3'	331	[25]
IpxAC. *lari*	5'TRC CAA ATG TTA AAA TAG GCG A 3'	296	[25]
IpxAC. *upsaliensis*	5'AAG TCG TAT ATT TTC YTA CGC TTG TGT G 3'	206	[25]
CmucLpxA	5'GTA GGC AAA AAT GAG TAA AAT TCA TCA TA 3'	381	[William G.
CsputLpxA	5'TAC TAT TGG AGA TGG CGG AAA AGT ATT TAG C 3'	222	Miller,
CfetLpxA	5'CGT TAG TTA CCG TCC AGA AGA AAA TAC A 3'	162	personal
ChelLpxA:	5'GAC AAA TTC ATT CTA GTG CAG TGA TT 3'	367	communicat
CcurvLpxA:	5'GCA AGA GTC ATC GGA AAC ACG CAA ATA 3'	242	ions]
IpxARKK2m (reverse)	5'CAA TCA TGD GCD ATA TGA SAA TAH GCC AT 3'		[23]
Con1	5'CAG TAT CGG CAA TTC GCT 3'		[26]
Con2	5'GAC AGT STC AAG GAT TTA CG 3'	306	[26]
Muc1	5'ATG AGT AGC GAT AAT TCG G 3'		[26]
HIP400F	5'GAA GAG GGT TTG GGT GGT G'3	735	[28]
HIP1134R	5'AGC TAG CTT CGC ATA ATA ACT TG'3		[28]

Table 2. Quality control strains included in the study.

Quality Control Strain	Collection Number
C. jejuni	NCTC 1168 (RM1862)
C. coli	RM2228
C. upsaliensis	RM3195
C. lari	RM2100
C. fetus	82-40 (RM15492)
C. curvus	525.92 (RM4077)
C. helveticus	ATCC 51209 (RM3228)
C. concisus	13826 (RM5485)
C. mucosalis	ATCC 43264 (RM4114)
C. sputorum	CCUG 20703 (RM4121)

3. Results

Macroscopical and microscopical analysis of the stool samples and the distribution of the gender and hospital status of the patients are shown in Table 3.

Table 3. Distribution of the patients according to gender, hospital status, macroscopical and microscopical analysis of the stool samples [n(%)].

Gender[1]		Hospital Status[2]		Macroscopical Analysis of the Stool Samples[3]							Microscopical Analysis of the Stool Samples[4]	
F	M	O	I	W	WP	L	LP	WBP	WB	LB	PNL(+)	PNL (-)
239	261	389	111	175	173	119	18	11	3	1	168	332
(52.2)	(47.8)	(77.8)	(22.2)	(35)	(34.6)	(23.8)	(3.6)	(2.2)	(0.6)	(0.2)	(33.6)	(66.4)

[1] F: Female, M: Male. [2] O: Outpatient, S: Inpatient. [3] W: Watery; WP: Watery with pus; L: Loose; LP: Loose with pus; WBP: Watery with blood and pus; WB: Watery with blood; LB: Loose with blood. [4] PNL (+): Polymorphonuclear leukocytes detected; PNL (-): Polymorphonuclear leukocytes not detected.

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Campylobacter was isolated from 31 (6.2%) patients of whom 18 were male and 13 were female. Of the 31 patients, 26 were out-patients and five were in-patients. PNLs were detected in 13 (42%) of 31 patients. More than 70% (n=22/31) of the stool samples collected from the patients who were infected with *Campylobacter* were either watery or watery with pus. *Campylobacter* spp. was not isolated from any of the healthy individuals.

Genus level identification of all strains that were identified as *Campylobacter* spp. by phenotypical methods was confirmed by PCR. Of 31 strains, 21 (67.7%) were identified as *C. jejuni*, nine (29%) as *C. coli*, and one (3.2%) as *C. concisus* by mPCR tests.

Isolation of *Campylobacter* species by filtration technique, culture method using Butzler agar and mCCDA under microaerophilic and hydrogen enriched atmosphere are shown in Table 4. Filtration method was superior to culture technique using mCCDA under microaerophilic atmosphere (p = 0.0240) whereas no significant difference was found among other methods.

Table 4. Recovery of *Campylobacter* species by the filtration technique and culture methods using different media and incubation atmosphere (n).

	Filtration	Butzler agar	mCCDA[1]	mCCDA[2]
C. jejuni (n=21)	21	20	18	18
C. coli (n=9)	9	9	7	6
C. concisus (n=1)	1	1	1	1
Total (n=31)	31	30	26	25

[1] Incubated under hydrogen-enriched atmosphere. [2] Incubated under microaerobic atmosphere.

Oxidase test, indoxyl acetate hydrolysis, nitrate reduction, H_2S strip test, growth at 42 °C and in 1% glycine were positive whereas H_2S production on TSI, growth at 25 °C and on Mac Conkey agar were negative for all of the strains. Hippurate hydrolysis test was found to be positive and negative for all of the *C. jejuni* and *C. coli* strains, respectively. *C. concisus* hydrolyzed hippurate but was negative for *hipO* gene. Catalase test was positive in all of the *C. jejuni* and *C. coli* isolates but negative in the *C. concisus* strain. Pyrazinamidase test revealed variable results for *C. jejuni* and *C. coli* strains. All of the *C. jejuni* and *C. concisus* strains were resistant to cephalotin whereas only one of the *C. coli* strains was susceptible to the antibiotic. All *C. coli* and *C. concisus* strains and 16 of 21 *C. jejuni* isolates were resistant to nalidixic acid (Table 5).

Table 5. Positive test results of biochemical characteristics according to the species (n).

Species	Oxidase	Catalase	Hippurate	Indoxyl acetate	Pyrazinamidase	Nitrate	H_2S strip	Urease	H_2S TSI	25 °C	42 °C	1% glycine	Amino-peptidase	Mac-Conkey	Cephalotin (R)[1]	Nalidixic acid (R)[1]
C. jejuni (n=21)	21	21	21	21	20	21	21	-	-	-	21	21	-	-	21	16
C. coli (n=9)	9	9	9	9	7	9	9	-	-	-	9	9	-	-	8	9
C. concisus (n=1)	1	-	1	1	1	1	1	-	-	-	1	1	-	-	1	1

[1] R: Resistant.

Of 31 isolates, 29 (93.5%), 17 (54.8%) and 1 (3.2%) were resistant to ciprofloxacin, tetracycline and erythromycin, respectively. Of 21 *C. jejuni* strains, 19, 13 and 1 were resistant to ciprofloxacin, tetracycline and erythromycin, respectively. All *C. coli* strains were resistant to ciprofloxacin and susceptible to erythromycin. Tetracycline resistance was detected in three *C. coli* isolates. *C. concisus* strain was resistant to ciprofloxacin and tetracycline but sensitive to erythromycin.

C. concisus was isolated from a 61-year-old woman. The patient had laparoscopic colesystectomy, diabetes, constipation, hypertension, cardiac dysrhythmia, atrial fibrillation, and hypothyroidy. She presented to the emergency unit with complaints of vomiting, fatigue, abdominal cramp, and bloody diarrhea three times a day. Serum C-reactive protein (CRP) level was elevated. PNLs were detected in microscopical analysis of the loose stool sample collected from the patient. *Salmonella*, *Shigella*, *Aeromonas*, *Plesiomonas*, *Yersinia*, and *Vibrio* spp. were not detected in the routine

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stool culture. The patient was discharged from the hospital after initiation of ampiric metronidazole and ciprofloxacin treatment.

4. Discussion

Campylobacteriosis is one of the most frequently detected bacterial foodborne infections worldwide. Two termotolerant species, *C. jejuni* and *C. coli*, have been reported to be responsible for the vast majority of the infections [7]. However, culture methods and incubation conditions generally used by the laboratories for the isolation of thermotolerant species do not to support the isolation of non-thermotolerant strains that are often susceptible to antibiotics included in the selective media and require prolonged incubation under hydrogen-enriched atmosphere. Thus, the occurrence of non-jejuni/coli strains of human origin is not well-recognized or underestimated. Lastovica *et al*. [8,13] recommended Cape Town protocol based on a filtration technique using non-selective medium and hydrogen-enriched atmospheric environment as an alternative to conventional culture method. The protocol was found to increase the isolation rate of *Campylobacter* spp. by three-fold compared to the direct culture on selective media [8]. Filtration technique was reported to be as efficient as culture method using mCCDA and more appropriate for the screening of all *Campylobacter* species and *Campylobacter*-like bacteria such as *Arcobacter* spp. [17]. In the present study, all of the 31 *Campylobacter* strains that were recovered from 500 patients were isolated by using Cape Town protocol. Of 31 strains, 30 were recovered by traditional culture on Butzler agar under microaerophilic atmopshere whereas 26 and 25 of the strains were detected on the mCCDA under hydrogen-enriched and microaerophilic atmospheric conditions, respectively. The filtration method was found to significantly increase ($p=0.024$) the isolation rate of *Campylobacter* spp. by 1.2 fold compared to the traditional culture method using mCCDA under microaerophilic atmosphere, whereas the recovery rates of *Campylobacter* by the Cape Town protocol and the conventional culture method using either Butzler agar under microaerophilic atmosphere or mCCDA under hydrogen enriched atmosphere were comparable.

In a study carried out in South Africa using Cape Town protocol, *C. jejuni* was the most frequently (32.3%) isolated species followed by *C. concisus* (25%) and *C. upsaliensis* (24%). Interestingly, species other than *C. jejuni* and *C. coli* accounted for approximately half of the *Campylobacter* species [8]. Vandenberg *et al*. [18] reported increased recovery of *C. upsaliensis*, *C. concisus* and various other emerging species using filtration method and incubating the media under hydrogen-enriched atmosphere. In another study that was carried out in Denmark including 11,314 stool samples collected from patients with diarrhea, *C. concisus* (3.9%) was isolated as high as *C. jejuni* and *C. coli* (4.8%) by filtration technique [15]. *C. jejuni* was the most frequently recovered species in the present study followed by *C. coli*. The two species were responsible for the great majority (96.7%) of the infections. Only one of the 31 strains was non-jejuni/coli, representing 0.2% of the patients and 3.2% of the *Campylobacter* species. The species distribution found in this study that revealed the predominance of *C. jejuni* and *C. coli* is comparable to the findings of the studies carried out in Turkey using classical culture techniques and incubation conditions that were optimal for the growth of thermotolerant species [29]. Moreover, the overall isolation rate of *Campylobacter* spp. that was 6.2% in this study is comparable to that of another study in which the prevalence was 5.4% [30]. Thus, the present study indicates that non-jejuni/coli species are rarely detected and represent the minority of *Campylobacter* species in Turkey.

Because of the long turnaround time of the culture method and fastidious nature of *Campylobacter* species, PCR technique has recently been attractive for the investigation of *Campylobacter* spp. in the stool samples. Bullman *et al*. [31] investigated the presence of *Campylobacter* spp. in the fecal samples of patients with diarrhea using culture technique and molecular methods. Together with the overall increase in the detection of the *Campylobacter* species, less frequently isolated species such as *C. fetus*, *C. upsaliensis*, *C. lari*, and *C. hyointestinalis* represented 10% of the samples that were negative by culture. The authors reported *C. ureolyticus* as the second most frequently (41%) detected species following *C. jejuni* (51%).

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Partly as a result of implementation of highly sensitive molecular techniques in the diagnosis, the role of emerging *Campylobacter* species in gastrointestinal diseases has been controversial because the species have also been detected in healthy individuals. In a study that investigated the distribution of *Campylobacter*, *Helicobacter*, and *Arcobacter* species in the stool samples of healthy volunteers and patients with diarrhea, *C. concisus* and various non-jejuni/coli species, including *C. ureolyticus, C. hominis*, and *C. gracilis* were reported not to be associated with acute gastroenteritis because of the failure to show any significant difference in detection between patients and the control group [32]. Similar findings were also reported by Inglis *et al*. [33], Tilmanne *et al*. [16], and Van Etterijck *et al*. [19]. On the other hand, Collado *et al*. [34] reported a significant difference in the prevalence of *C. jejuni* and *C. concisus* between patients with acute gastroenteritis and healthy individuals, a finding that supported the role of the two species in intestinal disease. Similar findings supporting the role of *C. concisus,* a human hosted species of which the primary colonization site is oral cavity, in gastroenteritis were reported in various studies as a result of failure to recover the species in healthy volunteers, ruling out the infections by the most frequently detected enteropathogens or taking into consideration the clinical findings [15,35,36]. Data supporting the role of *C. concisus* in gastrointestinal diseases such as inflammatory bowel disease have recently been accumulating via whole genome sequencing studies that revealed variation in the genomic content, affecting the pathogenic potential of the strains isolated from healthy subjects and patients with gastrointestinal system disease [11,37]. In the present study, *C. concisus* was recovered from the stool sample of a 61-year old patient who was admitted to the emergency department with complain of bloody diarrhea three times a day. Clinical findings, laboratory test results (increased serum CRP level, presence of fecal polymorphonuclear leukocytes), ruling out infections by the most frequently detected bacterial acute gastroenteritis agents such as *Salmonella*, *Shigella*, *Aeromonas*, *Plesiomonas*, *Vibrio* spp. and the detection of the agent only among the patient group were evaluated as indications that *C. concisus* was the etiology of diarrhea.

In routine clinical microbiology laboratories, genus and species identification of *Campylobacter* species are generally carried out by the use of phenotypic methods. In our study, a wide variety of biochemical tests were performed to investigate the utility of phenotypic tests for species identification. Hippurate hydrolysis test, one of the most frequently used tests that discriminates *C. jejuni* from other species, yielded false positive result for the *C. concisus* strain. In addition to false positive hippurate test results for the strains that were reliably identified as species other than *C. jejuni* by molecular tests, false negative results were also reported that brought the reliability of the test in question especially for epidemiological investigations that are critical to intervene appropriate preventive strategies [29,30]. Nalidixic acid susceptibility testing was one of the historical phenotypical methods that was used for species level identification of *Campylobacter* species. However, taking into account the high rate (26 of 31 strains) of nalidixic acid resistance among *Campylobacter* spp., it is thought that the method has lost its significance in species identification especially in regions where quinolone resistance is high. Similarly, indoxyl acetate and pyrazinamidase tests and the ability to grow on MacConkey agar yielded incompatible results with generally accepted biochemical profiles of *C. jejuni*, *C. coli*, and *C. concisus*. Because phenotypic identification is challenging due to biochemically inactive nature of *Campylobacter* species and due to the lack of definitive tests, the use of molecular techniques is recommended for surveillance and risk assessment studies that require prompt and accurate species level identification.

Increase in the antibiotic resistance among *Campylobacter* spp. is an urgent global public health threat. In 2021, the average level of ciprofloxacin resistance in the EU was reported at high levels in *C. jejuni* (64.5%) and *C. coli* (69.6%) human isolates. Tetracycline resistance was high (45.3%) in *C. jejuni* and extremely high (70.3%) in *C. coli* whereas erythromycin resistance very low (1.1%) and low (8.5%) in *C. jejuni* and *C. coli*, respectively [38]. According to The National Antimicrobial Resistance Monitoring System (NARMS) 2018 data, occurrence of ciprofloxacin, tetracycline and erythromycin resistance was 28.8%, 42.2%, and 2% in *C. jejuni* and 40.5%, 60.1%, and 13.3% *in C. coli* isolates of human origin in the USA, respectively [39]. Ciprofloxacin resistance was reported extremely high (over 70%) whereas tetracycline and erythromycin resistance were detected as 25% and 5.9% in

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Turkey, respectively [40,41]. In the present study, the rate of erythromycin resistance (3.2%) was lower whereas the rates of ciprofloxacin and tetracycline resistance were higher (93.5% and 54.8%, respectively) than those in the previous studies conducted in Turkey highlighting the increase in the resistance rates of the latter two antibiotics in Turkey.

5. Conclusions

The recent increase in discovery of novel species alongside with the rise in accumulating data that link emerging species to infections or intestinal diseases have brought some difficulties into routine diagnosis and species level identification of clinical *Campylobacter* strains. Routine culture techniques and phenotypic identification methods used in the diagnostic laboratories may remain inefficient for the diagnosis and identification of species other than *C. jejuni* and *C. coli*. Nevertheless, epidemiological investigation by use of a culture method that supports the recovery of emerging species in complement with molecular diagnostic techniques will contribute to the understanding of their roles in enteric diseases. The present study that included 500 patients with gastroenteritis and 100 healthy subjects is the first to investigate species other than *C. jejuni* and *C. coli* and to link *C. concisus* to acute gastroenteritis in Turkey. Additional studies in the field are warranted to clarify the significance of non-jejuni/coli species in Turkey. The findings of this study are thought to provide valuable insights to the field.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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